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APPLICATION OF GENOMICS AND PROTEOMICS IN JUTE AND ALLIED FIBER (JAF) IMPROVEMENT

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Abstract

About 95 % of Jute and Allied Fibers (JAFs) are produced by small scale farmers in Indo-China, south and south-east Asia. Majority of the high yielding varieties of JAF crops such as jute, kenaf and mesta have been developed so far using traditional breeding methods. However, serious limitations of the traditional approaches in developing new cultivars have urged the necessity of adapting to molecular techniques for the improvements of JAFs. This review aims in investigating the current status and future prospects of Genomic and Proteomic approaches in the improvement of JAF crops.

Keywords: *fibers, genomics, improvement, JAF, jute, proteomics*

Introduction

Traditionally, jute and its allied fibres (JAF) kenaf or mesta are used as packaging materials like twine, hessian, gunnysacks, wool pack, wallpaper, clothing, hats, rope carpet backing, upholstery and furnishing fabrics. However, this aspect has been receiving crippling beatings in economic terms in view of growing use of synthetics fibres (Swaminathan *et al*., 1961). Nonetheless JAF crops are alive and well and enjoying a resurgence because of their "naturalness" (Srvatanakul *et al.,* 2001). They are produced by small scale farmers in Bangladesh, India, China, Thailand, Nepal, Indonesia, Vietnam, Cambodia and Brazil. About 95 % of the total world jute production and 90 % of export of jute products are from five producing countries namely India, Bangladesh, China, Nepal and Thailand (Khatun *et al*., 1993). Recently jute diversified products appear to be promising to make jute once again a potentially viable constituent in the fast emerging global market economy. Thus, with the demand of diversification of jute products, the need of finer quality and yield stability from jute and allied fibre crop is gaining ground. United Nation declared 2009 as an International Year of Natural Fibre (IYNF). The IYNF will help to develop the efficiency and sustainability of JAF research and development industries that employ millions of people and provide economic development opportunities.

Biotechnology for fibre improvement

Two types of Jute are presently cultivated White jute (*Corchorus capsularis* L.) and Tossa jute (*Corchorus olitorius* L.), whereas allied fibre crops are Kenaf (Patsan: *Hibiscus cannabinus* L.); Roselle (Patwa: *Hibiscus sabdariffa* L.); Hemp (Sunnhemp: *Crotalaris juncea* L.); Ramie (Rheha: *Boehmeria nivea* L.); Agave (Sisal: *Agave sislana* Perr.) and Flax (Alsi: *Linum usitatissimum* L.). Jute fibre is extracted from the bark of the jute plant, which is popularly known as bast fibre crop in contrast to seed fibre from the cotton, leaf fibre from kenaf, vegetable fibre from roselle plant and stem fibre from flax, hemp and ramie plants. These fibres are basically eco-friendly, biodegradable, renewable, high frictional resistance and flexibility over synthetic fibre.

Genomics and Proteomics are currently the two key components of biotechnological research. First and foremost, the genome sequence provides a bird's eye view of the information needed for understanding the biology of model organisms (Tinker, 2002; Johnson, 2004); whereas, Proteomics in general deals with large scale determination of gene and cellular funtion directly at the protein level (Aebersold and Mann 2003). In recent years, researchers are getting involved in improving agronomic traits of jute and other fibre yielding crops through molecular approaches, which has however slowed down considerably since the whole genome sequence of jute is not yet available. Moreover, most of the small numbers of jute sequences deposited in Gene Bank are under characterized. Biotechnological approaches are therefore essential for developing varieties with the insertion of specific genes beside the traditional available approaches.

Basic concepts of genomics

Genomics or the study of an organism's entire genome is probably the most significant scientific achievement in the last decade of the 20th century and the beginning of the 21st century. Genomics combines three areas that focus on the science and technology of the organization of the genome (Johnson, 2004; Tinker, 2002). The first of these is *genetics*, the study of the particulate nature of inheritance. The second component of genomics is *automated laboratory tools* for high throughput DNA-, RNA- and protein analyses. The third component of genomics is *bioinformatics*, the application of information sciences to sequence-level genetic information and molecular genetics. It resides at the intersection of biology, mathematics, statistics, and computer sciences and uses information sciences to identify and align sequences, to predict putative gene function and structure, and predict how genes or their products interact to create genetic networks. Bioinformatics provides the tools that enable the molecular description of the genetic basis of phenotypes, and facilitates predicting phenotypes from gene sequences and associated information.

Currently available techniques in genomics research

Marker-assisted selection

Genomics offer a wide array of techniques for crop improvement. Marker-assisted selection (MAS) involves the transfer of a piece of DNA associated with a specific phenotype, using molecular markers to identify the DNA fragment and define its location

on the chromosome. Once the association between gene and phenotype has been established, then the tagged region can be transferred to successive generations without having to depend on generating the phenotype again. The most common molecular markers are restriction fragment length polymorphisms (RFLPs), simple sequence repeats (SSRs), amplified fragment length polymorphisms (AFLPs) and single nucleotide polymorphisms (SNPs).

Quantitative trait loci

A quantitative trait locus (QTL) is a region of a chromosome present in a population of segregating progenies generated from a biparental cross. It is associated with a specific phenotype, and its location is usually identified using flanking molecular markers. Often several QTLs are associated with the genetic control of a quantitative trait. Many QTLs have been identified in the literature (Yu, 2002), but very few have been used in applied crop improvement.

Gene expression analysis

Genomic technologies include powerful tools for the simultaneous study of expression of thousands of genes. Examining how expression varies with growth environment, tissues sampled and time of sampling provides insights on gene function and regulation. These can then be explored further through analysis of gene products, targeted environments, or other approaches (Zinselmeier *et al*., 2002).

Genetic transformation

Development of recombinant DNA technology in the 1970s has allowed genes unavailable through traditional breeding to be transferred across species. Genes may come from other plant species or other sources. Transformation requires identifying and isolating the desired gene, transferring the gene into the genome of the target plant, and then regeneration of a whole, fertile plant from the transformed tissue. The most widely used approach for transformation involves introducing the desired gene into the bacterium *Agrobacterium tumefaciens* (or, to a lesser extent *A. rhizogenes*).

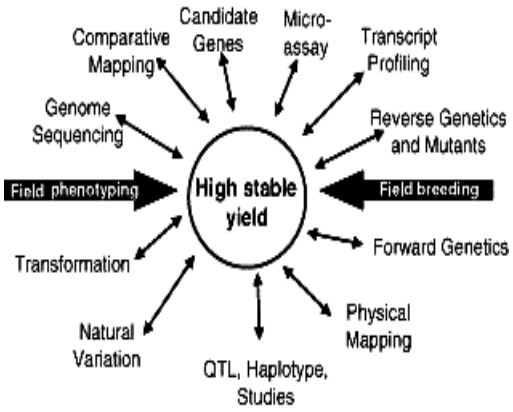

Fig. 1 Tools used in genomics (Edmeades *et al.,* 2005)

Forward and reverse genetics approaches

The standard forward genetics approach encompasses conventional plant breeding, and involves identifying the phenotype of interest and then searching for the genes that determine that phenotype. The reverse genetics approach relies on modifying the gene and then searching for the associated phenotype (Fig. 1). It includes the use of candidate genes and gene knockouts in populations that have been mutagenized by insertion elements (transposons), by the T-DNA of *A. tumefaciens* itself, or by exposure to chemicals such as EMS (Henikoff and Comai, 2003).

Expressed sequence tags (ESTs)

Expressed sequence tags *(*ESTs) are sequences obtained from either end of cDNA clones synthesized from anonymous messenger RNA molecules, are on average 250–500 bp in length, and are sequence fragments that permit the analysis of species-specific, temporal and spatial expression of genes. After clustering and comparison with previously known sequences, ESTs can be used to reconstruct the original messenger RNA, and to catalogue genes without the need for complete genome sequencing (Campos, 2003).

Genomics for fibre improvement

Significant improvements in the design and efficiency of molecular breeding will flow from the improved understanding of crop genetic resources that genomics provides. This includes co-linearity of genes, an understanding of chromosomal recombination, and an appreciation of how gene networks function. An array of genetic and physiological tools that focus on the discovery and validation of key processes, candidate regions and genes are now available. Exploitation of natural variation through conventional plant breeding methods and multi-environment testing remains the centerpiece of most major JAF breeding programs.

To improve the fibre quality currently used jute, flax and hemp varieties more knowledge about the molecular processes that underlie cell wall metabolism is needed. The characteristics of plant fibres are determined by the composition of the cell wall. The complexity of the biosynthesis and modification of the cell wall molecules is probably best illustrated by the number of genes involved. Carpita *et al.* (2001) calculated that 15 % (3800) of the *Arabidopsis* genes are directly involved in cell wall metabolism. DNA microarrays or DNA chips are quantitative tools for simultaneous analysis of gene expression profiles that can improve our understanding of the fundamentals of plant development.

Basic concept of proteomics

Proteomics is the large-scale study of proteins, particularly their structures and functions (Anderson and Anderson, 1998). The term proteomics was coined to make an analogy with genomics, the study of the genes. The word proteome is a portmanteau of protein and genome. The proteome of an organism is the set of proteins produced by it during

its life, and its genome is its set of genes (Aebersold and Mann, 2003).There is little doubt that proteomics will have great impact in all areas of the life sciences in the years to come. The task of studying the proteome has its share of challenges. On one hand it involves the sheer number of proteins that need to be identified on the other hand are the amino acids, the basic units of proteins are so small. One approach is by separating the proteins, breaking them into smaller pieces (small peptide units), and using mass spectrometers (MS) to, in effect, "weighs" each amino acid. Each type of amino acid has a unique mass, making identification relatively simple. By identifying and sequencing these smaller pieces, researchers can then easily detect the identity of the putative proteins they constitute (Blackstock and Weir, 1999).

MS based proteomics has its success buried in the availability of gene and genome sequence databases and technical and conceptual advances in several other related fields, most important being the development of the protein ionization methods (Aebersold and Mann, 2003). A list of commonly available recent techniques in proteomics has been included in Table 1. Electrospary Ionization (EI) and Matrix-Assisted Laser Desorption/Ionization (MALDI) are the two most common techniques used to volatize and ionize proteins or peptides for MS analysis. The mass analyzer is the central element of proteomics research.

Proteomics for fibre improvement

Most recent works on proteomics of fibers are currently restricted to model plants like *Arabidopsis*. Moreover the major focus and concentration of the resaerch is also restricted to the cell wall proteins (CWPs). These proteins are essential components of plant cell wall and are actively involverd in the modifications of the cell wall components, stucture, signalling and interactions with plasma membrane proteins on the surface of the cell. Minic *et al.* (2007) reported the contribution of proteomics in the identification and determination of the structural and functional aspects of CWPs. Such methods could prove to be extrermely valuable in similar CWPs proteins of fiber yielding plants like JAF. Recent research on proteomics of cell wall proteins (Jamet *et al*., 2006) highlight on the distinct identification of CWP family members in specific plant oragns, identification of new CWPs including those with no reported functions, characterization of CWPs through post-translational modifications and on proteins in general detected to be present on the cell wall. Many of these proteins may have signalling role or role in detecting pathogens, hence modifications of these proteins in the long run could result in development of new cultivars of fiber yielding plants resistant to diseases and with desirable quality of the fibers.

JAF improvement: Current biotechnological achievements

JAF crops are seen as "poor farmers" crops since they never enjoyed any advanced technological inputs unlike major food crops. A programme of genetic improvement of JAF using biotechnology was first initiated in 1996 by International Jute Organization (IJO). The major research priorities of IJO were the development of a transformation system and efficient tissue culture techniques, identification of appropriate genes for transformation, construction of a molecular map and development of fingerprinting techniques for JAF accessions. A number of researchers reported hybridisation between the two species *C. capsularis* and *C. olitorius* to be successful (Sinha *et al*., 2004).

DNA fingerprinting of jute genotypes and some promising lines from both *C. olitorius* and *C. capsularis* have been developed using RAPD, AFLP and SSR techniques (Basu *et al.* 2004; Hossain, 2002). RAPD markers were also used to examine phylogeny amongst kenaf species (Srivatanakul *et al.,* 2001) and flax; crosses have been made between 20 parents of different species of *Hibiscus*, and embryo rescue has yielded several plantlets from inoculated ovules.

Table1. Summary table of currently and commonly available techniques in proteomics research

Name of techniques	Common application(s)
1- + 2-D Gel Electrophoresis	Identify relative mass of a protein and its isoelectric points
X-ray Crystallography	Characterization of 3-D structure of peptides & proteins
Nuclear Magnetic Resonance (NMR)	Characterization of 3-D structure of peptides & proteins
Circular Dichroism (CD)	Elucidating secondary protein structures
Fourier Transform Infrared Spectroscopy (FTIS)	Elucidating secondary protein structures
Small Angle X-ray Scattering (SAXS)	Elucidating secondary protein structures
Tandem mass spectrometry (TMS) + Reverse Phase Chromatography (RPC)/ 2-DE	Protein identifications using database search tools
TMS + tagging technologies (TMT/ ICPL/iTRAQ)	Quantification of proteins and peptides
Mass spectrometry (no-tandem)/ MALDI-TOF, MALDI-MS	Protein identification by Peptide Mass Fingerprinting (PMF) Also used in MALDI-TOF MS protein profiling.
ESI-MS systems (LC-MS)	Analysis of relatively simple peptide mixtures Analysis of complex protein samples
ICP-MS + MeCAT - Metal Coded Tagging Technology	Ultra-sensitive quantification of proteins & peptides
Affinity Chromatography	Identification of protein-protein & protein-DNA binding reactions
Yeast Two Hybrid Techniques	Identification of protein-protein & protein-DNA binding reactions
Fluorescence Resonance Energy Transfer (FRET)	Identification of protein-protein & protein-DNA binding reactions
Surface Plasmon Resonance (SPR)	Identification of protein-protein & protein-DNA binding reactions
X-ray Tomography	Determination of labelled protein location
Software based image analysis	Automated quantification and detection of spots within and among gel samples

Recently, two varieties of kenaf resistant to anthracnose disease 'Everglades 41' and 'Everglades 71' have been developed by ARS researchers in Florida U.S. The recent developments in plant genomics and the availability of microarray technology are very much useful for better understanding of the molecular processes associated with the development of the bast fibre cells of flax stems (Ebskamp, 2002). Lipid transfer proteins (LTPs) and arabinogalactan proteins (AGP) transcripts were found well-correlated with stem segments during cell wall thickening phase of phloem fibre development; and chitinases, β-galactosidases, AGP, and LTPs were among the interesting transcripts enriched in specific stages of the developing stem in flax (Roach and Deyholos, 2007). These results indicate that similarity between the molecular mechanisms underlying phloem fibre development in flax and the gelatinous fibres of tension wood in trees, will lead to new targets and opportunities for breeding and could possibly become the final turning point for JAF.

JAF improvement: Future directions

Fibre qualities are increasingly important due to their global impact on textile manufacture, processing and end product value. Improvements of fibre quality and uniformity are likely to involve changes in fibre initiation, low lignin contain, elongation, diameter, maturation, strength and plant architecture. These changes will be made with knowledge gained from genomics and proteomics based trait genetic dissection, QTL definition, and functional genomic expression during the critical periods for fibre development.

These approaches are leading to an improved physiological understanding of fibre uniformity traits, their relationships to each other and to agricultural production. Another, growing concern about genetic vulnerability of the JAF gene pool to a wide range of biotic and abiotic hazards is exemplified by recent investigation of trends in yield improvement. These improvements can enhance the economics of production and fibre processing characteristics will ensure competitiveness in the market of this natural-renewable product with petroleum-derived synthetic fibres, and the livelihoods of millions of people worldwide.

Furthermore, with the menace and problems associated with synthetic plastics many countries are reverting back to the old biodegradable packaging products from JAF crops as an environmentally sustainable practice (Datta and Basu 2002; Datta *et al*., 2007). These would have tremendous prospect with respect to the growth of the cultivable areas for these crops in the developing and under developed countries in the near future. These would mean that there would be a great demand for new JAF cultivars that are fast growing, disease resistant, capable of surviving in the low input agri-environment of developing and under developed agriculture dependent countries and have substantial yield for the poor farmers.

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