

## India making strides in crop genomics

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Rice is the first food crop whose genome has been sequenced. Indian scientists were part of the international team, which sequenced the rice genome.

In the August 2005 issue of the journal *Nature*, members of a 10-nation International Rice Genome Sequencing Project (IRGSP) reported a highly accurate and "finished" map-based DNA sequence of the entire rice genome. This marks one step forward in rice improvement and breeding high yielding varieties. This international project led by Japan was launched seven years ago.

India, which has the maximum area in the world under rice cultivation (28 percent of its arable land) and produces about 130 million tons of rough rice every year, was a participant in the rice genome project. The Indian Initiative for Rice Genome Sequencing (IIRGS) was launched in June 2000 and was carried out at the University of Delhi, South Campus (UDSC), and the Indian Agricultural Research Institute (IARI) New Delhi with the support of the Department of Biotechnology and the Indian Council of Agricultural Research. The 28-member Indian initiative was jointly led by Dr Akhilesh K Tyagi of UDSC and Dr NK Singh of IARI and contributed to about 10 per cent of the global sequencing effort.

Different chromosomes/region of chromosomes were assigned for sequencing to various countries and IIRGS was assigned to deliver about 14 Mb of nucleotide sequence, spanning a region from 57.3 cM to 116.2 cM on the long arm of chromosome 11 of rice. The mandate was not only performing high throughput sequencing but also annotation of data and gene discovery.

To achieve this objective, at an estimated cost of Rs 48.83 crore (~ \$10 million), two independent research facilities were established at the Department of Plant Molecular Biology in UDSC and National Research Centre on Plant Biotechnology in IARI. These labs were equipped with performing high throughput sequencing as well as detailed annotation of the generated data.

By generating more than half a million individual sequences, Indian scientists assembled 16.5 million bases of the chromosome 11 coding for 2000 genes during the last five years thereby complementing efforts of more than 250 researchers from 10 participating nations. Besides Indian researchers, The \$-200 million project also included researchers from Japan, USA, France, Taiwan, China, India, South Korea, Thailand, Brazil and the UK.

Overall, the international consortium sequenced the 370 million base pairs of rice identifying the locations of over 35,000 genes that make the rice plant. Of the two most common rice varieties of the world, the team chose the 'Japonica' variety and now the Indian team plans to pitch in and complement the sequencing of the 'Indica' variety. The Indian government has already started a Rs 36-crore functional genomics project, which will help to identify/mine the useful genes. "India has a diverse germplasm and different ecological zones. This project has created a critical pool of trained scientists, infrastructure and capability to conduct genome wide research on a range of agronomically important crops", said Dr NK Singh, principal scientist and investigator, National Research Centre on Plant Biotechnology, IARI.

Previously published draft sequences of rice are shown to lack the coverage and accuracy required to identify the important genes. The emerging information from IRGSP has already led to the identification of agronomically important traits, e.g. genes for "green revolution", disease resistance, genes influencing growth habit to promote yield and photoperiod sensitivity genes to extend the range of cultivations of elite cultivars of rice. This could help feed an expanding world population at a time when restraints on agriculture are increasing. In the Indian context, the completed rice genome sequence and the genes now identified will help extend the studies on related cereals like wheat, maize, sorghum, bajra and ragi.

#### Tomato Genome Sequencing Initiative

India is also a part of the "International Solanaceae Genome Project (SOL)". Researchers from more than 10 different countries kick-started this 10-year initiative in late 2003 in Washington DC. The long-term goal of the SOL program is to create a network of map based resources and information to address key questions in plant adaptation and diversification.

Tomato is considered the model crop to study fruit ripening. Considering the importance of tomato it has been chosen by SOL as a model member of Solanaceae for sequencing of its genome and India has been allocated chromosome 5 for this project. The high degree of conservation in genome organization among Solanaceae members, would allow the information generated with tomato to be extended to other Solanaceae members in future.

Since scientists are sequencing only the euchromatin part of tomato genome, it is estimated that chromosome 5 consists of approximately 11 Mb of euchromatin. However, researchers estimate that the actual sequencing effort may require 10 percent additional sequencing and 20 percent overlap between neighboring BAC clones. The sequencing of the chromosome 5 is being carried out at University of Delhi South Campus (5 Mb), National Research Centre on Plant Biotechnology, IARI (5 Mb) and National Centre for Plant Genome Research, JNU Campus (2 Mb). All these labs are producing a high quality sequence of the gene rich euchromatin region assigned to them; then the sequences will be processed and annotated followed by the identification of agronomically useful genes from tomato genome.

The members of the Indian Initiative on Tomato Genome Sequencing have already confirmed 16 BAC clones from tomato chromosome 5 with the help of markers by PCR amplification, sequencing with marker-specific custom primers, end sequencing and fingerprinting. The shotgun libraries have been developed and high throughput sequencing has also started.

India's foray in the functional genomics domain (in crops like rice, tomato and sugarcane) would help identify genes for improvement in relation to water use efficiency, disease resistance and yield. In tomato, it also includes increase in shelf life and better nutrition. The demonstration of the potential by India during the last five years will also pave the way for improved food availability and diversification of the agro industry.

Rolly Dureha

#### Plants have a double line of defense

Plants are exposed to many different pathogens in the environment. Only a few of these pathogens, however, are able to attack a species of plant and "make it sick". If a particular pathogen is unable to attack a plant, that means that the plant is resistant to it - in other words, it cannot host the pathogen. This durable type of immunity of a plant to parasites is called nonhost resistance.

Although, in nature, nonhost resistance stops almost all parasitic attacks, it has been the subject of little research. Now scientists from the Max Planck Institute for Plant Breeding Research in Cologne have uncovered the molecular components of nonhost resistance and described this system of defense in a recent edition of the journal Science. In their findings, they draw parallels between the immune systems of plants and animals. This research could be central to the development of new "green" fungicides.

The Max Planck researchers were able to identify the gene known as PEN (penetration) as an important component of nonhost resistance. They isolated arabidopsis mutations, which are partially susceptible to powdery mildews. If these genes are defective, or if the protein they code is missing in the plant cells, the fungus can invade the leaf epidermis cells more frequently. For that reason the scientists looked particularly at the question of exactly which function the PEN2 protein has in the defense against pathogens.

The researchers also observed that when PEN2 is missing, the plants become more susceptible not only to grass powdery mildew fungi but also other pests - for example, the pathogens causing late potato blight. PEN2 is therefore a basic component of the plant's immune system with a broad range of effects.

However, if PEN2 is missing, the plant is not completely helpless against fungal diseases. There is still another line of defense, which they have to get through. If PEN2 is missing, the plant takes a drastic step: the cell dies together with its attacker, which protects the neighboring plant tissue from infection.

In this deadly line of defense, very different proteins play a key role, which identify molecular traits only present in parasites by using immune receptors both on the cell surface and inside the cell. Only if this second mechanism also fails can the originally non-virulent grass powdery mildew fungus colonize the plant.

The Max Planck research has demonstrated that the nonhost resistance of plants develops out of a defense system with at least two steps. These steps determine whether a plant is susceptible to a disease or not. The redundancy of the defense layers and the wide-ranging effects of PEN2 explain why, in nature, nonhost resistance is a durable and broadly effective defense mechanism. If a building block is missing from one defense layer, its function will be taken over by components of the next layer.

In further studies, the researchers hope to try to identify materials that are built up via PEN2 at the place of infection. They surmise that these materials could lead to the development of new kinds of "green fungicide" with a broad range of effects in the fight against plant diseases.

### **Loss of fear factor makes a timid mouse bold**

Researchers have identified a fear factor - a protein the brain uses to generate one of the most powerful emotions in humans and animals. The molecule is essential for triggering both the innate fears that animals are born with, as well as, fears that arise later in life due to individual experiences. Eliminating the gene that encodes this factor makes a fearful mouse courageous. The finding, the researchers say, suggests new approaches for drugs designed to treat conditions such as phobias, post-traumatic stress disorder and anxiety.

Working on mice, the scientists led by Howard Hughes Medical Institute investigator Eric R Kandel at Columbia University, found that the protein stathmin is critical for both innate and learned fear. Mice without stathmin boldly explore environments where normal mice would be hesitant, and unlike their normal counterparts, fail to develop a fear of cues that have been associated with electric shock. The scientists also found physiological changes in the brains of mice lacking stathmin that correlate to the behavioral changes they observed.

In their previous work, Kandel and his colleagues had determined the underlying mechanisms that encode fear in the brain. An indication that stathmin might contribute to fear came when they mapped the parts of the brain where the gene was most active. They found that stathmin was highly expressed not only in the amygdala, a region deep within the brain known to contribute to fear and other emotions, but also in other parts of the brain's fear circuitry.

To investigate stathmin's role in more detail, the researchers created mice lacking that gene and examined the brain activity in the lateral nucleus of their amygdalas. Recent work from other labs had shown that during fear learning, the connections

between the neurons in this part of the brain strengthen. In stathmin-deficient mice, however, the connections between these neurons remained virtually unchanged, despite repeated stimulation.

From a series of such related experiments, Kandel and his colleagues concluded that stathmin is required for both innate and learned fear and as drug target presents a unique opportunity.

### **Soy protein reduces effects of diabetes on liver**

A group of researchers from Mexico has discovered that a diet rich in soy protein may alleviate fatty liver, a disease which often accompanies diabetes. The high levels of insulin and insulin-resistance that accompany diabetes are often associated with fatty liver or hepatic steatosis, an untreatable condition that can lead to chronic liver disease and death. In this condition, large lipid-filled compartments accumulate in the cells of the liver due to an increase in production of fatty acids in the liver. The end result is an enlarged liver.

Following up research that indicated that eating soy protein reduces lipid production and prevents hyperinsulinemia (the loss of effectiveness of insulin), Dr Nimbe Torres, of the Instituto Nacional de Ciencias Medicas y Nutricion in Mexico, investigated the effects of a diet high in soy protein on the development of fatty liver associated with diabetes.

Dr Torres fed Zucker diabetic fatty rats that develop hyperinsulinemia and hepatic steatosis a diet of soy protein for 160 days. She found that the consumption of soy protein prevented the accumulation of triglycerides and cholesterol in the liver despite the development of obesity and hyperinsulinemia in the rats.

The effects of soy protein were due to a low expression of genes involved in the synthesis of fatty acids and triglycerides in the liver and these changes were due to a reduction in the transcription factors that control the expression of genes involved in lipid production, explained Dr Torres.

In addition, levels of a transcription factor involved in controlling genes involved in fatty acid breakdown, as well as its target genes, were increased in rats fed soy protein. Thus, feeding rats a soy-rich diet reduced the amount of fatty acid in their liver by not only reducing lipid production but also by increasing its breakdown.

Although further research is needed, Dr Torres believes that consuming soy protein could very well reduce insulin resistance, renal damage, and fatty liver, improving quality of life.