07/03/2018

To,
The Administrator
QEII Technicians Study Awards
773 Moonshine Road,
RD1, Porirua, 5381
Ph.: (04) 528 0808

Attention: Andrew Hutson

Dear Andrew,
Please accept this report for the completion of Queen Elizabeth II Technicians' Study Awards for 2017-2018. The grant was awarded for me to study molecular cytogenetics, which is an active area of research with broad applications in plant science, conservation and agricultural research.

Chromosome number and morphology form important characters when it comes to genetics, evolution and breeding programmes. As an island system, New Zealand has its own unique flora of which many are polyploids. FISH (fluorescent *in situ* hybridization) and GISH (genomic *in situ* hybridization) are powerful molecular cytogenetic tools that can be used to unravel the chromosomal intricacies of these unique New Zealand plants. The research group I am associated with at Massey University, Palmerston North studies the evolution of New Zealand plants using molecular techniques. Adding molecular cytogenetic data can add an important further dimension to our research.

As there are no courses in any of the New Zealand universities to learn FISH and GISH, I chose to learn the technique in a lab actively involved in molecular cytogenetic research, and to do so there is no other better place than the lab of Prof. Pat Heslop-Harrison at University of Leicester, England, who has done pioneering research in the field of molecular cytogenetics. I travelled to Leicester, England on 19th Oct. 2017 to start my twelve week training finishing on 19th Jan. 2018. The lab of Prof. Pat Heslop-Harrison is involved in extensive molecular cytogenetics of wheat and other plant species in various projects starting from breeding, phylogenetics to establishing conservation strategies, etc. I started my training on few British wheat varieties of which some carried rye translocations bearing resistance towards diseases. I was helped in the lab by Trude Schwarzacher, who is the first author of the book "Practical in Situ Hybridization" and works closely with Prof. Harrison. I also received help from other senior research students in the lab from time to time.

The basic steps started with germinating wheat seeds on filter paper, collecting and pre-treating root tips and fixing them in Cornoy's fixative. I then prepared metaphase spreads on microscopes slides and performed actual FISH and GISH experiments. To work more efficiently, the entire protocol was broken down into small parts to better manage time and the plant material (root tips). After completion of the experiment, slides were observed and

useful cells were photographed with a fluorescence microscope equipped with a digital camera. I also learned to process the photos in Adobe Photoshop and analyse them as per the objective of the set experiment. Although basic protocols are available in published literature, I really gained thorough knowledge about the intricate details of each step of FISH and GISH studies by being in the Heslop-Harrison lab and I am now prepared to implement these protocols in New Zealand.

In addition to learning lab techniques, I have established good contacts between the lab of Prof. Pat Heslop-Harrison and my Massey research group led by Dr. Jennifer Tate. Both Prof. Heslop-Harrison and Dr. Tate plan to work on a collaborative research proposal in the near future. The techniques learned will be utilized in the immediate future as part of a current Marsden Fund project (to Prof. William Lee, Landcare Research) that aims to understand consequences of genome diversification in native New Zealand flora. Dr Tate is an AI on this grant and will supervise a PhD student working on the genomics aspect of the project.

The training has surely raised my confidence to independently undertake molecular cytogenetic studies. These data will contribute to current research projects and allow further development of research proposals specifically aimed at improving the knowledge and understanding of the New Zealand plant chromosome evolution. This knowledge can have significant contributions not only to understanding our native flora but also to be useful in plant/crop breeding/improvement programmes. Also, I am sure that laboratories around New Zealand would take advantage of the expertise available to work on molecular cytogenetic projects and I would be happy to offer training to others in this area.

Molecular cytogenetics is an important area of research in both plant and animal science and this QEII award has allowed me to upskill my cytogenetic knowledge, particularly the molecular aspects. I am truly grateful for this opportunity and I thank members of the committee for awarding this technicians study award to me.

Regards,

Prashant Joshi

Appendix

Wheat varieties used in the study

Trimmer triticale
Skyfall
Nicodur
CDC Landmark
CDC Stanley
Relay

Microscope and Software used

Nikon Eclipse N80i fluorescent microscope equipped with a DS-QiMc monochromatic camera (Nikon, Tokyo, Japan).

NIS-Elements BR3.1 software (Nikon)
Each metaphase was captured with three or four filter sets: orange for Alexa 630 (far red), green for Alexa 594/xx (red), blue excitation for FITC/Alexa488/FAM and UV excitation for DAPI.

Images were falsely coloured, overlaid and the contrast adjusted with only those functions that treat all pixels of the image.

(either NIS-Elements or Photoshop)

Details about the technique learnt

1. Root tip germination, pre-treatment with ice cold water or alpha bromonapthline and fixation in Cornoy's fixative (Alcohol : Acetic acid - 3 : 1)
2. Preparation of buffers needed in FISH and GISH experiments
3. Digestion of root tips with enzyme mix at 37^0C, preparation of metaphase spreads on microscope slide
4. Selection of slides with better metaphase spreads and count for further experiment
5. Pre-treatment of slides to prepare them for the FISH/GISH
6. Preparation of probe for hybridization
7. Hybridization on Hi-Band bed moist chamber
8. Screening of slides and taking images of selected cells
9. Editing images in Adobe Photoshop and preparation of karyotype
10. Analyses and interpretation
11. Introduction to probe design using Next Gen sequencing data
12. I was briefed about the Nick translation protocol to label the DNA with fluorescent dye

Images of FISH/GISH of Wheat variety ‘Relay’ carrying wheat-rye translocation 1B/1R. Photograph is of a single cell captured in black and white under different wavelengths of light and then false coloured.

A. Merged image of MeC (Red), rye chromosome (Green) and DAPI stained metaphase (Blue). Red dots on the chromosomes are the anti-mouse antibody showing sites of DNA methylation. Green signal is of translocated rye chromsomes 1R onto wheat chromosome 1B. And finally blue signal is DAPI staining.
B. DNA methylation study using MeC anti-mouse antibody. The red dots on chromosomes indicate DNA methylations sites.
C. GISH using labelled rye DNA to locate the position of rye chromosome 1R on wheat metaphase spread. The rye chromosome was found to have replaced the wheat 1B.
D. Metaphase stained with DAPI