COTTON Innovate

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Molecular basis of leaf shape variation in cotton is decoded

Leaves are the biological factories in plants and shape of the leaf has a paramount importance in crop improvement. Leaf shape in cotton is known to have influence on plant and canopy architecture, lint yield, fibre quality, stress tolerance, and other production attributes. Though, modification of leaf canopy through manual or mechanical topping and pruning was suggested to improve the cotton yields but is not feasible on large scale cultivation. Genetic modification of leaf shape is a practically and economically viable approach to develop varieties with improved productivity. There are four major leaf shapes in tetraploid cotton viz., *normal*, *sub-okra*, *okra*, and *super-okra* which are easily distinguishable by simple visual observation. These leaf shapes are governed by a multi-allelic loci called *okra* (*L-D*$_1$) which has been mapped to short arm of chromosome 15-D$_1$. Another leaf shape *laciniate* in diploid cotton is similar *okra* type in morphology, maps to the orthologous diploid A-genome locus (*L-A*$_1$) on chromosome 2. Both loci, *L-D*$_1$ and *L-A*$_1$ in tetraploid and diploid cotton, respectively are known to act early in the developmental stage and once determined they remain constant through the crop life.

Major leaf shapes in cotton *(adapted from Andres et al., 2016)*

Using cytogenetic approach and biparental QTL mapping, the *L-D*$_1$ locus has been mapped to chromosome 15, 5.4cM from teleomere. Later, orthologous mapping of the *L-D*$_1$ locus to the sequenced D genome donor (*G. raimondii*) chromosome 2 has reduced the putative candidate gene interval to 337 kb carrying 34 putative genes. Through shuttle mapping using the orthologous *laciniate* (*L-A*$_2$) locus from *G. arboreum*, it was further reduced to 10 putative genes covering 112 kb. Andres and co-workers at North Carolina State University, USA have carried out a series of experiments to determine the molecular basis of leaf shape in cotton. They undertook fine mapping of *L-D*$_1$ locus using an association mapping panel and two sets of isogenic lines to further narrow down the region to 52 kb carrying four putative genes *viz., GhLMI1-D1a*, *GhLMI1-D1b*, *GhRLK1* and *GhHRA1*. Both *GhLMI1-D1a* and *GhLMI1-D1b* code for HD-Zip transcription factors, *GhRLK1* codes for a serine/threonine protein kinase and *GhHRA1* codes for a trihelix transcription factor. Through, expression analysis (Quantitative RT-PCR and RNA sequencing), they found equivalent expression of *GhLMI1-D1a* across leaf shapes and differential expression (up-regulation in okra and super-okra compared to normal and sub-okra) of *GhLMI1-D1b*. Though, sequencing of *GhLMI1-D1a* and *GhLMI1-D1b* revealed DNA polymorphisms within the loci, comprehensive interpretations based major nucleotide polymorphisms, gene expression differences across leaf shapes and association of markers with the phenotype have strongly indicated that *GhLMI1-D1b* is responsible for the leaf shape variation in cotton. The same was later confirmed by functional validation wherein specific knocking down of *GhLMI1-D1b* transcript through Virus-Induced Gene Silencing has produced normal leaf in an okra variety. Using a diverse array of genomic and molecular tools, research team lead by Dr. Vasu Kuraparthy have established that the leaf shape in cotton controlled by *L-D*$_1$ loci is governed by *GhLMI1-D1b* encoding an *HD-Zip transcription factor* homologous to the *LATE MERISTEM IDENTITY1 (LMI1)* gene of Arabidopsis.

This new discovery provides needed insights to the cotton researchers and open new avenues for developing cotton cultivars with ideal leaf shape for sustainable and profitable cotton production.

Suggested readings:

- Andres RJ, Bowman DT, Jones DC, Kuraparthy V (2016) Major leaf shapes of cotton: Genetics and agronomic effects in crop production. *J. Cotton Sci.*, **20(4):** 330–340.
- Andres RJ, Coneva V, Frank MH, Tuttle JR, Han SW, Samayoa LF, Kaur B, Zhu L, Fang H, Bowman DT, Rojas-Pierce M, Haigler CH, Jones DC, Holland JB, Chitwood DH, Kuraparthy V (2016) Modifications to a LMI1-like gene are responsible for the major leaf shapes of Upland cotton (*Gossypium hirsutum* L.) *Proc. Natl. Acad. Sci.*, **114(1):** E57–E66.

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