

Searching TILL high yielding jute is unearthed

*Jute fibres are sustainable, biodegradable alternatives to non-degradable plastic fibres. The cultivation of jute is hindered by the presence of only two cultivated varieties in *Corchorus* sp and their incompatibility for cross-breeding. Increasing the genetic diversity of jute is a priority, to increase the tolerance of this crop towards environmental stresses, as well as improving the quality of the fibres. With the sequencing of the jute genome, technological advances have made it possible to identify mutants with altered traits. The Basic and Applied Research on Jute (BARJ) Project at the Bangladesh Jute Research Institute under the leadership of Professor Maqsudul Alam use TILLING to screen a mutant population of jute to identify high-yielding varieties.*

Jute is cultivated in Bangladesh predominantly for the use of its fibres in manufacturing industries. Despite there being 100 varieties of jute, there are only two cultivated varieties: *Corchorus olitorius* and *Corchorus capsularis*. These two varieties cannot be inter-bred, nor cross-bred with wild

varieties due to incompatibility. This poses a problem for jute researchers who look to improve the yield and quality of fibres, as well as tolerance of plants to different stresses. The recent sequencing of the jute genome has opened up opportunities for researchers to find novel ways of improving the

quality of jute. A team of researchers working on the Basic and Applied Research on Jute (BARJ) Project, at the Bangladesh Jute Research Institute are using TILLING, a high throughput approach, to identify high-yielding jute varieties.

VARIETY IS THE ESSENCE OF LIFE

Every living organism is unique, because of its genetic composition. This means that the genes encoded in the DNA are unique for that particular organism. Every feature of an organism, such as its petal colour, or height, is controlled by distinct genes that contain sets of instructions to make proteins, which are chemicals made by cells. Without proteins, cells would not have a structure; proteins also control chemical reactions that result in myriad phenotypes (physical traits and characteristics) in the organism.



Corchorus olitorius.



Corchorus capsularis.

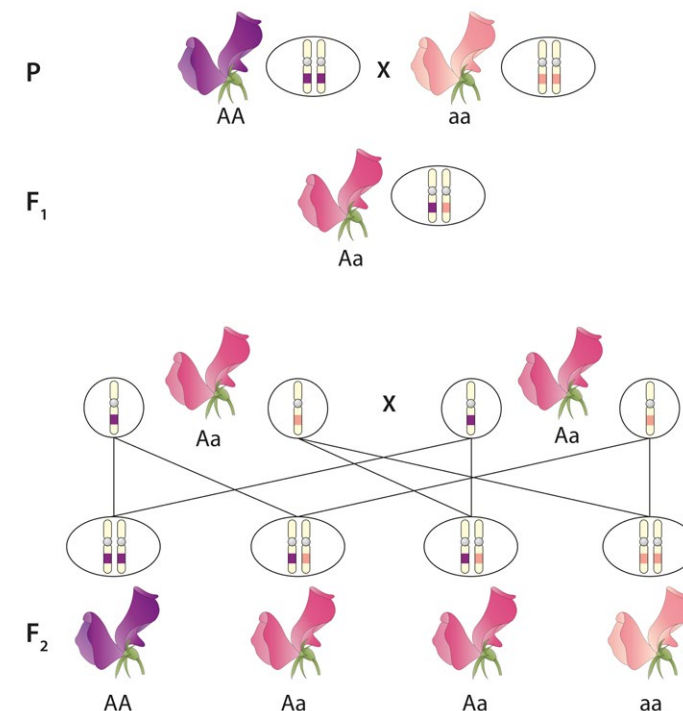
Phenotypes range from colour of tissues, height, biomass, as well as tolerance to different stresses. Varieties in every living species exist because of mixing of genes during breeding, and changes in the chemical structure of DNA when cells divide. This results in offspring having phenotypes that are a mixture of its parents' phenotypes, with some changes, be it a bird, a human or a tomato plant. In a population, therefore, there are a variety of different phenotypes for a particular organism. Through evolution, every genetic feature in the population of an organism is a result of changes that occur in its DNA. This ultimately results in genetic variations in a population of the same species – something that might be obvious in humans, but is also subtly present in plant populations.

PLANT BREEDING THROUGH TIME

When humans were hunter-gatherers almost two million years ago, food was obtained by gathering and hunting wild plants and animals. Over time, as populations increased and food was limited, humans discovered the art of cultivating plants for their food. This resulted in domestication of wild plants, and selecting them for suitable traits, so they could be more edible. For example, the bread wheat we know today is a result of plant breeding techniques over tens of thousands of years.

Plant breeding involves the selection of desirable traits in a plant by crossing (cross-pollinating) one variety of plant with another, resulting in a combination of the best traits. For example, crossing a plant yielding fleshier, bigger tomatoes with a plant tolerant to drought would result in bigger tomatoes in a drought-tolerant plant. By crossing, researchers have effectively mixed genes, resulting in changes in the DNA that yield plants with desirable traits, and removing less desirable traits.

While development of new plant varieties through traditional



TILLING is used to screen a population of jute to identify high-yielding varieties.

breeding has revolutionised agriculture, this does not occur at a rate fast enough for plants to cope with changes in the climate and the demand for food by a growing human population. Since the early 20th century, the discovery that chemicals or ionizing radiation can cause mutations in DNA has been an important tool for plant breeders.

MUTATION BREEDING

A mutation is a change in the DNA sequence (arrangement of four

an organism, through agents called mutagens. This discovery has been exploited by plant breeders for more than 80 years.

Mutagens are either physical agents such as ionizing radiation (like x-rays), or chemical, which are usually alkylating agents such as EMS (ethyl methanesulfonate) or sodium azide. Generally, physical mutagens produce large DNA deletions, while chemical mutagens result in point mutations, causing

TILLING (targeted induced local lesions in genomes) is a high throughput screening technology to identify mutant plants in a randomly mutagenized population.

important chemicals called bases) in an organism. This change can occur spontaneously, while cells divide, leading to slow variations through evolution of a species. However, the process can also be enhanced by inducing mutations in

single base changes in a DNA sequence. Application of these mutagens to plants results in the mutation of plant DNA. Through self-pollination the mutation follows through to subsequent generations. The mutagenized plants can show

distinct phenotypes, such as altered height, yield, or colour, and therefore a population can be screened to detect a particular phenotype of interest. These mutations are very rarely lethal, and the mutagens used are environmentally safe, posing no danger.

Mutation breeding has been used to generate new cultivated varieties of several crops. The newly generated plants can have a range of improved traits, such as higher yield, increased biomass, high protein content, tolerance to drought, and changes in flowering times. With advances in genomic technologies, researchers have combined mutation breeding with screening techniques to identify targeted mutations in select genes, yielding a phenotype.

TILLING: A POWERFUL GENOMIC RESOURCE

TILLING (targeted induced local lesions in genomes) is a high throughput screening technology to identify mutant plants in a randomly mutagenized population. Conventional TILLING is based on finding a mutation in a known gene. The mutagen generally used is EMS, to generate a parent population of

mutagenized plants. Subsequent generations are obtained to create a stable population of mutants, which is then screened using DNA technologies to find a mutation in the gene of interest. The plant corresponding to the obtained mutated gene is then examined for phenotypes.

TILLING can also be used in a different way, if pronounced phenotypes are observable. The population of mutagenized plants are screened

Researchers at the Bangladesh Jute Research Institute are using TILLING, a high throughput approach, to identify high-yielding jute varieties.

for specific phenotypes. Once these phenotypes carry through to subsequent generations, the gene responsible for the phenotype can be identified by locating the position of the gene in the genome, and subsequently, its identity. As TILLING poses no environmental risk and is not considered a transgenic technology, it has been a popular route to generate new plant varieties in many countries.

TILLING FOR JUTE

With the recent sequencing of the jute genome, the Basic and

Applied Research on Jute Project researchers have developed a TILLING population in jute through a carefully chosen method using EMS as the mutagen. A mutagenized population of jute was used to identify and group phenotypes into five different categories, of which increased plant height with altered stipulate colour was followed through to subsequent generations. A stable line was subsequently chosen (Robi-1) and was found to have higher yield, increased fibre quality and biomass compared with another high-yielding variety. Robi-1 shows increased disease resistance and

has a shorter lifecycle, thereby freeing the limited land for rice cultivation. Further analysis of the expression of genes in Robi-1 compared with its parental line showed that the expression of genes related to fibre biogenesis was altered in Robi-1. This correlates with increased lustre and density of fibres seen in Robi-1. Future work will identify the mutation of the gene that controls these phenotypes. This line has been released for commercial cultivation under the name BJRI Tossa Pat 8 (Robi-1).

Research Objectives

Decoding the jute genome data has opened up new possibilities for the basic studies of gene function and in the improvement of jute crops. Here we developed a TILLING (Targeting Induced Local Lesions in Genomes) population to create mutant alleles for functional genomes as well as development of desired plant traits.

References

Chen, L., Hao, L., Parry, M.A.J., Phillips, A. L., Hu, Y.-G. (2014) Progress in TILLING as a tool for functional genomics and improvement of crops. *Journal of Integrative Plant Biology*, 56, 425-443.

Kurowska, M., Daszkowska-Golec, A., Gruzka, D., Marzec, M., Szurman, M., Szarejko, M., Maluszynski, M. (2011) TILLING – a shortcut in functional genomics. *Journal of Applied Genetics*, 52, 371-390.

Personal Response

Once the mutation in Robi-1 has been identified, what will be the next step?

// We have used a phenotype screening method to identify a high yielding genotype i.e Robi-1, from the mutagenized population. A lot of mutations occur in the genome. Now we are working on that (phenotype to genotype). Identified or targeted mutated gene(s) will be validated by wet lab methods and also their function confirmed by gene knock-out methods. Finally targeted gene/s will be integrated into the targeted breeding. //

Has the TILLING population been used to identify mutations of specific target genes?

// We detected mutations in genes related to the fibre biosynthesis pathway as well as biotic and abiotic resistance in jute. Here we used a high-throughput sequencing approach combined by implementing either read percentage with non-reference nucleotide for single copy genes or minimum variance multiplier to set mutation prediction parameters for multiple copy genes. //



Left: O-9897; Middle: BJRI Tossa Pat 8(Robi-1); Right: JRO-524