

Peroxidases and lignification in xylem of Norway spruce

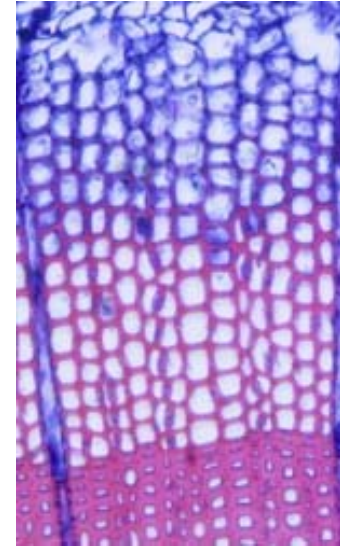
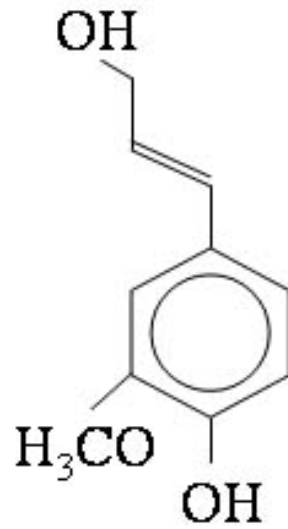
Kaisa Marjamaa
University of Helsinki

Class III plant peroxidases

- Multigene families eg. 73 in Arabidopsis (Welinder et al. 2002), 138 in rice (Passardi et al. 2004), over 150(?) members in poplar genome...
 - E.g. in Arabidopsis, 28-94% protein sequence identity between family members (Welinder et al. 2002)
 - Are able to catalyze oxidoreduction between hydrogenperoxide and of wide variety of phenolic substrates
 - Secreted to cell wall or vacuoles

 - Several proposed biological functions including catalysis of **oxidative polymerisation of monolignols in lignin biosynthesis**
-

Lignin and peroxidases in spruce wood



”Peroxidases have more functions than Swiss army knife”(Passardi et al. 2005)

So what’s required?

Ability to catalyze the oxidation of CA and/or lignin polymer.
Localization to lignifying cell wall.

Xylem peroxidases of Norway spruce

Three peroxidase cDNAs were cloned from lignifying xylem of 30 year old Norway spruce :

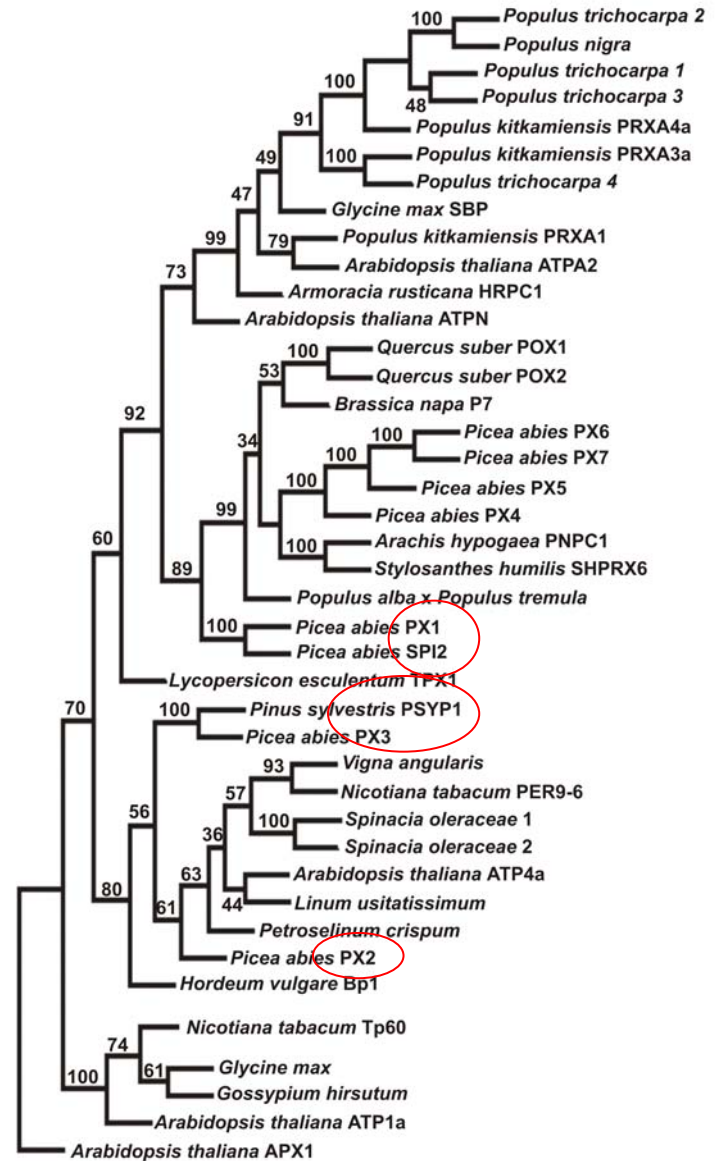
Gene	ORF	Protein	AA	MW kDa	pI	AA-sequence identities %	
						PX2	PX3
<i>Pa-px1</i>	1023	PX1	340	33.7	9.43	39.7	40.8
<i>Pa-px2</i>	1059	PX2	353	36.2	8.37	100	58.6
<i>Pa-px3</i>	1056	PX3	351	35.6	5.15	58.6	100

Xylem peroxidases of Norway spruce

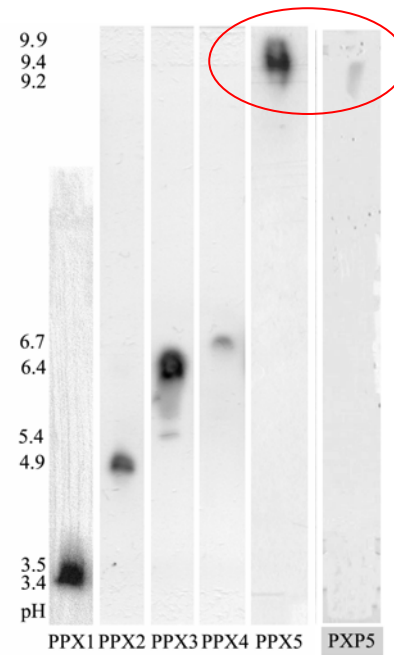
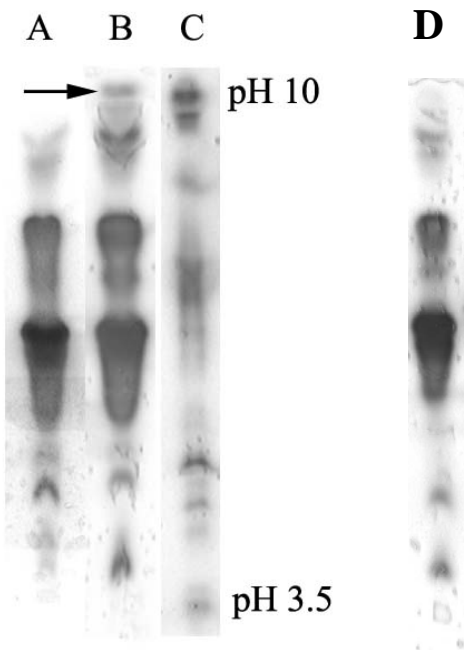
PX1: SPI2 72% similarity

PX2: PER9-6 78% similarity

PX3: PSYP1 80% similarity



Substrate preferences



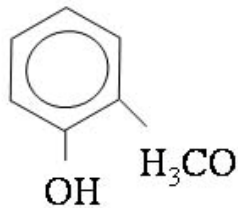
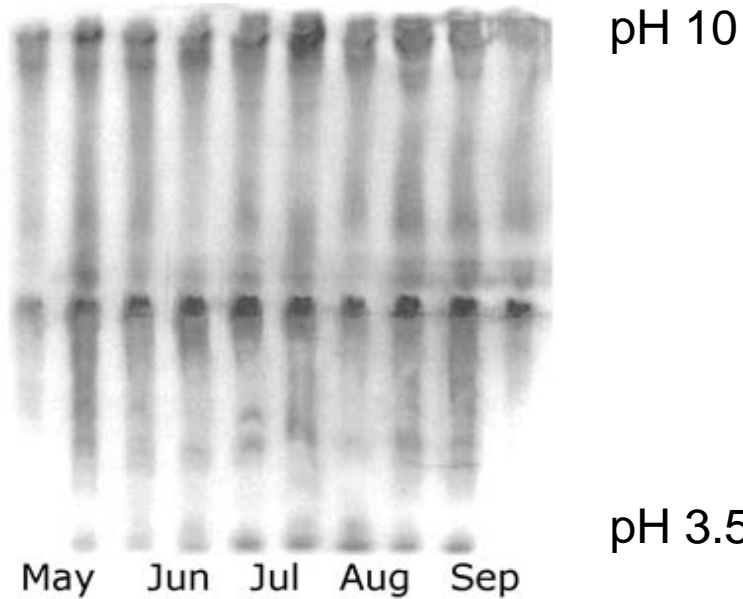
IEF gels from *C.roseus* hairy root extracts and *P.abies* xylem extract:

- A. *C.roseus* wild-type
- B. *C.roseus* with *Pa-px1*
- C. *P.abies* xylem extract
- D. *C.roseus* with *Pa-px2*

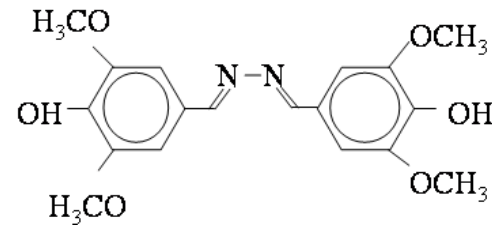
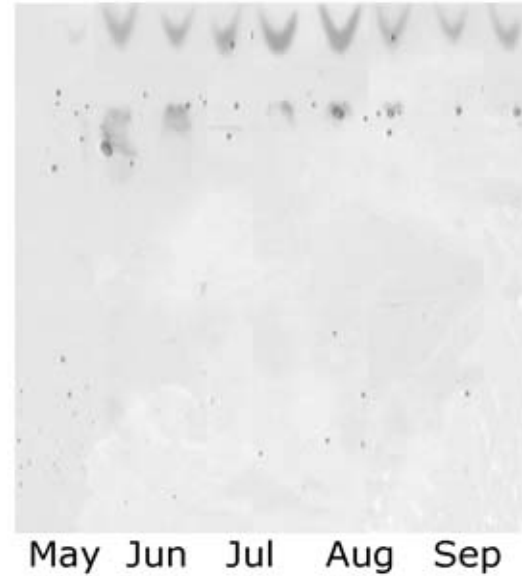
Fraction	CA	SA	p-CA
PPX1	137.5±18.8	23.9±0.4	3.4±0.3
PPX2	1.7±0.02	1.3±0.01	0.3±0.1
PPX3	22.3±0.6	2.6±0.4	2.7±0.6
PPX4	1.9±0.2	0.4±0.01	0.2±0.1
PPX5	27.3±11.1	5.7±0.9	0.8±0.03

Substrate preferences

Guaiacol



Syringaldazine



Substrate preferences

Peroxidases that are able to catalyze oxidation of syringaldazine are generally localized in developing xylem (Harkin and Obst, 1973, Christensen et al. 2001, etc.)

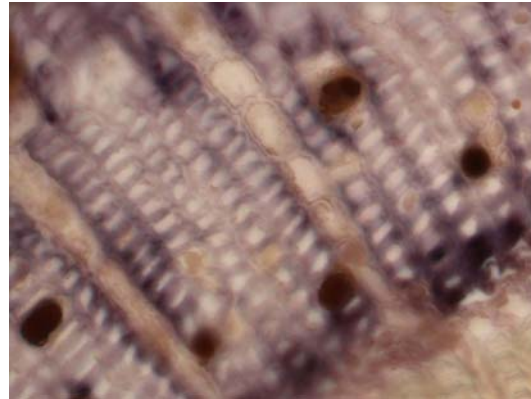
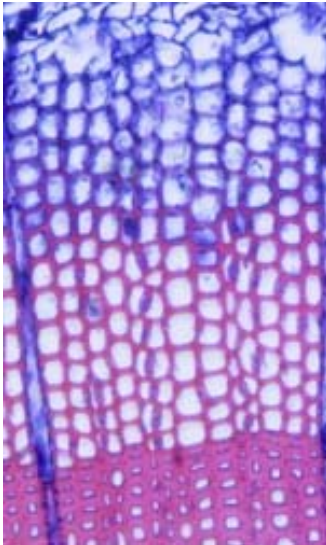
The cationic syringaldazine oxidizing peroxidase CWPO-C isolated from poplar callus culture was able to catalyse oxidation lignin polymers (Sasaki et al. 2004).



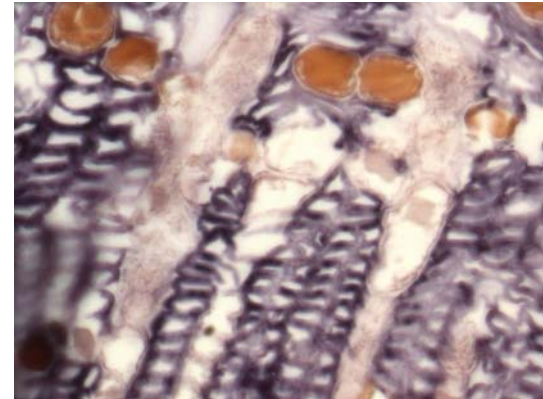
Spatiotemporal localization:

Expression in lignifying tracheids?

In situ hybridization of stem sections of spruce seedlings using full length DIG labelled RNA probes:

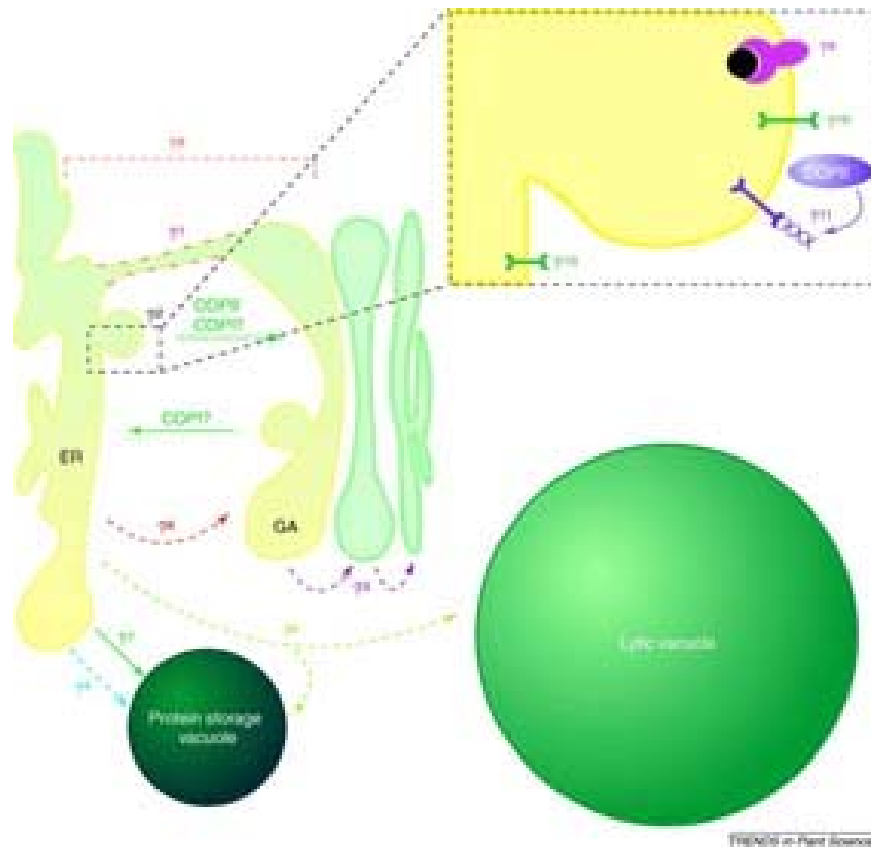


Pa-px1



Pa-px2

Spatiotemporal localization:



Hanton et al. 2006 (Trends in Plant Science, Vol. 11, No 7)

Spatiotemporal localization

Subcellular localization: cell wall or vacuole?

```
CLUSTAL X (1.8) multiple sequence alignment
HRPC      ---MHFSSSSTLFTFCITLIPLVCLILHASLS--DAQ----LTPTFDNSCPNVSNIVRDT
PX1      MARIKHSPGLTLQFQSVLITAVALMLWIQTL--DAQSCNGLSHHFYKSCPKAQAIKSM
PX3      ----MAKHITPLASVILCMFVIYGRAVHSL---PTPVAGLSWTFYSTSCPSLESIVRQR
PX2      ----MEKLMILCLFCTLWIACASRENVLTLSNDPPLVNGLSWTFYKSSCPKLESIVKQR
          *           .           *: ** .***. . *::.

HRPC      IVNELRSDPRIAASILRLHFHDCFVNGCDASILLDNTTSFRTEKDAFGNANS-ARGFPVI
PX1      VEDAVKKEARIAASLLRLHFHDCFVKGCDASLLDDNASFTGEKTAIPNKNS-LRGFEV
PX3      MGAYLSADITQAAGLLRLHFHDCFVQCGDGSVLLN-STSG--EQTTPPNLSLRAQAFKII
PX2      IDFYLKQDITQAAGLLRLHFHDCFVQCGDGSVLLAGSTSGPSEQGAPPNLSLRAKAFEI
          :   :   :   **.:*****:***.*:** .:*   *:: * .   :.* ::

HRPC      DRMKAAVESACPRTVSCADLLTIAAQQSVTLAGGPPSWRVPLGRRDSLQ-AFLDLANANLP
PX1      DKIKSNLEKACPGVVS CADILAVAARDSVAISGGPFWKVLLGRRDSRS-ASKSGANEDLP
PX3      NDIKQHV EAACSGIVSCADILALAARDSVAMAGGPFYPIPFGRDLSLTFANLSTTLANLP
PX2      NDIKSRVDKACKVVVSCADV TALAAKESVRAAGGPQYRIPLGRRDSLKFATQNVTLANLP
          :  :*  :: **   *****: :***:**   *** : : :***** * . : :**

HRPC      APFFTLPLQLKDSFRNVGLNRSDDLVALSGGHTFGKNQCRFIMDRLYN-FSNTGLDPDPTLN
PX1      APNSTHQ TLETKFKLQGLN-VVDLVALSGAHTIGLARCASFQRLYN-QTGN-KPDQTL
PX3      SPTSNTVLI SVLGPKGLT-FTDLVALSGGHTIGRSNCSSFQNRLYNSTTGISMQDSTLD
PX2      APSSKVTTLIKAFATKNLN-VTDLVALSGGHTIGIGHCTSF TDRLY-----PKQDTTLN
          :* . * : * . *****.*:* . * : :*** * **

HRPC      TTYLQTLRGLCLNG-NLSALVDFDLRTPTIFDNKYVYNLEEQKGLIQSDQELFSSPNAT
PX1      TTYLKQLRTVCPQTGTDNQTRPFDVPSPTKFDVNYKNNVAGKLLNSDEILYST-KGS
PX3      QNFAKNL YLTCTPTNT--SVNTNLDILT PNVFDNKYYVDLLENEQTLFTSDQSLYTD---T
PX2      KSFARLYTACPPKT--SSNTTVLDIRTPNVFDNKYYVDLMNRQGLFTSDQDLYSD---S
          .: : * ** . : * :*. ** :** :: : * : ** :*:: :

HRPC      DTIPLVRSFANSTQTFFNAFVEAMDRMGNI TPLTGTGQGIIRLNCRVVNSNSLLHDMVEV
PX1      RTAGFVKYTTNTTHAFKQFAASMIKMGNISPLTGFHGEIRKNCRRIN-----
PX3      RTRDIVKSFALNQLSFFQFVLSMLKMGQLDVL TGSEGEIRNNCWAANPSTYSIIDSEAS
PX2      RTKAI VNFALQDLFFEFKFAVAMVKMGQLNVLTGSKGEIRSNCVSNLASTSTVEVAEE
          * :*. :: . ** : * . : * :***: *** .:* ** *

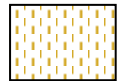
HRPC      DVFSSM----
PX1      -----
PX3      QESPSYSM--
PX2      DVIESYASFM
```

Spatiotemporal localization

Expression of signal peptide-GFP fusions in tobacco protoplasts

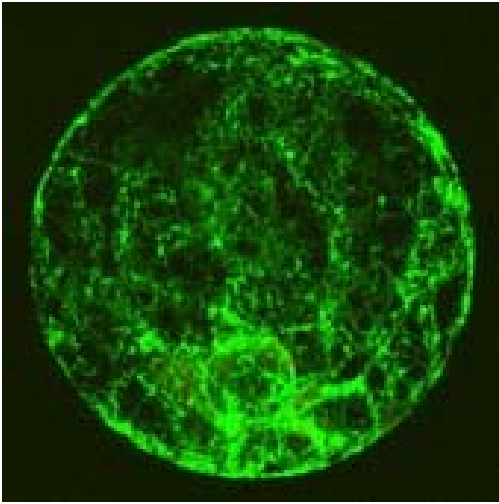


N-terminal secretion signal peptide

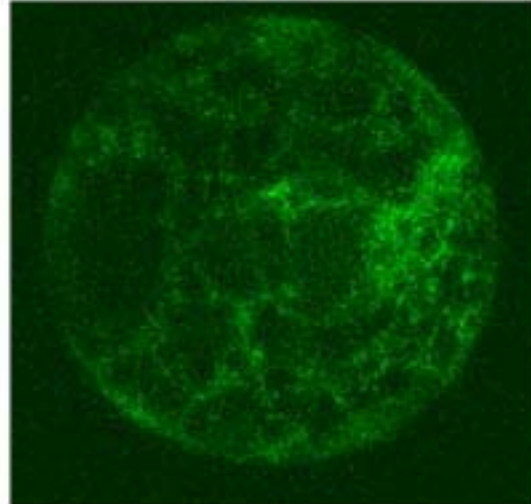


C-terminal putative signal propeptide

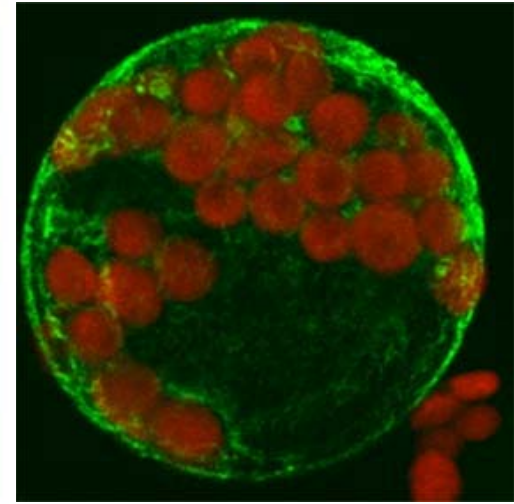
Spatiotemporal localization



PX1-GFP



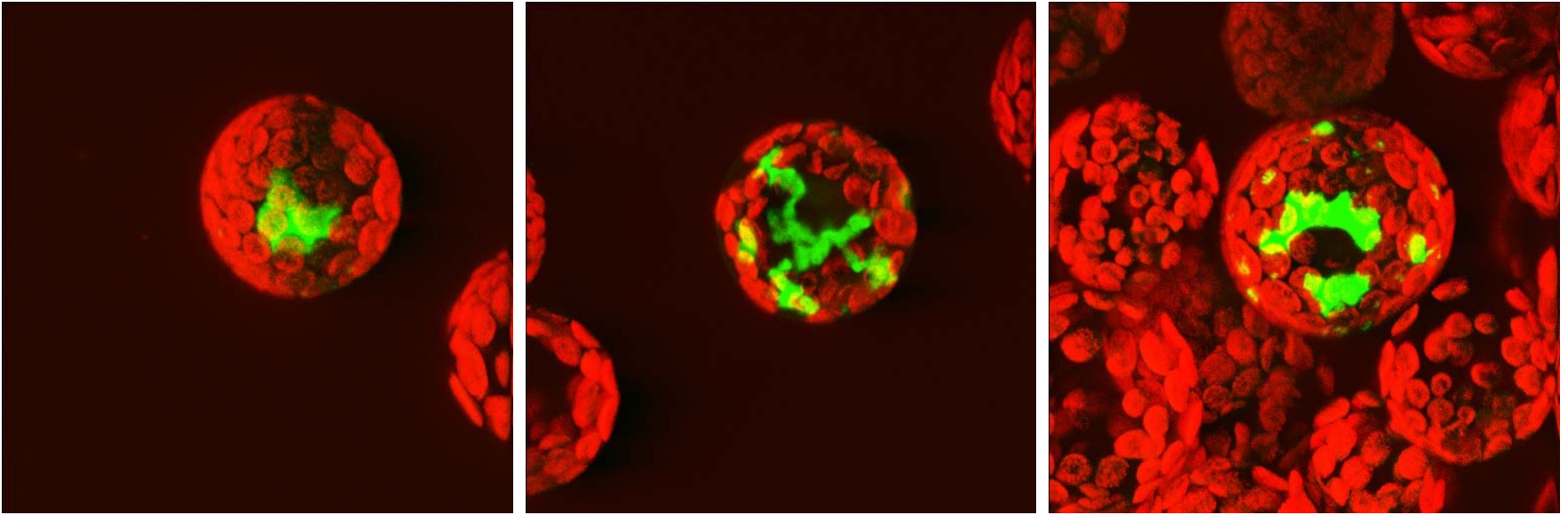
PX2-GFP



PX3-GFP

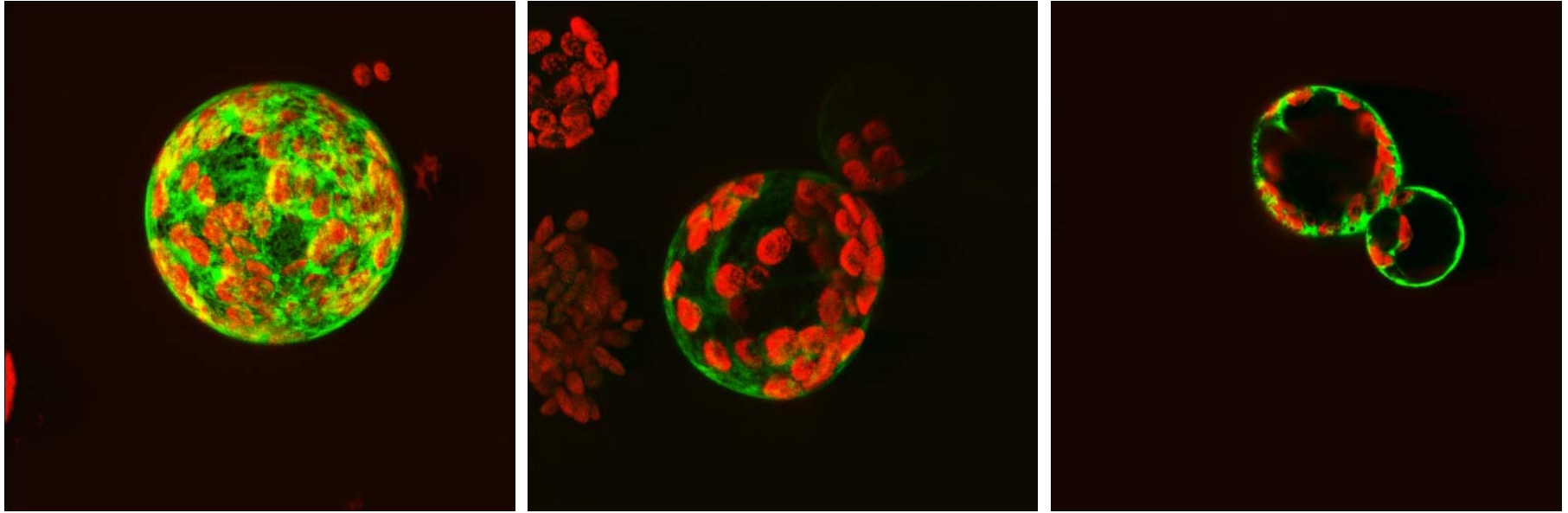
N-terminal secretion signal peptides directed GFP to ER

Spatiotemporal localization



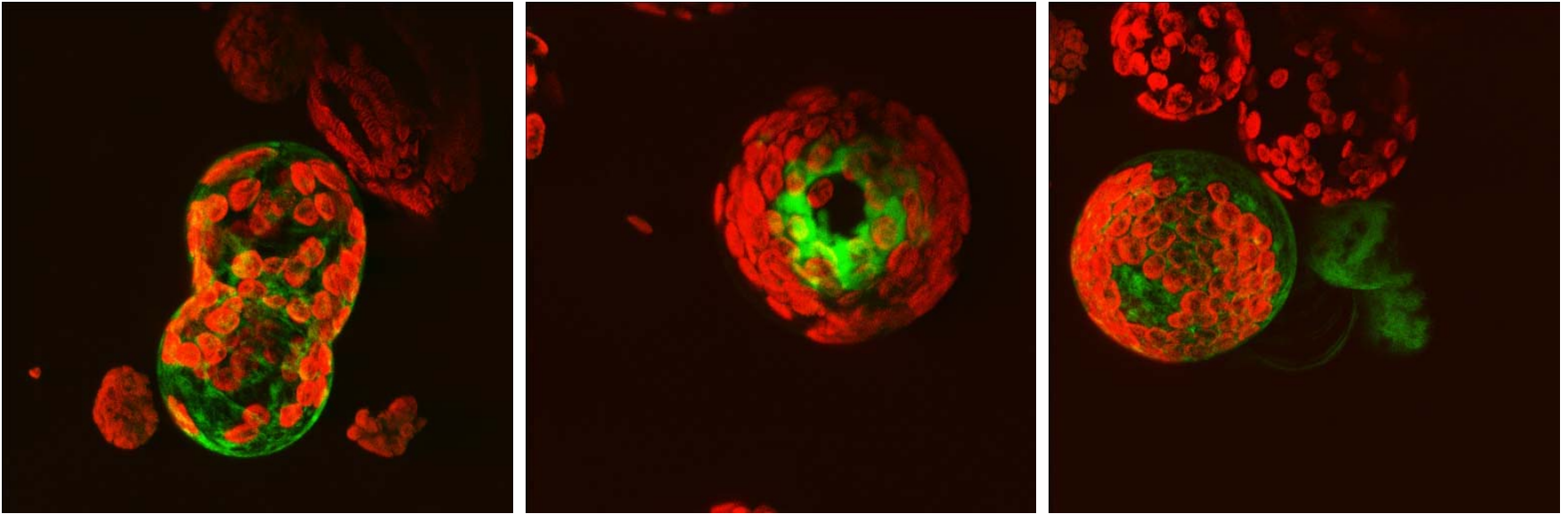
Tobacco protoplasts expressing PX3 N-terminal secretion signal peptide –GFP- C-terminal propeptide fusions two days after transformation

Spatiotemporal localization



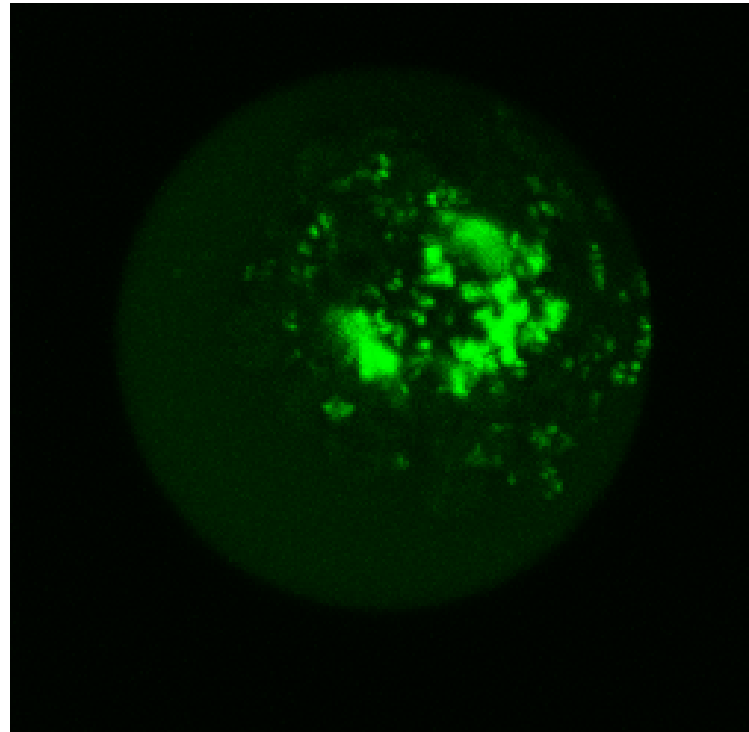
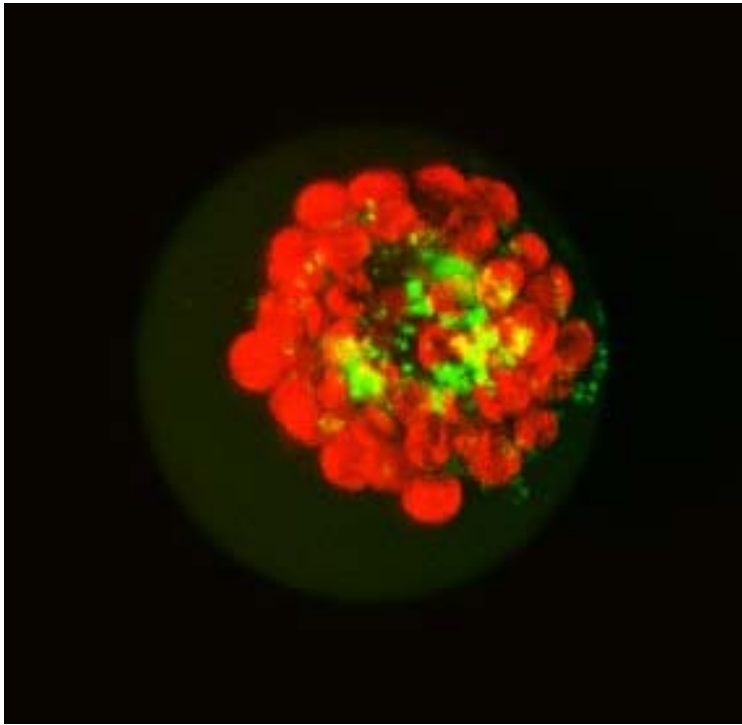
... four days after transformation

Spatiotemporal localization



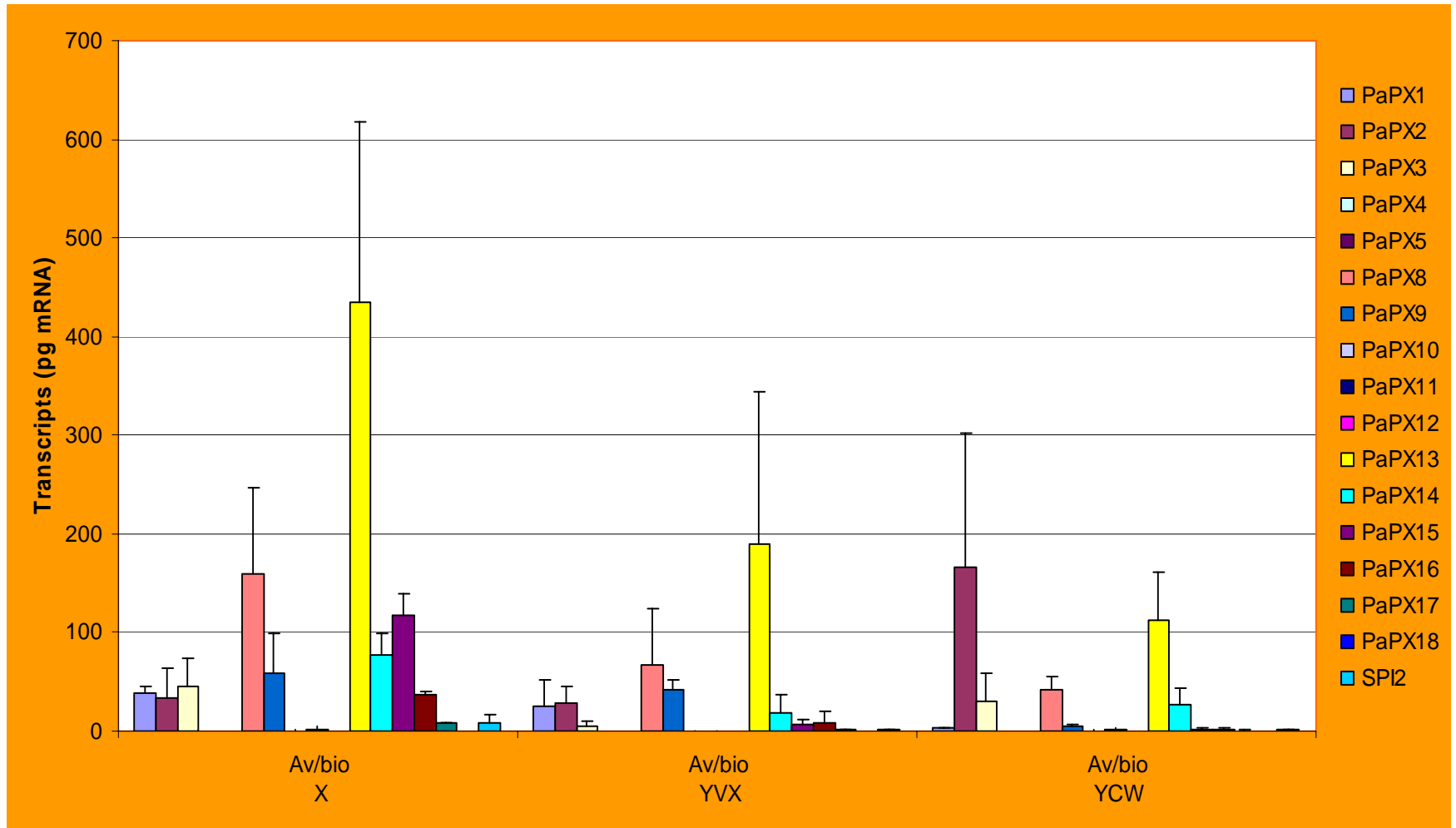
... six days after transformation

Spatiotemporal localization

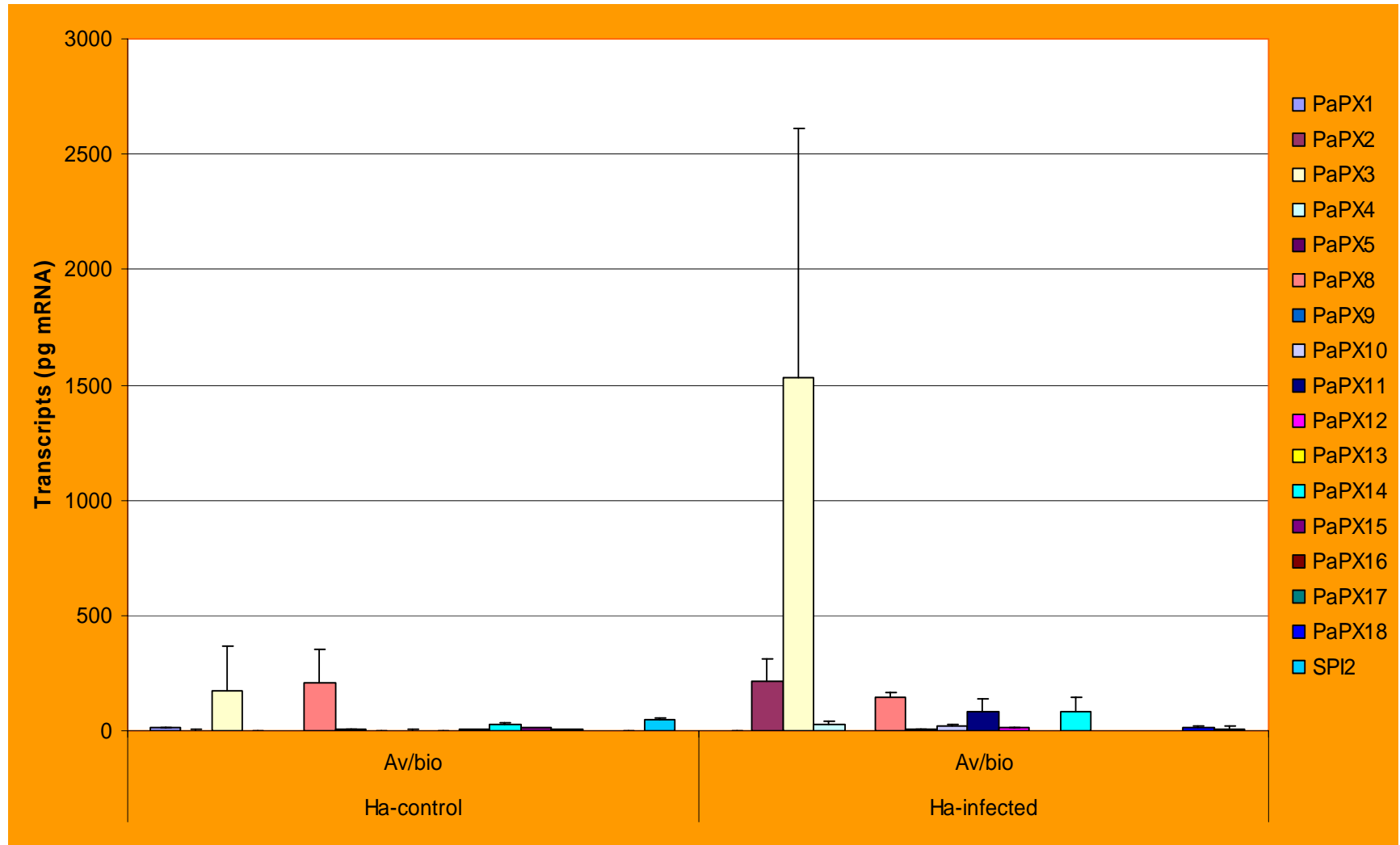


Tobacco protoplast expressing PX2 N-terminal secretion signal peptide –GFP- C-terminal propeptide fusions five days after transformation

Quantitative real-time PCR



Quantitative real-time PCR



Koutaniemi et al. 2006 (submitted)

Conclusions 1

- Spruce peroxidases *Pa-px1*, *Pa-px2* and *Pa-px3* are expressed at moderate levels in developing xylem of 30-year-old spruce trees
 - *Pa-px1* and *Pa-px2* are transcribed in developing tracheids of spruce seedlings
 - *Pa-px2* expression is high in compression wood of spruce seedlings
 - *Pa-px2* and *Pa-px3* expression is high in phloem of *Heterobasidion annosum* infected seedlings
-

Conclusions 2

- *Pa-px1* is translated to a highly basic peroxidase enzyme PX1, which is apparently transported via ER-Golgi to cell wall- Partially purified basic peroxidase isoforms from spruce xylem are able to catalyze oxidation of coniferyl alcohol (and one of them lignin polymer?)
 - In addition to N-terminal signal peptides, PX2 and PX3 amino acid sequences contain C-terminal signal peptides which affect their localization
-

Acknowledgements



Kurt Fagerstedt
Eija Kukkola
Mikko Lehtonen
Pekka Haapaniemi
(Dept. of Biological and
Environmental Sciences)

Taina Lundell
Kristiina Hilden
(Dept. of Applied Chemistry and
Microbiology)

Teemu Teeri
Sanna Koutaniemi
(Dept. of Applied Biology)

Heidi Holkeri
(VTT Technical Research Centre)

