Identification and characterisation of host factors involved in plant virus long distance movement

S. Chapuis, Sylvaine Boissinot, Véronique Brault, Frederic Revers, Véronique Ziegler-Graff

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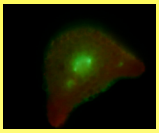
Potyvirus and polerovirus are two important genera of plant viruses responsible for serious damages on many agronomically important crops. They differ in the way they infect hosts : while potyviruses can invade all tissues of the plant, polerovirus infections are restricted to phloem cells (phloem parenchyma cells, companion cells and sieve elements). Therefore they represent two interesting models to study viral long distance movement in plants.

Long distance movement is an important step of the viral cycle because it leads to systemic infection of the host. After local multiplication, plant viruses move from the initially infected cell to the adjacent cells in order to reach the phloem and be unloaded into the sieve elements. Then, they are translocated to uninfected tissue where they can invade new sites. Interactions between host and viral factors that favor or restrict the long distance movement remain to be unravelled.

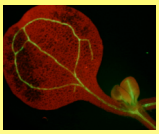
The aim of this project is to identify phloem specific genes, especially those from companion cells that are either deregulated by viral infection or that encode proteins which interact directly with viral proteins involved in phloem transport. To achieve this goal, we will isolate phloem companion cells using the Laser Scanning Microdissection (LSM) technology.

A useful tool :

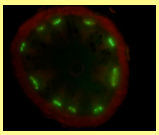
Transgenic *Arabidopsis thaliana* plants expressing GFP under the control of a companion cell specific promoter (AtSuc2) (Imlau *et al.*, 1999)



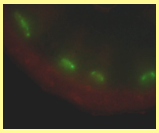
Section through the petiole



Cotyledon of a seeding



Section through the stem (x10)



Section through the stem (x20)

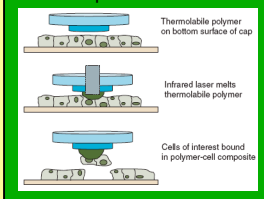
Several procedures have been developped to isolate particular cells/ tissues by LSM in order to extract specifically expressed RNA.

Plants/tissues	Aim of microdissection	Fixation	Embedding/Cutting	LCM	RNA Extraction method	References
phloem cells of rice	cDNA library	Acetone 100%	Tissue Tek OCT medium and cryosectionning	Laser Micro Cutter (SIGMA) (ultraviolet sectionning)	Lysis Buffer	Asano *et al.*, 2002
epidermal cells and vascular tissues of maize	cDNA microarray	Ethanol/Acetic acid (3:1)	Tissue Tek OCT medium and cryosectionning	Pix Cell II system (ARCTURUS) (infrared harvesting)	Absolutely RNA Microprep kit (STRATAGENE)	Nakazono *et al.*, 2003
embryos of *Arabidopsis thaliana*	cDNA microarray	no	OCT medium and cryosectionning	Pix Cell II system (ARCTURUS) (infrared harvesting)	Absolutely RNA Nanoprep kit (STRATAGENE)	Casson *et al.*, 2005
ovules, replums and stamen abscission zones of *Arabidopsis thaliana*	cDNA library, cDNA microarray	Ethanol/Acetic acid (3:1)	Paraffin and microtome	Pix Cell II system (ARCTURUS) (infrared harvesting)	PicoPure RNA isolation kit (ARCTURUS)	Cai *et al.*, 2006
phloem cells of *Arabidopsis thaliana*	cDNA microarray	Ethanol/Acetic acid (5:1)	Paraffin and microtome	Microbeam system (PALM) (ultraviolet sectionning)	Dynabeads oligo dT (DYNAL)	Deeken *et al.*, 2008

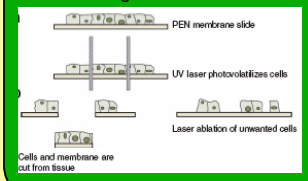
The most commonly used fixation method to isolate RNA is performed with ethanol/acetic acid.
Paraffin embedding is best way to obtain sections with high morphological integrity. However there seems to be a contreversy about the most efficient way to preserve RNA integrity (embedding in paraffin or cryosectionning).

There are 2 systems of LSM :
Espina *et al.*, 2006

- Laser capture



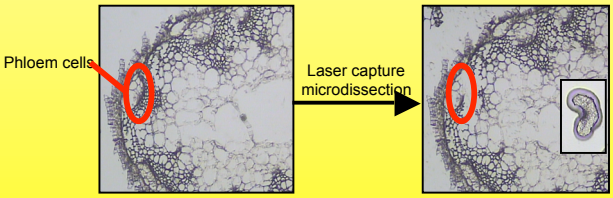
- Laser cutting



First trials of microdissection

- *A. thaliana* stems fixed in ethanol / acetic acid (3/1) and embedded in paraffin
- Laser capture microdissection with Veritas™ (Alphelys):

Slide for microdissection

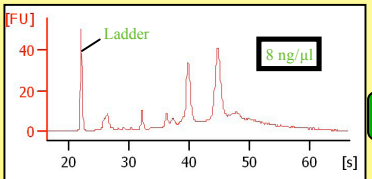


Section of *A. thaliana* stem (x 20)

Performance of microdissection relies on a balance between a good preservation of cells morphology and of RNA integrity.

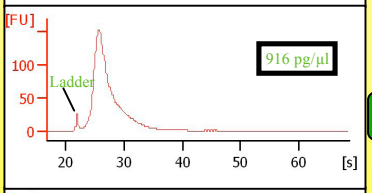
Analysis of RNA with Bioanalyzer (Agilent)

Total RNA of a handcut fixed petiole section. Sample only fixed.



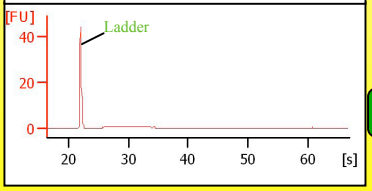
Good profil

Total RNA of stem sections from an entire slide. Sample mounted.



Degraded RNA

Total RNA of stem sections from an entire slide. Sample mounted.



No RNA

Electropherograms representing the quality of extracted RNAs.

Further technological improvements need to be pursued to maintain RNA quality, in particular reducing the paraffin embedding time and temperature by using a low melting point wax.

Chapuis, S., Boissinot, S., Brault, V., Revers, F., Ziegler-Graff, V. (2009). Identification and characterisation of host factors involved in plant virus long distance movement. In: 8ème colloque national de la SFBV (p. 1 p.). Presented at 8. Colloque national de la SFBV, Strasbourg, FRA (2009-07-08 - 2009-07-10).