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REVIEWS

Laser Assisted Microdissection, an Efficient Technique to Understand Tissue Specific Gene Expression Patterns and Functional Genomics in Plants

Vibhav Gautam · Ananda K. Sarkar

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Abstract Laser assisted microdissection (LAM) is an advanced technology used to perform tissue or cell-specific expression profiling of genes and proteins, owing to its ability to isolate the desired tissue or cell type from a heterogeneous population. Due to the specificity and high efficiency acquired during its pioneering use in medical science, the LAM technique has quickly been adopted for use in many biological researches. Today, it has become a potent tool to address a wide range of questions in diverse field of plant biology. Beginning with comparative transcriptome analysis of different tissues such as reproductive parts, meristems, lateral organs, roots etc., LAM has also been extensively used in plant-pathogen interaction studies, proteomics, and metabolomics. In combination with next generation sequencing and proteomics analysis, LAM has opened up promising opportunities in the area of large scale functional studies in plants. Ever since the advent of this technique, significant improvements have been achieved in term of its instrumentation and method, which has made LAM a more efficient tool applicable in wider research areas. Here, we discuss the advancement of LAM technique with special emphasis on its methodology and highlight its scope in modern research areas of plant biology. Although we put emphasis on use of LAM in transcriptome studies, which is mostly used, we also discuss its recent application and scope in proteome and metabolome studies.

Keywords Laser assisted microdissection (LAM) · RNA isolation · Gene expression · Genomics · Transcriptomics

V. Gautam · A. K. Sarkar (✉)
National Institute of Plant Genome Research (NIPGR), Aruna Asaf Ali Marg, New Delhi 110067, India
e-mail: aksarkar@nipgr.ac.in

Introduction

Gene regulation plays a crucial role in growth and development of plants, where control of gene expression in a specific biological process or under certain stress stimuli are aspects of prime research interest in plant science. Higher plants consist of different tissues with diverse cell types, in which every cell is unique in terms of its genetic, proteomic and metabolic make up. The plant kingdom, which consists of diverse kind of species ranging from lower plant bryophyte to higher angiosperms, possess approximately 40 different cell types which are likely to have varying gene expression profile, protein levels and metabolites [1]. It is noteworthy that two tissue or cell types may have various degrees of differences in terms of transcriptome, proteome and metabolome, due to transcriptional, post-transcriptional and post-translational regulation of gene function. Therefore, identification of the molecular variation in expression at genomic or proteome level is an important prerequisite for the understanding of complex biological and gene regulatory network present in plants.

Although the advent of microarray and next generation sequencing (NGS) technology and availability of genome sequences of several model organisms have facilitated the analysis of global gene expression studies, the challenge of tissue specific expression studies remained due to variation in complexity of both the genome and concerned tissue [2]. For comparative expression profiling, it is often necessary to isolate specific tissue or cell types without significant contamination with unwanted cells. One of the approaches is fluorescent activated cell sorting (FACS), where specific cell types are tagged with different marker reporters such as green fluorescent protein or yellow fluorescent protein etc. and followed by sorting or collection of marked cell

types separately [3]. In case of plants, FACS requires production of tissue- or cell-specific marker through the generation of transgenic plants, which is a tedious procedure and often falls short of knowledge of tissue specific promoters. This problem is further complicated by the fact that many plant cell types of interest, such as egg cell or cambium cells, are deeply embedded into a large plant body composed of strongly attached or immobile cells. In order to overcome the practical limitations of using marker-based approaches, use of laser assisted microdissection (LAM) has evolved as an efficient alternative and has been applied successfully in various aspects of plant biological research [4, 5].

LAM is one of the advanced forms of microscope based technique, which enables the isolation of specific cells of interest from a heterogeneous population. LAM permits rapid collection of desired cells or tissue selected on a computer screen from a heterogeneous mixture of cell populations or tissue sections mounted on specialised microscopic slides or culture plates. This is followed by isolation of biomolecules such as DNA, RNA, proteins or metabolites for comparative analysis. Availability of tissue specific marker is sometime helpful but not mandatory for LAM-based technique. During the advancement of this technique, the instruments for LAM have been patented and named by various manufacturers, mainly depending on the type of laser used and mode of sample collection (Table 1). Notwithstanding the basic similarity of LAM methods using various machines, there is no generalised protocol which is suitable enough for all plant species and varying cell types, and LAM still remains an evolving approach [6]. It has been a potent tool for pathological or diagnostic studies (such as isolation and study of cancer cells) for comparing the gene expression profile of infected and non-infected cells [7]. Gradually, the application of LAM has diverged and now it is used for studying many other tissue types for addressing various biological or developmental questions [8]. Owing to its ability to precisely isolate even single cell or small tissue, many of the plant biologist also used LAM to understand the tissue or cell-specific transcriptome in various model plants such as *Arabidopsis thaliana, Zea mays, Oryza sativa* etc [9–12]. LAM coupled with microarray or NGS helps in identifying tissue specific gene expression profile, which is often helpful for diagnostic purpose or for addressing specific biological question. Considering the broad-range applications of LAM in the field of life science, this review summarises the different aspects of LAM in plant biology and discusses major technical advancement achieved for various cell or tissue specific gene expression studies or related applications.

Principle and Instrumentation of LAM

Several instruments for LAM are commercially available for collection of a desired cell or tissue type from a heterogeneous population. These instruments are designed based on two major principles: laser capture microdissection (LCM) and laser cutting microdissection (LMD). In LCM, targets cells/tissues are cut using the pulsed infrared laser (700 nm–1 mm) [13]. LCM is a powerful technique for rapid isolation and collection of a homogenous population of cells from a heterogeneous population. It was invented for the first time at National Cancer Institute by Dr. Emmert-Buck and colleagues for studying the cancerous tissues [14]. It is based on an inverted light microscope coupled with a laser device to mediate the visualisation and collection of selected cells. One of the pioneering LAM devices is PALM microbeam manufactured by Carl Zeiss (Germany) (http://www.zeiss.co.in/microscopy/en_in/products/laser-microdissection/microbeam.html), which uses a sharp focussed beam of a UV laser to accurately cut and isolate the selected sample of interest. The PALM robot microbeam laser microdissection system (PALM) is able to

Table 1 Key features of LAM instruments manufactured by different companies

Name of the supplier	Model name (e.g.)	Major feature/technology used	Web link
Life Technologies	ArcturusXT LCM System	It uses UV laser for tissue microdissection; target tissue/cell is gently captured & collected in a Cap Sure LCM Cap by touch of a specialized membrane using IR laser.	http://www.lifetechnologies.com
Leica Microsystems	Leica LMD7000	It uses a UV laser for excision; sample capturing/collection is done by gravity fall into a tube.	http://www.leicamicrosystems.com
Carl Zeiss	PALMMicrobeam	It uses a UV laser for sample excision and laser based pressure catapulting & collection into a tube/cap.	http://www.zeiss.co.in
Olympus	MMI CELL CUT PLUS	It uses a low power UV laser for sample cutting and laser mediated pressure catapulting & collection of tissue.	http://www.olympusamerica.com/seg_section/

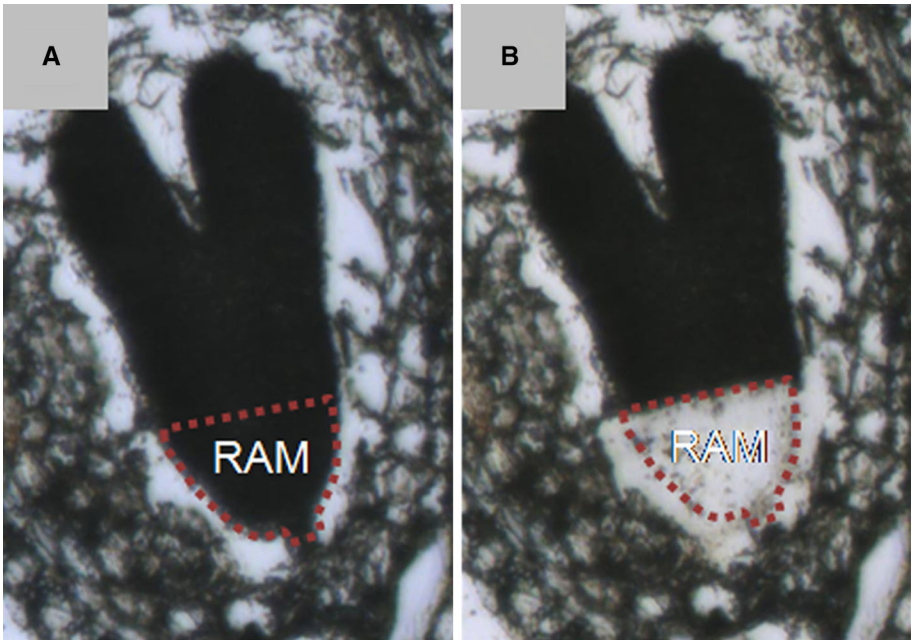

Fig. 1 LAM of *Arabidopsis* embryonic root apical meristem (RAM). **a** Uncut RAM indicated with *red dotted* area. **b** Empty *red dotted* area, showing RAM catapulted with LAM

generate a laser based photonic force which propels only the selected object (cells/tissue) of the slide upwards, due to which this technique is often referred to as laser microdissection and pressure catapulting (LMPC). Through catapulting the laser-cut tissue material is then lifted upwards to a collection tube cap, which contains a specialised buffer or oil into which the tissue will adhere to. The manufacturer claims that the PALM microbeam causes minimum damage to tissue and can be used for the cryo-sections, formalin fixed paraffin embedded (FFPE) materials and for fresh plant samples. A modified LCM system was manufactured by Leica Microsystems (Germany) which was named as LMD (http://www.leica-microsystems.com/science-lab/history/history-of-laser-microdissection/). In case of LMD the sample is cut using the UV laser (400–100 nm) which falls into the collection tube due to gravity [14]. There are two common types of commercially available models of laser microdissection: Infrared based laser capture system and ultraviolet laser-cutting system [14]. In LCM, cells are visualised through the microscope or on-screen and a thermoplastic film attached to a plastic cap is positioned such that it overlies the cells of interest. During on-screen sample visualisation, a low power IR laser is focused through the top of the cap which hits the thermoplastic transfer film that melts in localised region and surrounds the selected cells or tissue of interest. The film absorbs the laser radiation creating a non-damaging microdissection that guards the integrity of the captured material. Some of the commercially available IR LCM platforms are PixCell, Veritas and Arcturus XT systems. In contrast to LCM, LMD uses a narrow beam of UV laser to mark and excise the cell or tissue of interest, which are then directly deposited into the collection tube due to gravity [6, 15, 16]. LAM of *Arabidopsis* embryonic root apical meristem (RAM) tissue has been illustrated (Fig. 1).

Sample Preparation and LAM

Sample preparation from plant tissue is very important step before LAM. It involves tissue harvesting, fixation, processing, embedding, sectioning and slide preparation. Since plant cells possess cell wall of variable rigidity and a large central vacuole, the process of sample preparation is reasonably different for plant than animals. Therefore, tissue needs to be fixed in a suitable fixative and processed properly so that tissue or cells retain their morphology and biomolecules such as DNA, RNA and proteins etc. Tissue fixation is one of the most critical steps during sample preparation for LAM which may vary with tissue types. Depending upon the tissue types, proper fixative and a feasible approach are chosen (Table 2), which is equally important [17]. For most of the sample preparations, coagulative fixatives [18] such as acetone and alcohol or cross-linking fixatives [19] such as formaldehyde are used (Tables 2 and 3). These fixatives have the ability to crosslink the cytoplasmic proteins and lipids providing better tissue integrity. After fixation, tissues are gradually dehydrated and infiltrated with xylene or histoclearing agent. Later, tissue is infiltrated in wax or paraplast and embedded into wax or paraplast in a desired orientation. Generally 8–12 μm thick tissue sections are made from embedded tissue blocks, using microtome. Tissue sections are flattened on water and mounted onto charged slides or

Table 2 Summary of various tissue fixatives used in different protocols

Experiments	Tissue fixatives used	Plant tissue samples used	References
Identification of genes expressing during embryogenesis in *Arabidopsis* using cryosectioning	Ethanol:acetic acid (3:1) as fixative, sucrose (10–15 %) as a cryoprotectant	Embryos	Casson et al. [23]
Optimization of LCM for plant tissues	Ethanol:acetic acid (3:1), formalin:acetic acid: ethyl alcohol (3:1:1)	Embryos and shoot meristems	Kerk et al. [24]
Gene expression analysis of *Zea mays* (*Zm*) shoots apical meristem.	Chilled acetone (100 %)	Shoot & inflorescence meristems, & leaf primordia	Ohtsu et al. [55]
Comparison and isolation of RNA from paraffin embedded plant tissues	Ethanol (75 %) and acetic acid (25 %)	Immature embryos, root (for stele cells) and leaf	Takahashi et al. [25]
Comparison of gene expression and protein accumulation in pericycle cells of root (*Zm*)	Ethanol:acetic acid (3:1)	Root (for pericycle cells)	Dembinsky et al. [28]

Table 3 Comparison between sectioning of paraffin embedded tissue and cryo-sectioning for LAM

Cryo-sectioning	Sectioning of paraffin embedded tissue
It is generally preferred for molecular biological studies; it limits the use of various organic solvents which may affect cellular components of interest	Besides use in molecular biology, it is used to get a better anatomical understanding by preserving the tissue/cellular histology; many cellular components are still preserved
Usually 10–15 % sucrose is used as a cryoprotectant to prevent the ice crystal formation	Dehydration using a gradient of alcohol to prevent the tissue samples from getting shrinked
Optimal cutting temperature compound (OCT) embedded tissue sections are sectioned using a cryo-microtome at a relatively low temperature (normally 0 to -25 °C)	Paraffin embedded sections are cut using a rotary microtome at root temperature; thus, easier to handle
Sections cannot be stored for a longer duration	Sections can be stored at 4 °C under dry condition

charged synthetic membrane coated slides and dried on a hot plate. This helps the tissue to get stably fixed onto the slides. For free cells or cell cultures, embedding might not be required and cells can be directly collected from the cell smear on charged slides or cell culture on membrane coated Petri plates.

For LAM, slides with tissue or cell population are first observed under microscope and desired objects are identified. Use of appropriate objective has to be decided depending on the size of object to be collected. Cells or tissues of interests are monitored and marked on computer screen, marked area is cut with appropriate laser (depending on the manufacturer) to detach it from neighbouring tissues. This is followed by laser catapulting or collection of marked tissue in a collection tube or cap. For proteomic study, the use of UV laser is preferred in LAM [20]. This process is likely to vary with different LAM systems and respective softwares, and therefore, manufacturers' guides have to be followed. After LAM-based collection (in neutral oil or cell extraction buffer), desired tissues or cells may be stored at −80 °C for a short period or can be directly used for isolation of DNA, RNA or proteins. The entire schematic procedure for LAM has been described in Fig. 2.

A protocol by Scanlon et al. 2009 has used ice chilled 100 % acetone as a fixative; tissue dehydration was done using a mixture of acetone and xylene in different ratios depending upon the tissue type and followed by paraplast infiltration, and embedding (Table 2; Fig. 2). Using this coagulative fixative based approach, they have finally identified the genes involved in regulation of maize shoot apical meristem (SAM) through LCM-microarray as well as 454 sequencing-based functional genomics approach [21]. In addition, they have also used Farmer's fixative (3:1 ethanol: acetic acid) for studying the genes involved in maize SAM maintenance during various developmental stages (Table 2) in *Zea mays* [22]. Another protocol by Casson et al. 2005 have used cryosectioning of *Arabidopsis thaliana* embryo using cryomicrotome, which often produce better quality of RNA as compared to paraffin embedded samples (Table 2). They used ethanol acetic acid as a fixative and 10–15 % sucrose as a cryoprotectant followed by embedding using optimal cutting temperature compound for studying gene expression patter during embryonic development in *Arabidopsis thaliana*. After block preparation, samples were sectioned at a relatively low temperature, such as −25 °C (Table 3) [23]. Kerk et al. 2003 have described a method, where 3:1 ethanol: acetic acid (Farmer's fixative) was used as a fixative and tissue dehydration was done using the graded series of ethanol followed by ethanol: xylene series (Table 2). For embedding, they have used the paraplast and samples were

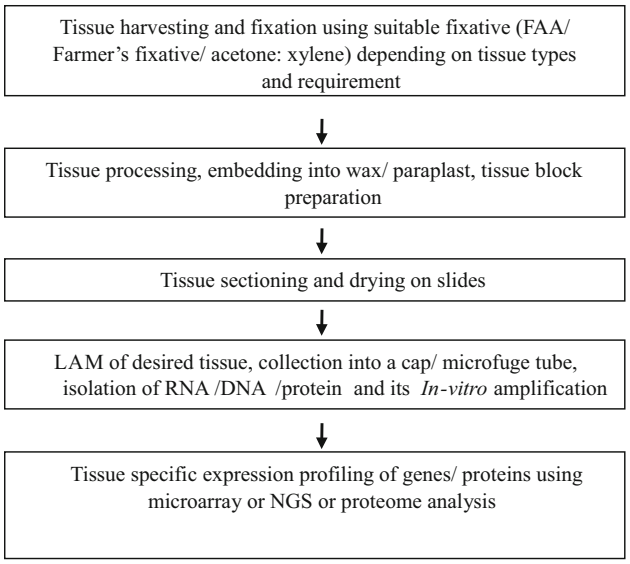

Fig. 2 Steps for LAM from fixed tissue sections and downstream application

sectioned using the rotary microtome. They also have optimised a protocol for various plants such as *Arabidopsis* and *Zea mays* (Table 2), and their results suggest that samples fixed using ethanol: acetic acid give better RNA quality with maximum RNA recovery [24]. A protocol by Takahashi et al. 2010 have described the use of 75 % ethanol/25 % acetate, 60 % ethanol/40 % acetate and 100 % ethanol or 100 % acetone as per the type of target plant tissues (Table 2; Fig. 2). The fixed samples were dehydrated with graded series of ethanol and were paraffin embedded; sections were done using the rotary microtome as in previously described protocols (Table 2). The authors claimed to have produced high quality RNA with good yield using described protocol, which also have recommended an optimum duration for tissue drying (at 40–42 °C) (Table 2). This suggests that post fixation tissue handling also affects RNA quality [25]. Kladnik laboratory (http://botanika.biologija.org/exp/protocols/) have used FAA (formaldehyde: acetic acid: ethanol) in a definite proportion as a fixative for fixing plant tissues for LAM (Table 2). For dehydration they have used graded series of tert-butanol in 95 % ethanol, followed by paraffin embedding and tissue sectioning (Table 2).

Fixation of plant samples for protein work requires precipitating fixative such as ethanol and methacarn [26]. The preferred way to study the proteome of the specific cell type is done by fixing the plant sample in 3:1 ethanol: acetic acid followed by cutting at low temperature using cryomicrotome to preserve the identity of the bio molecule of the interest [27, 28].

Keeping in mind majority of the described protocols, it appears that the quality of LAM-derived RNA depends largely on the handling during the experiments as well as on the concentration of the fixative, duration of incubation, embedding of the sample, processing of tissue sections etc. [10, 25] (Table 2). Tissue fixation and processing via paraffin embedding take a longer duration, which may affect the quality of the cellular biomolecules. Therefore, some research groups prefer the use of cryosectioning, which is performed at relatively low temperature for the recovery of better quality DNA or RNA or protein [29]. However, it is often not suitable for soft or tender tissue types, which may be damaged during the cryosectioning process. Various fixatives suitable for LAM of different plant samples have been summarised in Table 2 and a comparison between paraffin embedding and cryosectioning is shown in Table 3.

Table 4 List of some kits available for RNA amplification

Name of the kit	Basic features	Minimum amount of starting RNA
NuGEN amplification and labelling systems (range of kits, e.g. Applause/ Ovation AmpSystems)	Mostly based on their proprietary single primer isothermal amplification (Ribo-SPIA™) technology. Kit specific for FFPE may be more suitable for LAM-derived sample from formaldihyde fixed and paraplast embedded tissue (www.nugen.com)	500 pg
TransPlex Whole Transcriptome Amplification (Sigma-Aldrich)	Total RNA or whole transcript from various tissues/cells (plant/animal) can be efficiently amplified. It uses a "unique blend of quasi-random primers to ensure accurate transcriptome coverage and rapid amplification" (www.sigma-aldrich.com)	50 ng (at <5 ng/ µl)
Ambion RNA amplification or, MessageAmp II aRNA amplification kit (*Life Technologies*)	In this protocol, antisense amplified RNA (aRNA) by two times amplification of RNA using in vitro transcription (www.lifetechnologies.com)	0.1–100 ng

Note: We apologize for not being able to list all thesuppliers/companies/kits as its is beyond the scope

RNA Isolation and In Vitro Amplification of RNA Populations

Isolation of high quality RNA is a prerequisite for successful downstream experiments such as gene expression studies. Since LAM-derived tissue is often small in size, it remains a challenge to isolate good quality RNA. RNA

isolation from the LAM captured samples is done either by manual TRI-Reagent (/Trizol) based method or commercially available kit. A wide range of commercially available kits are used for RNA, DNA or protein isolation from LAM captured tissue or cells. Depending upon the need of the experiment a particular method of RNA isolation is selected. Commercially available kits such as *RNAqueous micro kit (Ambion)* isolates RNA from as less as ten cells (captured through LAM). On the other hands kits such as PicoPure RNA isolation (LifeTech) is capable of isolating the high quality RNA form 01-1000 LCM captured cells.

Depending on nature or size of target cell population and RNA isolation method, RNA isolated from LAM-derived tissue are often small in quantity. Therefore, for some downstream application such as NGS, it may sometimes be necessary to amplify LAM-derived RNA. Several kits and non-kits based methods have been designed to amplify the total RNA starting from the small fraction of RNA isolated through LAM. Commercial companies like NuGEN, Sigma and Ambion manufacture several kits, which are used for the amplification of the RNA starting from a amount as low as 5 ng. Depending on the downstream experiment, either it is microarray or NGS, a particular kit is selected. Details of few of the kits commonly used for total RNA amplification are shown (Table 4). Besides kits, few non-kit based methods have also been developed to amplify the total RNA population derived from LAM sample. One such method uses T7 RNA polymerase-based amplification to generate microgram quantities of amplified RNA [21, 30].

Application of LAM to Understand Tissue Specific Transcriptome in Various Biological Processes

The ability of LAM to isolate the specific cells or tissue from a heterogeneous population has been used as an efficient tool to address wide range of biological questions by isolating and profiling the biomolecules of interest [31]. LAM followed by microarray (LAM-M) and next generation sequencing (LAM-NGS) approaches have been used to identify the genes involved in specific developmental process. Due to reduced cost of NGS and advancement of technology, LAM-NGS is more frequently used for functional genomics, comparative genomics and study of plant-pathogen interaction in comparison to LAM-M. Casson et al. 2005 have studied the gene expression profile during embryogenesis in *Arabidopsis thaliana* using LCM-microarray (LCM-M) approach. Their study suggested that up to 65 % of the *Arabidopsis* genome is expressed in the developing embryo [23]. Spencer et al. 2007 have used LCM-M to perform the transcriptional profiling of the *Arabidopsis* embryo and have identified genes involved in different developmental stages during embryogenesis [9]. Application of LCM has not only remained restricted to *Arabidopsis* but also has widely been used for other model plants such as *Zea mays, Glycine max* [32], *Medicago truncatula* [33], *Oryza sativa* etc. [12]. Emrich et al. 2007 [34] have described the identification of novel genes in maize using LCM-454 sequencing approach and generated rare cell type specific transcripts. Recently LCM-454 pyro sequencing based study in Maritime pine (*Pinus pinaster*) has uncovered the transcriptomic status of the root tissue [35]. Using LCM-NGS-based approach, Takacs et al. 2012 have identified genes expressing at early as well as late stage of SAM development during embryogenesis in *Zea mays* [22].

LAM-NGS and LAM-M approaches have also been used to understand the plant-pathogen interaction [36–39] and to identify differentially expressed genes in infected plant cells. Ramsay et al. 2004 have used FAA for fixing the tomato roots infected with *Meloidogyne* spp. to study the *Cyclin* activity in infected cells and have shown the role of *Cyclin* genes in increasing cell size [40]. LAM-based approach has been used to harvest the cells during various stages of infections by a pathogen, which can be further used to study the genes involved in host-pathogen interaction and plant immune response.

LAM has also been used as a potent tool to study plant–microbe symbiotic association such as root nodule formation in plants induced by the symbiotic bacteria *Rhizobium*. Root nodules are specialised organs for the nitrogen fixation developed from roots of legumes upon infection with *Rhizobium*. To understand bacterial and plant gene expression pattern related to nodule development, LAM-454 sequencing approach has been used, which has identified genes involved in the symbiosis process between *Medicago truncatula* and its symbiont *Rhizobium meliloti* [41]. Another symbiotic association between fungus and roots of higher plants is termed as mycorrhizal associations. Arbuscular mycorrhizal association is one of the most common mycorrhizal associations where mycorrhizal fungus inhabits the cortex of plant root and obtains their nutrition from the plant host. People have improved protocol for tissue fixation and LAM-based RNA isolation from fungal colonised cortical cells, and have applied LAM-M or LAM-NGS to get better insight into gene expression pattern of individual cell types of mycorrhizal roots [33, 42].

Application of LAM in Proteomic Studies

Besides transcriptomic study, LAM coupled to MALDI (Matrix-assisted laser desorption/ionisation) is useful for conducting tissue specific proteomic studies in plants, specially, because transcriptome does not entirely correlate with proteome of the same cell [43]. In order to study the proteome of the specific cell or tissue, LAM is followed by

either MALDI-TOF/MS (Matrix-assisted laser desorption/ionisation-time of flight/mass spectroscopy) or 2D-PAGE [28, 44]. Using the LAM coupled to 2D PAGE (Two-dimensional gel electrophoresis), a proteome map of soluble protein extracts from pericycle cells has been generated which is useful for understanding the proteome level difference between the wild type and *rum1* mutant in maize having a severe root phenotype [45]. Using LAM coupled to 2D PAGE and MALDI, a reference proteome map for the maize root hairs has been generated. [16, 46]. Till date, only a limited number of experiments have been done in order to study the proteome status by using the LAM in plants. Unlike transcriptomic studies, the LAM-based proteomics study is not well established and there is no standard protocol to amplify a small amount of protein to be used for either MALDI-TOF/MS or 2D-PAGE. However, in order to enrich the LAM-derived protein samples, few kits are available to reduce the contaminating agents during the course of entire process (http://www.bio-rad.com/en-us/product/proteominer-protein-enrichment-kits).

Application of LAM in Metabolomics

Metabolites are considered as the broadcasting signals and therefore metabolomics is considered to provide a direct evidence for the physiological state of an organism or cell type. Although several techniques have been employed to analyse plant metabolites, the technical speciality of LAM provides a convenient approach for studying plant tissue or cell type specific metabolites [47]. The efficacy of LAM coupled with LC-UV/MS was evidenced in analysis of glucosinolate levels in *Brassica napus* seeds [48]. Similarly, LAM coupled with NMR/GC–MS has been used for metabolite profiling of *Arabidopsis thaliana* vascular bundles [49] and for estimating the phenolics in secretory cavities of *Dilatris pillanissi* [50]. In another study, LAM was used for analysing RNA, enzyme activity and metabolite profiling in white spruce (*Picea glauca*) [51]. In this study, a tangential cryosectioning approach using LAM was applied for getting large amount of cortical resin ducts and cambial zone tissues. This report showed differential expression of genes involved in terpenoid metabolism between cortical resin ducts and cambial zone tissues in response to methyl jasmonate. Following this, Abbott et al. 2010 have designed a LAM-based gene expression method for analysing metabolite levels and enzyme activities in woody plants as well [52]. Since metabolites are closely linked to the phenotype, physiology and genetic or proteomic make up of an organism, metabolomics data should be helpful in understanding the correlation between transcriptome, proteome and metabolome, and responses to the various kinds of stresses. Therefore, it can be considered as a potent tool for bridging the gap between a genotype and a phenotype [53].

Precautions to be Followed for LAM

To obtain good and reproducible results, LAM requires some basic precautions. There should be little time difference between tissue harvesting and initial fixation. If the downstream product is either RNA or protein, use of RNAase inhibitor or protease inhibitor is necessary in appropriate steps [25]. The concentration and duration of the fixation [10, 25] should be carefully followed in order to maintain the integrity of the tissue and biomolecule, which is essential to achieve good results in downstream experiments. Dehydration should not exceed beyond the standardised optimal time and temperature. If cryosectioning is chosen, proper mounting with appropriate cryoprotectant [54] and maintenance of temperature are essential so as to prevent cells or cellular molecules from getting damaged. If paraffin embedding is chosen, it should be done at optimal temperature (60 °C), as increase in temperature is likely to damage the tissue. During slide preparation for the LAM, slides should be dried at an optimal temperature and should be stored at 4 °C in a sealed RNase free rack, if cannot be used immediately [10, 25].

Practical Bottlenecks in LAM

LAM, which provides the basis for cell or tissue specific study, is one of the most potent tool but still has a few limitations; tissue handling being one of the most important precautions during the experiment. Tissues should be handled at relatively cold temperature with gloves to prevent the DNAse/RNAse contamination [55]. Sample preparation and LAM require a balance between tissue integrity and the activities of the biomolecules, which depends on the selection of the fixative. In case of cryosectioning, ice crystal formation in vacuolated cells affects the tissue integrity. To overcome this, cryoprotectant such as 10–15 % sucrose is often used [23]. In case of the paraffin embedded sample, tissue integrity is well maintained but the quality of DNA, RNA, and protein may get affected due to extended fixation protocols. This can be prevented by the selection of proper fixative, depending upon the tissue type [25.]. LAM-based RNA isolation can be performed by any of the commercially available kits which should fulfil the requirement for downstream purpose such as total RNA isolation, small RNA isolation etc. While performing the RNA amplification, orientation of the RNA should be taken care of after certain rounds of amplification which may generate either sense or antisense amplified RNA. Depending upon the kit or amplification protocol, orientation of the amplified strand may be altered. These things should be carefully monitored if microarray analysis is to be performed in the downstream application [21].

Conclusions and Future Perspectives

Today, when much of the technological advancement in the area of plant biology has taken place, LAM is still acting as an efficient tool coupled with microarray and NGS analysis. LAM-based functional genomics approach is currently being used to address a wide range of biological questions pertaining to the various aspects such as developmental biology [23], plant-pathogen interactions [36, 37], plant physiology related studies [56], tissue specific proteome studies etc. Because of its ability to isolate a specific cell type from a population, it paves the way to link many of the ongoing studies on cellular relationships, comprehensive genomic and proteomic dataset [28]. Recently, with combination of NGS, LAM produced transcriptome data sets for specific tissue or cell types [57]. Moreover, LAM approach has been widely applied for a wide range of plants and various tissue types to perform comparative study of expression and functional genomics. In future, it can be an indispensable tool to study the cell signalling in plants, nutrient transfer, transcription, cell wall modifications and defence against pathogen [55]. Although, the application of LAM at this stage is prevalent in the study of tissue or cell-specific gene expression profiling, we expect more advancement in the method related to LAM-based proteome, metabolome and epigenome study in near future.

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