FIGURES

Figure 1. Detection of XSPs with an Antiserum Raised against Xylem Sap of Cucumber.

Proteins in xylem sap from young cucumber plants were separated by SDS-PAGE and blotted onto a nitrocellulose filter. The filter was stained with non-immune serum (lane 1) or immunostained with the antiserum (lane 2) or with amido black (lane 3). Molecular mass markers (kDa): Phosphorylase b (94); bovine serum albumin (67); ovalbumin (43); carbonic anhydrase (30); trypsin inhibitor (20.1); and α-lactalbumin (14.4).

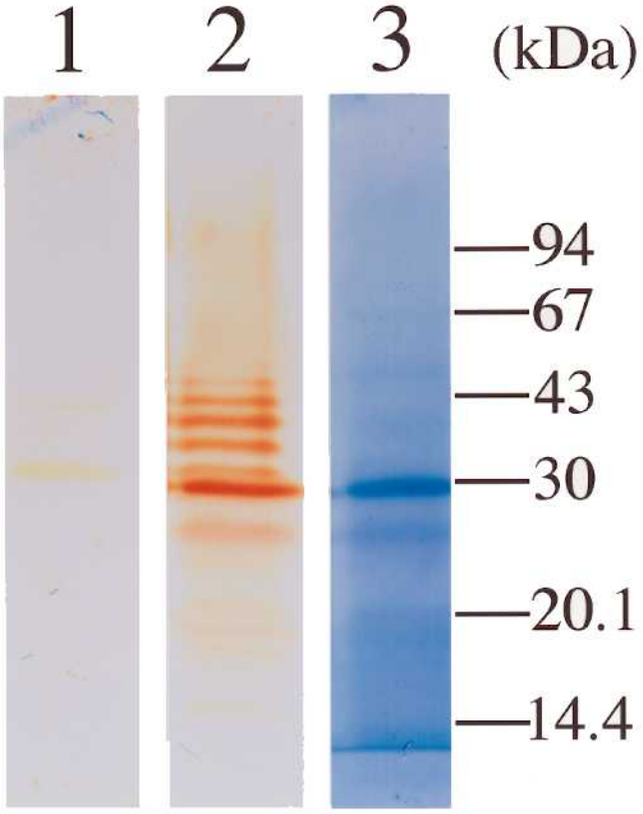

1
2
3
(kDa)
94
67
43
30
20.1
14.4

Figure 2. Characterization of Cloned XSP-cDNAs.

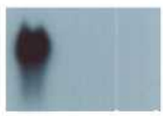
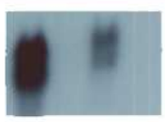
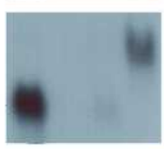
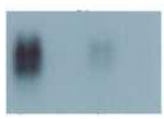
Clone	Size of RNA (kb)	Expression Root Leaf Stem Seed	Sequence Determined (kbp)	Homologue
XSP 1	1.2		0.4	None
XSP 4 (CRGRP-1)	1.4		0.2	Glycine-Rich Protein
XSP 5	1.4		0.3	None
XSP 6	1.3		0.3	Cystatin
XSP 9	0.8		0.2	None
XSP 10 (CRGRP-2)	2.0		0.2	Glycine-Rich Protein
XSP 15	0.7		0.2	Glycine-Rich Protein
XSP 16	0.8		0.8	Lectin
UBIQUITIN				

Figure 3, Deduced Amino Acid Sequences of CRGRPs.

Green lines indicate signal sequences identified by the PSORT II program. Blue lines indicates non-glycine-rich domain. Yellow lines indicate glycine-rich domain.

CRGRP-1

MAIFRTFSFGFLLLVSLGLASATRSLLTYDPPHHSVYDDHNTKVGYGRDH 50

HDQPYGGGVGASGGYGAGAGSGYGGVGYEHDHHDGYERDHDRSYGGSAGG 100

GYGVGAGSSLGGSGYGNVDHGVGYSNGGSGGYGAGVGSDLGGSGYGSGNG 150

GQVEVEMVI

CRGRP-2

MAISKTLSFGFLLLVSLGLASAARSLLSYDIPPHRSGYDNYDHPVVNPKV 50

GYEHDRRDGYYHDRDHHDAPYGGGAGGGYGAGAGAGSSLGGSGYGSGGGG 100

GSGYGGVGNHEVGYGSGGGGGYGAGVGSDLGGSGYGSGGGGGTGGGYGDL 150

GGRGKGYGSGGGGGSGYGGRGDHGVGYGSGGGGGYGSGVGGGAGVVDHGV 200

GYGSGGGGGAGSGYGGSKGYGGGSGGGGGAGYGGGAHGSGYGSGGGAGSG 250

EEGGYDGGYAP

Figure 4. Genomic DNA Gel Blot Analysis of *CRGRP-1* and *-2* Genes.

Genomic DNA (10 μg/lane) was isolated from cucumber cotyledons and digested with the restriction enzymes *Bam*HI (lane 1), *Eco*RI (lane 2) and *Hind*III (lane 3). Fragments were separated in a 1% agarose gel, blotted and allowed to hybridize with a ^{32}P-labeled *CRGRP-1* probes.

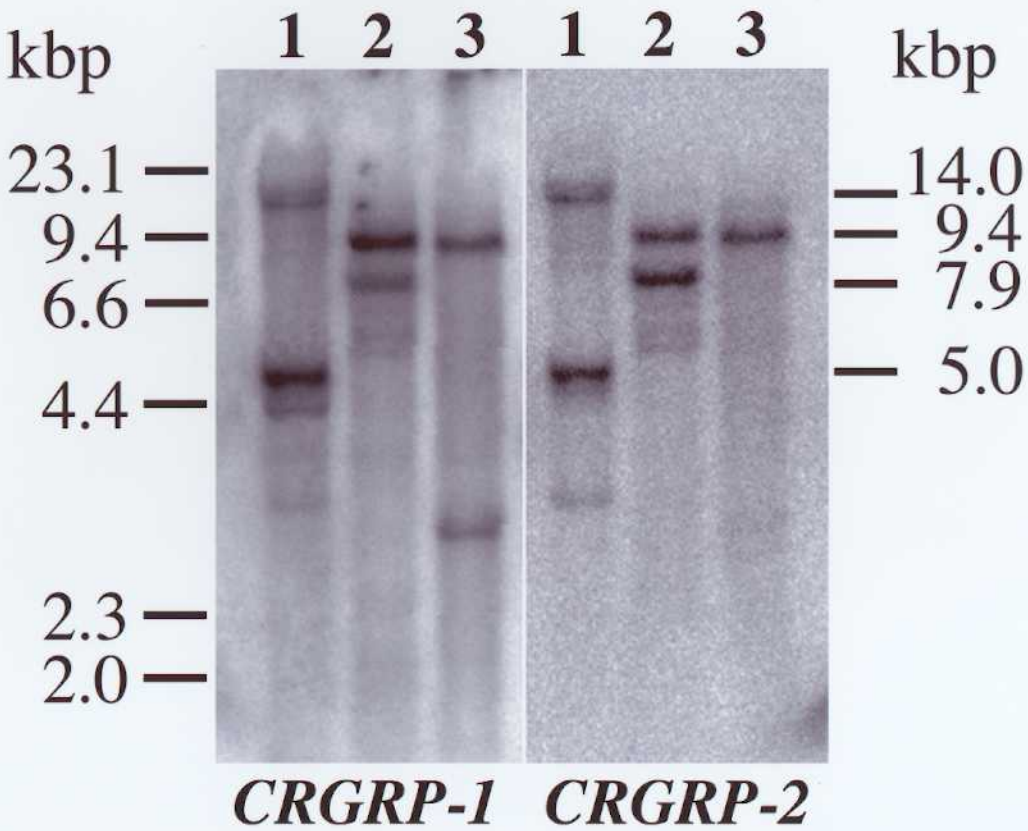

kbp
1 2 3 1 2 3
kbp
23.1
9.4
6.6
4.4
2.3
2.0
14.0
9.4
7.9
5.0
CRGRP-1 CRGRP-2

Figure 5. RNA Gel Blot Analysis of Transcripts of *CRGRP* Genes from Various Organs.

Total RNA (5 μg/lane) isolated from root (lane 1), mature stem (lane 2), mature leaf (lane 3), apical shoot including shoot apex, immature stem and immature leaf (lane 4), and whole aboveground organs (lane 5) of 1-month-old plants, and poly[A]$^+$RNA (2 μg /lane) extracted from total RNA of whole aboveground organs of 1-month-old plant (lane 6) were applied to 1.5% agarose gel. After the electrophoresis, RNA was blotted and allowed to hybridize with ^{32}P-labeled *CRGRP-1*-specific probe (A), or ^{32}P-labeled *CRGRP-2*-specific probe (B), or ubiquitin probe as a control (C and D). (A) to (C), exposed for 2 days, (D), exposed for one day.

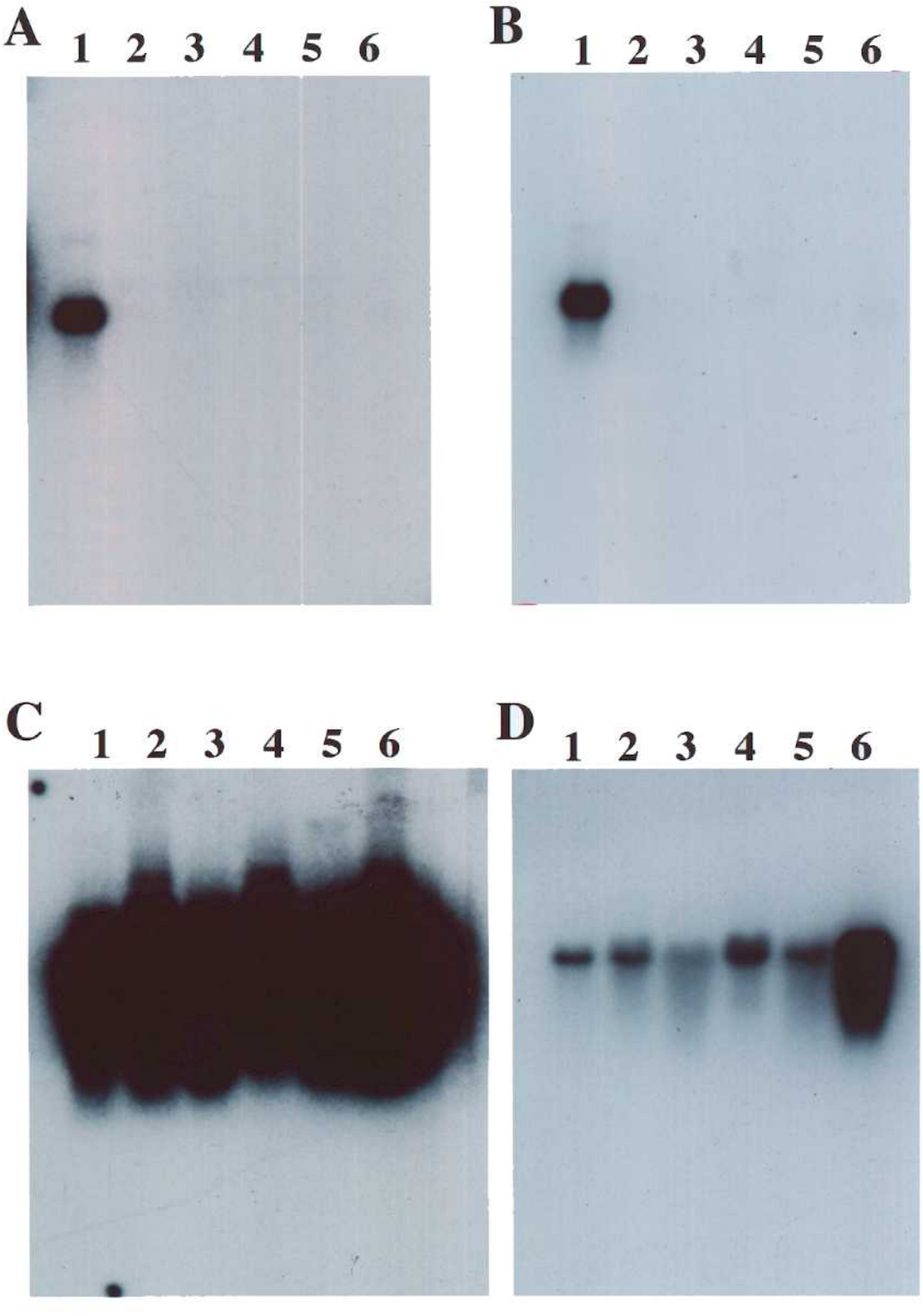

A
1 2 3 4 5 6
B
1 2 3 4 5 6
C
1 2 3 4 5 6
D
1 2 3 4 5 6

Figure 6. Morphological Observations of Formation of Root Primordia.

Hypocotyls after 0-4 days in culture were fixed, embedded in plastic resin and sliced at 2-μm thickness. Sections were stained with toluidine blue and observed under a light microscope. On the second and third days (B and C), root primordia were observed in vascular bundles (arrowheads). On the fourth day (D), adventitious roots emerged from the epidermis of the hypocotyl and vascular tissue was observed (arrow).

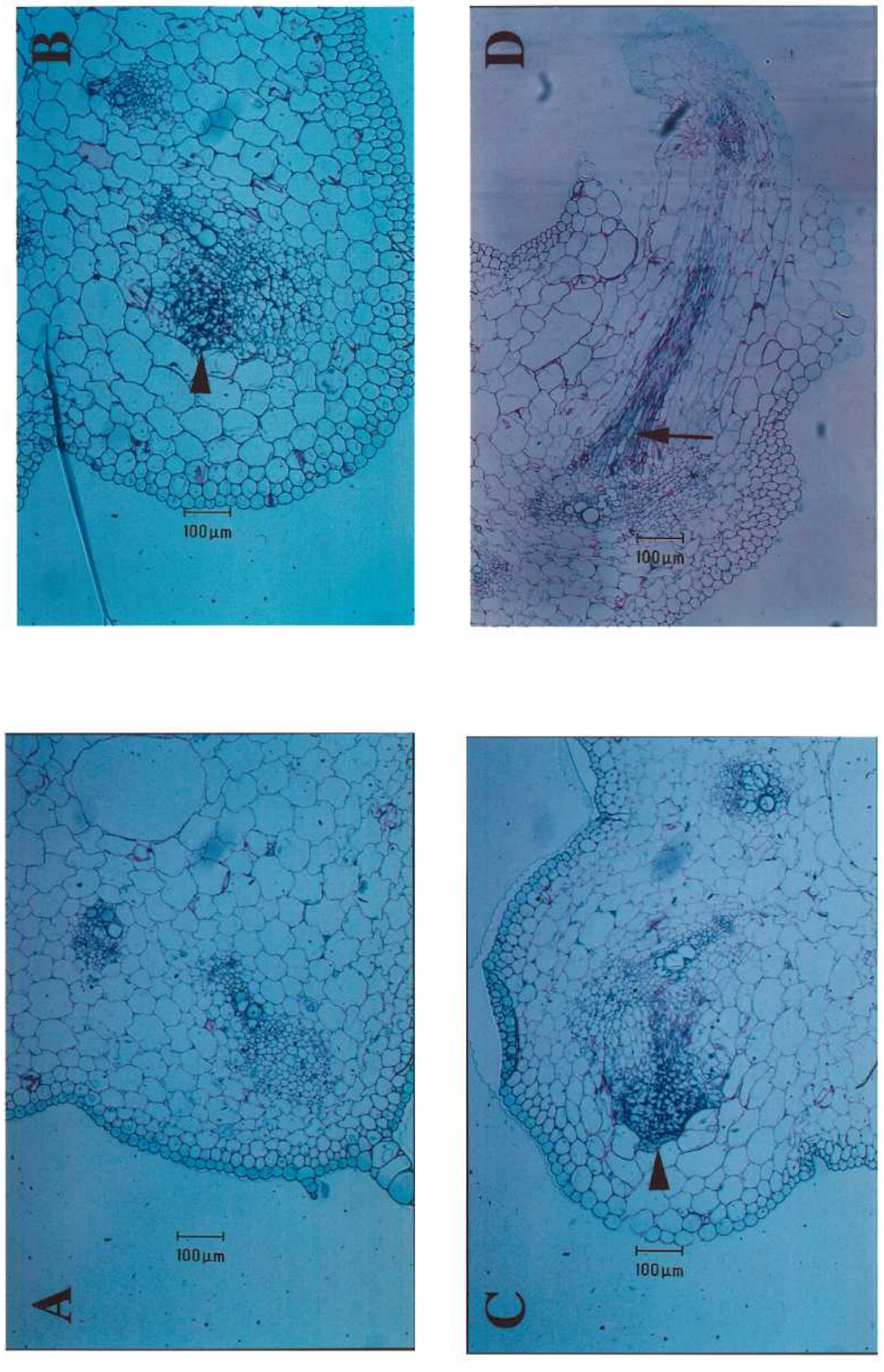

B
100µm
D
100µm
A
100µm
C
100µm

Figure 7. Expression of Transcripts for CRGRP-1 and CRGRP-2 during the Formation of Adventitious Roots.

Total RNA (10 μg / lane) from hypocotyls after 0-15 days in culture was subjected to RNA-gel blot analysis and probed with ^{32}P-labeled cDNAs for *CRGRP-1* (A) and *CRGRP-2* (B).

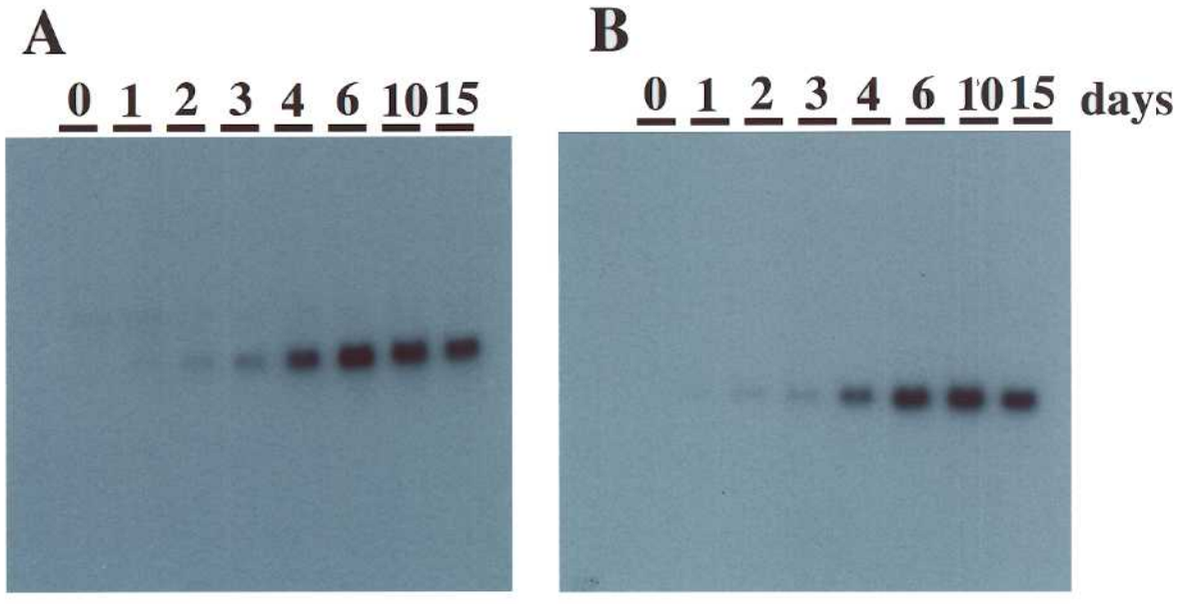
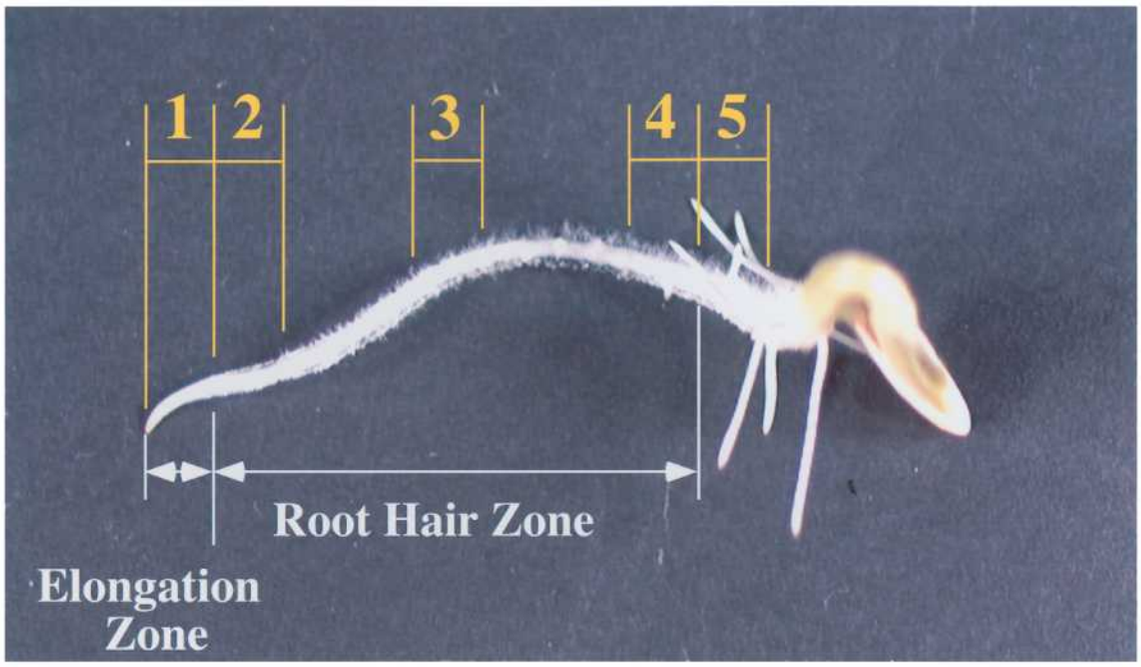
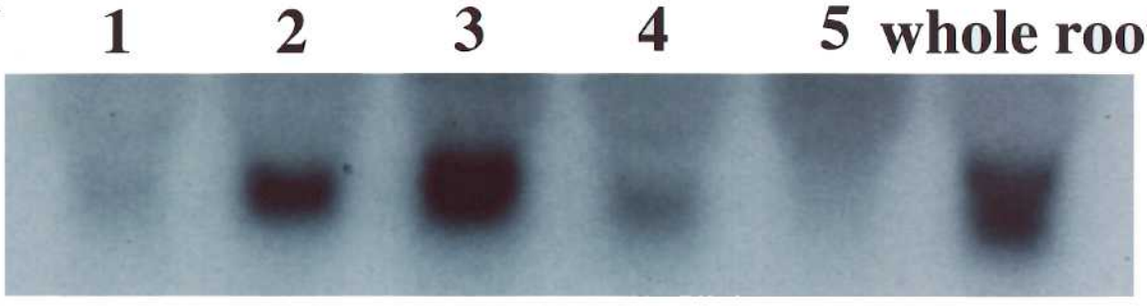

A
0 1 2 3 4 6 10 15
B
0 1 2 3 4 6 10 15 days

Figure 8. Expressions of Genes for CRGRP-1 and CRGRP-2 in the Tap Root.

Total RNA (10 μg) was extracted from five parts of the tap roots of five day-old seedlings (numbered 1 to 5, as indicated) and from whole roots and then subjected to RNA-gel blot analysis with cDNAs for CRGRP-1 (A) and CRGRP-2 (B) as probes.


1
2
3
4
5
Root Hair Zone
Elongation Zone

A


1
2
3
4
5 whole root

B

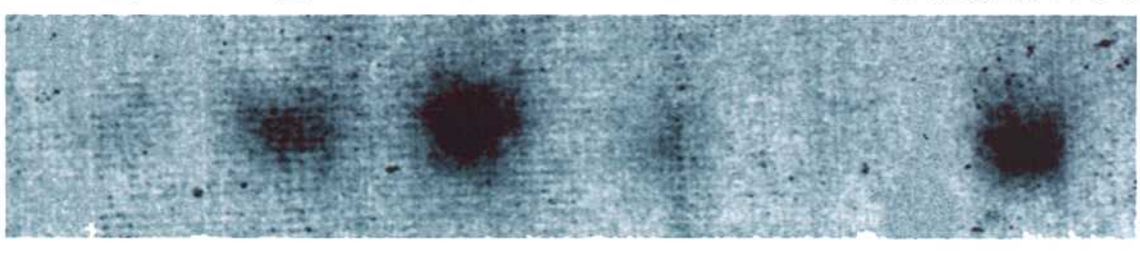

1
2
3
4
5 whole root

Figure 9. Localization of Transcripts of *CRGRP* genes in Cross Sections of Cucumber Roots.

In situ hybridization was performed with whole *CRGRP-1* cDNA as the source of sense and antisense probes. Sections were prepared from Technovit 7100-embedded tissues. (A) to (D), micrographs of cross-sections of the root-hair zone of the tap root; (E) and (F), micrographs of cross-sections of the younger part of a lateral root.

(A), (D), and (E) show results of hybridization with the antisense RNA probe.

(B) and (F) show results of hybridization with the sense RNA probes, as controls.

The section in (C) was stained with toluidine blue.

Bars in (D), (E) and (F) indicate 10 μm, and those in other panels indicate 100 μm. Arrows indicate the parenchyma cells stained with the antisense probe.

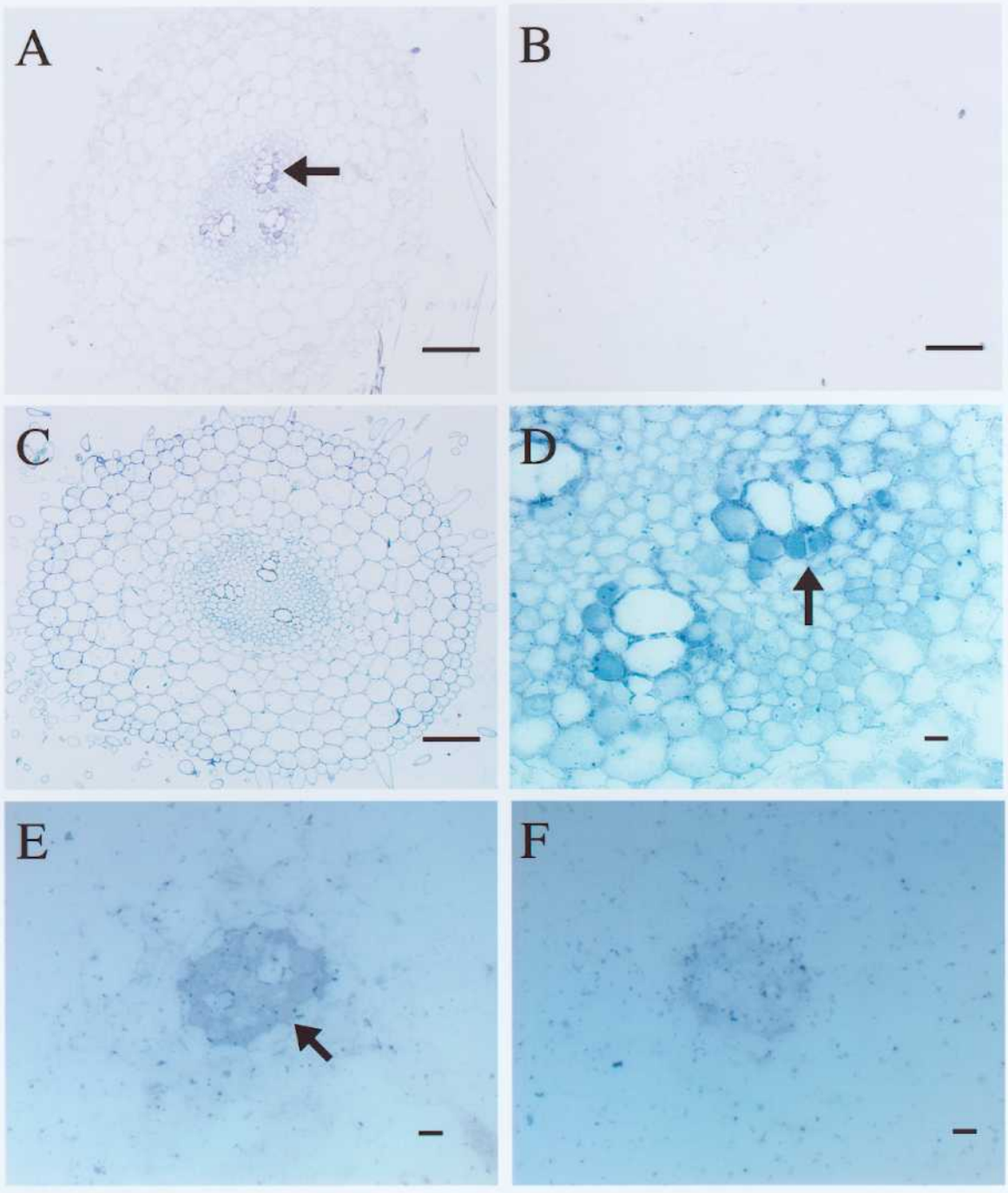

A
B
C
D
E
F

Figure 10. Immunoblot Analysis of CRGRPs and GRP1.8.

E. coli-produced GRP1.8 (lane 1), freshly collected xylem sap (lane 2; 5 μl) and proteins extracted from the root (lane 3; 6 μg), stem (lane 4; 3 μg) and immature leaf (lane 5; 1 μg), expanded leaf (lane 6; 1 μg) and apical shoot (lane 7; 12 μg) of 1-month-old plants were subjected to immunoblotting with the CRGRP-specific antiserum (A) and with antiserum raised against GRP1.8 (B).

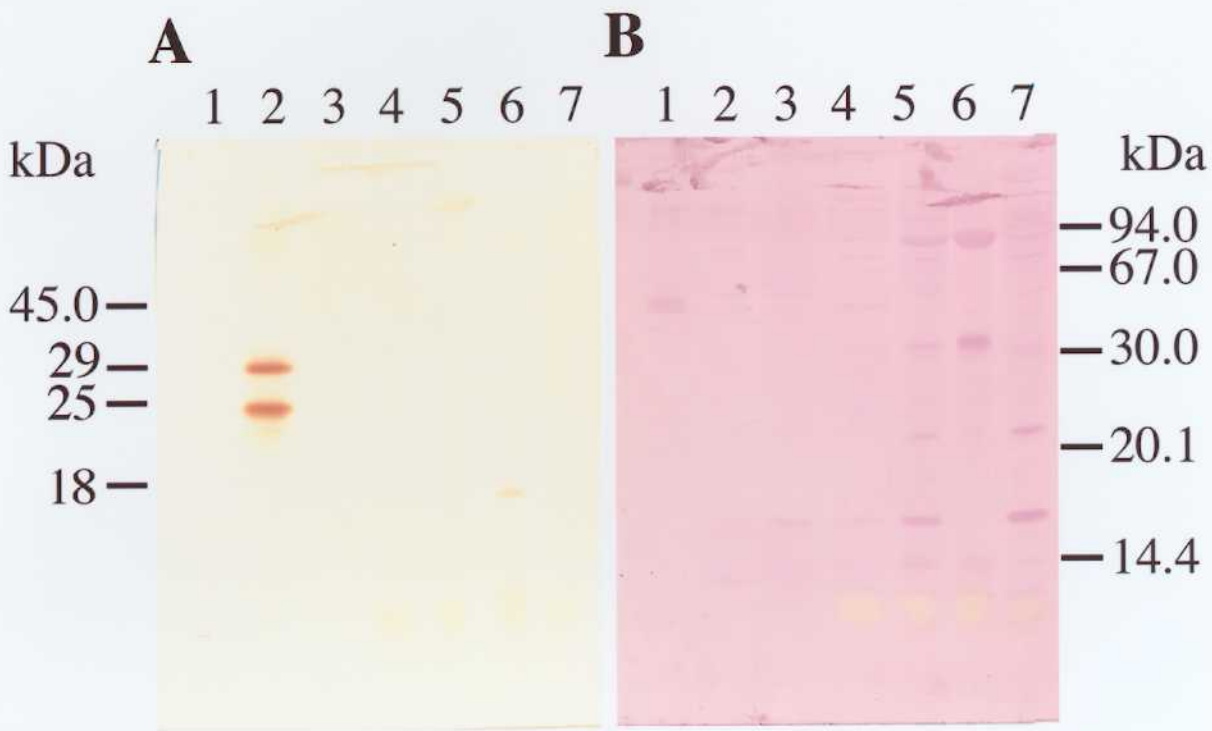

A
B
1 2 3 4 5 6 7
1 2 3 4 5 6 7
kDa
kDa
45.0
29
25
18
94.0
67.0
30.0
20.1
14.4

Figure 11. Immunolocalization of CRGRPs in the Expanded Leaf.

Sections of a mature expanded leaf were allowed to react with CRGRP-specific antiserum and with non-immune serum as a control. (A) and (C), CRGRP-specific antiserum; (B), non-immune serum; (D), dark-field micrograph of the section in (B).

Bars indicate 100 μm. Arrows indicate the wall of a metaxylem vessel stained with the antiserum.

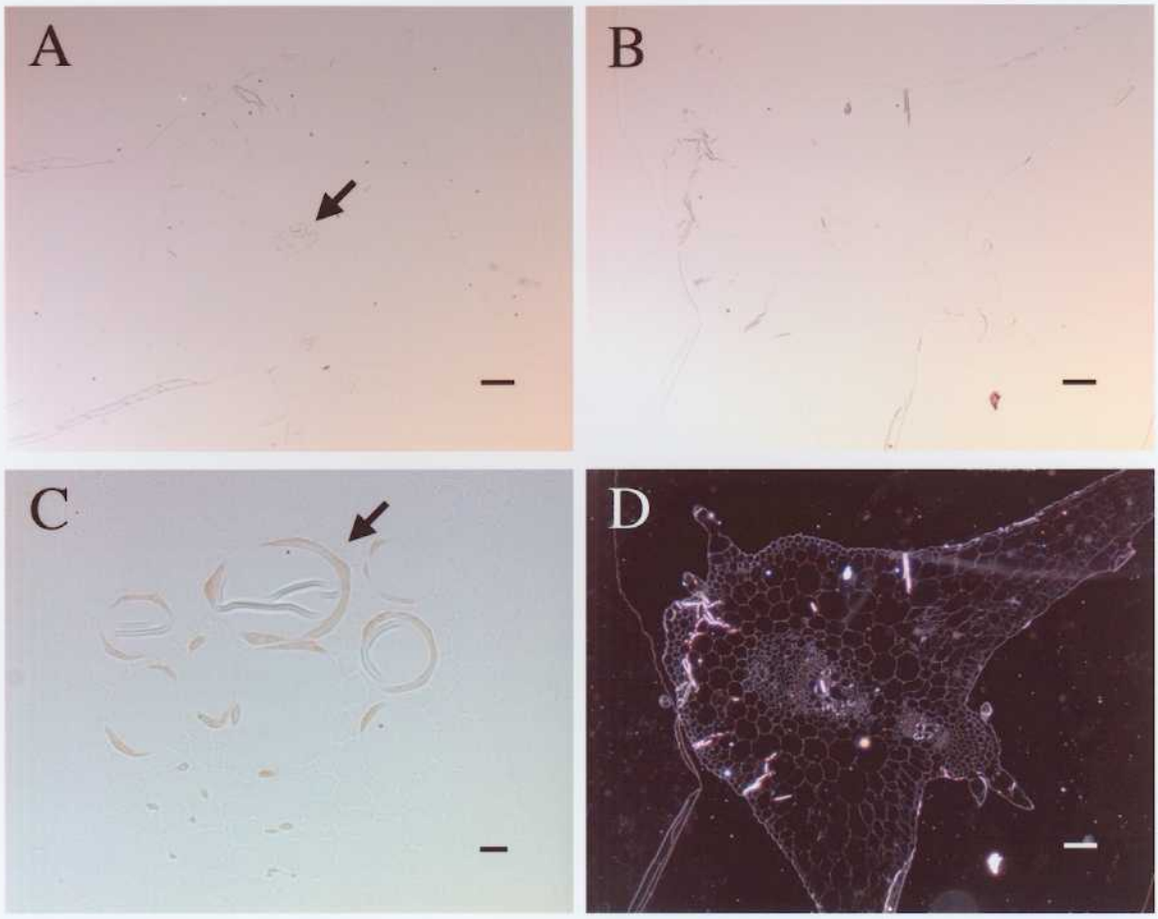

A
B
C
D

Figure 12. Immunolocalization of CRGRPs in the Stem.

Sections of stem were reacted with CRGRP-specific antiserum and non-immune serum as a control.

(A), (C), (E) and (F), stained with CRGRP-specific antiserum; (B), stained with non-immune serum; (D), dark-field micrograph of the section in (B); (G) staining with phloroglucinol-HCl.

Bar in (F) indicates 10 μm; bars in the other panels indicate 100 μm.

Arrows and arrowheads indicate, respectively, the wall of a metaxylem vessel and the cell walls of perivascular fibers stained with the specific antiserum.

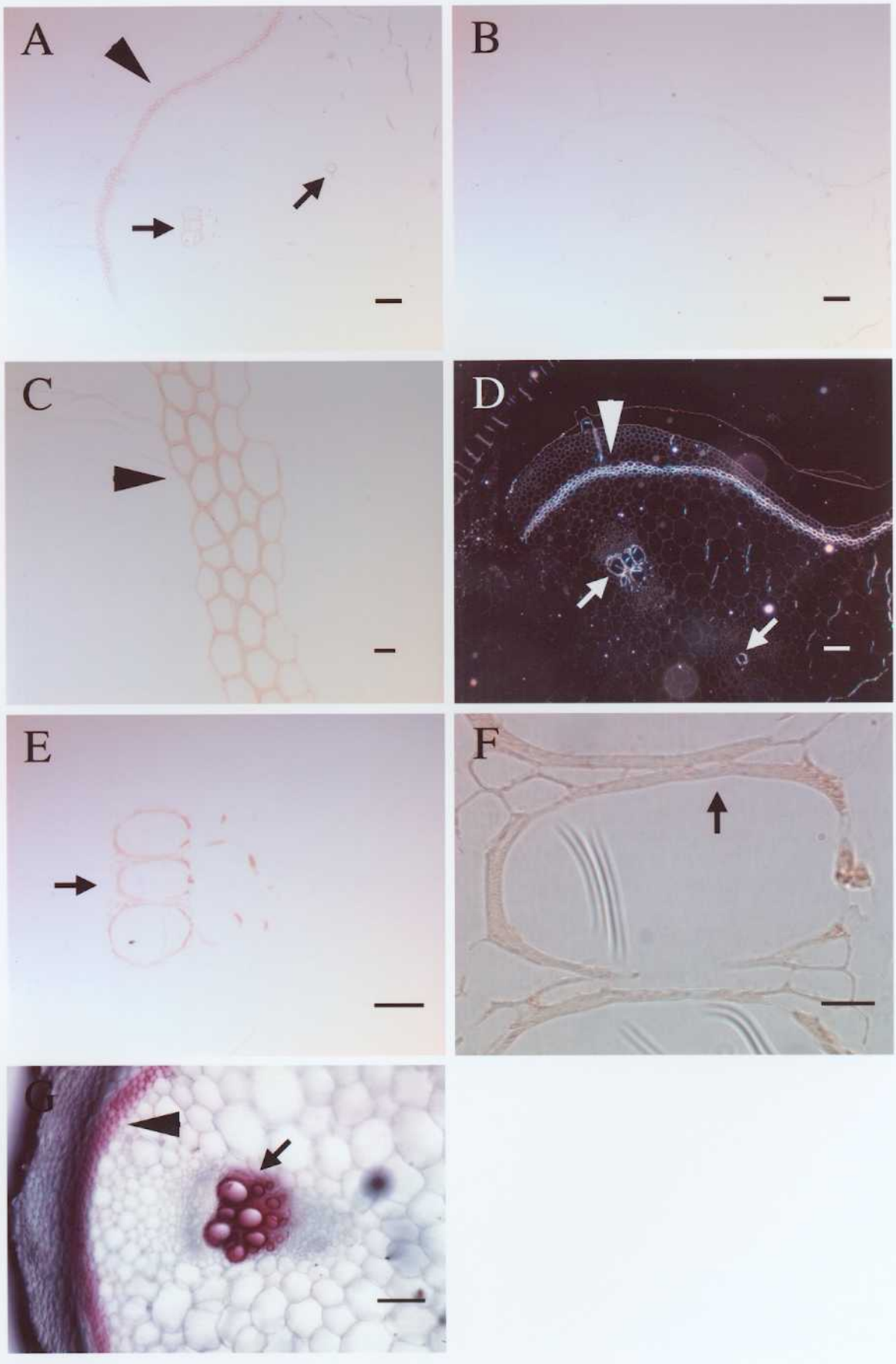

A
B
C
D
E
F
G

Figure 13. Immunolocalization of CRGRPs in the Root.

Sections of the basal part, (A) through (D) and the younger part, (E) through (H), of the tap root, were reacted with CRGRP-specific antiserum and non-immune serum as controls; (A), (C), (E) and (G), CRGRP-specific antiserum; (B) and (F), non-immune serum; (D) and (F), dark-field micrographs of sections in (B) and (H), respectively.
Bars in (A), (B), (D), (E) and (H) indicate 10 μm; bars in (C), (F) and (G) indicate 100 μm. Arrows indicate the walls of metaxylem vessels stained with antiserum.

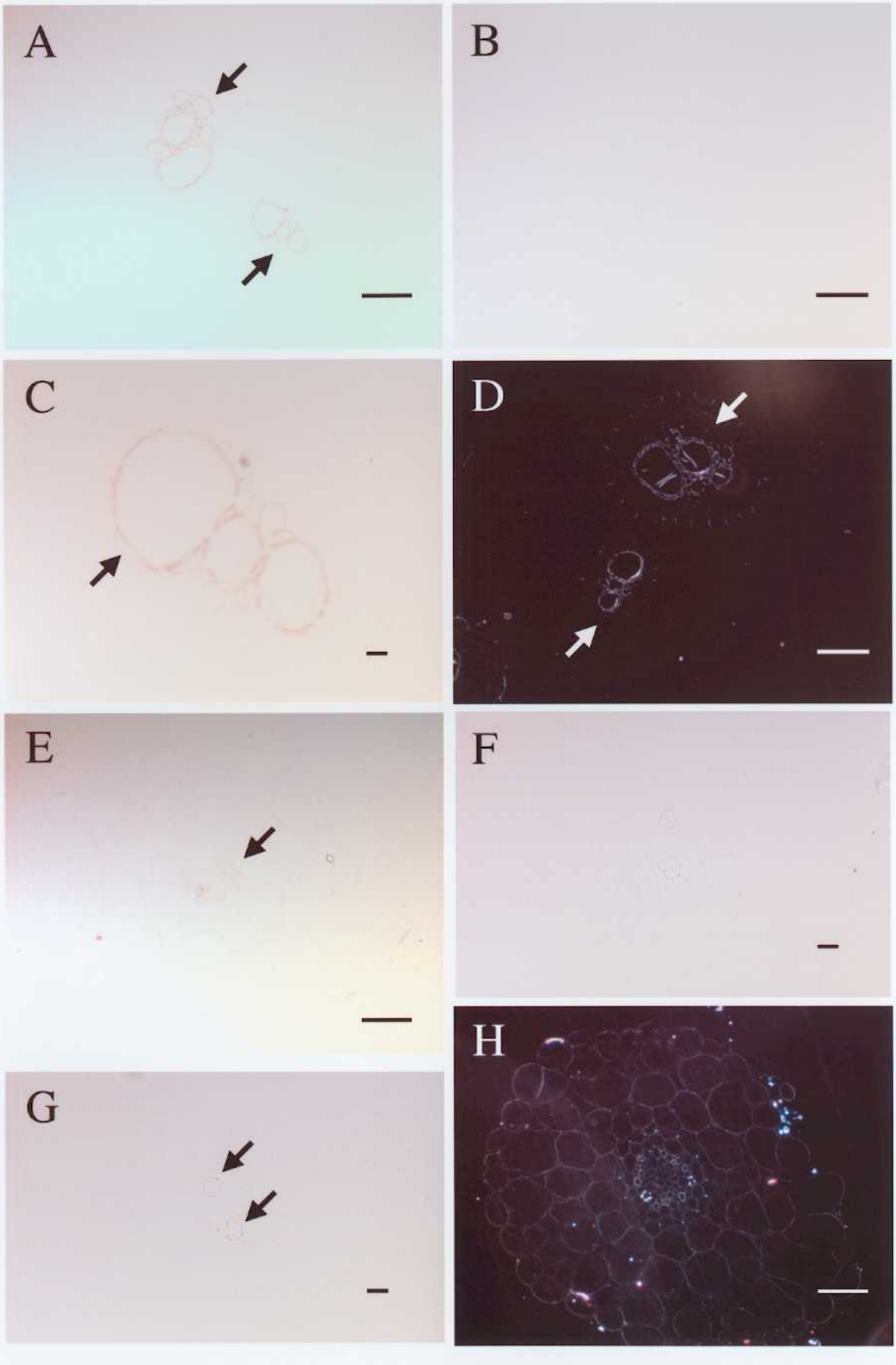

A
B
C
D
E
F
G
H

Figure 14. Immunolocalization of CRGRPs in SDS-Treated Blocks of Stem.

Blocks of the stem were thoroughly washed, with sonication, in a hot solution of SDS and then sections were prepared and reacted with CRGRP-specific antiserum. Sections of treated tissues are shown in (B) through (E); non-treated tissue is shown in (A). (A) and (B), Stained with CBB R-250; (C) to (E), Stained with CRGRP-specific antiserum. Bars in (A) through (C) indicate 100 μm; in (D) the bar indicate 50 μm; and in (E) the bar indicates 10 μm. Arrows and arrowheads indicate the walls of metaxylem vessels and the cell walls of perivascular fibers, respectively.

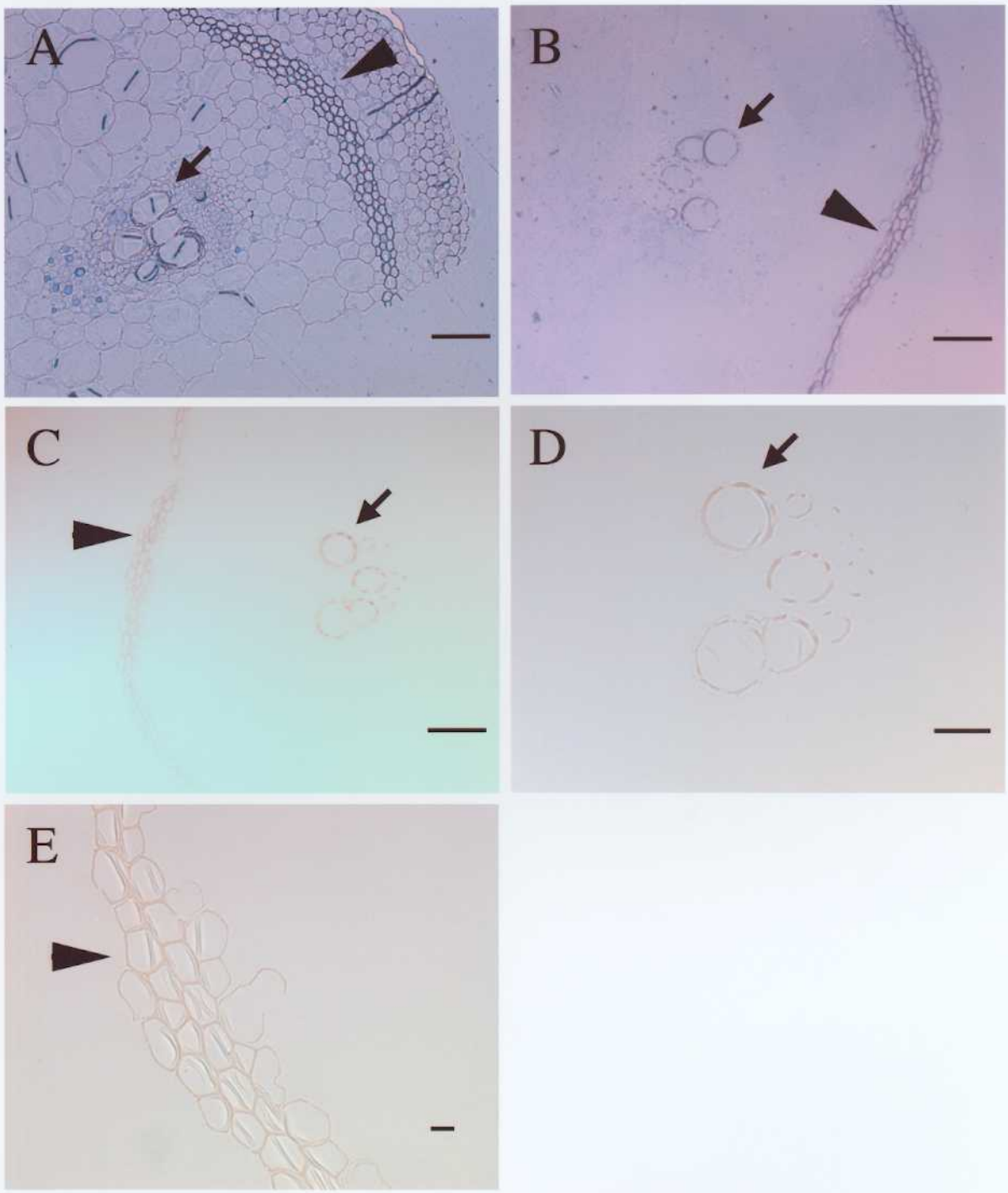

A
B
C
D
E

Figure 15. Immunolocalization of CRGRPs in Hypocotyls of Kidney Bean.

Sections of the middle part, (A) through (D), and the basal part, (E) through (H), of hypocotyls of kidney bean were reacted with CRGRP-specific antiserum and non-immune serum as a control. (A), (C), (E) and (G), CRGRP-specific antiserum; (B) and (F), non-immune serum; (D) and (F), Dark-field micrographs of sections in (B) and (F), respectively. Bars in (C) and (G) indicate 10 μm; bars in other panels indicate 100 μm. Arrows and arrowheads indicate, respectively, the walls of metaxylem vessels and the cell walls of sclerenchyma cells that were stained with the specific antiserum.

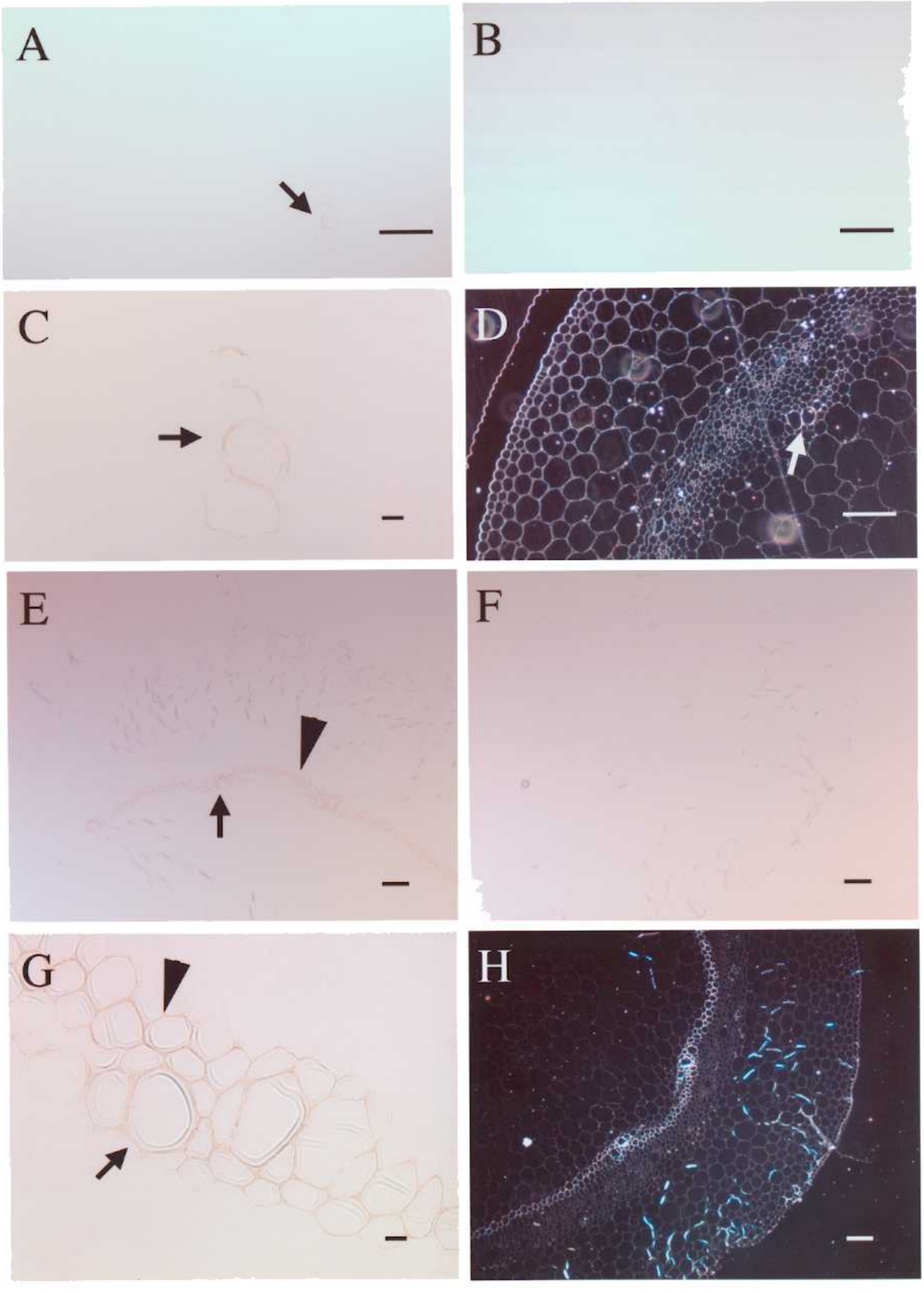

A
B
C
D
E
F
G
H

Figure 16. Movement of Water and CRGRPs in Root.

CRGRPs, which was synthesized at xylem parenchyma cells, might be secreted into apoplastic space of xylem parenchyma in a process mediated by their signal sequences. Water in soil was absorbed by root-hair and flowed across cortex (inside and outside of the cells), endodermis (only inside of the cells), xylem parenchyma (mainly outside of the cells) and then loaded into xylem vessels. CRGRPs in apoplastic space might be ridden on the water flow, and also loaded into xylem vessels, since the expression of CRGRP genes was predominant in the root-hair zone, a major site of the absorption of water.

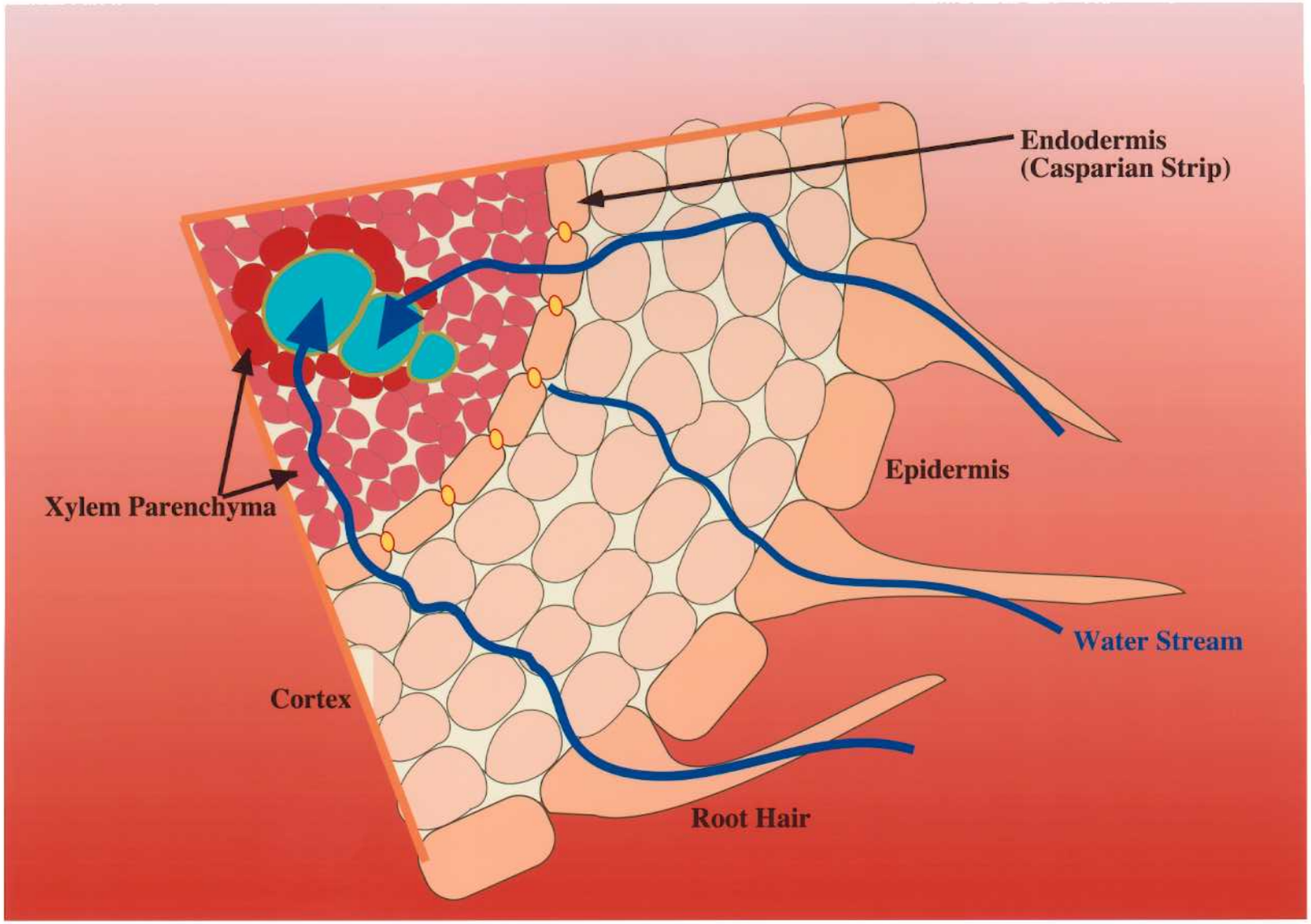
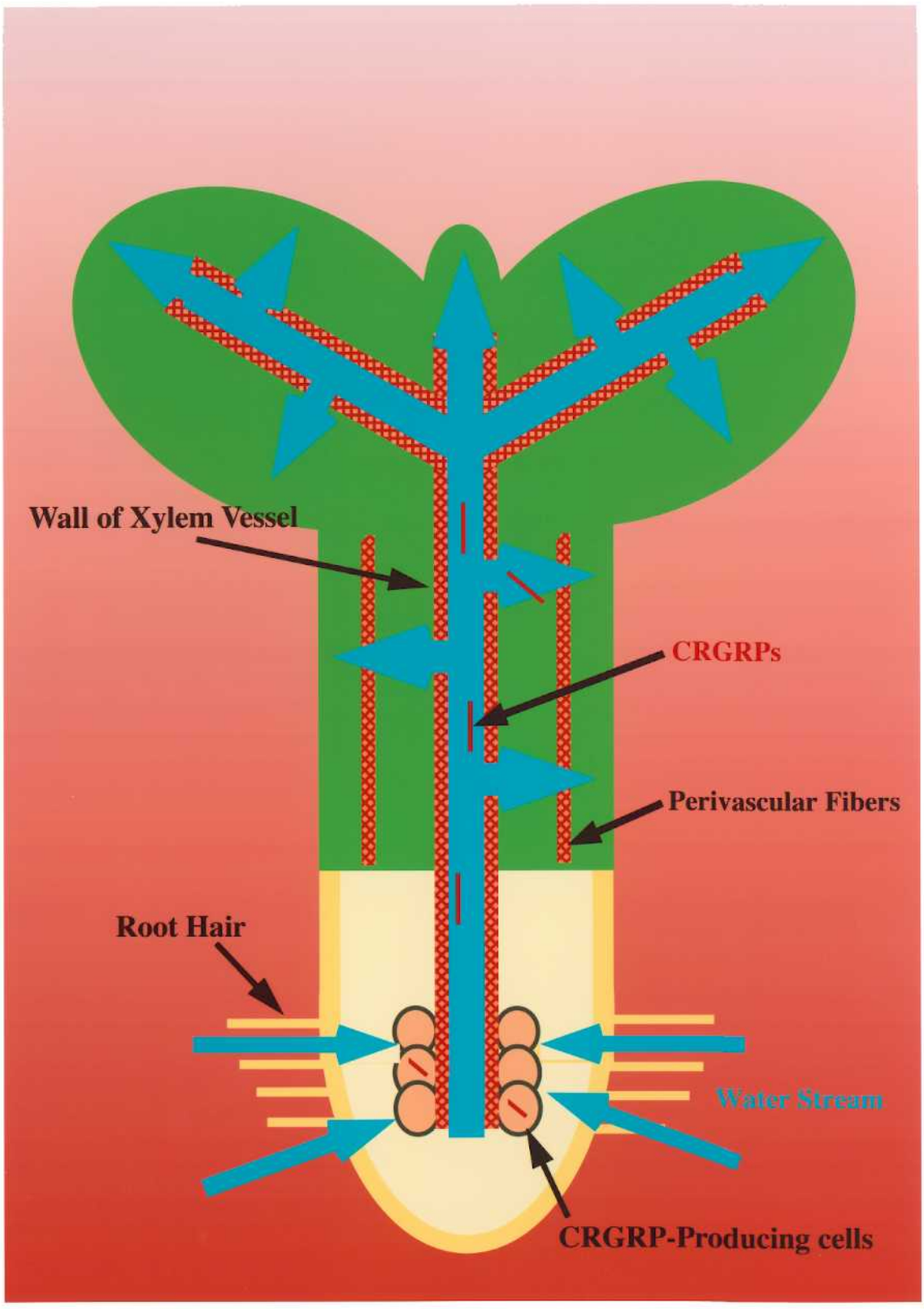

Endodermis
(Casparian Strip)
Epidermis
Xylem Parenchyma
Water Stream
Cortex
Root Hair

Figure 17. Systemic Delivery of CRGRPs from Root.

CRGRPs were produced at xylem parenchyma and then loaded into xylem vessels. They are systemically delivered to the shoot and gradually accumulated at the wall of xylem vessels.

Xylem vessels are composed of dead cells, and are impossible for them to repair themselves. Moreover, because the water current in the shoot is directed to the outside from the inside of xylem vessels, it is also difficult for xylem vessels to be supplied with materials from the neighbor cells beyond them, against the current of water. Therefore, the flow of xylem sap may be a convenient system for the maintenance of the walls of xylem vessel.


Wall of Xylem Vessel
CRGRPs
Perivascular Fibers
Root Hair
Water Stream
CRGRP-Producing cells