1-PAGE ABSTRACT
(Mr. K. Premananda)
“Map-based cloning and characterization of TARANI a global regulator of Arabidopsis development”

Forward genetic screen was performed in Arabidopsis thaliana to isolate novel genes involved in leaf development. The tarani (tni) mutant was selected for further study based on its unique cup-shaped lamina with +ve Gaussian curvature. We show that the larger size of tni leaves is due to rapid growth rate due to excess and prolonged cell division. We monitored the front of the receding cell division zone as a function of time and showed that the shape of the front is more concave compared to wild type, leading to positive curvature. Application of gibberellic acids (GA) synthesis inhibitor rescued the positive curvature of tni suggesting a role for GA in maintaining leaf flatness. Overexpression of cell cycle inhibitor KRP2 also flattened the leaf, confirming a role of cell division. The floral organs and seed are also larger in the tni mutant. Besides growth, tni trichomes are hyper-branched which usually happens when there is more endoreduplication. We found that the nuclei of tni trichomes are larger than wild type nuclei, suggesting increased DNA content. Genetic interaction studies showed that TNI works independent of other trichome branching genes such as with TRYPTICHON and FURCA1.

Map-based cloning showed that tni is positioned on left arm of the 3rd chromosome. Using molecular markers, we narrowed down to interval to a 65 kb region, which codes for 19 genes. Sequencing several of them revealed a G→A transition at the 3rd intron - 4th exon junction of At3g20630 gene. RT-PCR analysis showed the presence of an additional full-length transcript with extra un-spliced 3rd intron. Overexpression of this un-spliced variant in wild type plants produced phenotypes like hyperbranched trichomes and cup-shaped leaves; plus additional phenotypes like organ fusion and organ polarity defects. Complementation and allelic tests confirmed that TNI codes for AtUBP14, an ubiquitin protease.

The tni plants have longer stem and roots which grow at faster rate compared to wild type. Confocal microscopic analysis of mature embryos showed that both shoot (SAM) and root apical meristems (RAM) of tni plants are larger in size. In RAM, the numbers of quiescent center (QC) cells and stem cells have increased in tni plants. The tni inflorescence and flowers are bigger than wild type in size. Also the degree of axillary shoots has increased in the tni plants. Overexpression of the splice variant of TNI produced undifferentiated callus-like structures in the shoot apex and in hypocotyl. All these phenotypes show that TNI is involved in meristem proliferation.

The tni siliques produced many un-fertilized ovules and shrunken and malformed seeds suggesting gametic and/or embryo lethality. We observed that tni embryos were mis-patterned at various stages of development. Following the cell division pattern shows that cells arising from the ‘basal cell’ of the embryo take apical cell fate in tni embryos. The topmost cell of the suspensor, which is also the precursor cell of RAM, is not specified as hypophysial cell in several tni embryos.

In the forward genetic screen, we isolated another mutant called tooth (tth), which has deeper serrations at the leaf margin and narrower leaves compared to wild type. It has been mapped to the longer arm of the 2nd chromosome. Genetic interaction studies show that tth is not allelic to other serration mutants such as serrate and mir164a.