Figures

Figure 1. Development-dependent gene expression in cucumber roots. Total RNA (10 μg/lane) extracted from roots of 4, 6, 8, 10, 12, 14, 16, and 18-day-old seedlings at dusk was subjected to RNA gel blot analysis. The transcripts were probed using cDNA for full length *XSP30*, partial *CRGRP-2*, and ubiquitin. The ubiquitin cDNA was isolated from cucumber roots, where it is expressed constitutively.

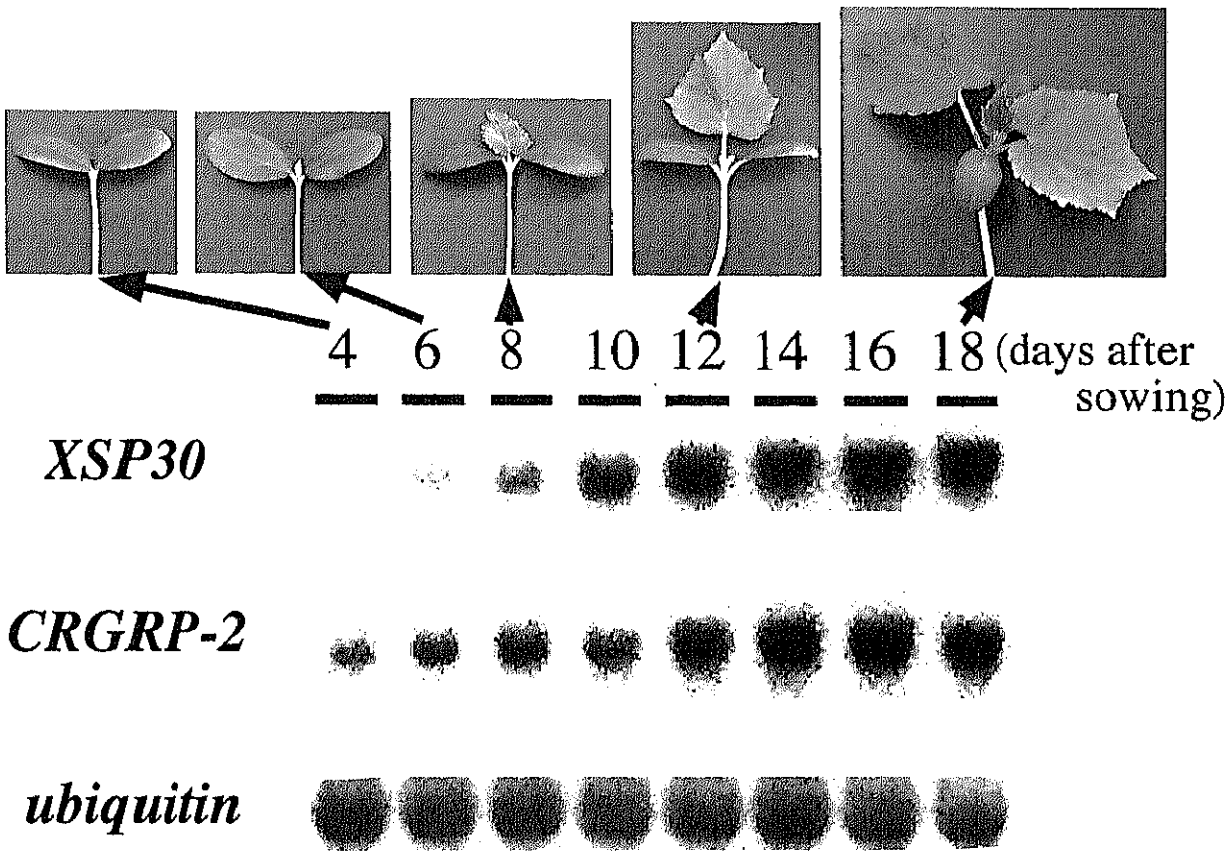

4 6 8 10 12 14 16 18 (days after sowing)
XSP30
CRGRP-2
ubiquitin

Figure 2. Time course of the expression of *XSP30* and *CRGRP-2* under a 16 h light photoperiod. Cucumber plants were grown under a photoperiod (16 hL/8 hD). Total RNA (10 μg/lane) was extracted from the roots of seedlings every 4 h, beginning on the 11th day after sowing. Samples were subjected to RNA gel blot analysis with cDNA for XSP30 (open circles), CRGRP-2 (closed circles), and rRNA (triangles) as probes. Quantified data are shown in (B). The periods of light and dark are indicated as shaded and black bars, respectively.

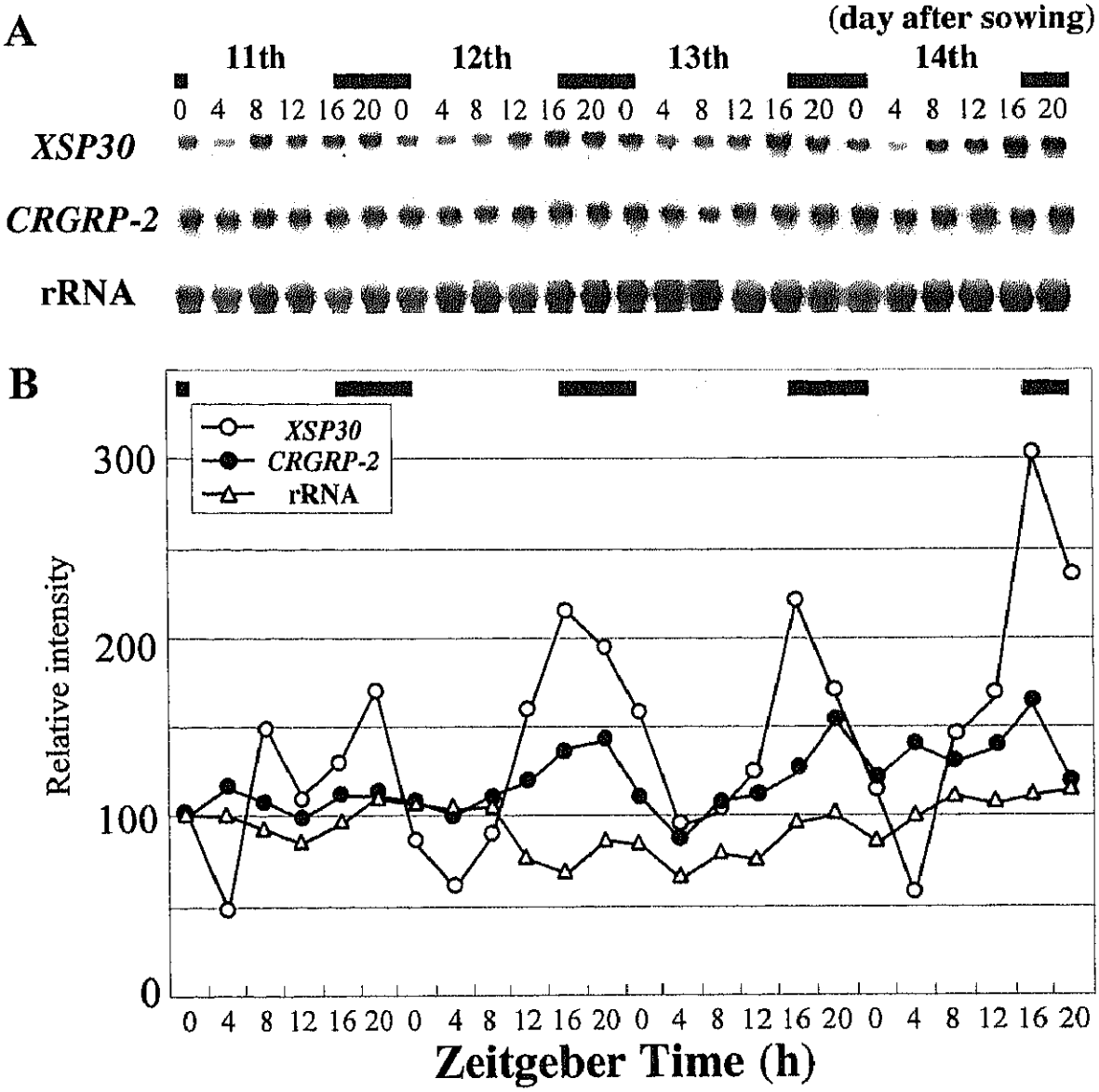
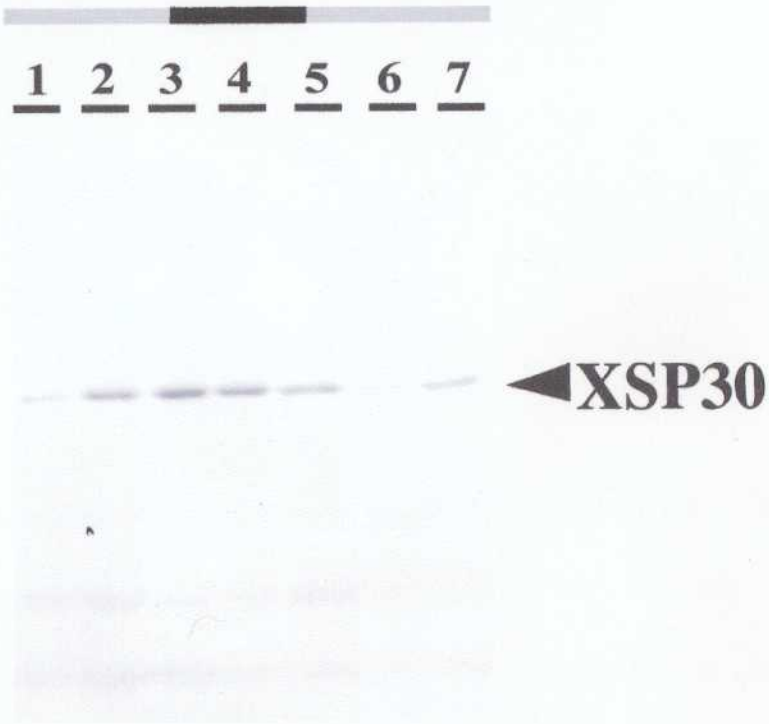

A
(day after sowing)
11th
12th
13th
14th
0 4 8 12 16 20 0 4 8 12 16 20 0 4 8 12 16 20 0 4 8 12 16 20
XSP30
CRGRP-2
rRNA
B
XSP30
CRGRP-2
rRNA
300
200
100
0
Relative intensity
0 4 8 12 16 20 0 4 8 12 16 20 0 4 8 12 16 20 0 4 8 12 16 20
Zeitgeber Time (h)

Figure 3. Detection of XSP30 protein in xylem sap. Xylem sap was collected from cucumber stems at 4-h intervals. Samples were collected at midday (lane 1), 4 h before dusk (lane 2), at dusk (lane 3), at midnight (lane 4), at dawn (lane 5), 4 h after dawn (lane 6), and at midday (lane 7). The proteins in equal volumes of sap (5 μl/lane) were separated by SDS-PAGE, and detected with XSP30 antiserum on nylon membranes (A), or the proteins were stained with silver stain (B). The periods of light and dark are indicated as shaded and black bars, respectively.

A



B

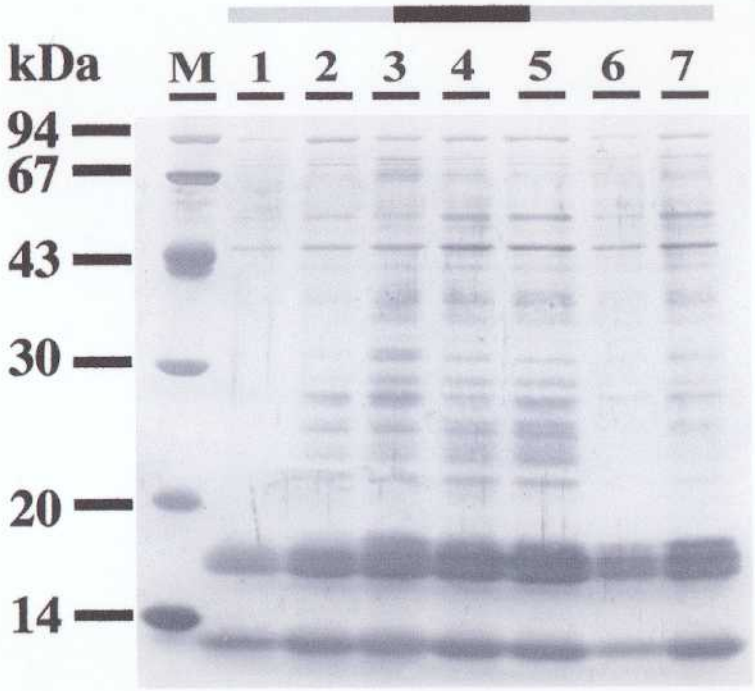
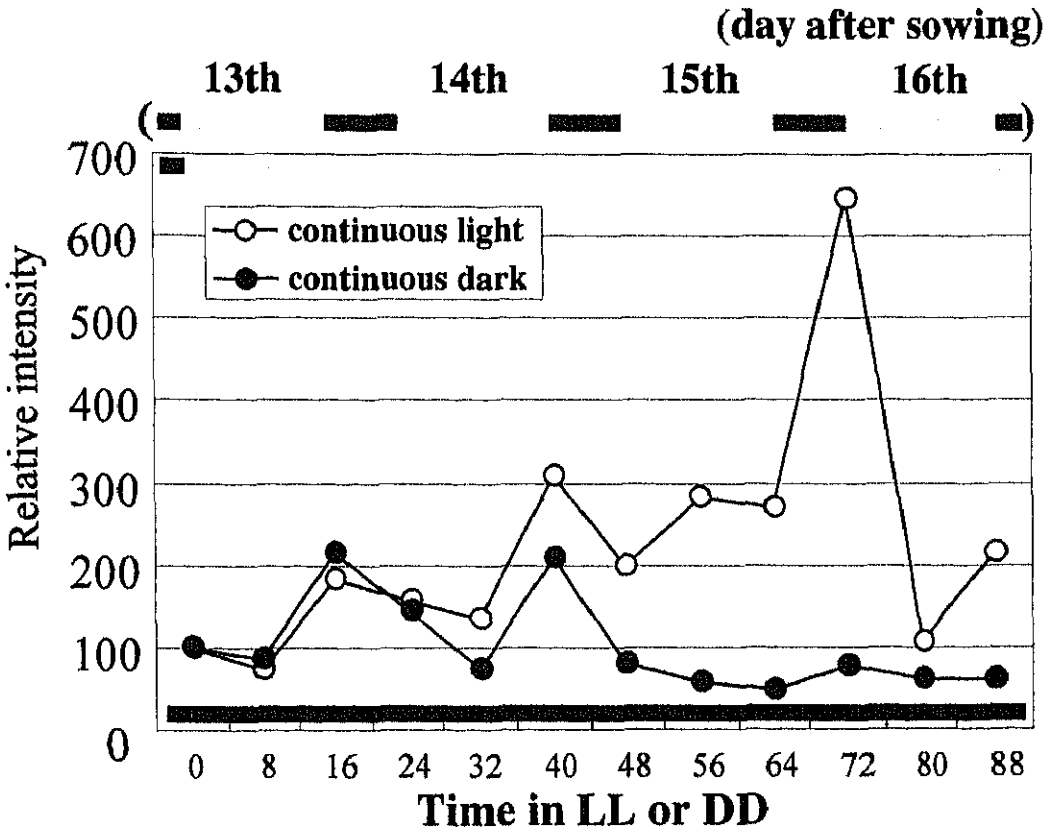


Figure 4. The expression of *XSP30* and *CRGRP-2* under continuous light or dark. Thirteen-day-old seedlings grown under a 16-h light photoperiod were transferred to continuous light (open circle) or dark conditions (closed circle). Total RNA was extracted from roots of treated seedlings every 8 h. The transcripts were probed with cDNA for XSP30 (A) or CRGRP-2 (B), and quantified data were collected. The shaded and black bars indicate the original periods of light and dark, respectively.

A


(day after sowing)
13th
14th
15th
16th
Relative intensity
700
600
500
400
300
200
100
0
continuous light
continuous dark
0
8
16
24
32
40
48
56
64
72
80
88
Time in LL or DD

B

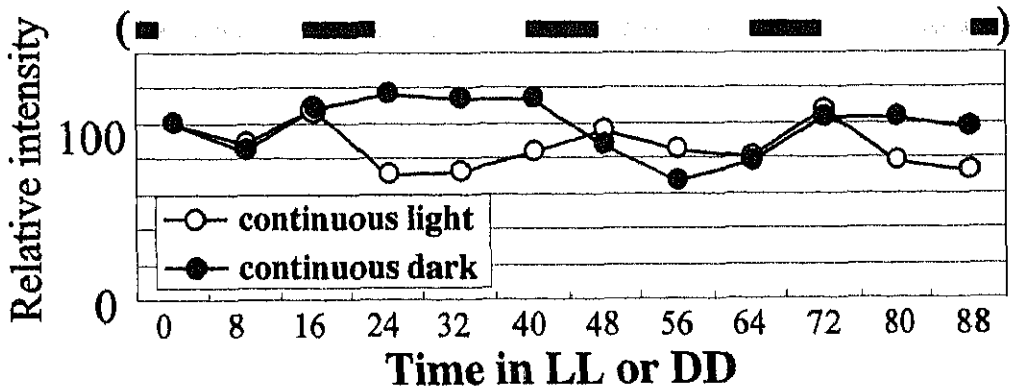
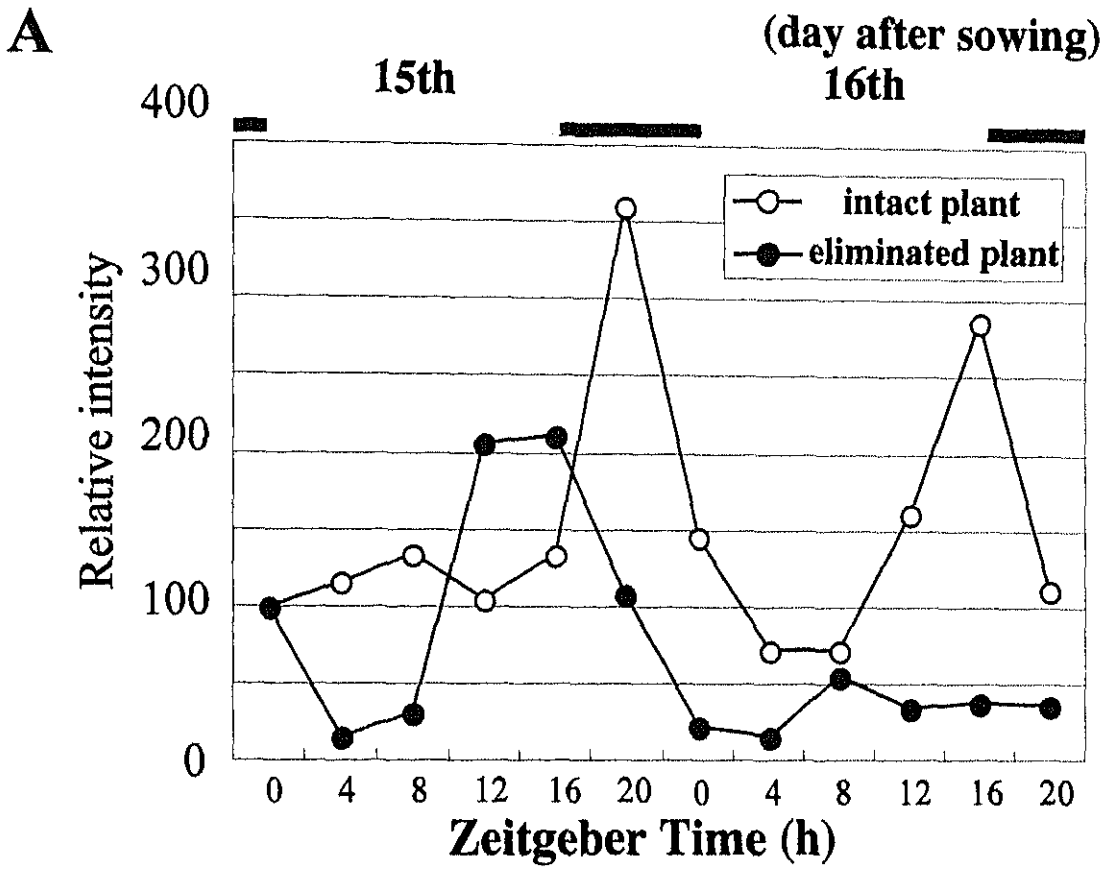

Relative intensity
100
0
continuous light
continuous dark
0
8
16
24
32
40
48
56
64
72
80
88
Time in LL or DD

Figure 5. Effect of the elimination of aboveground tissues on *XSP30* and *CRGRP-2* expression in roots. Total RNA (10 μg/lane) was extracted every 4 h from the roots of 15-day-old cucumber plants, grown under a 16-h light photoperiod, from which the shoot was removed at the middle of the hypocotyl (closed circles) or left intact (open circles). Root samples were subjected to RNA gel blot analysis with cDNA for XSP30 (A) and CRGRP-2 (B). Quantified data are shown. The periods of light and dark are indicated as shaded and black bars, respectively.


A
(day after sowing)
15th
16th
400
300
200
100
0
Relative intensity
intact plant
eliminated plant
0 4 8 12 16 20 0 4 8 12 16 20
Zeitgeber Time (h)

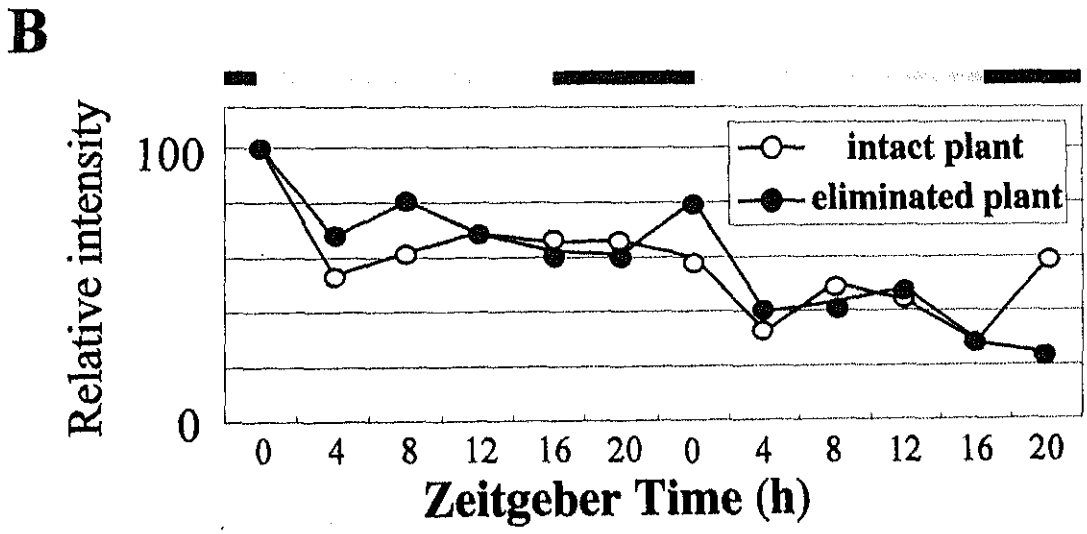

B
100
0
Relative intensity
intact plant
eliminated plant
0 4 8 12 16 20 0 4 8 12 16 20
Zeitgeber Time (h)

Figure 6. Effect of the elimination of leaves on the expression of *XSP30* and *CRGRP-2* in roots. Cotyledons plus the shoot apex (open circles and open triangles) or mature first leaf (closed circles and closed triangles) were removed from 13-day-old seedlings, and the roots were collected every 8 h for RNA gel blot analysis. The transcripts were probed using cDNA for XSP30 (A) and CRGRP-2 (B) and quantified data are shown. The data shown as circles and triangles were obtained in independent experiments. The periods of light and dark are indicated as shaded and black bars, respectively.

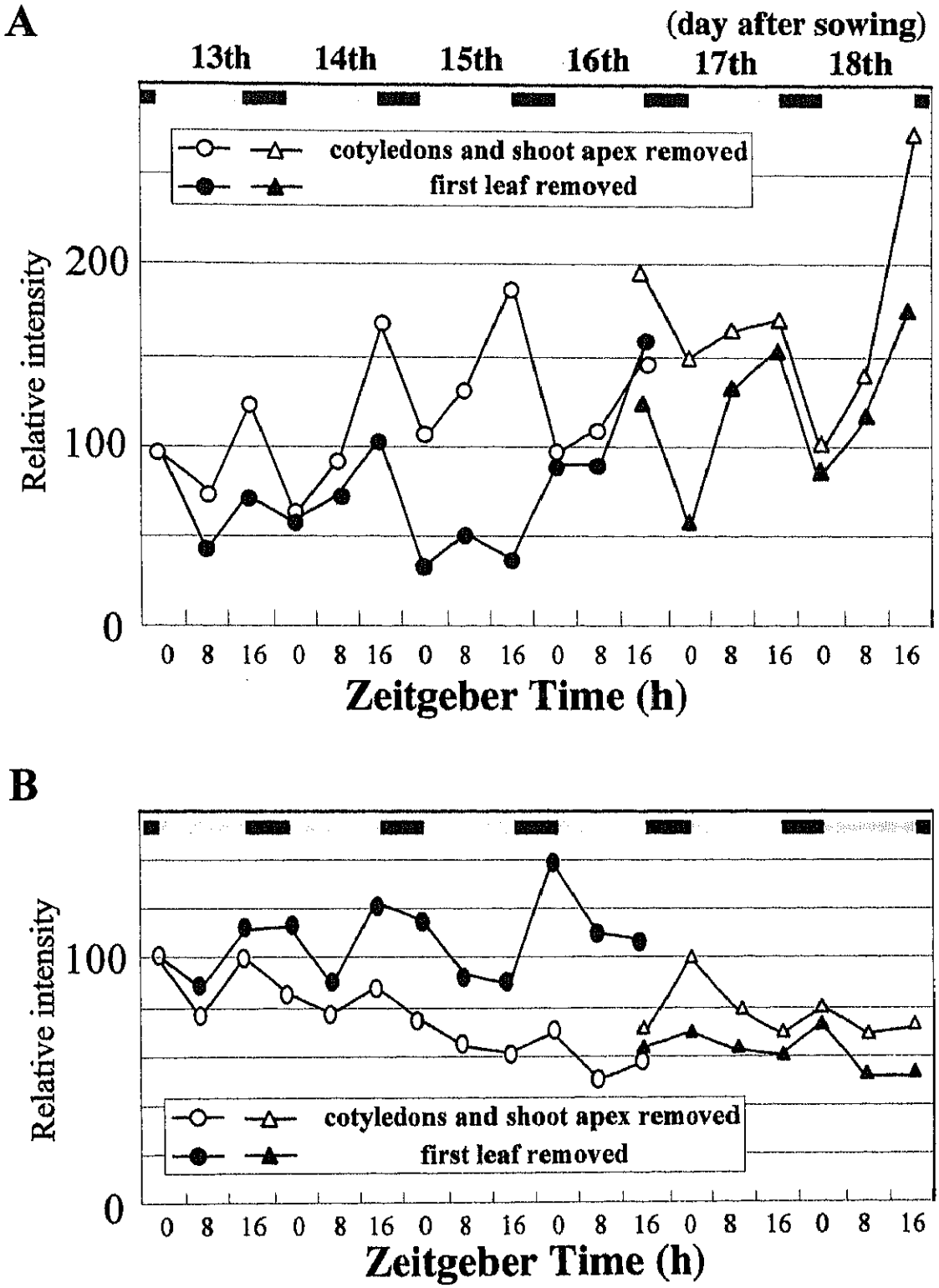
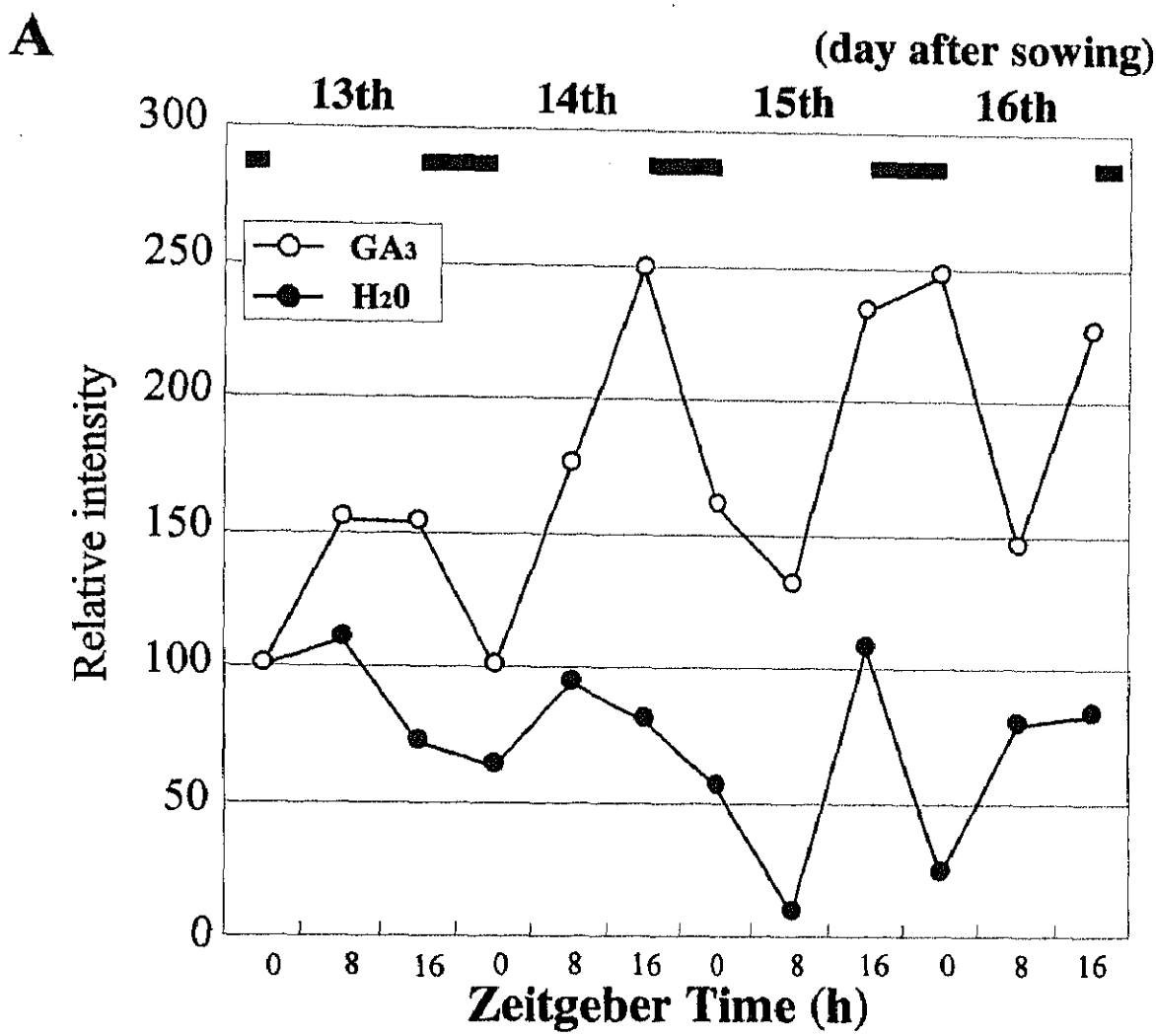

A
(day after sowing)
13th
14th
15th
16th
17th
18th
cotyledons and shoot apex removed
first leaf removed
Relative intensity
200
100
0
0 8 16 0 8 16 0 8 16 0 8 16 0 8 16 0 8 16
Zeitgeber Time (h)
B
Relative intensity
100
0
cotyledons and shoot apex removed
first leaf removed
0 8 16 0 8 16 0 8 16 0 8 16 0 8 16 0 8 16
Zeitgeber Time (h)

Figure 7. Effects of gibberellin on *XSP30* and *CRGRP-2* expression in roots.

Mature first leaves were removed from 13-day-old seedlings, and 2×10^{-4} M GA_3 (open circles) or water (closed circles) was applied every 2 days to the cotyledons and shoot apex; roots were collected every 8 h for RNA gel blot analysis. The transcripts were probed using cDNA for XSP30 (A) and CRGRP-2 (B) and quantified data are shown. The periods of light and dark are indicated as shaded and black bars, respectively.


A
(day after sowing)
13th
14th
15th
16th
300
250
200
150
100
50
0
Relative intensity
GA3
H20
0
8
16
Zeitgeber Time (h)

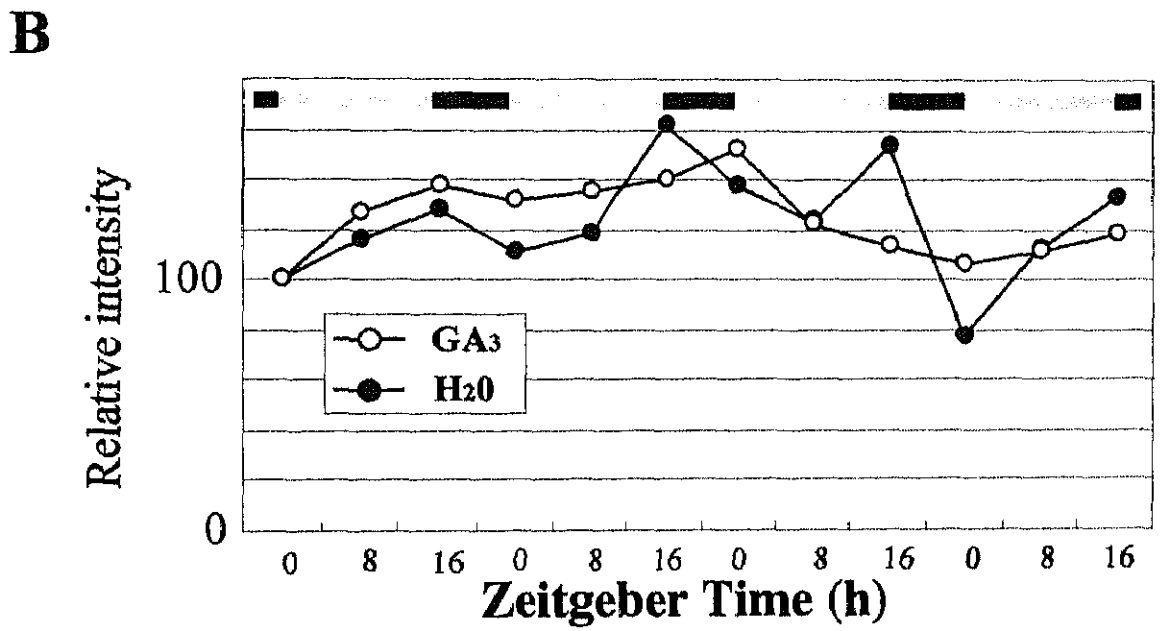

B
100
0
Relative intensity
GA3
H20
0
8
16
Zeitgeber Time (h)

Figure 8. Effect of uniconazole-P and gibberellin on *XSP30* and *CRGRP-2* expression in roots. 10^{-4} M uniconazole-P (closed circles) or 10^{-4} M uniconazole-P plus 2×10^{-4} M GA_3 (open circles) was applied to the shoot, including the cotyledon, first leaf, and shoot apex, of 13-day-old seedlings; roots were collected every 8 h for RNA gel blot analysis. The transcripts were probed using cDNA for XSP30 (A) and CRGRP-2 (B) and quantified data are shown. The periods of light and dark are indicated as shaded and black bars, respectively.

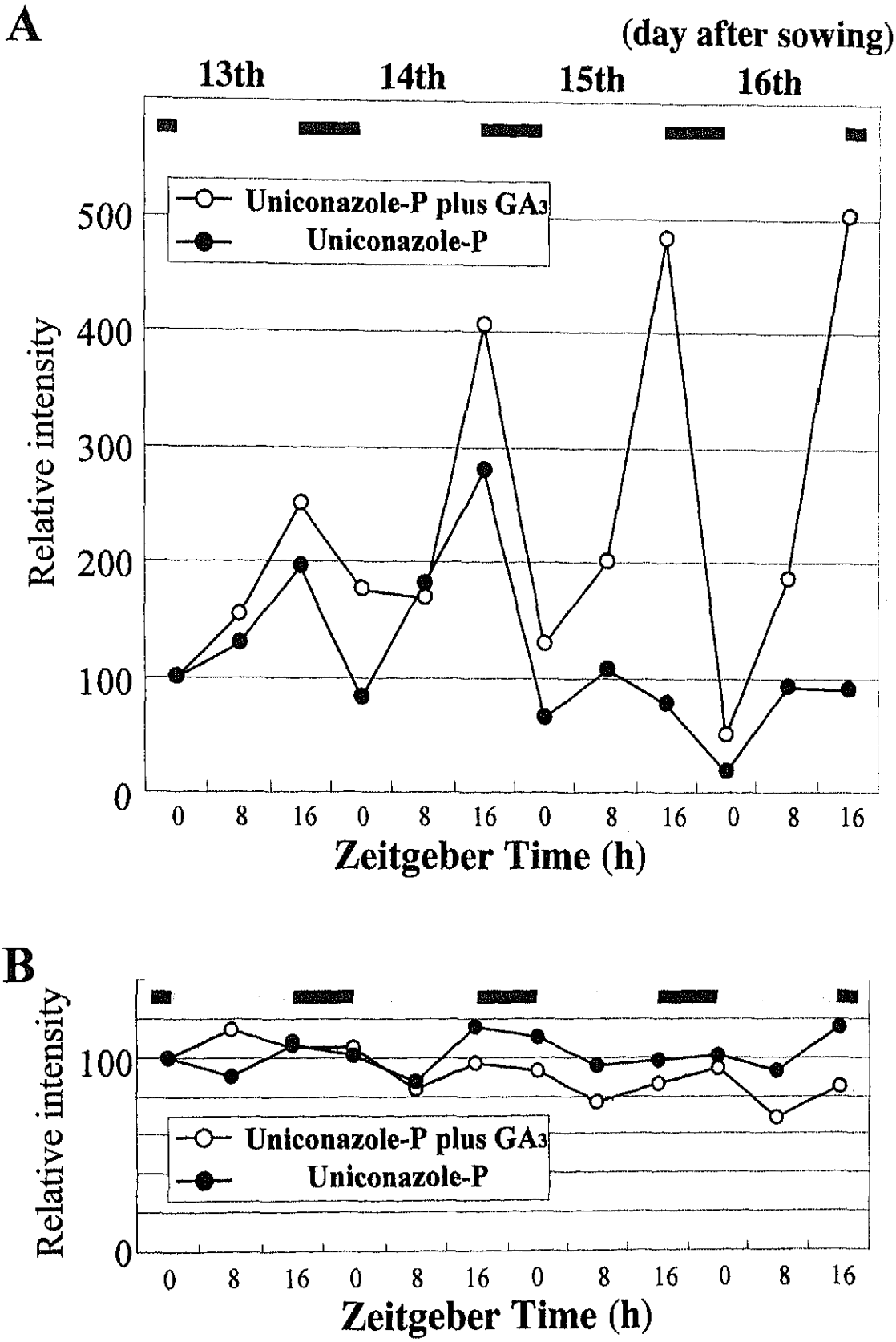
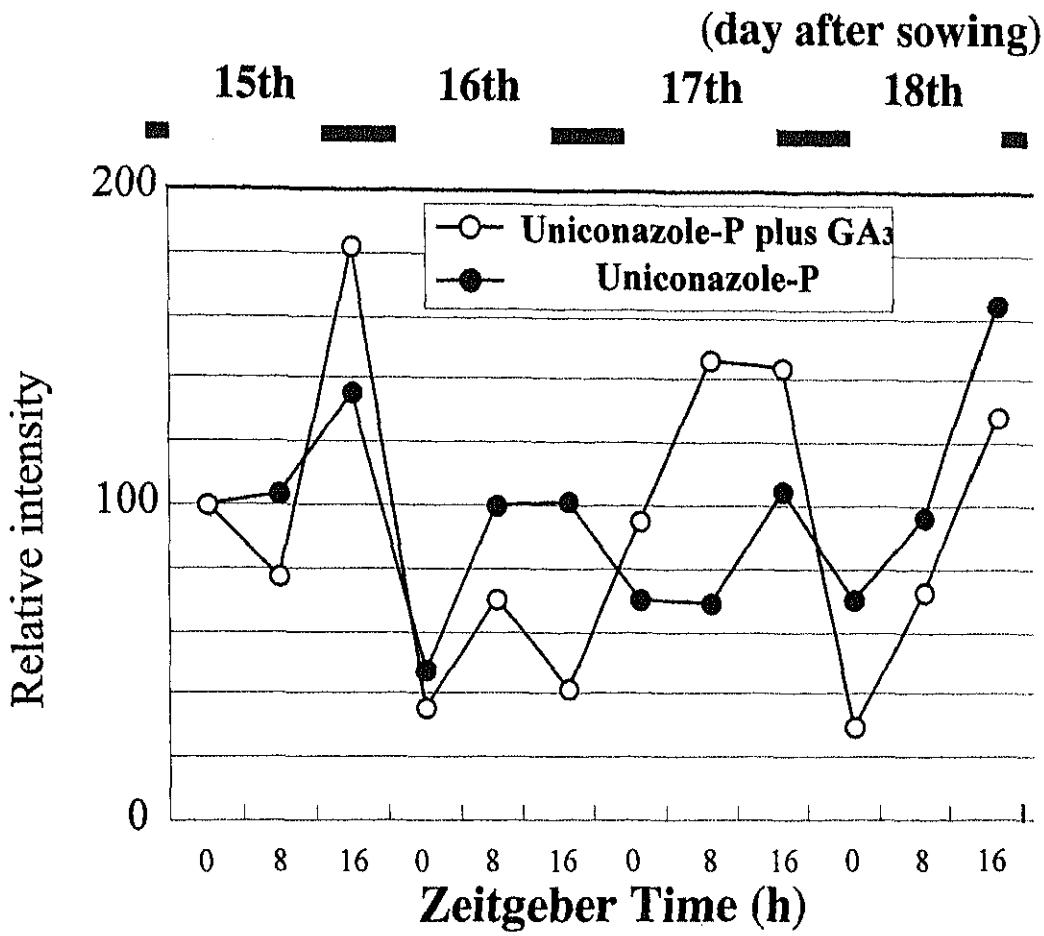

A
(day after sowing)
13th
14th
15th
16th
Uniconazole-P plus GA3
Uniconazole-P
Relative intensity
500
400
300
200
100
0
0 8 16 0 8 16 0 8 16 0 8 16
Zeitgeber Time (h)
B
Relative intensity
100
0
Uniconazole-P plus GA3
Uniconazole-P
0 8 16 0 8 16 0 8 16 0 8 16
Zeitgeber Time (h)

Figure 9. Effect of exposing the roots to gibberellin on *XSP30* and *CRGRP-2* expression in roots. The shoots of 13-day-old seedlings were pretreated with 10^{-4} M uniconazole-P. Distilled water (solid circles) or 10^{-4} M GA_3 (open circles) was applied to the roots of 15-day-old seedlings; the roots were collected every 8 h for RNA gel blot analysis. The blots were probed with cDNA for XSP30 (A) and CRGRP-2 (B) and the quantified data are shown. The light and dark periods are indicated as shaded and black bars, respectively.

A


(day after sowing)
15th
16th
17th
18th
200
100
0
Uniconazole-P plus GA3
Uniconazole-P
Relative intensity
0 8 16 0 8 16 0 8 16 0 8 16
Zeitgeber Time (h)

B

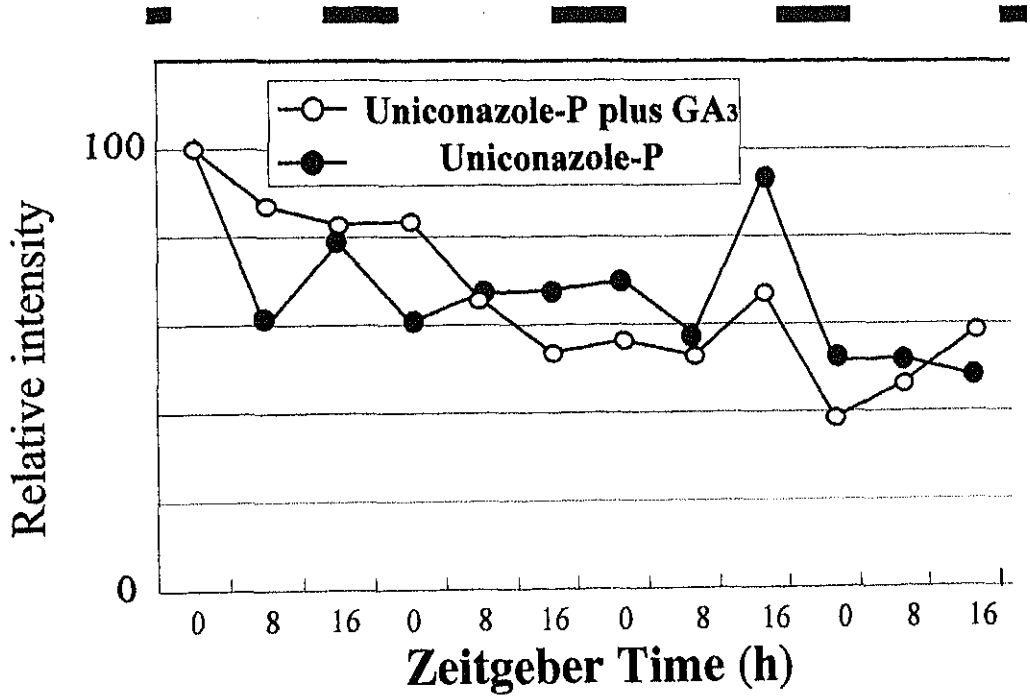

100
0
Uniconazole-P plus GA3
Uniconazole-P
Relative intensity
0 8 16 0 8 16 0 8 16 0 8 16
Zeitgeber Time (h)

Figure 10. *XSP30* Promoter sequence. The underlined portions are the 1) ARFT, 2) GT1CONSENSUS, 3) EPELEE4, 4) MYBGAHY, and 5) CIACADIANLELHC motifs.


-820 TGTTGCCACTTCTGACTCAAATTATATATTATCTTCTTAACTTCTGTTATCTCTTGTCTATAAATGTCAATATTATGTCTCACCATCGGTTCCTTGTAAC -721
-720 AATATTTATCCCAAAATTTATCATCACATTATACCCCATAATCTTTCGTAAGAGATATTATCCTAGAAAAGTATGCCTATAGGGTTGGAAAATCTTTCTA -621
-620 TTAAGTAGGTACAAAAGTAGTGAAATAAAATTCAAATTTATATGTTTACTTGCATGGCCAATGTAACAACAAAAAATAATAATGTAGAAACATGGTCTAT -521
-520 ATTAAATAATAAATAGAGTAAAATAAATCAAAATATTTATAAATATAGCAATATTTTACTTTTTACTTACGATAGATCGTGGTTGACTAATTTCTATGTT -421
-420 TTAATAGGTCTATATATAATAATTTAAAGATAATAAATTGTGATATTTTGTTATATTTATAAATATTTTTAATCACATTTAAAACAAATTTCCATTCACT -321
-320 CCACTAAACTATGAATTTCATCCTAATTACCACTGGCAAAATGTAACAAAAAGTTCAAACCAATGGGGAGAGTTTATGTGTATACTCATCCCCATAAACA -221
-220 ACCCCATCCATTGGAAGAACAATTATGGATTTGTGGATTCATCATTCCCCACTACAATAATTTTTTTTGTGATCCTTTTTAATTAGAATAATAATGCTTG -121
-120 TTCCATTCATAATTGTCCACTGGGCTAGCCTTTCGTTGTATTTTGTGTGTATATATACCCCTTCATTCTAGCCCAACTTTGTCAAACAAATATATATAGT -21
-20 CGAATTAAAGTAATCAAAGGA +1
① ② ③ ② ④ ⑤

Figure 11. Transgenic hairy roots induced by infection with *Agrobacterium tumefaciens R1000*. The growth of transgenic hairy roots from the 7-day-old cotyledon of cucumber plant was induced by infecting them with *Agrobacterium tumefaciens R1000* containing P_{XSP30}::*GUS* construct plasmids. The transgenic hairy roots were photographed in 3 weeks after infection.

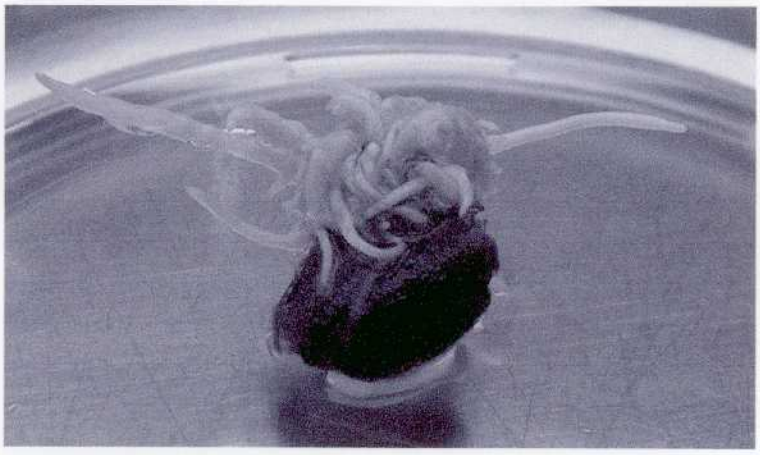
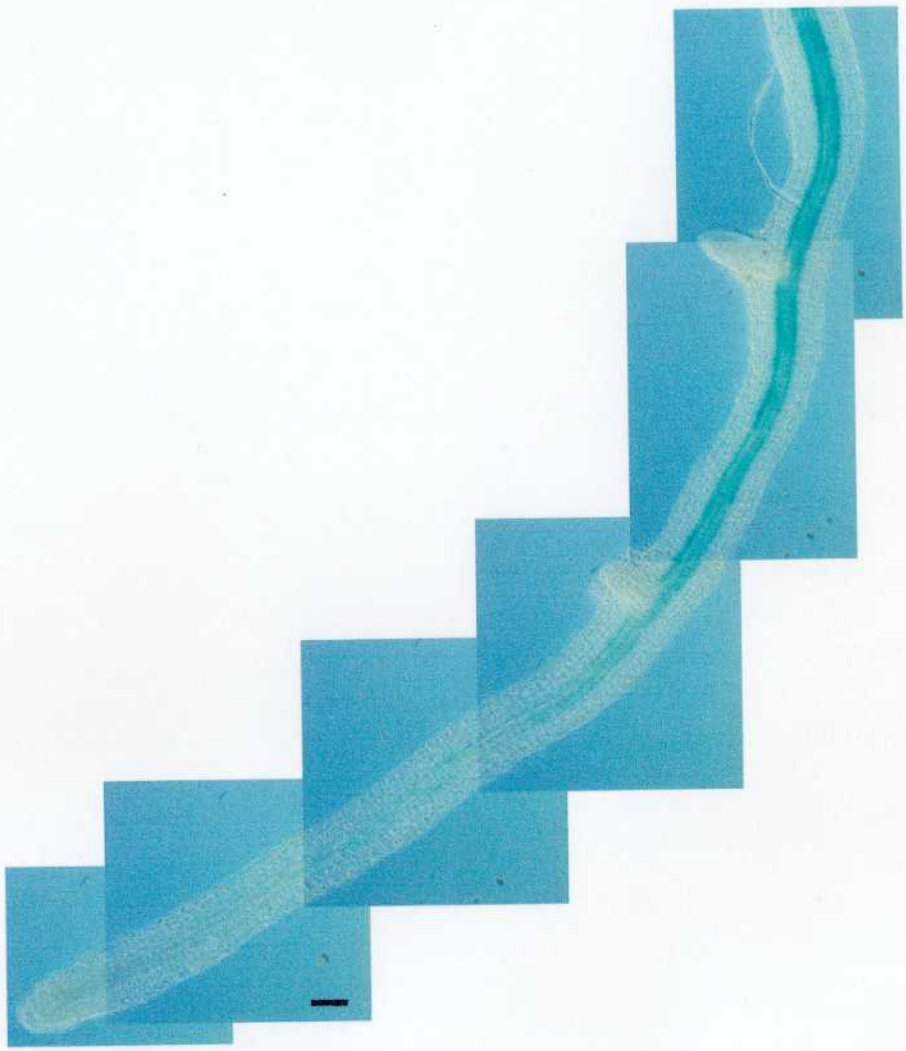
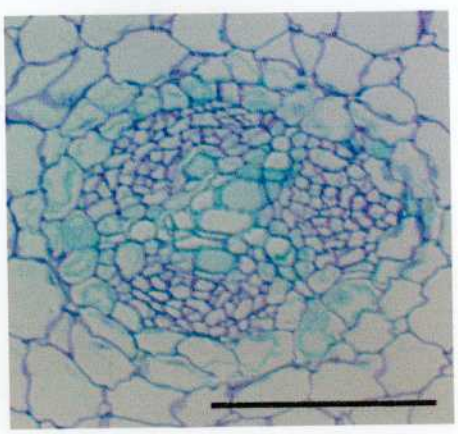


Figure 12 GUS activity in P_{XSP30}::*GUS*-transgenic hairy roots. GUS activity was observed in the central region of mature roots, but not in the root cap (A). GUS-stained transgenic roots were embedded in Technovit 7100, and thin serial sections were stained by toluidine blue (B) or were left unstained (C). The central cylinder is shown. Scale bars indicate 100 μm. Arrow and arrowhead indicate GUS-staining in the xylem parenchyma and pericycle cells, respectively.

A



B



C

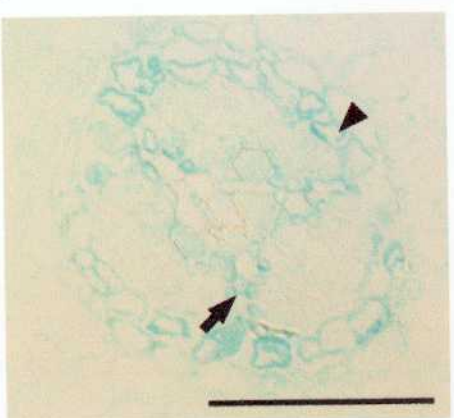
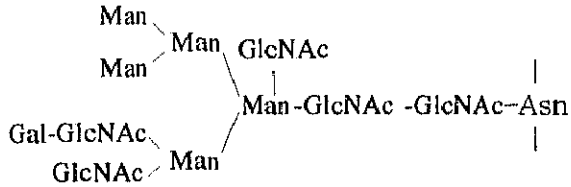
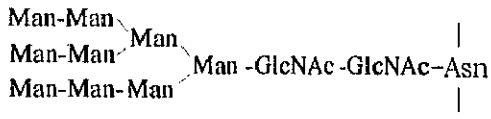


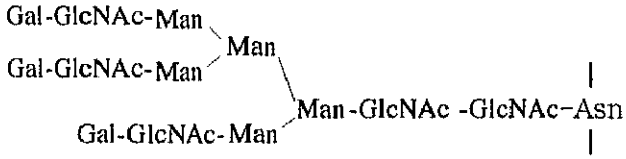
Figure 13. Structure of *N*-linked oligosaccharide chain of glycoprotein.



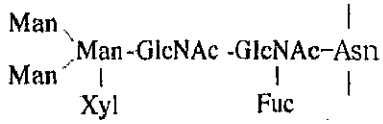
ovalbumin (Yamashita et al., 1978)



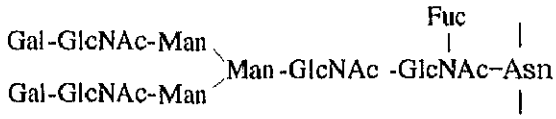
Soybean agglutinin (SBA) (Dorland et al.,1981)



asialofetuin (Nilsson et al., 1979)



Soybean peroxidase (Gray et al., 1996)



γ globulin (Oda et al., 2000)

Gal (galactose)	Xyl (xylose)
GlcNAc (*N*-acetylglucosamine)	Fuc (fucose)
Man (mannose)	

Figure 14. Lectin activity of XSP30. Ovalbumin (lane 1) and asialofetuin (lane 2) (1 μg each) were separated by SDS-PAGE, blotted onto nitrocellulose sheets, and stained with amido black (A) or reacted with anti-XSP30 antiserum (B), xylem sap followed by anti-XSP30 antiserum (C), or RCA (D).

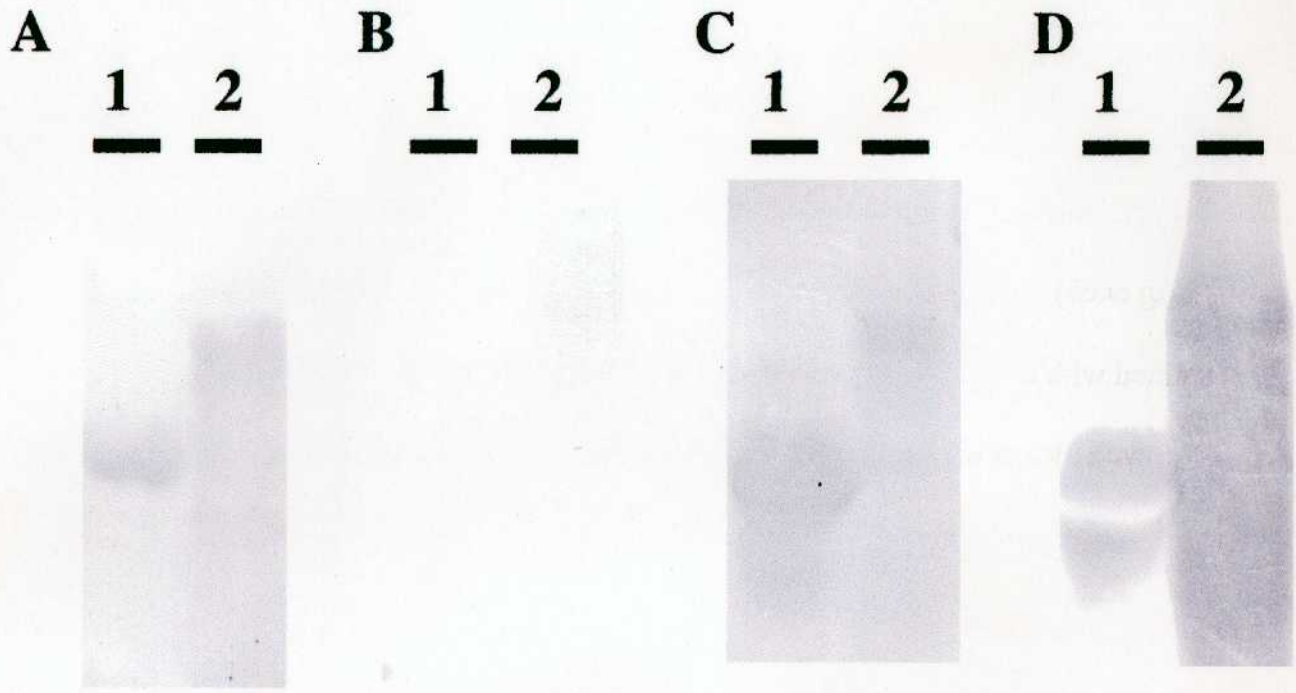

A
1 2
B
1 2
C
1 2
D
1 2

Figure 15. Binding activity of XSP30 to asialofetuin lacking galactose. Asialofetuin (lane 1) and asialofetuin whose terminal galactose was removed by galactosidase treatment (lane 2) (1 μg each) were separated by SDS-PAGE, blotted onto nitrocellulose, and then stained with amido black (A) or analyzed by lectin blot with XSP30 (B) or RCA (C).

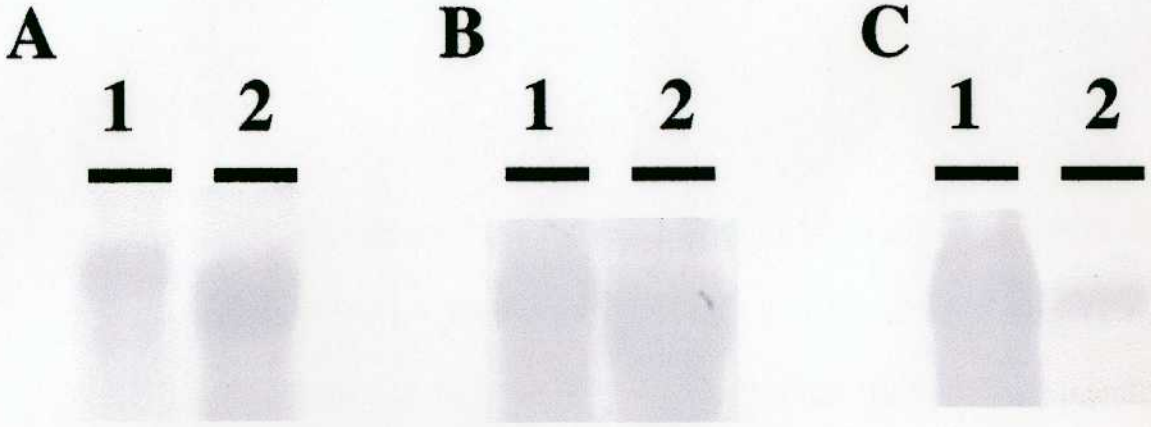

A
1
2
B
1
2
C
1
2

Figure 16. Binding activity of XSP30 to high-mannose-type glycoproteins.

Soybean agglutinin (lane 1), soybean peroxidase (lane 2) and γ -globulin (lane 3) (1 μg each) were separated by SDS-PAGE, blotted onto nitrocellulose, and stained with amido black (A) or analyzed by lectin blot with XSP30 (B) or Con A (C).

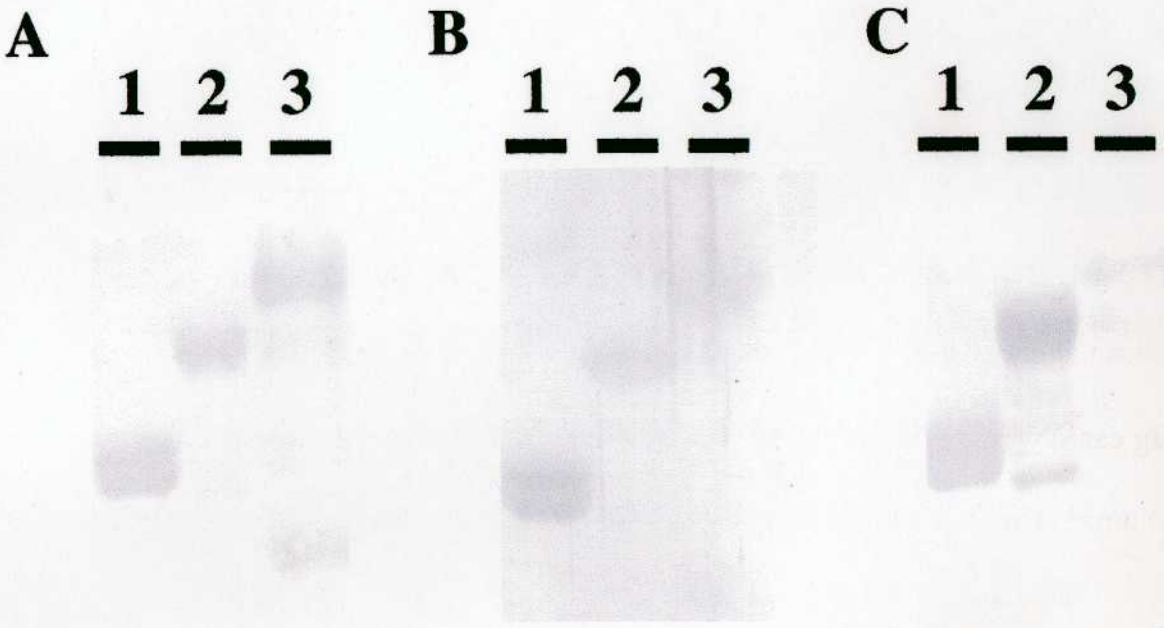

A
1 2 3
B
1 2 3
C
1 2 3

Figure 17. Inhibition of XSP30 lectin activity by oligo-*N*-acetylglucosamine. Soybean agglutinin (1 μg) was separated by SDS-PAGE and blotted onto nitrocellulose, and XSP30 binding activity was examined in the absence (lane 1) or presence of 1 mM (lane 2) or 0.1 mM (lane 3) tri-*N*-acetylchitotriose or 1 mM (lane 4) or 0.1 mM (lane 5) di-*N*-acetylchitobiose

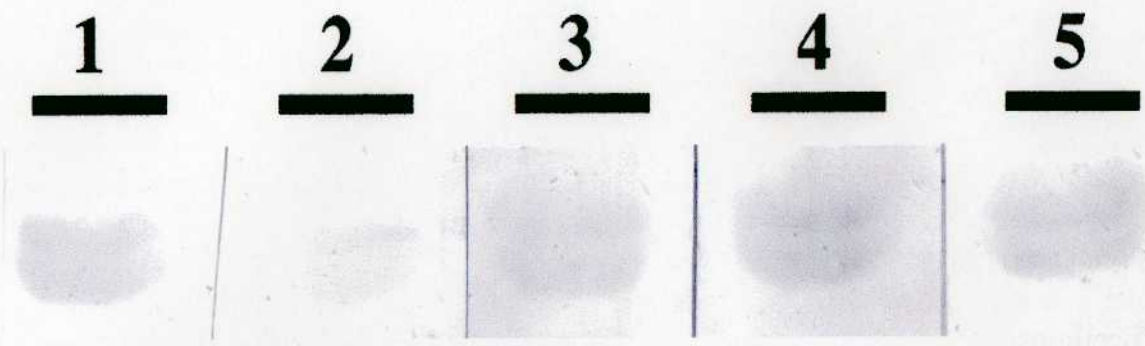
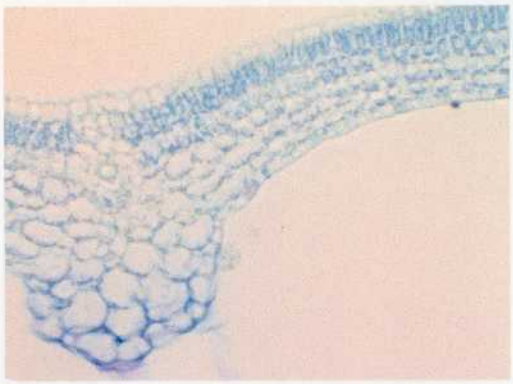
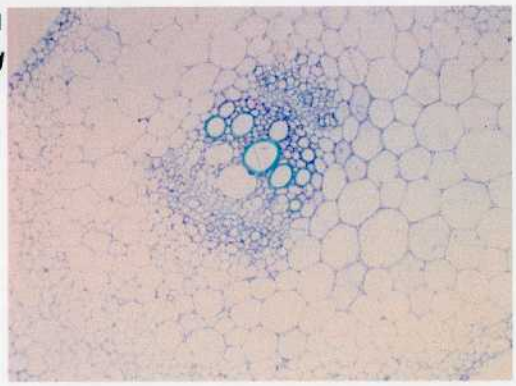

1
2
3
4
5

Figure 18. Binding of XSP30 to cucumber leaf and stem tissue sections. Leaf (A-D) and stem (E-H) sections were stained with toluidine blue (A and E), or allowed to react with anti-XSP30 antiserum (B and F), or xylem sap followed by anti-XSP30 antiserum (C, D, G and H). Proteins in sections in D and H were digested by proteinase K before the xylem sap and anti-XSP30 treatments. Scale bars indicate 100 μm.

A

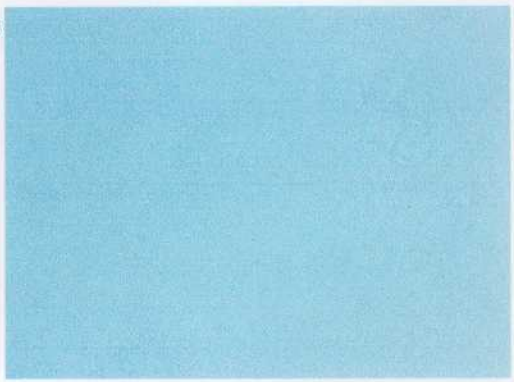


E

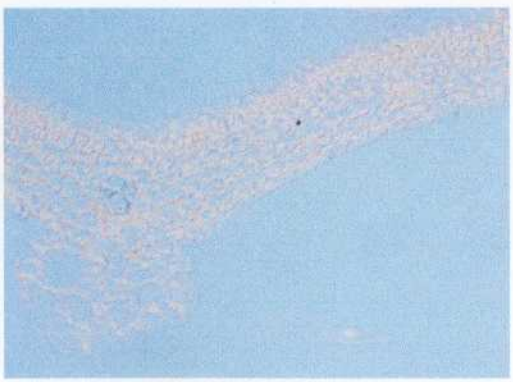


B

F



C



G

D

H

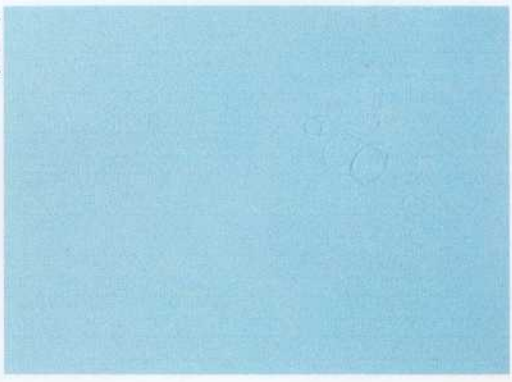
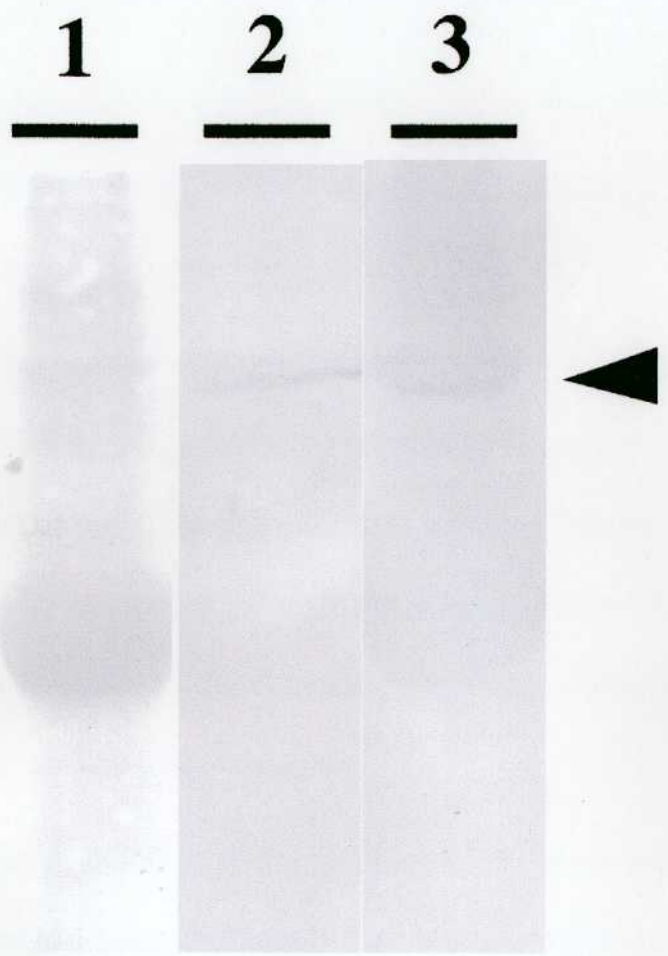


Figure 19. XSP30 binding to proteins in cucumber leaf particulate fraction. Proteins in particulate fraction of cucumber leaves were separated by SDS-PAGE, blotted onto nitrocellulose, and stained with amido black (lane 1), or allowed to react with anti-XSP30 antiserum (lane 2) or xylem sap followed by anti-XSP30 antiserum (lane 3). Arrow indicates non-specific staining.


1
2
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