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Microdissection to Isolate Vascular Cambium Cells in Poplar

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Vascular cambium is the lateral meristem producing xylem cells inwards and phloem cells outwards in plant stem. Thus, in trees, the quality and quantity of wood is a result of highly regulated developmental process depending initially on the vascular cambium cell production. The availability of accurate transcriptomics technologies based on high coverage sequencing raises the level of expectations on tissue sampling to a very high degree. What is the benefit of top-level transcriptomics in wood formation studies if we are using these technologies on raw tissues, mixing cells at the organ level or even higher scale? The presented work describes a nine-step procedure, from standing tree to isolated ray and fusiform cells from cryolyophilized tangential sections of the poplar cambial zone. The aim of this paper is to present a step by step procedure including advices on how to select the optimal tree, how to fell the tree while securing its physiological parameters, how to cryolyophilize and microdissect under binocular, presenting the time schedule of the whole process and RNA analysis.

Keywords fusiform cambial cells, microgenomics, *Populus* spp., ray cambial cells
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1 Introduction

Vascular cambium is the secondary meristem which initiates wood formation in trees. Vascular cambium is formed by two types of cell from which different tissues are derived in the stem: fusiform cambial cells (FCC), which become mainly fibers and xylem vessel elements; and ray cambial cells (RCC), which become parenchyma cells (Larson 1994). The way vascular cambium produces layers of tissues, phloem outwards and xylem inwards from FCC ultimately determines how saps will be transported in the tree (Sperry et al. 2008). However, understanding RCC develop-

ment is also crucial as rays provide photoassimilates, water and other signal molecules to the xylem (Lachaud et al. 1999). Since the vascular cambium is a key actor in wood production, it fundamentally affects the properties and quality of the wood (Fahn 1990, Catesson 1994). In order to understand the physiology of wood formation it is necessary to investigate the cambium at the cell level. However, little is known about the formation and organization of vascular cambium, and the molecular mechanisms underlying the cambial cell functioning is also poorly understood despite recent studies (see Matte Risopatron et al. 2010, Spicer and Groover 2010) for recent reviews. Gaining access to the cambial zone tissues in the stem without adversely affecting the transcriptome has been a major hurdle in forest research.

In general, the more powerful the analytical technique in molecular biology, the more accurate must be the sampling. New technologies make transcriptome profiling at the cell level possible. Such high resolution molecular analyses are complicated by problems associated with sample collection, i.e. access to tissues or cells of interest, acquisition of suitable sample size, and the limited sensitivity of the expression assays (Freeman et al. 1999). However, resolution at the cell level between different cell types is becoming more widely used and is adding to our understanding of gene regulation at the cellular level (Ohtsu et al. 2007). The analysis of individual tissues and single cells eliminates the dilution effect due to complex tissue mixtures, and allows the discovery of very small differences among distinct cell types (Kehr 2003). Various techniques have been developed for this purpose, which differ according to the nature of the plant sample (Galbraith and Birnbaum 2006). For example, by cell sorting (Becker et al. 2003, Birnbaum et al. 2003), by keeping cells in their biological context until sampling by micropipeting (Karrer et al. 1995, Brandt et al. 1999, Elge et al. 2001, Brandt et al. 2002), by forceps micromanipulation (Rottloff et al. 2009), and laser microdissection techniques (Asano et al. 2002, Kerk et al. 2003, Nakazono et al. 2003, Inada and Wildermuth 2005, Ohtsu et al. 2007). Tissue preparation necessitates a trade-off between the preservation of histological detail and the recovery of nucleic acids from the harvested cells (Kerk et al. 2003). Chemical fixation of specimens and paraffin embedding, used with a laser microdissection technique, is possible but adversely affects RNA quality and quantity when compared with frozen tissues (Serth et al. 2000, Gillespie et al. 2002, Parlato et al. 2002, Perlmutter et al. 2004, Portillo et al. 2009).

In the present paper, we propose the microdissection of cryolyophilized tissues as an adapted technique to isolate highly vacuolated meristematic cells (Catesson 1990) from vascular woody plants. Originally, this cell microdissection technique was used for metabolite and enzymatic assays (Jones et al. 1977), and for measuring the organic acid and potassium content of foliar cells (Outlaw and Lowry 1977). A similar technique has been used to isolate soybean nodule tissue and was proven to minimize any disturbance to cell metabolism (Oresnik and Layzell 1994). More recently, in woody plants, serial sectioning and cryolyophilization has been used to measure hormone concentration gradients across the developing secondary vascular tissues of Scots pine (Uggla C., Magel E., Moritz, T. and Sundberg, B., unpublished data). In the present report, cryolyophilization and microdissection are used together, and adapted to a cell microdissection technique for transcriptome analysis. Previous experiments (Hertzberg et al. 2001, Schrader et al. 2004) have shown that transcript extraction from vascular cambial tissue is possible starting from cryosections, when combined with PCR amplification of primer-tagged cDNA. We applied RNA linear amplification by *in vitro* transcription technique (Van Gelder et al. 1990) to cambial cell samples. Our results show that RNA amplification makes analyses possible and generate various transcript profiling depending on types of vascular cambium sampling.

Formerly, we published a general study that compared gene expression between FCC and RCC (Goué et al. 2008) without detailing the microdissection procedure. The purpose of the present paper is to share our hand-microdissection technique used for cambial cells and to illustrate the urging necessity of working on homogeneous samples. We aim at developing a procedure where the geometrical constraints of cell layers in tangential sections in the cambial zone will be considered before sampling; where the hydraulic

consequences of tree felling on sap fluxes will be taken into account during sampling; where consequences of tissue freezing and thawing will be prevented after sampling; where microdissection of cell types will be possible at room temperature under a simple binocular; and finally where recent transcriptomics techniques can be applied on selected samples.

2 Materials and Methods

The complete procedure can be described in nine steps. **a**) select a tree with optimum stem diameter; **b**) avoid air in the xylem during tree felling, **c**) freeze stem samples at felling and never thaw consecutively, **d**) cut frozen blocks in cambial zone, **e**) cryosection tangential sections in the cambial zone, **f**) cryolyophilize cambial zone sections, **g**) microdissect RCC and FCC from lyophilized cambial zone sections, **h**) linearly amplify mRNA from cell-type enriched samples, and finally **i**) convert mRNA into cDNA.

2.1 Tree Felling

Sample collection was done on the 25th June 2002 from a 15-year old hybrid poplar, 'Boelare' (*Populus trichocarpa* × *P. deltoïdes*) with a diameter of about 50 cm (**step a**), growing at Beuxes, Moulin de Bariteau, Dominique Meese†, France. The tree was felled one half-slab (10 cm) higher than breast height (1.4 m) and the top of the remaining standing stem was cut into 6 slabs (**step b**) from the stem circumference, with dimensions of approximately 20 cm longitudinally, 15 cm tangentially, and 7 cm radially (Fig. 1). The slabs were snap-frozen in liquid nitrogen in the field (**step c**) before returning to the laboratory where the frozen material was further cut into about one thousand blocks (**step d**), each 1 cm longitudinally × 0.5 cm tangentially × 0.5 cm radially. 200 blocks were used for each sampling.

2.2 Tissue and Cells Sampling

Each block was fixed on the holder with O.C.T. (TissueTek, Agar Scientific, England) and cut into three 25 μm-thick longitudinal-tangential cryosections (**step e**) with a cryomicrotome (HM 505E, Microm Laborgeräte, Walldorf, Germany). Sections were collected on microscope cover slips

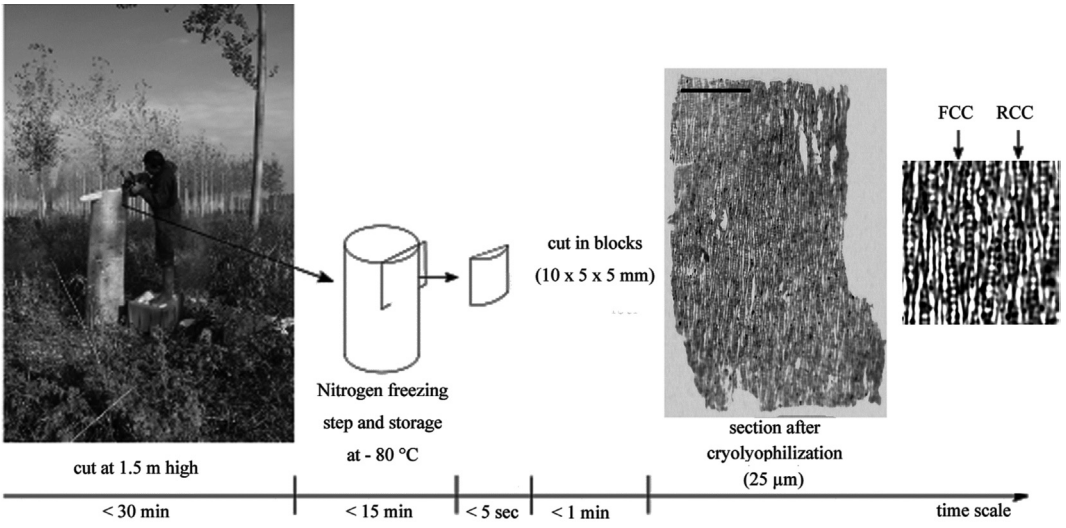

Fig. 1. Sampling procedure from tree to cryolyophilized sections of cambial zone. Bar = 400 μm. Sections are 25 μm thick.

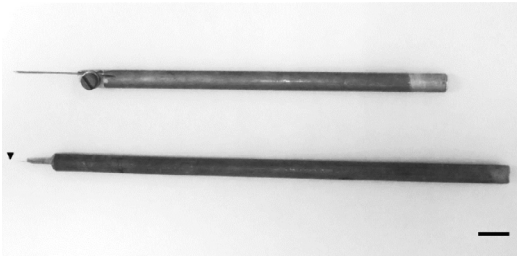

Fig. 2. Hand-tools used in manual microdissection. Top: steel needle tool; bottom: marten's hair tool. Black arrow indicates the marten's hair. Bar = 1 cm.

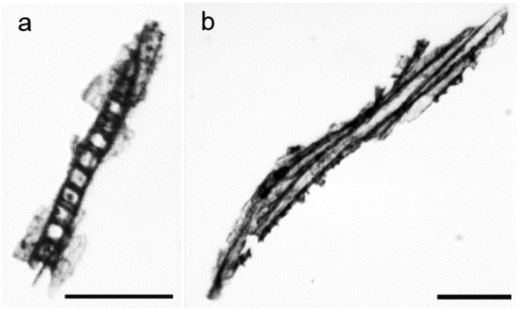

Fig. 3. Cryolyophilized cambial cell sections and microdissected cambial cells, (a) RCC and (b) FCC. Bar = 100 μm.

(24×24 mm) kept below 0°C, stacked together on 35 mm petri dishes, partially sealed, and transferred in a liquid nitrogen pot to a –30°C freezer to be freeze-dried (**step f**). The freeze-dryer was connected to a vacuum pump gauge and a –120°C fry ice trap (Hampp et al. 1990) and the samples were freeze-dried for 2 weeks until the pressure in the freeze-drying chamber stabilized. The completely dried samples were then removed and allowed to warm up slowly to room temperature.

Part of these cryosections were saved and kept as whole sections in a desiccator for further analysis. Remaining cryosections were microdissected by hand (**step g**) under a stereomicroscope (Stemi 2000-C, Zeiss, Germany) using magnification from ×100 to ×500 in a room with constant humidity (max. 40%). Cells were excised from one hundred lyophilized sections using sterilized dissecting instruments consisting of a steel handle holding a sharpened needle. Cells were collected by using a marten's hair stuck to a steel handle (Fig. 2). Dissected cells (Fig. 3) were collected into a 1.5 ml autoclaved tube kept in silica-gel container during the procedure. However, because it is impossible to see microdissected cells in the tube with the naked-eye, it is recommended that the tube be centrifuged before opening. We isolated 2000 FCC and 4000 RCC for each mRNA extraction.

Scraped cambial zone was sampled according to Micheli et al. (2002) after bark removal on frozen and lyophilized stem portions. Microdissected cells, tissue sections and scraped tissues were compared in order to study the effect of sampling precision on gene expression.

2.3 Transcript Isolation and RT-PCR

Messenger RNAs were isolated from three different samples: 1) from lyophilized and scraped cambial zone (CZ); 2) from cryolyophilized sections of CZ; and 3) from microdissected cambial cells (CC). Purification of mRNA from all three sample types was performed using Dynabeads mRNA DIRECT^{TM} Micro kit (Dynal Biotech Lake Success, NY, USA). Tissue cryosections and microdissected CC were used for two independent repetitions of mRNA extractions. Transcripts were amplified (**step h**) by using two-round amplification of MessageAmp^{TM} II aRNA kit (Ambion, USA) followed by reverse transcription (**step i**) with a Superscript II according to the manufacturer's instructions (Invitrogen). Nucleotides integrity was checked on gel.

The candidate genes in the present study were selected according to the results from a microarray experiment on vascular cambium (Goué et al. 2008). Six candidate genes were selected, which are referred to as 'annotated clones' in PopulusDB (http://poppel.fysbot.umu.se/index.html) and as 'gene models' in the JGI database (http://www.phytozome.net/poplar), namely a lipid transfert protein, *Pt-HPS.3* (A013P67, POPTR_0001s00410), a dirigent family protein, *Pt-DRR206.6* (A047P55, POPTR_0001s10120), a fasciclin-like arabinogalactan protein, *Pt-FLA14.6* (A054P41, POPTR_0012s14510), a phosholipase D, *Pt-PLD1.2* (A062P40, POPTR_0001s19360), a photosystem II 10 kDa polypeptide PsbR, *Pt-PP1.1* (A078P57, POPTR_0011s14550) and a

Table 1. Primer sequences and PCR conditions.

Primer name	Primer sequence (3'–5')	Tm (°C)	PCR cycles	Amplicon size (bp)
Pt-FLA14.6 s	CTCCAGCACCAGCACCTGAA	55	30	239
Pt-FLA14.6 as	GGATTCGATCCGACTCTGCAA			
Pt-DRR206.6 s	GTGGGTTCTACTTCCATGAC	54	30	403
Pt-DRR206.6 as	ACAACTGAATATCAACACGAAG			
Pt-HPS.3 s	AACGTCACCATTGGCTCACC	54	26	194
Pt-HPS.3 as	GGCATTGGAAGTCTTTGGGAAC			
Pt-PLD1.2 s	GAGAATGCTGCATGGTTCCAG	54	28	174
Pt-PLD1.2 as	AAATGCATCCATGCGGTGAG			
Pt-PP1.1 s	GTCGTTCGAGGAAGTCCAAG	54	28	231
Pt-PP1.1 as	GCCCAGATCAACAAACCAGT			
Pt-XTH9.2 s	TGATTGGGCTACACAAGGTGGCCGTGTC	60	20	200
Pt-XTH9.2 as	GCTGATGGCTCTGGTGCGCATTGAGC			

xyloglucan endotransglycosylase/hydrolase protein, *Pt-XTH9.2* (G095P92, POPTR_0013s14860). The candidate genes were amplified with Platinum Taq polymerase (Invitrogen, CA, USA) according to the manufacturer's instructions using 25 ng of nucleic acids with gene-specific primers (Table 1). cDNAs extracted from scraped CZ were used as PCR controls.

2.4 Characterization of Cambial Cells

The surface areas of the FCC and RCC were measured from cryolyophilized cambial zone sections (see above) using images taken from a stereomicroscope (Leica MZ12, Leica Microsystems, Germany) connected to a digital camera and processed with Image J software (www.rsb.info.nih.gov/ij/). To estimate the weights of the CC we proceed as following. Twenty cryolyophilized sections of cambial zone were weighed (W) and scanned. Then, the total cell number (N) as well as the numbers of both cell types (Nfcc and Nrcc) were counted on one tenth of the images. The final weights (Wfcc and Wrcc) were obtained with the formula: $Wfcc = (W \times Nfcc)/N$ and respectively for Wrcc using Nrcc. Using this cell weight estimation, the cell number in the scraped cambial zone sample was estimated using its scraped sample weight. After RNA extraction and amplification, an estimation of mRNA was estimated per cell type.

3 Results

3.1 Characteristics of Cell Types

The application of the technique described in the present paper has provided new information concerning CC characteristics of hybrid poplar. At the end of the procedure, even though cells might have been slightly deformed by cryolyophilization, cell morphology was well conserved to set up cell characteristics and to perform homogeneous cell microdissection. The average longitudinal length of a FCC was found to be 255 μm (range 71–603 μm) and the average height of an uniseriate ray of RCC was found to be 188 μm (range 38–627 μm). We also determined the tangential dimension of CC to be 19 μm enabling us to calculate an estimate of cell surface area. The relative proportion of FCC and RCC is based on the area occupied by both CC types in a tangential plane. The surface of FCC and RCC is 78.4% ± 5.5 and 21.6% ± 5.5, respectively. Finally the estimation of the dry weight of a FCC is 65 ng and 10 ng for a RCC.

The molecular characteristics of cambial cells were calculated on a population of 2500 microdissected cells. RNA amplification is a required step when starting from small amounts of material. Gel-analysis has shown that the average length of molecule size is approximately 500 base pairs (Fig. 4). After 20 cycles of linear amplification, the amount of cDNA is estimated to be 1 μg; and

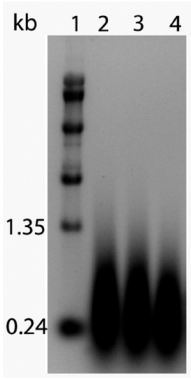

Fig. 4. Analysis of the quality and integrity of nucleic acids. Electrophoresis (1% denatured agarose gel) of aRNA samples labeled with digoxigenin. RNA ladder (1). Messenger RNAs come from RCC (2), FCC (3) and sections of vascular cambium (4).

two rounds of RNA linear amplification generate approximately 10 μg of aRNA. Our sampling strategy made it possible for us to estimate the amount of mRNA in each cell type, to be 3 pg in a FCC and 1.6 pg in a RCC.

3.2 Validation by Candidate Genes

The validation presented here was conducted by RT-PCR on heterogeneous and homogeneous samples. The RT-PCR showed a specific expression of six genes according to cell type and sampling methods (Fig. 5). Care was taken to select genes with referenced clones having similar GC contents (around 40%) to avoid bias even though we were not able to exclude any differences due to cell-type in specific samples. There is no difference in expression between scraped cambial zone (CZ) and cryolyophilized CZ sections except for the lipid transfer protein Pt-HPS.3 with a higher expression in scraped CZ compared to cryolyophilized CZ sections. In the case of FCC preferential expressed genes (Fig. 5a), preferential expression is clearly shown for the three genes *AGP15*, *Pt-DRR*, and *Pt-XTH9.2*. Similarly, RCC preferential gene expression is observed (Fig. 5b) except for a photosystem II polypeptide PsbR (Pt-PP).

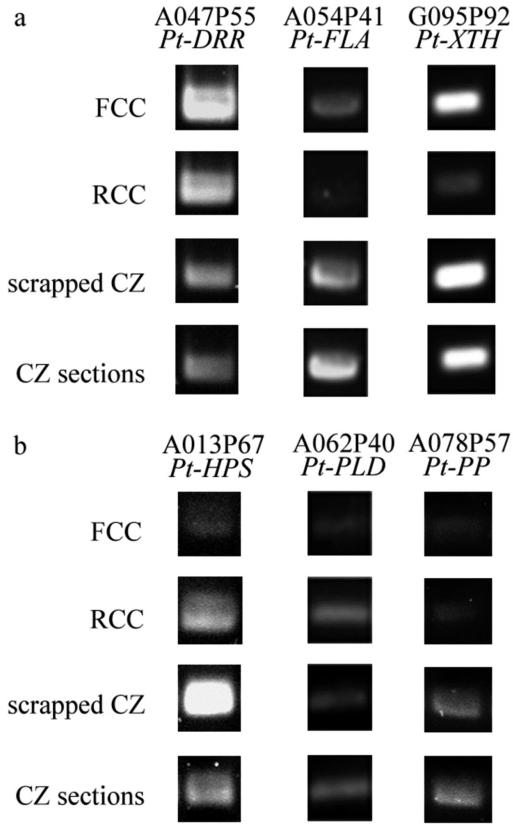

Fig. 5. RT-PCR for six poplar clones described with their accession number and encoded protein description. (a): A047P55, dirigent protein; A054P41, fasciclin-like arabinogalactan protein; G095P92, xyloglucan endotransglycosylase/hydrolase protein 9 and (b): A013P67, lipid transfer protein; A062P40, phosholipase D; A078P57, photosystem II 10 kDa protein.

4 Discussion

The cryolyophilized microdissection technique is relatively easy to set up in any standard laboratory. However, it requires dexterity from the manipulator despite the limit of dissection by hand is generally as few as 2 μm (Outlaw and Zhang 2001). Thus, manual microdissection can be performed on CC with a tangential length of 19 μm which is their shortest dimension in this plane.

4.1 Cell Characteristics

There are striking variations in the dimensions and volumes of CC and their derivatives, which depend on parameters such as species, individual, tissue, localization within the tissue, age, and environmental conditions. Investigations on CC dimensions aid our understanding of wood-forming processes because CC size, or more precisely FCC size, has been shown to determine fiber and tracheary cell size (Bailey 1920, Ajmal et al. 1986). The average longitudinal length of a FCC was found to be 255 μm in our experiment. It is slightly below the estimation done on previous work on *Populus* spp. that reported FCC length to be 490 μm (range 350–660 μm) (Bailey 1920), and in other dicotyledons, between 300 μm and 350 μm (Catesson 1980). This can be due to the weak deformation that occurs at the lyophilization step. We also found the tangential dimension of CC to be 19 μm enabling us to calculate an estimate of cell surface area and the relative proportion of FCC and RCC, based on area occupied by both CC types in a tangential plane. This character appears to be species-specific. For the hybrid poplar 'Boelare', the proportion of RCC is estimated to be around 22%, which is comparable with the results of previous studies on *Juglans regia* (19%) and *Prunus persica* (33%) (Bartwal et al. 1983).

The whole procedure is time-consuming but the use of linear amplification gained from microdissected cells reduces the number of required cells. Further on, we will follow the nine-steps description of the procedure to discuss each step of the work.

4.2 Selecting Trees with Optimum Stem Diameter

Cambial cells, either FCC or RCC are known to have radial dimensions of about 10 μm when active (Catesson 1990, Iqbal 1990). The purpose of the method was to collect FCC and RCC as whole cells. To achieve this, the effect of the geometry of the stem radius on the thickness of the cambial section is of utmost importance. In theory, the stem radius of sampled tree should be infinite to ensure that no CC is lost during the flat cryosectioning step. In other words, to ensure that cryosection in the tangential-longitudinal plane is flat, the stem radius should be infinite. Geometrically, with an infinite stem radius, the arc of the cambial section is equal to its chord (the chord here being its width in the tangential direction), where arc = alpha × R, and chord = 2 × R × sin(alpha/2); alpha being expressed in radians, and R being the radius of the stem in meters. Obviously an infinite stem diameter is an impossibility; nevertheless, we had to select as large a diameter standing tree as possible in order to ensure that this geometrical limitation would be negligible. In our case, a 50 cm stem diameter led to a 0.004% difference between the arc and the chord on the tangential-longitudinal plane, instead of a theoretical zero difference. This is a critical, technical consideration when attempting to acquire an homogeneous sample, as the curvature of the tissue will affect the quality of a section taken from it: i.e. while cambial cells may be in a meristematic stage in the center part of the section, they may already be showing signs of differentiation at the periphery of the section, and vice-versa. This geometrical aspect has to be considered at the experimental design stage.

4.3 Avoid Air in the Xylem during Tree Felling

A second important point is to limit hydraulic flux in the xylem zone during stem sampling and before freezing in liquid nitrogen. When the tree is felled, even during the brief period when it is lying on the ground, atmospheric evaporative demand and stomatal conductance remains and generates a negative water potential in the stem (Tyree and Ewers 1991). Within only a few minutes of felling, therefore, air enters very rapidly into the stem through the cut base and produces large movements of water and sap in the xylem vessels and in the cambial zone of the felled tree. According to the cohesion-tension theory, a change in transpiration should result in the essentially instantaneous onset of changes in the flow and xylem tension (Sperry et al. 2008). Since these water movements might drastically change the location of mobile molecules, such as mRNA, in the cambial zone, and might affect any

subsequent results, we had to cut the tree above the position at which the samples were to be taken, which was at breast height. This procedure ensures that the CZ subsequently cryolyophilized and microdissected from these trunk portions are free of air, and that limited movement of water and sap does not modify the localization of mRNA in the different cell types.

4.4 Freeze Stem Samples at Felling, Cut Frozen Blocks, Cryosection Tangentially and Never Thaw until Lyophilization

From the top of the remaining standing stem after felling, the outer trunk portions were then cut and immediately frozen (Fig. 1). Blocks surrounding cambial zone were cut frozen and because tissues frequently split at the xylem expansion zone when frozen in liquid nitrogen, only those blocks that included the complete phloem-cambium-xylem structure were used for cryosectioning. During the procedure, the frozen stem samples, isolated blocks and tissue sections never thawed and remained in the solid phase throughout. Keeping cells as close as possible to their normal biological and physiological environment can be challenging when transcriptome analysis is conducted. The use of liquid nitrogen to freeze material is a key point in the procedure as this rapidly stops all water movements in the sampled tissues. Special care was taken to maintain sub-zero temperatures during all procedural steps in order to prevent any such thawing that might cause the formation of crystalline ice and the consequent degradation of nucleic acids and metabolites within the cells.

4.5 Microdissected RCC and FCC from Cryolyophilized Cambial Zone Sections

Although the freezing procedure is considered the best way to yield an adequate quantity of high quality mRNA, it is not widely used because of the potential of forming ice crystals that may destroy cell structure upon thawing. Tissue sections are so thin that special care must still be taken in order to avoid thawing occurring in those steps prior to cryolyophilization. Thawing may occur on tissue sections within just a few seconds at room temperature. The cell microdissection techniques developed here suppresses the critical thawing steps by applying the lyophilization procedure to frozen tissue sections, which naturally avoids the formation of ice crystals. It makes the long lasting step of hand-microdissection possible at room temperature.

FCC and RCC were isolated (Fig. 3) from cryolyophilized tangential sections in a constant humidity room because an appropriate level of humidity limits the amount of static electricity in the ambient air of the dissecting room and so facilitates the collection of dissected cells.

4.6 Linearly Amplify mRNA from Cell-Type Enriched Samples

Our results indicate a difference in transcript amounts in FCC vs RCC, 3 pg vs 1.6 pg. Previous investigations have also found differences in the amount of transcripts according to cell type, and shown that care should be taken to normalize the yield when designing experiments that rely on quantitative comparisons between different cell types (Kerk et al. 2003). To that end, we isolated 2000 FCC and 4000 RCC for each mRNA extraction. Previous transcriptome profiling, based on cell isolation, has been conducted on 10 cells (Kryvych et al. 2008); 150 cells (Asano et al. 2002); 2000 cells (Inada and Wildermuth 2005); and up to 10 000 cells (Nakazono et al. 2003). It is important to perform a cell-type transcriptome analysis on an optimized amount of cells in such a way that results for a so-called ‘average cell’ are achieved without bias due to there being too few cells (Kawasaki 2004). The technique of nucleic acid amplification is now making cell level investigations technically feasible and it has been suggested that transcriptome analyses on populations of several hundreds to thousands of cells would give a realistic representation of what happens in tissues, organs, and ultimately in the whole plant (Nygaard et al. 2005).

4.7 Convert mRNA into cDNA

RT-PCR results indicate that gene expression is changing according to the precision of the sampling. Interestingly, our RT-PCR results show that a gene showing an expression in scrapped CZ or in tissue sections is not reflecting what is happening in FCC only but can also reflect an expression occurring preferentially in RCC. However, our results clearly show that intensity of expression is greater if the preferential expression is in FCC rather than RCC. This is hypothetically due to the fact that differential expression is generally better observed where preferential expression occurs in FCC rather than in RCC for two reasons. The first one is due to the proportion of FCC vs RCC that varies within a tree and amongst species (Lev-Yadun and Aloni 1995) and is 78% ± 5 of FCC in our case study. Secondly, our results also indicate that the quantity of messenger RNA would be higher in FCC than in RCC and then add to the unbalanced ratio between the 2 types of cell within the cambial tissue. Finally, when considering genes known to be expressed in the cambial zone, although the patterns of expression were similar in scraped CZ and in CZ sections, they do not reflect what occurs inside the tissue. If a gene of interest is specifically expressed in FCC or RCC, then expression is observed both in scraped cambial zone and in cambial zone sections. Caution should therefore be taken when investigating wood formation, and especially vascular formation, if using heterogeneous samples since the level of expression may reflect preferential expression in either FCC or RCC.

4.8 Producing Cell-Type Enriched Samples

When sampling material for cell microdissection, the possibility of contamination of the sample (Nakazono et al. 2003) can never be entirely eliminated, whatever sampling technique is used (Laval et al. 2002). Based on careful observations of their histological sections, Ornstein et al. (2000) estimated each dissection by laser cell microdissection to contain more than 95% of the desired cells, which is similar to our findings with lyophilized cryosection microdissection. Moreover, the technique that we have developed has the advantage of maintaining the vicinity of cell of interest intact, whereas laser cell microdissection can cause carbonization of the cell's vicinity. Intercellular communication is another area that could be investigated experimentally by lyophilized cryosection microdissection of vascular cambium, since plasmodesmata have been shown to be well represented in the tangential walls of RCC and in the radial and transverse walls between FCC, between RCC, and at both cell types junctions (Catesson 1990, Fuchs et al. 2010).

5 Conclusion

The approach of combining cell microdissection and transcriptome analysis is a valuable tool for the investigation of cell-specific expression in plants and is expected to highlight our understanding of wood formation in regards to recent transcriptomics technologies such as RNAseq (Marguerat and Bähler 2010). It can be adapted to diverse kinds of tissues and especially to wood tissues. This technique can be widely used in plants and can be adapted to the analysis of other features such as cell wall components (Angeles et al. 2006), metabolomes and proteomes (Wienkoop et al. 2004) and then contribute to the understanding of the physiology of the entire organism.

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