

# An 'AWSAR' to Devise a Protocol for Laser-based Excision of Microscopic Woody Apple Bud Meristem for Tissue-Specific Expression Analysis

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Apples from the hills of Shimla region of Himachal Pradesh are freighted nationwide and abroad. Being a resident of the hills, it had always been figurative as well as exciting for me to see that how this local produce is moved from hills to the plains. Therefore, it is a common sight on Shimla-Chandigarh National Highway-22 to see the trail of trucks carrying the apple-packed cartons from orchards to other parts of the country. But, this time these were apple buds packed in dry ice instead of fruit cartons, which were being carried by me from these hills to National Institute of Plant Genome Research (NIPGR), New Delhi as part of my DST-SERB N-PDF research project work.

Apple (*Malus × domestica*), being a major temperate fruit, is consumed as a rich source of phytochemicals worldwide. In the remote hill areas, where the transport facilities still remain poorly developed and income sources are limited, this fruit is the only source of income for some local and adjoining communities involved in maintenance, harvest, packaging and transport. Apple tree is a temperate woody perennial. It does not bear any fruits for the first few years of its life which is known as the juvenile phase of its life. After the completion of juvenility, the reproductive events which involve setting of flowers and bearing of fruits take place perennially for the rest of its life. The tree undergoes various developmental reprogramming according to change in seasons. Being smart enough to cope with the harsh winter conditions, standing snow-covered at an altitude

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\* Dr. Swati Verma, Post Doctoral Fellow from National Institute of Plant Genome Research, New Delhi, is pursuing her research on "Possible Role of Small RNAs in Regulating Gene Expression during various stages of Bud Dormancy Release in Apple." Her popular science story entitled "An 'AWSAR' to Devise a Protocol for Laser based Excision of Microscopic Woody Apple Bud Meristem for Tissue Specific Expression Analysis" has been selected for AWSAR Award.

of 2276 meters above sea level and still managing to serve a visual-delight to onlookers, these temperate trees plan their lifecycle wisely and undergo developmental reprogramming to bear the environmental changes. This involves slowing down of the metabolic events and shedding leaves to conserve energy for the next vivacious phase of their life. This vivacious phase, known as the reproductive phase, embraces setting of flowers and fruits and is initiated consecutively after breaking of dormancy. The appropriate onset of the aforesaid reproductive event encompass dormancy only if the chilling, light and temperature parameters are properly met by these trees. Apart from the rest of the tree organs instrumental for life, a bigger responsibility to contrive this tendency is mostly controlled by the self-perpetuating microscopic units of life on the apical and axillary shoot buds, called the shoot meristems. The meristems are basically niches of actively dividing cells which are capable of giving rise to different cell types.

I was in constant touch with one of my MSc Professor, now heading the ICAR-NBPGR, regional station Shimla to kindly keep me informed about sample stages of apple I needed to collect. Since the trees are very much sensitive to environmental signals, they bloom early if summers onset early. This time the summers arrived early and it was the time to pack the bags and head to the queen of hills, Shimla to collect the apple buds. Looking at me amazed and curious for why I was collecting the small pieces of wood (apple bud samples), I had a brief conversation with a few local people collecting forage for their livestock and wood for fuel from nearby apple orchards. These people following a lineage of worshipping local deities said in surmise in their local dialect, '*lagam reo devta naraaj ho ryan, hyun kam pad reo, ta karin ye baar garam jhik ho ryo ta seb ne lag ryan*'. Being a native, I somehow managed to understand what they were saying. They meant that there has been a substantial reduction in snowfall since last some years because the deities have been furious and, therefore, apple production was getting affected due to prevalent scorching temperatures in the hills. Scrupulously, they were not wrong since these are wisely planned means by ancestors to conserve natural resources and forests in name of the fear of local deities and Gods. Throughout the year, all the major festivals of these tribes tend to fall around the seasonal environmental changes for celebrating the local temperate produce. Being a part of the research community of a country where roughly 70% of population resides in small remote villages, alongside studying the effects of global temperature elevation on staple food crop production, it is of utmost importance to understand the effects of global climate changes on the regional temperate commercial crop production. Apple is a crop accounting for 90% of the total commercial horticultural production of the hill state of Himachal Pradesh and is consumed as a major temperate crop worldwide. This temperate horticultural crop serves as an appropriate model for studying the molecular events encompassing this seasonal reprogramming in the woody apple buds, which are the heart of meristematic activity.

The abovementioned events of dormancy breaking are initiated after perception of environmental signals and accordingly a particular gene is switched off or turned on to modulate the overall protein expression of the plant cell as per the needs of plants. In this process, apart from mRNAs which code proteins, the small non coding miRNAs play important regulatory roles by cleaving their mRNA targets or rendering a protein non-functional. We are interested

in deciphering the role of small non-coding miRNAs in regulating bud dormancy release in the meristem tissue of apple. For this, firstly we were required to devise a protocol for excision of the microscopic meristematic tissue from apple bud. But, apple has a woody bud containing a woody meristem, so devising a protocol for its excision was the first hurdle. We could have carried out the studies in easy to grow model plants like *Arabidopsis* for which some protocols of tissue specific excision are already available but it was impossible to replicate and investigate the seasonal events, which we are particularly interested in studying, in these annual species.

The first step was fixing the sample's molecular details in the stage at which they were excised. This was done using various organic solvents. After bringing them back to host lab at NIPGR, New Delhi, aldehyde-based reagents were used for crosslinking of the proteins. This was followed by removing the traces of water from tissue by dehydrating and infiltrating it with gradients of alcohol-based reagents. The next step was embedding the buds in wax and making wax blocks using specific molds which could facilitate sectioning of the buds for making fine thin micrometer thickness chips of the tissue. For this, firstly the tissue was acquainted with wax infiltration for some days at an ambient temperature which could facilitate wax infiltration without damage to cellular components and the overall cell morphological details. The buds were cut to fine sections using a microtome, a tool used to cut extremely thin slices (sections) of a material.

We deployed Laser Capture Microdissection (LCM) for the excision of cells of interest from the apple bud sections. LCM involves excision of cells from a particular mixed population of cells using a laser beam. The LCM excised apple meristem tissue was utilised for total RNA isolation. Since the host lab of my N-PDF research project headed by Dr Ananda K Sarkar at NIPGR, New Delhi is quite experienced in working with LCM of plant tissues, some empirical hit-and-trial-worthy modifications and availability of the related materials, chemicals and equipment in the lab helped in devising a protocol which could facilitate the process for woody apple buds. Till date, none of the research work addresses isolation of miRNA from microscopic cell/tissue types of tree species. We have placed a protocol in place for this kind and similar related analysis of a hard to excise microscopic woody tissue.

The protocol would also help in overcoming the hurdle of immediate unavailability of stage-specific samples throughout the year due to their perennial lifecycle. Because now the tissue can be fixed and sectioned, the cells of interest can be excised from a mixed population using LCM, the nucleic acids and other cell components can be isolated, preserved and analyzed as per the need of the experiment at any time of the year. The research is likely to help in elucidating the role played by miRNAs in regulating the events of dormancy breaking in temperate woody perennials.

The time is apt to address the effects of global warming on temperate woody perennial plant crop productivity which are subject to maxim adverse effect of global warming. You are able to understand the problems of a particular geographical area very well which you have been rooted to. This research project is an absolute 'AWSAR' for me to get a scientific insight into the modulation of gene expression during bud break process and helped me to put a tissue specific excision and RNA isolation system in place for further expression analysis.