

**PROCEEDINGS OF THE WORKSHOP ON “METHODOLOGIES FOR QTL AND
ASSOCIATION MAPPING”**

- Date: 23rd December 2010
- Venue: Institute of Forest Genetics and Tree Breeding
- Funding Agency: Department of Biotechnology, GoI
- Participants: The participants of the workshop included
- Director, IFGTB
 - Group Coordinator Research, IFGTB
 - All Heads of Division
 - Principal Investigator, Co Investigator, research scholars and field assistants of the project
 - Research Scholars from Division of Plant Biotechnology and Genetics and Tree Breeding Division
- Partners:
- a. Dr. S. Chinnaraj, TNPL, Karur,
 - b. Shri. Manjunatha, MPM, Bhadravathi
 - c. Shri. Wison Kennedy, TAF CORN, Trichy
- Guest Speakers:
1. Dr. K. Gurumurthi, Former Director, IFGTB, Coimbatore
 2. Dr. K.V. Bhat, Principal Scientist, NBPGR, New Delhi
 3. Prof. M. Maheswaran, CPMB, TNAU, Coimbatore
 4. Dr. M. Raveendran, Associate Professor, CPMB, TNAU, Coimbatore
 5. Dr. Vimal Kothiyal, Scientist F & Head, Forest Products Division, Forest Research Institute, Dehradun

The orientation workshop on “Methodologies for QTL and Association Mapping” was conducted to provide an insight into the recently initiated project titled “*Genetic improvement of Eucalyptus through mapping and tagging of QTLs/ Genes*” funded Department of Biotechnology with total budget outlay of ₹ 191.84 lakhs for a period of five years.

In view of the importance of forestry resources and need for biotechnological interventions to compliment the conventional improvement and conservation strategies, Department of Biotechnology, Government of India had launched a mission program on “Biotechnological interventions for improvement, conservation and utilization of forestry resources”. Under the sub-mission II on tree improvement, *Eucalyptus* was identified as a model system for functional genomics, wood quality, plant architecture, metabolic pathway engineering and others. IFGTB was identified for coordinating the program and developing a network program involving industry, forestry geneticist and molecular biologists.

The workshop was inaugurated and the welcome address was given by Shri. T.P. Raghunath, Group Coordinator Research, IFGTB. In the morning session, the on-going activities of the institute was presented by Dr.Gurudev Singh, Scientist F and Head, Genetics and Tree Breeding Division, IFGTB.



Dr. Sivakumar, Scientist D, Division of Genetics and Tree Breeding, IFGTB elaborated on the *Eucalyptus* improvement program conducted by the institute followed by a discussion on

breeding plan of eucalypts was discussed. He briefed the house on the initiation of the program and detailed the efforts taken on the first generation and second generation improvement of *E. camaldulensis* and *E. tereticornis*. Details on clonal selections and their growth performance and wood characteristics were presented.

Dr. Gurumurthi suggested that the Eucalyptus improvement program has to be carried out as a coordinated program incorporating the strengths of all the ICFRE institutes and other organizations working on eucalypts to enable supply of improved material to the Industries. Dr. Krishna Kumar, Director, IFGTB replied that All India Coordinated



Project on Eucalypts was coordinated by IFGTB and similar programs for other species has been developed and the proposal had been submitted to Ministry of Environment and Forests, GoI.

Dr. Yasodha, Scientist E and Technical Co-ordinator of the project presented the background, the ongoing activities and the role of the partners in the project. Dr. Gurumurthi enquired regarding the wood specific consumption (amount of fresh wood required for the production of 1 ton of pulp) from the paper and pulp industries in India. Dr. Yasodha replied that such data was not available from the industries but assured that data for calculating % pulp yield in Indian Scenario based on the annual report from industries will be referred and facts compiled. Dr. S. Chinnaraj, TNPL offered to provide the data from TNPL. Dr. Gurumurthi suggested that such data would help in highlighting the importance of such high through put programs for genetic improvement

to the policy makers. Dr. K. Gurumurthi enquired about the suitability of the software used for genetic map construction and Dr. Bhat expressed that the Mapmaker and QTL Cartographer are routinely used for such purposes.

Dr. K.V. Bhat, Principal Scientist, NBPG, New Delhi delivered the lecture on “***Marker Assisted Breeding for Genetic Improvement – an Overview***”. The lecture provided a brief introduction

on the theory and advantages of marker assisted breeding (MAS) in crop plants for early selection of superior genotypes. He detailed on the different marker systems used for MAS and emphasized that microsatellite markers (SSR) were the best suited for such studies. He also



expressed that the markers need to be tightly linked to target loci and markers like SSR are much useful when present within a gene. He discussed on the new high throughput technique used for DNA isolation and genotyping of large number of samples. He also mentioned about the importance of marker in breeding program which involves Marker assisted backcrossing, pyramiding and combined approach. He gave few examples of important genetic traits screened by markers and used in early selection for crop plants like maize and added that networking project involving various institutes are being carried out in wheat against rust resistance. Dr. Gurumurthi enquired regarding the time scale for developing markers by IFGTB and Dr. Bhat replied that even in crop species several years were dedicated towards developing basic marker information. He expressed that if the whole genome sequencing of the target species was made available in public domain, it would accelerate the process of developing MAS in tree species.

Dr. Yasodha informed that the eucalypt genome has already been sequenced and the annotation is presently in progress and the sequence data would be available by 2011.

The next lecture on “*QTL mapping and its application in plants*” was delivered by **Prof. M. Maheswaran, CPMB, TNAU, Coimbatore**. He elaborated on the principles of quantitative

genetics, conventional breeding and molecular breeding. Further, he detailed the house on the different theoretical and practical aspects of QTL mapping highlighting the concepts of major and minor effect genes, additive, dominant and epistatic QTLs. He



summarized about the types of markers available and its characteristics and the requirements for QTL mapping, importance of SSR markers in QTL and also the importance of phenotyping and statistical packages for efficient QTL mapping. He stressed on the importance of generating mapping population and cautioned that populations generated for a specific trait should never be used for other traits which would lead to identification of dubious and non reliable markers. While explaining the importance of phenotyping, he stressed on the fact that major time should be spent for phenotyping. He highlighted the statistical problems including identification of false positive and false negative in detecting QTL. He also detailed the different softwares used for the QTL mapping for identification of potential QTLs.

A lecture on “*Importance of pheotyping in genetic mapping of plants*” was given by **Dr. M. Raveendran, Associate Professor, TNAU, Coimbatore**. He briefed on the QTL technology by

which one could increase the yield and reduce the gap between potential yield. He also expressed that if perfect markers are available then it is possible to increase the yield using MAS. The importance of phenotyping in MAS programs were highlighted with several



examples. He explained the methods for automated phenotyping for research on drought tolerance. He stressed on the fact that lack of accurate phenotyping facilities as one of the important factors for the slow progress in MAS. He identified the following areas which requires consideration during both QTL and Association mapping

1. Precise measurements, failing of which would result in false association
2. Limited missing data
3. Sample size, which should be as maximum as possible
4. Across locations, the study should be carried out with adequate number of replications and environment.

Dr.Vimal Kothiyal, Scientist F and Head, Wood Products Division, FRI, Dehradun gave a detailed lecture on “*Near Infra-Red Specroscopy (NIR) for high throughput analysis of wood samples*”. He detailed about the principle and characteristics of NIR spectrum and its application in analyzing wood samples. He explained in detail about the wavelength and calibration set used in developing different NIR models, which are useful in analyzing unknown

samples. Examples from eucalypts and Acacia were given to highlight the use of this technology for indirect analysis of cellulose and lignin. Dr. Gurudev Singh enquired about the level of confidence in identification of species using NIR.

Dr. Kothiyal replied that about 100 to 200 samples have to be taken as reference to get the threshold value for identification of unknown species. Dr. Gurumurthi enquired regarding the models used to analyze hybrid wood samples and Dr.



Kothiyal replied that the reference library for both species needs to be created to derive threshold value and analyze hybrid wood samples. It was further added by Dr. Modhumita, Scientist E, IFGTB that Dr. Kothiyal was a Co Principal Investigator in the project handling the objective of wood analysis using NIR. It was stated that samples of parent populations was sent for developing the model and in future, both parents and hybrid progenies of same age will be sent for estimation of cellulose and lignin content. A discussion on development of multisite model for reliable phenotypic data was held in detail and Dr. Gurumurthi expressed that precise data of wood samples using NIR would be a key factor in identification of reliable markers in the present project.

The final panel discussion was held with views present by the project partners representing TAF CORN, TNPL and MPM. Dr. Gurumurthi delivered a lecture on the present status and requirement of the paper and pulp industries in India. He explained that only 4 to 5 efficient clones are used by most of the paper industries and there was no back up breeding program and lack of financial support to develop alternate clones for use by the industries. He also discussed

on the increase in pulp yield/ ton of wood and expressed that for 1% increase, there is an estimated profit by ₹ 2crores. He urged the industries and institutes to take up breeding programs targeting other important traits to facilitate genetic improvement of the species.



Shri. Wilson Kennedy, TAF CORN enquired regarding the development of gall wasp resistant and alkaline resistant hybrid clones under this program and Director, IFGTB clarified that the present project does not target these traits and future programs will be initiated to take up other traits. However, he appraised the house on the other programs of the Institute focusing on developing gall tolerant germplasm. Shri. Manjunatha, MPM detailed on the use of *E. camaldulensis* clones for large scale planting by MPM. He expressed that the resources could be used for future breeding program. Similarly, Dr. Chinnaraj, TNPL detailed on their activities and offered the support of TNPL in proximate analysis of wood samples. Dr. Modhumita expressed that the wood samples would be analyzed using NIR spectroscopy since the project involved large number of samples (~650). However, it was explained that short listed hybrid clones with superior quality can be sent to TNPL for additional analysis like kappa number, pulp yield etc.

Subsequently, the component Principal Investigators detailed on the progress made in the project and also highlighted the anticipated outcome after completion of the program. Dr. Nagarajan, Scientist E and Head, Division of Plant Biotechnology, IFGTB detailed on the hybridization schedules under the program and explained that all targeted crosses were completed and the

hybrid progenies are being raised in the nursery. Dr.Gurumurthi enquired about the status of such programs in forestry species and Dr. Yasodha clarified that till now, in India, QTL mapping has been done only in agricultural crops and this is the first initiative



in forestry species and that we are one generation away from the wild *Eucalyptus* species. She also explained the research in the component on genotyping where in SSRs from other eucalypt species are being targeted for cross amplification. Dr. Modhumita explained the efforts in progress on phenotyping of samples and also mentioned that the samples are also analyzed for microfibril angle at CSIRO, Australia using SilviScan. The research targets in the component on Association mapping was also explained.

It was suggested by the Director, IFGTB that an annual appraisal of the project can be conducted with the project partners to strengthen the link with the industries. Dr. Bhat was requested to provide technical support to the group for achieving the defined objectives. Dr.Gurumurthi



concluded that the project is a good initiative and wished that outcome of the project have to be realized within the period by achieving all objectives identified.

Dr. Krishna Kumar summed up the outcome of the panel discussion and requested the partners to provide continuous support to the program. The workshop ended with the formal vote of thanks.

Workshop Recommendations

1. Well characterized contrasting phenotypes as parents are essential for successful QTL-Marker associations
2. Accurate and precise phenotyping for the selected traits should be carried to enable QTL mapping.
3. Saturated genetic map with codominant markers like SSRs should be developed for the localization of QTLs
4. Continuation of controlled hybrid generation program in eucalypts is essential for the release of clones with specific end uses.
5. Subpopulation structure should be considered for association analysis
6. Avoid same mapping population for identifying QTLs for multiple traits which can lead to spurious associations.