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Expression of Root-Specific Genes in *Phaseolus vulgaris* L.

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The roots of higher plant are involved in the acquisition of water and nutrient, and anchorage of the plant. Plant roots are formed from a root meristem derived from the hypophysis and adjacent cells of the embryo proper by a regulated program that specifies the timing of cell division, orientation of the plane of cell division, and the extent of cell expansion (Steeves and Sussex, 1989; Dolan *et al.*, 1993). The apical meristem of the root is arranged into three primary meristem tissues: the protoderm, ground meristem, and procambium. These primary meristems by means of cell division and cell expansions give rise to cell files that develop into the epidermis, cortex, endodermis, and vascular cylinder, respectively (Dolan *et al.*, 1993; Aeschbacher *et al.*, 1994). Furthermore, root development is fairly uniform and reiterative fashion with no significant developmental transition (Esau, 1977). All stages of root development are apparent at all times. Thus, roots represent a useful organ for pattern formation and cell differentiation in plant (Schiefelbein and Benfey, 1991).

We are interested in the mechanisms controlling root development in higher plants. We analyzed endogenous cytokinins and change of protein pattern in the root development of *P. vulgaris* seedlings (Kim *et al.*, 1992; Choi *et al.*, 1993). Cytokinin levels were maintained homeostatically during root development (Kim *et al.*, 1992). Two-dimensional polypeptide gel electrophoresis showed that about 200 polypeptides were detected and 14 polypeptides were changed differentially in the coures of the root development (Choi *et al.*, 1993). The changes in protein pattern resulted from the change of certain gene expression. One approach to study molecular mechanisms involved in bean root development, we also isolated and characterized cDNA clones of mRNA expressed preferentially in the roots of bean seedlings (Choi *et al.*, 1996a; Choi *et al.*, 1996b). The present review summarizes the results obtained in these investigations.

THE ROOT-SPECIFIC GENES IN PLANTS

In spite of the unique developmental characteristics of the root, little is known about the genetic regulation of root development. Since root-specifically expressed genes may play a role in root development, some investigations have focused on molecular investigation of root-specific genes (Table 1).

The expression of genes during root development and the location of specific gene products within roots using *in situ* hybridization and β-glucuronidase (GUS) reporter gene expression provides clues about the understanding the function of those genes. In some of these root-specific genes, tissue-, or cell-specific expression was investigated. A root-specific lectin gene, *BLc3*, was expressed specifically in the outer cell layers of barley root (Lerner and Raikhel, 1989). Expression of *TobRB7*, encoding for a putative membrane channeling protein, was restricted to the root meristem and central cylinder region of tobacco (Conkling *et al.*, 1990; Yamamoto *et al.*, 1991). *γ-TIP* gene, encoding tonoplast intrinsic protein was expressed in the root cells of the elongation region (Ludevid *et al.*, 1992). *ZRP4*, encoding for an O-methyltransferase, was expressed in the endodermis of maize roots (Held *et al.*, 1993). *RH2*, encoding for a pathogenesis-related protein, was expressed in the epidermis of pea roots (Mylona *et al.*, 1994).

Secondary roots are initiated internally in the pericycle of the vascular cylinder and penetrate the surrounding tissues. *HRGPnt3*, encoding for an isotype of hydroxyproline-rich glycoprotein, *RSI-1*, encoding for a cysteine-rich protein, and *LRP1*, containing the conserved amino acid sequence of the zinc binding site of the activation domain of the protein kinase C family of proteins, were specifically expressed in emerging lateral roots of tobacco, tomato and arabidopsis, respectively (Keller and Lamb, 1989; Taylor

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Table 1. Summary of Genes Expressed Preferentially in Root.

Species	Gene	Tissue expressed[a]	Deduce protein[b]	Reference
Alfalfa	*A9*	NT	Proline-rich protein	Winicov and Deutch (1994)
Arabidopsis	*AHA2*	NT	Plasma membrane H^+-ATPas	Harper *et al.* (1990)
	AR25	NT	40S ribosomal protein	Shimizu *et al.* (1994)
	AR34	NT	F_1-ATPase γ-subunit	Shimizu *et al.* (1994)
	AR56	NT	Human factor XI	Shimizu *et al.* (1994)
	ARST1	NT	Kinase	Hwang and Goodman (1995)
	AtRB7	NT	Membrane Channel Protein	Yamamoto *et al.* (1990)
	LRP1	NT	Zinc binding	Smith and Fedoroff (1995)
	β1-tubulin	NT	β-Tubulin	Oppenheimer *et al.* (1988)
	γ-TIP	Elongation zone	Tonoplast intrinsic protien	Ludevid *et al.* (1992)
Barley	*BLc3*	Root cap and outer cell layer	Lectin	Lerner and Raikhel (1989)
	Ids2	NT	Dioxygenase	Okumura *et al.* (1994)
Bean	*PVR3*	Cortical cell	Lipid transfer protein	Choi *et al.* (1996b)
	PVR5	Cortical cell	Proline-rich 14 kDa protein	Choi *et al.* (1996a)
Brassica	*DR34*	NT	Pathogenesis-related protein	Park *et al.* (1993)
	R103	NT	Rupin-like protein	Park *et al.* (1993)
Carrot	*DcPRP1*	NT	Proline-rich Protein	Ebener *et al.* (1993)
Cowpea	*Ext3*	Root hair	Extensine	Arsenijevic-Maksinovic *et al.* (1997)
	Ext26	Root hair	Extensine	Arsenijevic-Maksinovic *et al.* (1997)
Cowpea	*Ext127*	Root hair	Extensine	Arsenijevic-Maksinovic *et al.* (1997)
Ice plant	*MCR23-1*	NT	Germin like protein	Michalowski and Bohnert (1992)
Maize	*MR19 NT*	NT	α-tubulin	Montoliu *et al.* (1989)
	ZRP3	Cortical cell	Proline-rich protein	John *et al.* (1992)
	ZRP4	Meristematic region	0-Methyltransferase	Held *et al.* (1993)
	pZSS1	NT	Asparagine synthatase	Chevalier *et al.* (1996)
Pea	*RH2*	Epidermis	Pathogen related protein	Mylona *et al.* (1994)
Soybean	*SbPRP1*	Apical and elongation region	Proline-rich protein	Suzuki *et al.* (1993)
	SbPRP2	Cortical cell	Proline-rich protein	Ye *et al.* (1991)
Rice	*COS6*	NT	Unknown	de Pater and Schilperoort (1992)
Rice	*COS9*	NT	Unknown	de Pater and Schilperoort (1992)
	Rcc2	Elongation zone and root cap	Proline-rich protein	Xu *et al.* (1995)
	Rcc3	NT	Proline-rich protein	Xu *et al.* (1995)
Tobacco	*salT*	NT	Unknown	Claes *et al.* (1990)
	HRGPnt3	Pericycle and endodermis	Hydroxyproline-rich glycoprotein	Keller and lamb (1989)
	RB7	Meristem and centeral cylinder	Membrane Channel Protein	Conkling *et al.* (1990)
Tomato	*RSI-1*	Lateral root	Unknown	Taylor and Scheuring (1994)
	LeRse-1	Root hair	Mannitol dehydrogenase	Lauter (1996)
		NT	lysyl-tRNA synthetase	Gritch *et al.* (1997)

[a]resultes from *in situ* hybridization. NT: not test,
[b]protein deduced from cDNA.

and Scheuring, 1994; Smith and Fedoroff, 1995).

Expression of several root-specific genes was regulated by environmental conditions. In tomato, mannitol dehydrogenase (*LeRse-1*) gene is specifically expressed in the root hair (Lauter, 1996). Interestingly, expression of the *LeRse-1* gene is induced in the root

hair by exposure of the shoot to light. During the *Rhizobium* infection of legume roots, root-specific expression of *ENOD12* was induced (Scheres *et al.*, 1990). In contrast to *ENOD12*, root hair-specific expressions of extensin genes, *ext3*, *ext26* and *ext127*, are reduced by the *Rhizobium* infection in cowpea (Arsenijevic-Maksimovic *et al.*, 1997).

ISOLATION AND CHARACTERIZATION OF ROOT-SPECIFIC GENES IN *P. vulgaris*

In general, it is accepted that plant growth and differentiation involve selective expression of specific genes required for the specific developmental processes (Kuhlemeier *et al.*, 1987). Consequently, quantitative and qualitative differences of gene expression were detected in the distinct types of tissue and in the various stages of development (Woodson, 1987; Stabel *et al.*, 1990).

In this respects, we constructed the cDNA library using the poly(A)$^+$ RNA from the roots of 3 to 7 days old bean (*P. vulgaris* L. cv ChungJu Jaelae) seedlings. We had attempted to isolate root-specific cDNA clones from the cDNA library by a differential screening strategy. The potential positive plaques were analyzed for root-specific expression by determining the ability of the ^{32}P-labelled inserts to hybridize with total RNA prepared from the root, stem, and leaf tissue of *P. vulgaris* seedlings. From about 43,000 plaques, several distinct cDNA clones were confirmed that their mRNAs were accumulated specifically in the root (Fig. 1). Among them, we chose *PVR3* and *PVR5* to characterize further because of the relatively high abundance of the corresponding mRNAs.

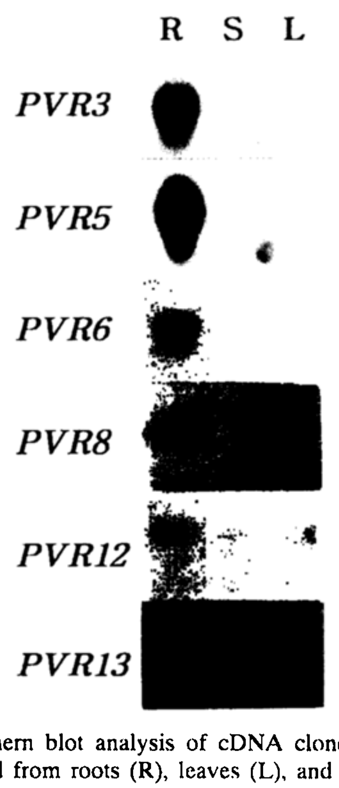

Fig. 1. Northern blot analysis of cDNA clones with total RNA isolated from roots (R), leaves (L), and stems (S) of *P. Vulgaris.*

The *PVR3* cDNA sequence is 544 nucleotides long, excluding poly(A)$^+$ tail, and an open reading frame begins with an ATG initiation codon at nucleotide 25 and ends with a stop codon TAG at nucleotide 331 (Fig. 2). The predicted peptide sequence consists of 102 amino acids with a calculated molecular mass of 11.14 kDa. A putative signal peptide of 25 amino acids was identified in the amino-terminal region by using the method of von Heijne (1986). The PVR3 protein, excluding the putative signal peptide, is 77 residues long, corresponding to a molecular mass of 8.6 kDa, with a predicted isoelectric point of 6.7. Comparison of the deduced *PVR3* polypeptide sequence with the polypeptide sequences of previously cloned genes indicates that *PVR3* may encode an ns-LTP-like protein (Choi *et al.*, 1996b). The highly conserved spacing of the eight cysteine residues, one of the characteristics of ns-LTP is also found in the PVR protein. The presence of a putative secretory signal peptide at the N-terminus and the small molecular mass (9 kDa) are also common features of these

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                        TAGTGAATCATAATATTGGTAGTG ATG GAA GCA TAC AAG AAG AAG GTG ATG    51
                                                  M   E   A   Y   K   K   K   V   M     9
ATT GTG GTG ATG ATG GTG TTG GGC ATT GTG ATG AGT GGA TCC AAT GCG CAG   102
 I   V   V   M   M   V   L   G   I   V   M   S   G   S   N   A   Q     26
TAC TCG TTT TGT CGC ATG CCC AAA GAT GGG TTG AAG TCA TGC TTA GCA AGT   153
 Y   S   F   C   R   M   P   K   D   G   L   K   S   C   L   A   S     43
GTG AGT GGG GAC AAC CCT GTT GAT CCC ACC TCT GAT TGT TGC TTA GCC ATT   204
 V   S   G   D   N   P   V   D   P   T   S   D   C   C   L   A   I     60
GCA AAG GCT GAT CTG CAG TGC TTC TGC CGC TAC AAG GAT TCG GGA TTG CTC   255
 A   K   A   D   L   Q   C   F   C   R   Y   K   D   S   G   L   L     77
TCT ATC TAT GGT GTT GAT CCC AAC AAA TGC ATG GAA CTC CCT GTT AAG TGC   306
 S   I   Y   G   V   D   P   N   K   C   M   E   L   P   V   K   C     94
AAG GTT GTC GAC TCT TTC CAC TGC TAG AGGTGGCTCTCAGTAGTTATGTACTCTTTAA   364
 K   V   V   D   S   F   H   C   *                                    102
TTGCCCTGTTTTATGTTTTTTTTTCATGGTTTCCAATGTTATGGACCAAGTATGCTTCTTTGTTTGT   431
TTGTTTGTTGGTGTTCTTGTAATGATGTGATTTGTTCTTTAGTTTTGGCTAGTTCCTGTGTACATCT   498
TATTTTCGTTCGTTGTTGGTAATGTACTTGTCCTCTTGTTTTCTTTAAAAAAAAAAAAAAAAAAAA   565
AA                                                                  567
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Fig. 2. Nucleotide sequence and deduced amino acid sequence of *PvLTP* (A). The termination codons was indicated by asterisk. The putative signal peptide sequence is double underlined. Bold italics indicate the conserved cysteine residues of ns-*LTP* genes.

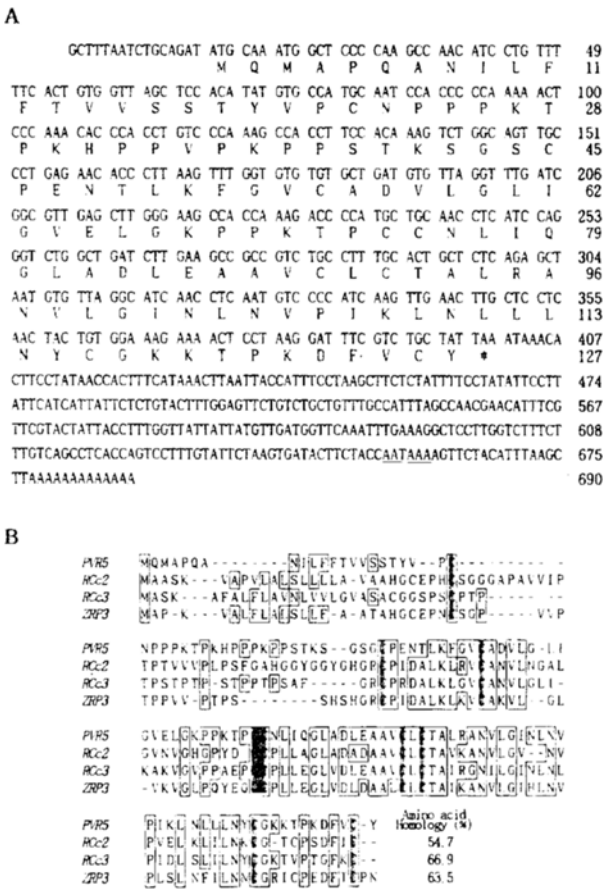

Fig. 3. Nucleotide and deduced amino acid sequence of *PvPRP* (A). The termination codon is indicated by the asterisk. The putative polyadenylation signal is underlined. Comparison of amino acid sequence of the PvPRP protein with 14 kDa proline-rich proteins from root of other plant species (B). ZRP3 from maize root (John *et al.*, 1992); RCc2 and RCc3 from rice root (Xu *et al.*, 1995). Nine conserved cysteine residues were shaded in boxes (Choi *et al.*, 1996a).

polypeptides. So, we designated the cDNA clone encoding ns-LTP as *PvLTP*.

In *PVR5* cDNA clone, the longest open reading frame is 381 bp long starting at the ATG initiation codon at position 17 and terminating with a stop codon TAA at nucleotide 398. A putative polyadenylation signal sequence (AATAAA) is found 24 nucleotides upstream of the poly(A)$^+$ tail. The predicted polypeptide consists of 127 amino acids with a calculated molecular mass of 13.5 kDa (Fig. 3). Comparison of the PVR5 amino acid sequence with sequences available in databases revealed that the protein shares significant homology with other proline-rich proteins of 14 kDa: ZRP3 from maize, RCc2 and RCc3 from rice, DC2.15 from carrots, SAC51 from oilseed rape pod, and CR14KDA from madagascar periwinkle (John *et al.*, 1992; Xu *et al.*, 1995; Aleith and Richter, 1990; Coupe *et al.*, 1993; Hotze *et al.*, 1994). SAC51 mRNA accumulates preferentially in the dehiscense zone of pod. DC2.15 mRNA temporarily accumulated during the initiation of somatic embryogenesis. CR14KDA was induced by a change from the growth medium to a solution of 8% sucrose. The predicted polypeptides of these PRPs show no significant homology with cell wall proline-rich proteins, including the hydroxyproline-rich glycoprotein (HRGP) and proline-rich protein (PRP) previously described (Cassab and Varner, 1988; Showalter, 1993). Furthermore, the proline repeat motif of HRGPs and PRPs are not present in these PRP polypeptides. In the highly homologous C-terminus region of these PRP proteins, nine cysteine residues are present at conserved locations. From these results, *PVR5* was renamed *PvPRP*.

CORTICAL CELL-SPECIFIC EXPRESSION OF *PvLTP* AND *PvPRP*

Although precise *in vivo* role of LTPs remains to be unknown, LTPs were defined by their ability to bind and transfer of phospholipid between membranes *in vitro*. ns-*LTPs* have been reported from higher plants, including both mono- and dicots. They comprise a homogeneous family of basic polypeptide and contain eight cysteine residues at conserved positions. Recently, crystallographic study of maize LTP showed that eight conserved cysteines form four disulfide bridges as follows: Cys^{4}-Cys^{52}, Cys^{14}-Cys^{29}, Cys^{30}-Cys^{75}, and Cys^{50}-Cys^{89} and a tunnel-like hydrophobic cavity, which is large enough to accommodate a long fatty acyl chain, is present (Shin *et al.*, 1995). Using the maize ns-LTP as a template, a model of the three-dimensional structure of the PvLTP protein was analyzed with Silicon Graphics using the program Modeler (Quanta/Charmn). The three-dimensional model of PvLTP resembles that of maize ns-LTP. As shown in Fig. 4, the model of PvLTP is superimposable with that of the maize ns-LTP. Four α-helices; Helix 1 (residues 12 to 21), Helix 2 (residues 29 to 33), Helix 3 (residues 39 to 52), and Helix 4 (residues 59 to 62) were connected by three short loops, and a long C-terminal region was present. The eight conserved cysteines form four disulfide bridges. A tunnel-like hydrophobic cavity is also present in the PvLTP protein (Fig. 4B). The volume of the hydrophobic cavity was calculated to be approximately 210Å^3, which is large enough to accommo-

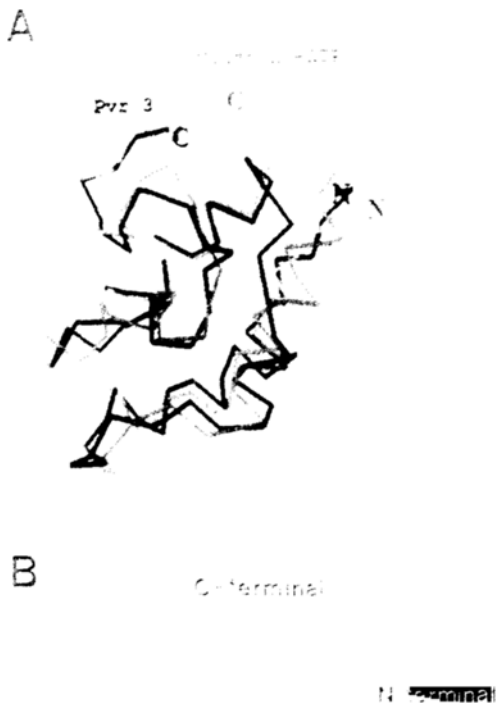

Fig. 4. Three-dimensional models of PvLTP protein. Superimposition of the three-dimensional models of PVR3 and maize ns-LTP (A). Three-dimensional model of PVR3 protein showing presence of the tunnel-like hydrophobic cavity (B) (Choi *et al.*, 1996b).

date a long fatty acyl chain (Choi *et al.*, 1996b).

The expression patterns reported for the different plant ns-*LTP* genes were found to be displayed its own peculiar pattern of gene expression (Fleming *et al.*, 1992; Kader, 1997). *In situ* hybridization analysis of *LTP* genes showed that expression of each *LTP* gene is mainly restricted to defined cell layers, generally peripheral tissue such as epidermis of leaf and floral organs and protoderm cells of embryo (Sossountzov *et al.*, 1991; Sterk *et al.*, 1991; Clark and Bohnert, 1993; Gausing, 1994; Kotilainen *et al.*, 1994; Thoma *et al.*, 1994). Expression of *ltp1* and *E2* is specifically restricted to aleurone layer of barley and anther tapetum layer of *B. napus,* respectively (Foster *et al.*, 1992; Skriver *et al.*, 1992). Another interesting feature of *LTP* expression is that all of the plant ns-*LTP* genes studied to date were shown to be expressed only in the aerial parts of the plant and shown little or no expression in the root tissue (Vignols *et al.*, 1994; Canevascini *et al.*, 1996). Therefore, it was suggested that an ns-LTP isoform might be expressed specifically in the root tissue (Bernhard *et al.*, 1991). Root-specific expression of *PvLTP* cDNA is consistent with this hypothesis.

In bean seedlings, *in situ* hybridization showed that expression of *PvLTP* was detected in the root cortical cells, but not in the epidermal tissue. And the cortical cell-specific expression of *PvLTP* was regulated developmentally (unpublished data). Although *in vivo* role of *PvLTP* is remained to be unknown, from the *in situ* hybridization result of *PvLTP,* it is suggested that *PvLTP* may play a role in development of cortical cell of the root in the bean seedlings.

Since roots have an indeterminate growth pattern, consecutive stages of root development can be observed in a single longitudinal axis. Northern blot analysis showed that *PvPRP* expression was highest in the root tip, and decreased with distance from the root tip. However, the *PvPRP* expression of root segments including the region where secondary roots developing was higher than that of elongation region (Choi *et al.*, 1996a).

In situ hybridizations to longitudinal section of the roots of bean seedling confirmed the results obtained with RNA gel blot analysis. Expression of *PvPRP* was also restricted to cortical cells and high in cortical ground meristem of the roots of bean seedlings and decreases in abundance in the elongation region of the root (Fig. 5). In contrast to *PvLTP*, level of *PvPRP* expression was low in the region of proximal meristem. The pattern of *PvPRP* expression during lateral root development is similar to that observed in primary root. Since the organization of cells in the lateral roots is similar to that of the primary root, this is not surprising.

Expression of *PvPRP* was gradually decreased as cortical cell developed. This decrease may reflect a change in the function of the cortical cells, so that the PvPRP protein is no longer required, or it is possible that a sufficient level of PvPRP protein has been accumulated, and no further synthesis is required. Among the *PRPs* showing significant amino acid sequence homology with *PvPRP, ZRP3* from maize and *RCc2* and *RCc3* from rice was also expressed in the root (John *et al.*, 1992; Xu *et al.*,

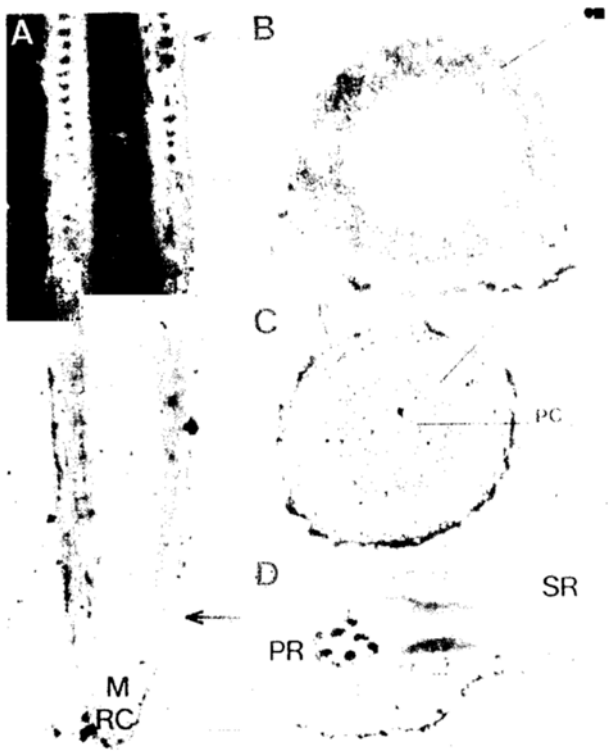

Fig. 5. Expression of *PvPRP* in the bean root. Dark-field photograph of a longitudinal section hybridized with the antisense *PvPRP* mRNA probe (A). Dark-field photographs of a transverse sections made at positions indicated by arrows in panel A and hybridized with the antisense *PvPRP* probe (B and C). The position of the root cap (RC) and apical meristem (M) are indicated. Localization of *PvPRP* mRNAs by *in situ* hybridization to longitudinal section of laterial root (D). PR, primary root; SR, secondary root. The bar equals 100 μm (Choi *et al.*, 1996a).

1995). Spatial expression of *ZRP3* in maize root is similar to that of *PvPRP* in the bean root (John *et al.*, 1992). It is suggested that these root-specific cDNA clones encoding PRP isolated from rice, maize, and bean belong to a gene family that may be evolutionally conserved among many plants (Xu *et al.*, 1995; Choi *et al.*, 1996a). Therefore, it is suggested that these PRP proteins may be involved in some aspect of the differentiation of the cortical cells (Choi *et al.*, 1996a).

FUTURE PERSPECTIVE

In general, it is accepted that root-specific genes may participate in developmental processes of the root. Many of the fundamental developmental processes must be similar to those of other plant organs (Aeschbacher *et al.*, 1994). Even if the precise roles of root-specific genes remain to be unknown and these genes are not directly involved in the root development, the temporal and spatial expressions of these genes still be useful marker for the root development. The true *in vivo* role of *PvLTP* and *PvPRP* remains to be established. Further experiments to clarify function of these proteins are next research steps.

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