VATIS UPDATE
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Highlights

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UNITED NATIONS ESCAP
Economic and Social Commission for Asia and the Pacific

APCTT
ASIAN AND PACIFIC CENTRE FOR TRANSFER OF TECHNOLOGY

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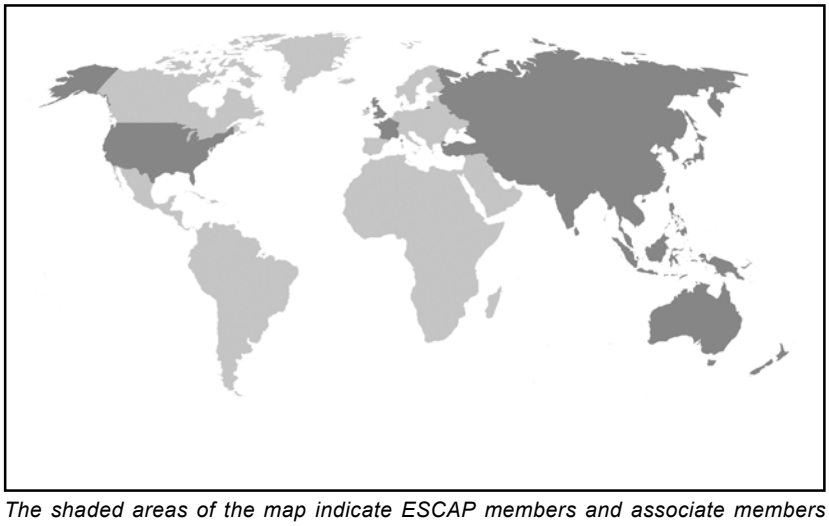
The **Asian and Pacific Centre for Transfer of Technology (APCTT)**, a subsidiary body of ESCAP, was established on 16 July 1977 with the objectives: to assist the members and associate members of ESCAP through strengthening their capabilities to develop and manage national innovation systems; develop, transfer, adapt and apply technology; improve the terms of transfer of technology; and identify and promote the development and transfer of technologies relevant to the region.

The Centre will achieve the above objectives by undertaking such functions as:

- Research and analysis of trends, conditions and opportunities;
- Advisory services;
- Dissemination of information and good practices;
- Networking and partnership with international organizations and key stakeholders; and
- Training of national personnel, particularly national scientists and policy analysts.



The shaded areas of the map indicate ESCAP members and associate members

Cover Photo

Soybean tissue cultures that have been inoculated with agrobacterium

(Credit: BASF, Germany)

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*** Value Added Technology Information Services**

IN THE NEWS

How biotechnology benefits farmers and the developing world

The biotech industry boosted farming across the globe to the tune of almost US$65 billion during the period 1996 to 2009, according to the latest analysis published in the *International Journal of Biotechnology*. The amount is the increase in net farm income, the farm level benefit after paying for the seed and its biotech traits. The study's authors – Mr. Graham Brookes and Mr. Peter Barfoot of PG Economics Ltd., the United Kingdom – estimate that almost half of that was derived by farmers in the developing world. They investigated the economic impact at the farm level of agricultural biotechnology, looking at yields, key costs of production, direct farm income, indirect (non-pecuniary) farm-level income effects and impacts on the production base of the four main crops of soybeans, corn, cotton and canola. It is estimated that biotech has added 83 million tonnes and 130 million tonnes to global production of soybeans and corn, respectively.

Net farm-level economic benefits amounted to almost US$11 billion in 2009 alone. The estimate of benefits amounting to US$65 billion is based on the assumption of average levels of weed and pest pressure. If the assumptions assume extremes of low weed and pest pressure in all years and high weed and pest pressure in all years, then the farm level benefits from using biotech in agriculture during the period studied would fall within a range of about US$58 billion to US$73 billion. (Source: www.physorg.com)

India to fund organizations interested in stem cell research

The Indian Council of Medical Research (ICMR) would fund organizations interested in stem cell research and therapy from January 2012, said ICMR Director-General Mr. V.M. Katoch. However, the funding would be subject to monitoring by a regulatory body for possible unethical application that would attract punishment, including imprisonment. "Each state every year will get three stem cell research centres approved by ICMR at least for five years so that the maximum capacity of stem cell research is achieved at the state level,'' Mr. Katoch stated. Presently, bone marrow transplant and cord blood transplant for haematological diseases are approved by a joint committee of ICMR and Department of Biotechnology of the Government of India. (Source: www.moneycontrol.com)

Malay genome mapped, better drugs to come

Universiti Teknologi Mara (UiTM), Malaysia, has mapped the Malay ethnic genome, paving the way for specific medicines to be developed for the native population of Southeast Asia. Malaysian Biotechnology Corp. estimates this to be a market of over 200 million people and worth at least US $657 million per year. "The Malay genome applies not just to Malaysians but Indonesians, Southern Thais and the Filipinos. It allows us to develop specific medicine to be prescribed individually, which will be more effective", stated UiTM Chief Executive Dr. Mohd. Nazlee Kamal at the unveiling of the completed genome data in UiTM campus. UiTM mapped the Malay genome at a cost of US$49,270 from July to December 2010. It has now embarked on sequencing Orang Asli DNA. "The DNA of Malaysian aborigines are the second oldest after African (natives). But many tribes are in danger of dying out," stated Prof. Mohd. Zaki Salleh who heads the genome project in UiTM. (Source: www.themalaysianinsider.com)

Philippines releases eight new rice varieties to farmers

The Philippine Rice Research Institute (PhilRice) recently released eight new early maturing varieties that may help provide farmers more options on suitable and better rice varieties to plant on their rice fields. Ms. Thelma Padolina, Division Head of PhilRice Plant Breeding and Biotechnology Division said the National Seed Industry Council (NSIC) approved the release of the eight early maturing varieties after demonstrating good performance in field trials. Developed for irrigated and rain-fed lowlands and saline-prone areas, the new PhilRice varieties mature in 108-118 days and include six inbreds, a hybrid and an inbred glutinous rice.

Tubigan 22 variety, maturing in 108 days when direct-seeded and 115 days when transplanted, showed a higher yield of about 18 per cent than the check variety, PSB Rc82. Multi-location adaptability trials also showed that Tubigan 22 could yield about 6 t/ham with a yield potential of about 11 t/ha and 8 t/ha under transplanted and direct-seeded conditions, respectively. It is resistant to blast, sheath blight, bacterial leaf blight and white stem borer. Malagkit 5, a glutinous rice variety that matures in 122 days, has moderate resistance to whiteheads, brown planthopper, green leafhopper and yellow stem borer. A variety with maximum 7 t/ha harvest, it is slightly aromatic when cooked. Mestiso 29, a hybrid with high yields in dry and wet seasons, has an average yield of 7 t/ha. With a maximum yield of 11 t/ha and 113 maturity days, the variety has resistance to white and yellow stem borers, brown planthopper and green leafhopper. In rain-fed lowland and drought-prone areas, Sahod Ulan 2 yielded about 30 per cent over the check variety, PSB Rc14. Averaging at 3 t/ha, it matures in 110 days and is resistant to blast, bacterial leaf and sheath blights, white and yellow stem borers, and green leafhopper. Sahod Ulan 10 has a yield of 3 t/ha, or 5.4 per cent over the check variety, UPL R17. On-station trials have shown that the variety matures in 118 days and is resistant to blast, bacterial leaf blight and stem borer.

Among the varieties for saline-prone and coastal areas with moderate salinity, Salinas 6 exhibited the highest average yield of about 4 t/ha. The variety could yield up to 6.5 t/ha in favourable conditions. It matures in 113 days and is resistant to blast, bacterial leaf blight, stem borer, brown planthopper and green leafhopper. Salinas 7 has an average yield of 3 t/ha and matures in 111 days. The long and slender-grained variety is resistant to blast and the only new variety that resists tungro. Salinas 8 had the least average yield among the three varieties for saline-prone areas – an average yield of 3 t/ha. It matures in 117 days and is resistant to bacterial leaf blight and stem borer. (Source: businessmirror.com.ph)

Pakistan's efforts to enhance agriculture productivity continue

The Pakistan government has taken a number of measures for making agriculture competitive and profitable through enhanced crop production on sustainable basis both at the farm and country levels, according to a press release issued by the Ministry for Food and Agriculture. The government has adopted a policy that biotechnology would provide powerful tools for sustainable development of agriculture in Pakistan, ensuring food security and poverty alleviation at the rural level. Biotechnology could play a significant role in meeting the needs of an increasing and expanding rural and urban population in the new millennium. Thus, the government has planned to introduce hi-tech seeds (hybrid and Bt cotton seeds) in the country with the collaboration of multinational seed companies such as Monsanto. (Source: www.dailytimes.com.pk)

First cell-based flu vaccine plant in the United States dedicated

The first United States facility to use a faster and more flexible technology to make influenza vaccine was dedicated as part of an initiative that could provide vaccine supplies sooner in an influenza pandemic. The plant in Holly Springs, North Carolina, can create vaccine using cultured animal cells instead of the conventional process of using fertilized eggs. It is a public-private partnership of the United States Department of Health and Human Services (HHS) and Novartis Vaccines and Diagnostics Inc. The partnership will run under contract for at least 25 years.

The dedication means that during an influenza pandemic, the facility can produce 25 per cent of the cell-based influenza vaccine needed in the United States for use under authorization by the Food and Drug Administration (FDA). In addition, cell-based technology used in this facility may be adapted to produce vaccines for other known and emerging infectious diseases in an emergency. The United States joins several European countries with the capability to manufacture cell-based influenza vaccines on a large scale. In addition to partnering to bring cell-based flu vaccine and adjuvant technologies to the United States, HHS and Novartis are partnering with Synthetic Genomics Vaccines on new technologies to shorten the vaccine manufacturing timeline by optimizing vaccine virus seed strains used for flu vaccine production. (Source: www.marketwatch.com)

MARKET NEWS

Camson, Dutch varsity tie-up to produce bio-friendly crops

Camson Biotechnologies, India, and Wageningen University, the Netherlands, have entered into an agreement to develop disease-resistant and bio-friendly fruits and vegetables. The tie-up will help garner a large share of the cash crops segment, pegged at US$813 million in India and slated to grow at 25-30 per cent per year. According to Mr. Dhirendra Kumar, Managing Director of Camson Biotech, the technological tie-up will pave the way for a biotech revolution for developing hybrid seeds that can be used to satisfy the hunger situation across the globe. Wageningen University is globally renowned for its expertise in implementing Cisgenesis to arrive at superior produce. It was in the news recently for its pioneering work in developing disease-resistant potatoes.

Through Cisgenesis, Camson hopes to develop new cultivars that are strongly tolerant to biotic and abiotic stress. In Cisgenesis, hybrid seeds are developed from genes of the same plant or from those belonging to the same species. For instance, a hybrid seed could be developed from the genes of two unrelated chilli plants. Cisgenic plants are considered as safe genetically modified organisms because they do not use the genes from unrelated species, as in the case of transgenic crops. (Source: www.thehindubusinessline.com)

Global insulin pumps market to reach US$1.2 billion by 2017

The global insulin pumps market, valued at US $724.3 million in 2010, is predicted to grow at a compound annual growth rate (CAGR) of 7.9 per cent to reach US$1.2 billion in 2017. According to research and market reports, this market will be driven by a rapid increase in the prevalence of diabetes, availability of reimbursement, positive clinical outcome in studies on the use of insulin pumps and advantages offered by insulin pumps to niche patient groups. The United States remains the largest insulin pumps market, driven by high penetration of insulin pumps and availability of reimbursement. The United States insulin pumps market, valued at US$518.5 million in 2010, is expected to grow at a CAGR of 8.5 per cent to reach US$915.4 million by 2017. (Source: www.bloomberg.com)

Cancer Trails Australia ties up with Clininvent Research

Cancer Trials Australia (CTA) has entered into a collaboration with India-based Clininvent Research to leverage complementary capabilities in selected areas of clinical development and offer services spanning Phases I to IV to clients in Australia and India. The combination of CTA's experience in Phase I clinical trials – which has led the company to become a major centre for such studies in Australia – and Clininvent's proven track record of managing multisite trials, recruiting patient in large numbers, optimum GCP and protocol design compliance are expected to ensure scale and competitive advantage for both the partners. Mr. Marcus Clark, CEO of CTA, said: "We expect that this collaboration will be highly advantageous to our clients as it allows for a seamless transition from a Phase I centre to a network that includes sites in both India and Australia. With both CTA and Clininvent collaborating to offer trial design, ethics and governance management, site initiation and monitoring, there would be significant efficiencies achieved for client companies." (Source: www.bioportfolio.com)

Janssen in discovery deal with Molecular Partners

Johnson & Johnson's Janssen Biotech Inc., the United States, is taking its discovery pact with Switzerland's Molecular Partners a step further. Building on their pact to use Molecular Partners' small protein Designed Ankyrin Repeat Protein (DARPin) tech platform, Janssen is picking up four options on new treatments found for immunological conditions. Janssen could get up to US $200 million total for each option, with a double-digit royalty stream available on each.

"We see enabling power of the DARPin platform as a compound engine for us and our partners to generate pioneering multi-specific compounds delivering true patient benefit," said Mr. Christian Zahnd, CEO of Molecular Partners. Molecular

Partners has the rights to any programmes that Janssen passes on. The company's small protein drug platform has been getting attention lately. (Source: www.fiercebiotech.com)

HTDS to merge with Canadian biotech firm

Hard to Treat Diseases (HTDS), China, focused on the development and licensing of proprietary technologies, has plans to merge with a Canadian biotech company that deals with blood transfusion products. The Canadian company is involved in validating a product that will reduce the major side-effects related to transfusions of donated blood or haemoglobin-based blood substitutes. The market value for blood transfusions on the North American market is currently in excess of US$14 billion. According to HTDS, currently, there is a shortfall of blood available for transfusions and serious harmful side effects exist in the use of stored donor blood; specifically, the loss of nitric oxide (NO) from the recipients' blood creates a risky condition known as vasoconstriction. This biotech company plans to demonstrate in pre-clinical tests that its product can circumvent the loss of NO from blood as well as improve the shelf-life of stored blood for up to 42 days. (Source: www.biospectrumasia.com)

Glycos starts operation in Malaysia

Glycos Biotechnologies, an emerging biochemical company based in the United States, has signed definitive agreements with Bio-XCell of Malaysia for establishing the company's industrial biochemical plant and biotechnology research and development (R&D) facility in Malaysia. Glycos plans to focus much of its initial R&D efforts on creating isoprene to support Malaysia's rubber industry. "For Malaysia, isoprene is a strategic product because of the large domestic latex industry and where we plan to focus much of our R&D efforts," said Mr. Richard Cilento, CEO of Glycos Biotechnologies. "With the construction underway, our long-term strategy includes further expansion in Malaysia and across Southeast Asia forming joint venture partnerships with existing petrochemical, oleochemical and biofuel producers, and to partner with end market players in advanced biochemicals development," he added. (Source: www.biospectrumasia.com)

Bayer extends tie-ups in Singapore

Bayer HealthCare Singapore, a subsidiary of Bayer AG, Germany, has announced additional US$11.5 million worth of investment for enhancing its expertise in qualifying research and development (R&D) activities in Singapore to improve early diagnosis and treatment outcomes of cancer patients. In partnership with the National University of Singapore (NUS), National University Health System (NUHS), SingHealth and A*STAR's Singapore Bioimaging Consortium (SBIC), Bayer HealthCare will launch five new projects to investigate novel approaches to diagnose and treat cancers, including those that are highly prevalent in the region. The partnership will help to accelerate Bayer HealthCare's drug discovery and development in Asia. The collaboration is also part of Bayer HealthCare's US$15.9 million investment in joint research projects with Singapore-based universities, hospitals, research institutes and companies over six years. Bayer HealthCare will work closely with Singapore's Biomedical Sciences Industry Partnership Office (BMS IPO), as it expands its R&D activities in Singapore. (Source: www.biospectrumasia.com)

Biocon's first high-end bio-manufacturing and R&D facility

Biocon, India, recently held a project commencement ceremony for its biopharmaceutical manufacturing and research and development (R&D) facility in Bio-XCell, a custom-built biotechnology park and ecosystem in Johor, Malaysia. In the first phase, Biocon proposes to invest approximately US$161 million in this facility, targeted to be operational by 2014. The investment is the largest for the Malaysian healthcare biotechnology sector thus far. This facility will serve the global requirements for Biocon's range of biosimilar insulin and insulin analogues for diabetes treatment, being commercialized by Pfizer Inc., stated Ms. Kiran Mazumdar-Shaw, Chairman and Managing Director of Biocon. The project will also focus on R&D and production of other biopharmaceutical products later. The plant will be built to the cGMP standards of the United States Food and Drugs Administration (FDA). Biocon had signed a Memorandum of Understanding with Malaysian Biotechnology Corp. a year ago to establish the facility in Bio-XCell.(Source: www.expresspharmaonline.com)

GENOMICS

Cannabis genome mapped

Scientists in Canada have sequenced the genome of *Cannabis sativa,* the plant that produces both industrial hemp and marijuana, and in the process revealed the genetic changes that led to the plant's drug-producing properties. Mr. Jon Page, a plant biochemist and Adjunct Professor of biology at the University of Saskatchewan, explains that a simple genetic switch could be responsible for producing tetrahydrocannabinolic acid (THCA), the precursor of the active ingredient in marijuana. "The transcriptome analysis showed that the THCA synthase gene, an essential enzyme in THCA production, is turned on in marijuana but switched off in hemp," Mr. Page stated.

Researchers compared the potent Purple Kush marijuana variety with 'Finola' hemp grown for seed production. Hemp lacks THCA, but contains cannabidiolic acid (CBDA), a non-psychoactive substance. "Detailed analysis of the two genomes suggests that domestication, cultivation and breeding of marijuana strains has caused the loss of the enzyme (CBDA synthase) that would otherwise compete for the metabolites used as starting material in THCA production," said Mr. Tim Hughes, co-leader of the project and a professor at the Department of Molecular Genetics at University of Toronto. This means that over thousands of years of cultivation, hemp farmers selectively bred *C. sativa* into two distinct strains – one for fibre and seed and one for medicine. The researchers expect that sequencing the *C. sativa* genome will help answer basic questions about the biology of the plant as well as furthering development of its myriad applications. (Source: www.sciencedaily.com)

Discovery of gene could improve efficiency of molecular factories

The discovery of a new gene by researchers at Michigan State University (MSU), the United States, makes more-efficient molecular factories possible in the future. The researchers led by Ms. Katherine Osteryoung, an MSU plant biologist, discovered clumped chloroplasts (CLMP1), a new class of proteins. CLMP1 plays a major role in helping chloroplasts, which carry out photosynthesis, to separate when the chloroplasts divide. The newly identified proteins are also critical in the perpetuation of chloroplasts during cell division.

Studying mutant *Arabidopsis thaliana* plants that failed to produce CLMP1, Ms. Osteryoung saw that the chloroplasts had nearly completed the division process, but had they failed to separate, remaining connected to each other through thin membranes. "The mutant plants had chloroplasts that appeared like clusters of grapes," she said. In normal plants, chloroplasts are separated and distributed throughout, enabling them to move freely to maximize photosynthesis. "In the mutant, where the chloroplasts remain bunched together, they cannot move around as freely, which probably impairs photosynthesis. The discovery of CLMP1 helps explain how plants have evolved mechanisms to promote chloroplast division and dispersal and avoid clumping," she noted.

In normal plants, the separation and distribution of chloroplasts also helps ensure that, when cells divide, each daughter cell inherits about half of the chloroplasts. Further investigation showed that CLMP1 is required for this normal inheritance of chloroplasts during cell division, Ms. Osteryoung said. Since genes closely related to CLMP1 are also present in crop plants, the new research could lead to improvements in soybeans, corn, wheat and other food crops. (Source: www.medicalnewstoday.com)

Complementary DNA libraries of first lizard genome sequence

In the United States, scientists at Indiana University (IU) Bloomington's Centre for Genomics and Bioinformatics are credited with constructing the complementary DNA (cDNA) libraries for the first-ever genome sequence of a non-bird reptile, the North American green anole lizard *(Anolis carolinensis)*. Mr. John Colbourne, the Centre's Director and Mr. Zachary Smith, research scientist, state that the Centre provided the cDNA resources that were used to discover and characterize the transcripts – the ribonucleic acid copies of a DNA sequence – of green anole lizard genes as expressed under various conditions. The sequences produced by the Centre's resources helped

determine the structures of *A. carolinensis* genes, including alternative transcripts that produce different forms of proteins.

Scientists are particularly interested in the first reptile genome sequence for understanding the evolution of the amniotic egg and of vertebrate evolution more generally, as the evolution of the amniotic egg allowed vertebrates to conquer terrestrial environments. Among amniotes, genome sequences had not been available for non-avian reptiles. Working in a team of 50 scientists, Mr. Colbourne and Mr. Smith created a number of normalized cDNA libraries from a variety of *A. carolinensis* tissues for sequencing, to facilitate the annotation of genes. The researchers report that close to 100 conserved, non-coding elements in the human genome are derived from remaining mobile elements in the lizard genome.

The first analysis of the *A. carolinensis* genome microchromosomes uncovered a complete lack of isochores, regions of the genome with high or low concentrations of the nucleotides guanine and cytosine, which give human chromosomes a distinct banding pattern. The lizard's sex chromosomes appeared to have, like humans and unlike birds, XX and XY chromosomes: males have two identical ZZ sex chromosomes, while females have two different ZW sex chromosomes. (Source: www.physorg.com)

Tool for identifying subtle changes in gene expression

In a truly challenging task, the FANTOM5 Consortium, an international collaboration headed by scientists at the RIKEN Omics Science Centre in Japan, is striving to profile the regulation of gene expression in every known human cell type. "We expect to generate in the order of 3,000 or more [datasets] for this project," says Mr. Masayoshi Itoh, a RIKEN scientist involved in the effort. "This will capture the majority of human cell types, tissues and cancer subtypes," he adds. The work will benefit greatly from HeliScopeCAGE, a sensitive expression analysis technique developed recently by FANTOM5. Previous FANTOM studies used techniques based on 'cap analysis of gene expression' (CAGE), which enables quantification of messenger RNAs (mRNAs) transcribed from active genes by generating short DNA 'tags' that can be analysed by sequencing. Conventional CAGE relies on polymerase chain reaction (PCR). However, PCR can introduce biases into libraries by preferentially amplifying some molecules – a serious impediment to the accurate measurement of gene expression.

HeliScope, an instrument developed by Helicos Biosciences for analysing individual molecules of DNA, allows researchers to get accurate results from as little as 100 ng of material (the mRNA content of approximately 20-100 thousand cells) and chart differences in gene expression levels ranging across five orders of magnitude. The researchers noted that the observed differences between different cell types correlated with previous findings. HeliScopeCAGE even revealed changes in thousands of genes that had been overlooked by older platforms. Mr. Itoh points out that their technique proved highly quantitative, based on trial experiments with different concentrations of control templates, and yielded consistent data in run after run. (Source: www.scoop.it)

'Genome mining' technique streamlines discovery from nature

A newly developed method for microscopically extracting ("mining") information from genomes could represent a significant boost in the search for new therapeutic drugs and improve science's understanding of basic functions such as how cells communicate with one another. The research technique created in the United States jointly by scientists at the Scripps Institution of Oceanography and the Skaggs School of Pharmacy and Pharmaceutical Sciences (of University of California San Diego) and their co-researchers taps powerful laboratory instruments to trace promising chemical compounds back to their genomic roots.

The technique uses mass spectrometry, a mass analysing tool that deciphers the size and make-up of molecules, to reveal core structural details of genomes. With only very small amounts of crude sample material, the mass spectrometer is able to fragment an unknown peptide into individual amino acid building blocks, so that the researchers can then map those to the genome level. Knowing such minute data provides scientists a way to connect the natural chemicals

produced by organisms back to the enzymes that construct them. These "biosynthetic pathways" are considered prized information in the search for new pharmaceuticals to treat diseases. Using the new method, the scientists have already discovered two new classes of peptides. (Source: www.bioresearchonline.com)

Analysis of mammals reveals 'dark matter' of the genome

An international team of researchers report to have discovered the vast majority of the so-called "dark matter" in the human genome, by means of a comprehensive comparison of 29 mammalian genomes. The researchers have pinpointed the human genome parts that control when and where genes are turned on. This map is a critical step in interpreting the thousands of genetic changes that have been linked to human disease. Early comparison studies of the human and mouse genomes led to the discovery that the regulatory information that controls genes dwarfs the information in the genes themselves. These mysterious regulatory sequences have been referred to as the dark matter of the genome. The new study enlisted a menagerie of mammals – rabbit, bat, elephant, etc. – to unravel these mysterious genomic elements.

In the United States, researchers from the Broad Institute, the Washington University's Genome Institute and the Baylor College of Medicine's Human Genome Sequencing Centre have sequenced the genomes of 29 placental mammals. The researchers compared all these genomes, looking for regions that have remained largely unaltered across species. The new map reveals almost 3 million previously undetected elements in non-coding regions that have been preserved across all mammals, and whose disruptions appear to have an association with diseases. These findings could yield a deeper understanding for disease-focused studies, which look for genetic variants closely tied to disease. The scientists found almost 4,000 previously unknown exons (DNA segments that code for protein), more than 1,000 new families of RNA secondary structures with diverse roles in gene regulation, and 10,000 highly conserved elements that may be involved in protein production. (Source: www.eurekalert.org)

PROTEOMICS

Largest protein interaction map

Researchers have built a map that shows how thousands of proteins in a fruit fly cell communicate with each other. This is the largest and most detailed protein interaction map of a multicellular organism, showing how about 5,000, or a third, of the proteins cooperate to keep life going. "For me, and hopefully for researchers studying protein interactions, this map is a dream come true," says Mr. Spyros Artavanis-Tsakonas a cell biology professor at Harvard Medical School, the United States, and senior author of the study.

Humans and fruit flies are both descended from a common ancestor, and in most cases, both species still rely on the same ancient cellular machinery for survival. In that respect, the fruit fly's map serves as sort of a blueprint, a useful guide into the cellular activity of many higher organisms. Understanding how proteins behave normally is often the key to their disease-causing behaviour. Mr. Artavanis-Tsakonas and his colleagues provide the first large-scale map of this population of proteins. The map, though not yet fully complete, reveals many of the relationships these myriad proteins make with each other as they collaborate, something which, to date, has been to a large degree an enduring mystery to biologists. (Source: www.proteomicsnews.com)

New way to target shape-shifting proteins

Researchers at the University of Leeds, the United Kingdom, have identified a molecule that can stop the formation of long protein strands, known as amyloid fibrils, that cause joint pain in kidney dialysis patients. The discovery could lead to new methods to identify drugs to prevent, treat or halt the progression of other conditions in which amyloid fibrils are involved, including Type II diabetes and Alzheimer's and Parkinson's diseases. The researchers – from Leeds' Astbury Centre for Structural Molecular Biology and Faculty of Biological Sciences – found that an antibiotic known as Rifamycin SV was able to prevent the protein â2microglobulin (â2m) from forming into fibrils.

â2m accumulates in renal dialysis patients and forms fibrils within the joints, causing extreme pain and arthritis. By using a specialized analytical technique called ion mobility spectrometry-mass spectrometry (IMS-MS), the researchers were able to see at what stage of the process Rifamycin SV prevented amyloid fibril formation.

In their normal, folded state, proteins are unable to link together to form long fibrillar assemblies, but if they unfold, they expose areas where they can bind to each other. Initially they form small groups of two, three or four proteins, and then these link into long strands that twist together to form fibrils. Most analytical techniques can only show the mass of the protein or its make-up in terms of amino acids, neither of which changes as the protein unfolds. Others are unable to look at individual molecules within complex mixtures. However, IMS-MS can measure the mass and shape of a protein, allowing researchers to watch the unfolding process, the aggregation into small groups and the assembly into the fibril, and to find which of these species is able to bind a ligand and stop the assembly process. (Source: www.eurekalert.org)

Scientists create the largest synthetic protein

A team of chemists at Vanderbilt University, the United States, has designed and successfully synthesized the largest human-designed protein, a variant of a protein that nature uses to manufacture the essential amino acid histidine. The new protein is more than twice the size of the previous record holder, created by researchers in 2003 at University of Washington, the United States. The synthetic protein, designated FLR, validates a new approach that the Vanderbilt scientists have developed to design functional artificial proteins substantially larger than previously possible. “We now have the algorithms we need to engineer large proteins with shapes that you don’t see in nature. This gives us the tools we need to create new, more effective antibodies and other beneficial proteins,” said Mr. Jens Meiler, an associate professor of chemistry at Vanderbilt who led the effort.

Their success provides new support for a controversial theory about protein evolution called the gene duplication and fusion hypothesis. The advantage of small proteins is that they can evolve rapidly in response to changing conditions, but larger proteins can perform more complex functions. Nature found a way to get both advantages by selecting small proteins that can interact with other copies of themselves to form larger proteins, called dimers. Once useful dimers have been created, the gene that coded for the original protein is duplicated and fused to form a new gene that can directly produce the dimer. After it is created, the dimer gene is gradually modified by natural selection to make it more efficient or develop new functions. Because their halves are identical, dimers have a large degree of symmetry. By taking these symmetries into account, the Vanderbilt group was able to substantially reduce the amount of computing time required to create the FLR protein: it took just 10 days of continuous processing to find the most stable configuration.

About 120 amino acids was the upper limit of designing proteins “*in silico*”, on computer using sophisticated protein modelling software. The new protein contains 242 amino acids: the scientists overcame the limit by modifying the widely used protein engineering platform ROSETTA, so that it can incorporate symmetry in the design process. To check the accuracy of their design, the researchers synthesized the DNA sequence that produces the protein, inserted it in *Escherichia coli* bacteria and confirmed that they produced the protein and it folded properly. The FLR protein assumes a 3-D shape called a TIM barrel, which is found in 10 per cent of proteins and is particularly prevalent among enzymes. It is formed from eight beta strands that are surrounded by eight alpha helices arranged in a hexagonal shape like a tiny barrel. (Source: www.proteomicsnews.com)

An invention unravels mystery of protein folding

An invention at Oak Ridge National Laboratory (ORNL), the United States, with the capacity to quickly predict 3-D structure of proteins could have huge implications for drug discovery and human health. While scientists have long studied protein structure and the mechanism of folding, this marks the first time they are able to predict

computationally the 3-D structure independent of the size of the protein. Because the invention also determines possible intermediate states in the protein folding process, it provides a clearer picture and could open doors to designing new medicines for neurodegenerative diseases that are caused by incorrectly folded proteins.

Proteins often adopt a 3-D structure that allows them to carry out their designated function, but such a structure has provided a computationally challenging task. Using the fundamental insights of the protein structure, dynamics and function, the ORNL invention discloses a unique computational methodology to explore the conformational energy landscape of a protein. "One of the main advantages of this approach is that it follows the natural intrinsic dynamics of the protein and by promoting the relevant dynamical modes allows rapid exploration of the folding pathway and prediction of the protein structure," said Mr. Pratul Agarwal, inventor of the method and a member of the ORNL Computer Science and Mathematics Division. In the area of drug development, Mr. Agarwal expects this discovery to help in the development of treatments with little or no side effects. (Source: www.humanhealthandscience.com)

Protein as the villain in pancreatic, brain cancer growth

Tumours of the head and neck in humans represent a molecularly diverse set of cancers, but relatively few proteins have actually been shown to drive the disease at the molecular level. The protein CPEB4 appears to start off legions of genes that spur the growth of pancreatic and brain cancer, and possibly other kinds of tumours, scientists in Spain have discovered. Knowing what CPEB4 is capable of also makes it a drug target to watch, according to the researchers from the Institute for Research in Biomedicine (IRB) and the Institut de Recerca Hospital del Mar, who are studying potential CPEB4 inhibitors that could become cancer treatments.

Specifically, the researchers are looking at brain and pancreas tissue in mice with human cancer cells determined that expression of the protein in an incorrect site triggered an expansive process, leading, in turn, to the expression of unsuitable amounts of so-called normal genes and the resulting tumours. They also realized that CPEB4 is found in tumour cells but not in healthy ones. They were able to reduce tumour size by as much as 80 per cent by decreasing CPEB4 levels in cancer cells. CPEB4 is thus offering the potential of a highly targeted cancer treatment with a small number of side-effects, the researchers note. (Source: www.fiercebiotechresearch.com)

Quantitative chemical proteomics reveals new potential drug

In search of new targets for individualized diagnosis or therapeutic intervention, researchers at Technische Universitaet Muenchen, Germany, performed a kinase-centric chemical proteomics screening and quantified 146 kinases across 34 head and neck squamous cell carcinoma (HNSCC) cell lines via intensity-based label-free mass spectrometry. Statistical analysis of the profiles revealed strong inter-cell line differences for 42 kinases ($p<0.05$). Loss of function experiments using small interfering RNA (siRNA) in high- and low-expressing cell lines identified kinases including EGFR, LYN, NEK9, JAK1, WEE1 and EPHA2 involved in cell survival and proliferation. EGFR inhibition by the small molecule inhibitors lapatinib, gefitinib and erlotinib and also by siRNA led to strong reduction of viability in high-expressing (but not low-expressing) lines confirming EGFR as a drug target in 10-20 per cent of HNSCC cell lines.

Similarly, high, but not low, EPHA2-expressing cells showed much reduced viability concomitant with down-regulation of AKT and ERK signalling following EPHA2 siRNA treatment or EPHA1-Fc ligand exposure, suggesting EPHA2 as a novel drug target in HNSCC. This notion is underscored by immunohistochemical analyses showing that high EPHA2 expression is detected in a subset of HNSCC tissues and is linked to poor prognosis. Given that dasatinib, the approved pan-SRC family kinase inhibitor, is also a very potent inhibitor of EPHA2, these findings may lead to new therapeutic options for HNSCC patients. Importantly, this study employs a generic strategy and therefore is of more general utility for the identification of new drug targets and molecular pathway markers in tumours. This may ultimately lead to a more rational approach to individualized cancer diagnosis and therapy. (Source: www.wzw.tum.de)

MEDICAL BIOTECH

Human creates humanized mice

Ms. Sangeeta Bhatia, Professor of health sciences technology and electrical engineering at the Massachusetts Institute of Technology (MIT), the United States, has developed and implanted tiny artificial human livers into mice, creating "humanized" mice that could increase speed and reliability in the process of screening new candidate drugs. Ms. Bhatia and her colleagues packed human liver cells and mouse skin cells in a polymer scaffold and nourished them with a cocktail of biochemicals to build the artificial livers. They then implanted these artificial human livers into the bodies of healthy mice. The artificial livers displayed human liver functions for several weeks, synthesizing the same proteins and enzymes that normal human livers produce, closely mimicking human physiology.

"We think this is a considerable step towards creating implantable livers for patients," stated Ms. Bhatia. A key issue is scale-up: at least 1,000 times more cells would be needed for patients. Further, the transplants must function for many years. The humanized mice developed by Ms. Bhatia and her co-researchers might serve as efficient models, perhaps superior to the best available mice models, to screen or test candidate drugs. Many drugs are broken down by the liver and an assessment of a candidate drug needs to take into account how liver cells interact with the drug's by-products. A humanized mouse with a liver that behaves just as a real human one would thus yield more reliable results than other mice. (Source: www.telegraphindia.com)

Human heart cells that can be paced with light created

At Stanford University, the United States, a multidisciplinary team of researchers led by Dr. Oscar Abilez has for the first time engineered human heart cells that can be paced with light using a technology called optogenetics. According to the researchers, in the near term the advance will provide new insight into heart function. In the long term, however, the development could lead to an era of novel, light-based pacemakers and genetically matched tissue patches that replace muscle damaged by a heart attack. To create the light-responsive heart cells, the researchers inserted DNA encoding a light-sensitive protein, channelrhodopsin-2 (ChR2), into human embryonic stem cells. ChR2 is sensitive to a very specific wavelength of blue light and regulates tiny channels in the cell surface. ChR2 also controls the flow of ions into the cell. For heart cells, the primary ion is sodium. When ChR2 is illuminated by the right wavelength of blue light, the channels open to allow an influx of electrically charged sodium into the cell, producing a contraction. The researchers thus transformed the optogenetically engineered stem cells into cardiomyocytes unlike any others – those that respond to light.

After creating the cells in a laboratory dish, Dr. Abilez next turned to Ms. Ellen Kuhl, Associate Professor of mechanical engineering and senior author of the study, to test the new cells using a computer simulation of the human heart. They injected the light-sensitive cells in various locations in the heart and shined a virtual blue light on them to observe how the injections affected contraction as it moved across the heart. "With these models we can demonstrate not only that pacing cells with light will work but also where to best inject cells to produce the optimal contraction pattern," Ms. Kuhl explained. The long-term goal is a new class of pacemakers. (Source: www.physorg.com)

Research aims to prevent kidney failure from diabetes

The enzyme arginase-2 plays a key role in kidney failure, and blocking the action of this enzyme might lead to protection against renal disease in diabetes, according to researchers in the United States. "We believe these arginase inhibitors may be one of the new targets that can slow down the progression of, or even prevent the development of, end-stage renal disease," stated Mr. Alaa S. Awad, Assistant Professor of nephrology, Penn State College of Medicine.

Researchers tested two different sets of diabetic mice to try to prevent kidney failure. They gave one set of mice – genetically diabetic – a potent arginase inhibitor; the other set of mice – induced

to be diabetic – were genetically unable to produce arginase-2. Both sets of mice showed no signs of kidney failure during the test period. The body naturally produces varieties of arginase. The liver produces arginase-1, while the kidneys produce arginase-2 that leads to kidney failure. The researchers did not detect arginase-1 in the kidneys of the mice. One of the symptoms of diabetic nephropathy is albuminuria – losing protein via urine. The researchers found that the mice protected from arginase-2 were also protected from albuminuria. People with diabetes and diabetic nephropathy often experience low levels of nitric oxide – a vital compound in cardiovascular function – because arginase steals the common precursor, L-arginine, that the nitric oxide needs. This causes cardiovascular problems. Arginases have been implicated in heart disease but had not been connected to diabetic nephropathy prior to this research. (Source: news.biocompare.com)

Method for raising antibiotic yields

A new method to increase the amounts of antibiotics produced by bacteria could help improve the yields of these important compounds also in commercial production, besides being valuable in the discovery of new compounds. In a work carried out at the John Innes Centre, the United Kingdom, Prof. Mervyn Bibb and collaborator Dr. Koji Yanai from a Japanese laboratory discovered 36 repeating copies of one gene cluster in a strain of *Streptomyces* that had been repeatedly selected to overproduce the antibiotic kanamycin. "This suggested to us that controlled and stable amplification of antibiotic gene clusters might be possible, and that if it was, it would be a valuable tool for engineering high-yielding commercial strains of bacteria," said Prof. Bibb. The researchers then went on to identify the components within *Streptomyces* responsible for creating the repeating clusters that led to kanamycin overproduction. These consist of two DNA sequences that flank the gene cluster and a protein, known as ZouA, that recognizes the two sequences and replicates them.

The system may also uncover new antibiotics. A number of *Streptomyces* species have had their entire genomes sequenced, and many more are expected. Researchers have been able to identify other gene clusters within these sequences with unknown products. It is likely that many of these 'cryptic' gene clusters produce potentially new antibiotics, but at an undetectable level or only under specific environmental conditions. Using the gene cluster amplification system, it would be possible to amplify these cryptic gene clusters, identify their products and potentially discover new antibiotics for the battle against resistant superbugs. (Source: esciencenews.com)

Bio-engineered protein shows promise in haemophilia therapy

A genetically engineered clotting factor that controlled haemophilia in an animal study offers a novel potential treatment for human haemophilia and a broad range of other bleeding problems. Researchers in the United States took the naturally occurring coagulation factor Xa (FXa), a protein active in blood clotting, and engineered it into a novel variant that safely controlled bleeding in mouse models of haemophilia. "Our designed variant alters the shape of FXa to make it safer and efficacious compared with the wild-type factor, but much longer lasting in blood circulation," said study leader Mr. Rodney A. Camire, a haematologist at the Children's Hospital of Philadelphia and an Associate Professor of Paediatrics in the Perelman School of Medicine at the University of Pennsylvania. The shape of FXa changes when it interacts with another clotting factor that is made available following an injury, and this increases the protein function, which helps stop bleeding.

"The variant we have developed puts FXa back on the table as a possible therapeutic agent," said Mr. Camire. Because of its shape, naturally occurring (wild-type) FXa is not useful in therapy, as normal biological processes shut down its functioning very quickly. By custom-designing a different shape for the FXa protein, researchers give it a longer period of activity, while limiting its ability to engage in unwanted biochemical reactions such as triggering excessive clotting. When infused into mice with haemophilia, the FXa variant reduced blood loss after injury. Further studies in large animal models are needed to determine the efficacy of this approach in treating haemophilia patients who have developed inhibitors, or even in checking uncontrolled bleeding in other clinical situations. (Source: www.sciencedaily.com)

Pawpaw shows promise in fighting drug-resistant tumours

The pawpaw tree (*Asimina* sp.) that bears the largest fruit native to North America may help fight cancer. In Purdue University, the United States, researchers have found in the bark of the tree compounds that have shown initial success in fighting some drug-resistant cancers. The study has shown that the pawpaw compounds not only are effective in killing tumours that are resistant to anti-cancer agents but also seem to have a special affinity for such resistant cells. "Tumour cells that survive chemotherapy treatments often recover with increased resistance to the agent used in the original treatment programme as well as to other related drugs," said Dr. Jerry McLaughlin, Professor of Pharmacognosy in Purdue's School of Pharmacy and Pharmacal Science. One of the most common mechanisms that cancer cells use to circumvent the anti-cancer agents is to develop a "pump" that is capable of pushing anti-cancer agents out of the cell before they can kill it. These pumps are called P-glycoprotein mediated pumps, named for the type of protein used to construct and operate them.

One of the tricks currently attempted in treating cancer patients is to flood the body with other compounds to keep the pump engaged, and then administer high doses of an anti-cancer agent, hoping that some of it will be able to stay in long enough to kill the cancer cell. Dr. McLaughlin, whose research group has identified more than 40 pawpaw compounds with anti-cancer properties, discovered a series of the compounds, called annonaceous acetogenins, that were capable of killing cancer cells that used the pump mechanism. He then studied the cytotoxic or cell-killing effects of one of the compounds, called bullatacin, on human mammary cancer cells. The study compared bullatacin's effects on standard, non-resistant cancer cells and on multidrug-resistant cells. Bullatacin was found to preferentially kill the multidrug-resistant cells by inhibiting the production of adenosine triphosphate (ATP), a compound that works to release energy in a cell and is essential for all cell processes. Though the pawpaw compounds also inhibited ATP production in non-cancerous cells and non-resistant cancer cells, those cells were not affected as dramatically, Dr. McLaughlin stated. If proven effective in animals and humans, the compounds may be used to treat multi-drug resistance in a variety of cancers. (Source: www.eurekalert.org)

New way to grow synthetic collagen

In a major advance for cosmetic and reconstructive medicine, scientists at Rice University, the United States, have unveiled a new method for making synthetic collagen. The new material forms from a liquid in as little as an hour. It has many of the properties of natural collagen and may prove useful as a scaffold for regenerating new tissues and organs from stem cells. "Our work is significant in two ways," said Mr. Jeffrey Hartgerink, Associate Professor of chemistry and bioengineering. "Our final product more closely resembles native collagen than anything that has previously been made, and we make that material using a self-assembly process that is remarkably similar to processes found in nature." Collagen, the most abundant protein in the body, is a key component of many tissues, including skin, tendons, ligaments, cartilage and blood vessels. Biomedical researchers in the burgeoning field of regenerative medicine, or tissue engineering, often use a combination of stem cells and collagen-like materials in their attempts to create laboratory-grown tissues that can be transplanted into patients without risk of immunological rejection.

Despite the abundance of collagen in the body, deciphering or recreating it has not been easy for scientists. One reason for this is the complexity collagen exhibits at different scales. For example, just as a rope is made of many interwoven threads, collagen fibres are made of millions of proteins called peptides. Like a rope net that can trap and hold items, collagen fibres can form 3-D structures called hydrogels that trap and hold water. "Our supramolecules, fibres and hydrogels form in a similar way to native collagen, but we start with shorter peptides," explained Mr. Hartgerink. However, Mr. Hartgerink states that it is too early to say whether the synthetic collagen can be substituted medically for human or animal-derived collagen, but it did clear the first hurdle on that path; the enzyme that the body uses to break down native collagen also breaks down the new material at a similar speed. (Source: bioengineering.rice.edu)

AGRI BIOTECH

New research finding will protect vital global crops

A team of researchers led by Prof. Bruce Fitt, now at the University of Hertfordshire, the United Kingdom, reports a new form of resistance to the damaging pathogen that causes light leaf spot in oilseed rape – one of the world's most important crops. The team describes a research project carried out at Rothamsted Research, under the Institute of Arable Crops Research, which looked at diseases of oilseed rape in the United Kingdom and came up with new findings about crop resistance that impacts the global bid to protect arable crops from disease. Results indicate a novel form of resistance in *Brassica napus*, a specific variety of oilseed rape, mediated by a single so-called "R gene." R genes are important for plant resistance to pathogens and they work in various different ways. In this case, the R gene produces a protein inside the plant that can limit pathogen asexual reproduction – which occurs regularly during the cropping season – but allows sexual reproduction – which generally occurs only once a year – and so significantly reduces the chances of a light leaf spot epidemic developing during the crop growing season. (Source: www.sciencedaily.com)

Hybrid rice varieties offer benefits to growers

Researchers at the United States Department of Agriculture (USDA) and their colleagues have developed new rice varieties that offer new options for growers and expanded market opportunities for the rice industry in the country. Agricultural Research Service (ARS) scientists at the Dale Bumpers National Rice Research Centre in Stuttgart, Arkansas, and the Rice Research Unit in Beaumont, Texas, developed the new varieties, in collaboration with researchers at Texas A&M University, University of Arkansas and Clemson University, and the International Rice Research Institute (IRRI) in the Philippines.

Geneticists Ms. Anna McClung and Mr. J. Neil Rutger (now retired) at the ARS research centre in Stuttgart are among the collaborators involved in developing the new rice varieties. Mr. Rutger helped develop JES, an aromatic, soft-cooking, long-grain rice suited for the market predominantly filled by imports. A Jasmine-style rice, JES has higher yields, is 5 inches shorter and matures a week earlier than Jasmine 85, a variety currently grown for this market. Charleston Gold, another aromatic rice, was derived from Carolina Gold (an heirloom variety that was the basis for establishing the United States rice industry) and genetic material from the Philippines and India. It has excellent yields, disease resistance and good cooking quality. This cultivar may lend itself to production under organic conditions. While conventional long-grain varieties are grown on more than 75 per cent of the rice acreage in the United States, there is interest in developing cultivars that possess specific qualities required for certain value-added markets. (Source: www.sciencenewsline.com)

New insight into plant immune defence

Researchers from the University of Edinburgh, the United Kingdom, and Syngenta AG, based in Switzerland, have identified an important cog in the molecular machinery of plant immunity – a discovery that could help crop breeders produce disease-resistant varieties to help ensure future food security. The results of the research, led by Prof. Gary Loake from the University of Edinburgh, may have implications for treating human immune-related disorders as well.

Plants are under constant attack from disease-causing organisms and to protect themselves they have developed a simple immune system. One defence mechanism is to trigger threatened cells to die and so remove the food source from the invading pathogen. Prof. Loake and his team have uncovered the happenings inside the cells and, in doing so, have put in the spotlight an enzyme called NADPH oxidase. When a plant is attacked by a bacterium or a fungus, there are various ways in which the plant perceives this attack. One of the common responses is to trigger the production of nitric oxide (NO) and a class of molecules known as 'reactive oxygen intermediates' (ROIs) that includes things like hydrogen

peroxide and free radicals. Besides being very toxic to the invading organism, NO and ROIs are key to encouraging cells to die if they are threatened. NADPH oxidase comes into the picture because it is critical for the production of ROIs. The team found that there is a feedback loop where, as the levels of NO go up, NADPH oxidase is altered by the addition of an NO molecule to its structure so that it doesn't work so well. This causes the level of ROIs to drop and cell death tapers off. (Source: esciencenews.com)

Researchers coax rice to take up and keep more iron

On the back of a groundbreaking scientific discovery, researchers from Flinders University in Australia are pushing ahead with a plan to create super rice that could potentially combat nutrient deficiencies in third world countries. Working under the guidance of Associate Professor Mr. James Stangoulis, doctoral student Ms. Bianca Kyriacou is leading a research mission to increase the iron content of rice grains in a bid to eradicate nutrient deficiencies, such as anaemia. So far, her research has produced a genetically modified rice grain containing up to four times more iron than conventional rice, using the plant's own abilities to acquire more iron from soil, which is in turn transported to the grain. With the 'proof of concept' stage an official success, Ms. Kyriacou said the next step is to grow subsequent generations of the iron-rich rice to determine whether the plant is capable of producing the same results year after year.

Ms. Kyriacou said her research – a collaboration with three universities in South Africa and the University of Melbourne – was unique in that it does not 'trick' the plant into thinking it lacks iron. "The plant already has the ability to extract nutrients for its own benefit but we have human requirements from these plants. So what we have done is to modify the expression levels of the plant gene to enhance its natural transport mechanism of carrying nutrients from soil to grain," Ms. Kyriacou stated. "This whole chain of events makes the plant do what it is already capable of doing – we are just improving the efficiency of that process." As an extension of her project, scientists from the International Rice Research Institute (IRRI) will begin field trials in the Philippines using a similar super grain. (Source: www.physorg.com)

Gene controlling flowering boosts energy production from sorghum

A team at Texas AgriLife Research, the United States, has now discovered a gene that regulates flowering in sorghum. "For energy crops, we want to prevent plants from flowering so they accumulate as much biomass as possible for bioenergy/biofuels production," said Dr. John Mullet, AgriLife Research biochemist. The researchers identify a gene in sorghum that controls flowering in response to day length, and we discovered that the gene is regulated by the plant's internal 'clock' and light enabling the plant to flower at approximately the same date each growing season.

Early researchers identified four genes – called Ma1 through Ma4 – that control flowering time in sorghum. In the current study, the research team identified the gene in sorghum that corresponds to Ma4-1. In some sorghum genotypes, there are mutations in that gene that inactivate the gene and cause plants to flower early. But when the gene is active, the plants flower late. Sorghum breeders have been using this variation in the activity of the gene corresponding to Ma1 in breeding programmes for years to fine-tune when their hybrids would flower. A planting test of sorghum phenotypes with an active form of Ma1 and other genes in this pathway could be delayed in flowering for up to 200 days compared with the usual 60 days for a grain-type sorghum. Breeders can now use molecular markers to assist in the design of sorghum hybrids that flower at optimal times accelerating the process of breeding high-yielding grain, sweet and energy sorghum hybrids. (Source: www.eurekalert.org)

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