

NATIONAL SYMPOSIUM

On

Transgenic Crops in India: Progress and Challenges
March 16-17, 2016

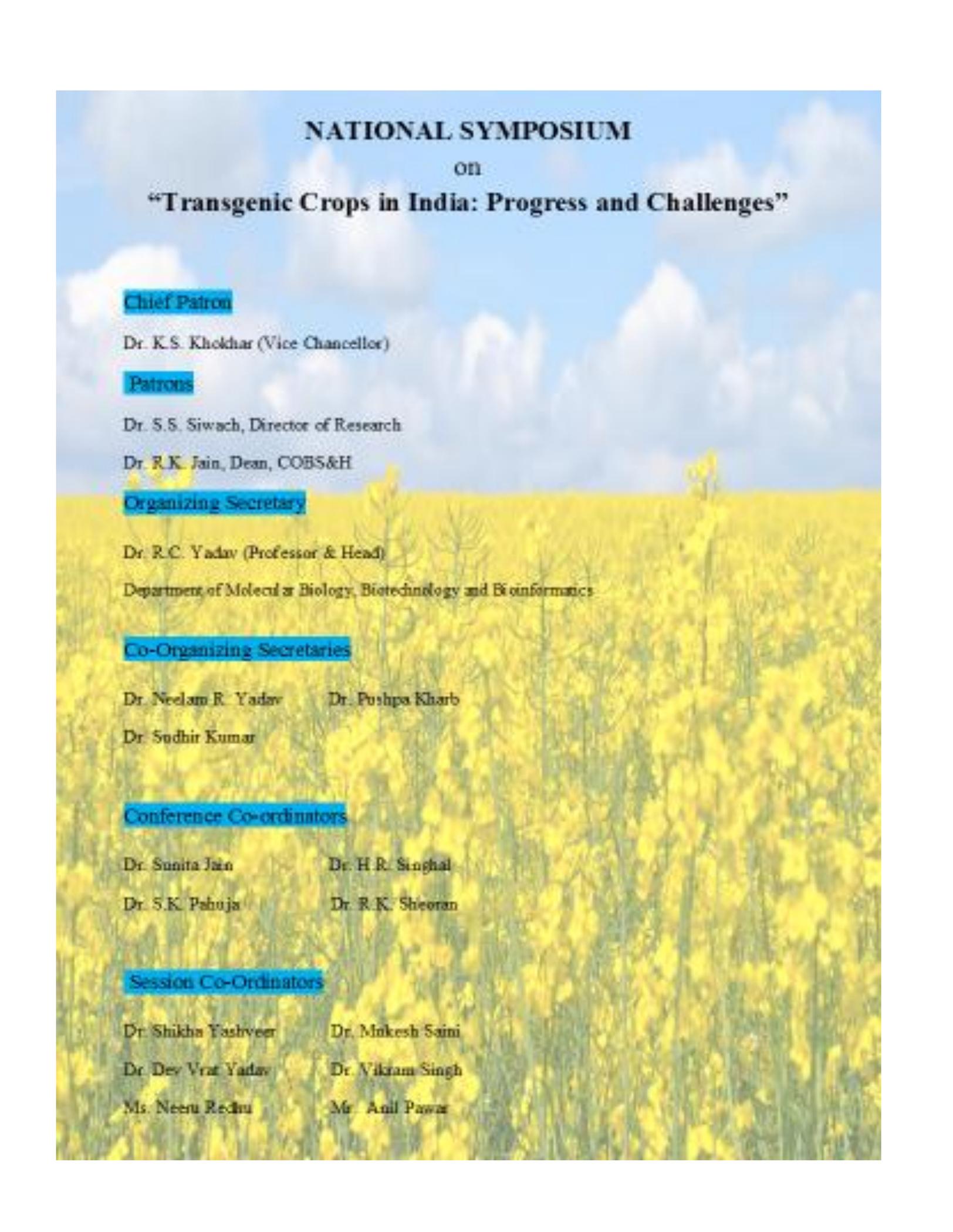


Organized by

*Department of Molecular Biology, Biotechnology & Bioinformatics,
CCS Haryana Agricultural University, Hisar*

&

Society for Plant Biochemistry and Biotechnology, New Delhi



NATIONAL SYMPOSIUM
on
“Transgenic Crops in India: Progress and Challenges”

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भारतीय कृषि अनुसंधान परिषद्
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KRISHI BHAWAN, DR. RAJENDRA PRASAD ROAD, NEW DELHI-110001



MESSAGE

I am highly pleased to know that the Department of Molecular Biology, Biotechnology and Bioinformatics, College of Basic Sciences & Humanities, Chaudhary Charan Singh Haryana Agricultural University, Hisar in collaboration with Society for Plant Biochemistry and Biotechnology (Part of Silver Jubilee Celebration of SPBB) is organizing a National Symposium on 'Transgenic crops in India: Progress and Challenges' on March 16-17, 2016.

Global population, which was only 1.7 billion at the turn of the century in 1900, is now 7.2 billion, expected to climb to 9.6 billion by 2050, and will be close to 11 billion at the end of this century in 2100. Globally, 870 million people are currently chronically hungry and 2 billion are malnourished. Therefore there is a great need to increase crop productivity, by at least 60% or more by 2050 with an improved and sustainable use of lesser resources – less land, water, fertilizer and less pesticides along with new challenges associated with climate change.

It is now evident that conventional crop technology alone will not allow us to feed the fast growing population. Biotechnology can supplement the conventional crop technology to achieve this target. The global hectareage of transgenic crops has increased more than 100-fold from 1.7 million hectares in 1996 to 181.5 million hectares in 2014 making transgenic crops the fastest adopted crop technology in recent times. This impressive adoption rate speaks for itself, in terms of its sustainability, resilience and the significant benefits it delivers to both small and large farmers as well as consumers. On an average GM technology adoption has reduced chemical pesticide use by 37%, increased crop yields by 22%, and increased farmer profits by 68%. India cultivated a record 11.6 million hectares of Bt cotton planted by 7.7 million small farmers with an adoption rate of 95% in 2014. The farm income enhanced from Bt cotton by US\$16.7 billion in the twelve year period 2002 to 2013.

Transgenic crops are essential but are not a panacea and adherence to good farming practices, such as rotations and resistance management, are a must for transgenic crops as they are for conventional crops. Therefore there is need for a balanced, safe and sustainable approach, using the best of conventional crop technology (well adapted germplasm) and the best of biotechnology (appropriate GM and/non-GM traits) to achieve sustainable intensification of crop productivity on the 1.5 billion hectares of cropland globally.

The present symposium is a timely step towards better understanding of transgenic technology and bringing further awareness in the general masses.

I wish the symposium a great success.

(J.S. Sandhu)

14th March 2016
New Delhi

**CHAUDHARY CHARAN SINGH HARYANA AGRICULTURAL UNIVERSITY HISAR,
HARYANA (INDIA)**



Prof. K.S. Khokhar
Vice-Chancellor



Message

It is a matter of immense pleasure for me that the Department of Molecular Biology, Biotechnology and Bioinformatics, College of Basic Sciences & Humanities, in collaboration with Society for Plant Biochemistry and Biotechnology (Part of Silver Jubilee Celebration of SPBB) is organizing a National Symposium on ‘Transgenic crops in India: Progress and Challenges’ on March 16-17, 2016.

World population is likely to grow to ~10 billion by 2050 with major increases in developing countries including India. Global warming and associated adverse changes in climate, temperature and precipitation, loss of productive land to rising sea levels, salinization, urbanization, soil erosion and environmental problems associated with high-input agriculture are being realized as major threats to sustainable food production. In the last several decades, plant breeding and allied agricultural sciences have led to the impressive gains in crop productivity. Many scientists feel that in major crop species such as rice and wheat a yield plateau has reached and there is a need to complement conventional breeding methods with modern biotechnological tools to break this plateau.

Genetic transformation is one of the most powerful interventions ever developed to tackle the problems of food and nutritional security in a sustainable way. Benefits of the technology include improving yield, reducing use of pesticides and toxic chemicals, decreasing soil erosion and diminishing agriculture’s carbon footprint. Commercial cultivation of transgenic crops started in the early 1990s. The main GM crops soybean, maize, canola and cotton currently under commercial cultivation are for herbicide tolerance and insect resistance. Transgenic crops are now commercially planted on about 181 million hectares in some 28 developed and developing countries. Bt cotton has been one of the widely adopted and effective transgenic crops grown worldwide. In India, Bt cotton was approved for commercial growing in 2002 and its adoption rate by the farmers is more than 90%. The country has witnessed a shift in average national fibre yield from less than 300 kg lint per hectare to 500 kg lint per hectare within 10 years of the large scale adoption of Bt cotton hybrids.

In every country, the prescribed bio-safety requirements such as food toxicity and allergenicity, cross-pollination, effect on non-target beneficial organisms etc. are to be fulfilled before a transgenic product is approved for commercialization.

The present symposium is a timely evaluation of the technology and a step towards further safe utilization of the technology for sustainable and green agriculture.

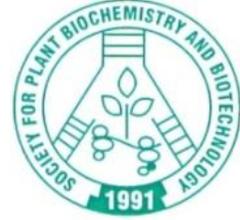
I wish the symposium a great success.



K. S. Khokhar



Dr. S.L. Mehta
President, SPPB
Ex-VC, MPUAT,
Udaipur
Ex-DDG(Edu),ICAR



I am extremely happy that under the auspices of the Society for Plant Biochemistry and Biotechnology and Chaudhary Charan Singh Haryana Agricultural University, Hisar is organizing on 16th and 17th March a National Symposium on Transgenic Crops in India: Progress and Challenges" which also happens to be part of Silver Jubilee celebration of the Society. The theme chosen is very important because in many crops yield plateau has reached and further improvement in productivity will come from use of new technology and tools. Development of transgenic crops gives hope for meeting the challenges of food and nutritional security for our increasing population. Already tremendous increase in production of cotton through use of Bt cotton seeds is well known and it has improved income of the farmers.

Indian agriculture is high influenced by climate change specially rainfall and temperature. This year almost scanty rainfall has raised alarm bells about resilience of Indian agriculture. To meet the newer challenges of increasing crop productivity and profitability, it is important to try and use all options available. Transgenic technology is one such important technology which has tremendous benefits and can help to mitigate climate change effects. However, except cotton, we at present are not able to utilize the benefits. This symposium provides opportunity to discuss the present state of knowledge and how do we move ahead keeping bio-safety of the product upfront. There is also need to properly articulate the potential of transgenic crops in proper perspective. I am confident that the galaxy of scientists who would assemble will deliberate on some of the new technology options and give recommendation which could form the basis for policy options. I am confident that under your leadership the Symposium will be a resounding success.

S.L. Mehta



राष्ट्रीय पादप जैव प्रौद्योगिकी अनुसंधान केन्द्र
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March 11, 2016

Message

This is silver jubilee year of the establishment of Society for Plant Biochemistry and Biotechnology (SPBB) and it is great to be part of the "National Symposium on Transgenic Crops in India: Progress and Challenges" being organized on this occasion jointly by SPBB and Chaudhary Charan Singh Haryana Agricultural University (CCSHAU), Hisar on March 16-17, 2016. After its introduction about two decade ago, the transgenic or GM crop technology has been deployed worldwide on more than 100 million hectares and has the potential to revolutionize crop production but it has been a subject of intense debate due to strong opposition by certain sections of the society. So far Bt-cotton has been the only GM crop allowed for commercial cultivation in India and despite the success of Bt-cotton in almost doubling the production and making India a net exporter of cotton, commercial release of GM food crops such as Bt-brinjal and GM-mustard have faced stiff opposition from within the ruling establishments. Although science academies and mainstream scientists have come out in support of the technology, some NGOs and conservative organizations have opposed the introduction of GM crops and governments have found it difficult to decide one way or the other. Although, India has an elaborate system for the biosafety evaluation and regulation for commercial release of GM crops, there is a long-standing demand for setting up of an independent Biotechnology Regulatory Authority of India for a transparent single window regulation of GM crops.

It is commendable that the SPBB has taken up this important issue for deliberation in the National Symposium and it is hoped that useful recommendations will emerge at the end of this two-day symposium. On behalf of the SPBB and on my own behalf I would like to extend heartiest welcome to all the participants of the National Symposium and thank the organizers, particularly Dr. Ram. C. Yadav and Dr. R. K. Jain for their untiring effort under the guidance of CCSHAU VC Dr. K.S. Khokhar and SPBB president Prof. S. L. Mehta. I wish the National Symposium a grand success.

(Nagendra Singh)
Secretary, SPBB



भा.कृ.अं.प.-राष्ट्रीय पादप जैव-प्रौद्योगिकी अनुसंधान केन्द्र
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डॉ. तिलक राज शर्मा

परियोजना निदेशक

Dr. T.R. Sharma

FNA, FNASc, FNAAS

JC Bose National Fellow

Project Director



March 8, 2016

Message

Crop improvement is a continuous process for increasing crop production world over. It has more relevance in present context when there is a great threat on agricultural crops because of Global Climate Change and emergence of new strains of insect-pests and diseases. Genetic engineering is one of the important and promising technologies of developing new crop varieties. It has been used extensively for the improvement of crop varieties for herbicide tolerance, insect resistance and nutritional improvement. Transgenic crops are being grown on an area of about 177 million hectare world over. In India, *Bt* cotton is the only crop being cultivated commercially since 2002 and presently cover 90% of the area under cotton cultivation. Since then, cotton production has gone up more than 2½ times. Any adverse effects of *Bt* cotton on human or animal health has not been reported. Therefore, the technology is well proven where ever it has been used.

I am happy to know that Haryana Agricultural University Hisar and Society for Plant Biochemistry and Biotechnology (SPBB) is organizing a National Symposium on *Transgenic Crops in India: Progress and Challenges* on March 16-17, 2016 as a part of Silver Jubilee Celebrations of SPBB. The topic of the symposium is very relevant in present context, because of a countrywide debate on GM crops. I am sure that some action points will come out from the deliberations during the symposium, which will be helpful for the policy makers and general public.

I wish grand success to the symposium.


(T R Sharma)

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Chaudhary Charan Singh Haryana Agricultural University, Hisar



Dr. S.S. Siwach
Directorate of Research



I am happy to know that Department of Molecular Biology, Biotechnology & Bioinformatics Chaudhary Charan Singh Haryana Agricultural University (CCS HAU) is organizing National Symposium on “Transgenic Crops in India: Progress and Challenges” on March 16-17, 2016 at CCS HAU, Hisar.

The fundamental discoveries of Darwin and Mendel established the scientific basis for plant breeding and genetics at the turn of the 20th Century. Similarly, the integration of advances in biotechnology, genomic research and molecular marker application with conventional plant breeding practices has created the foundation for molecular plant breeding, an inter-disciplinary science that is revolutionizing 21st century crop improvement. The plant breeding paradigm has been enormously successful on a global scale, leading to green revolution and the recent commercialization of transgenic crops. It is generally believed that the most of the crops especially wheat and rice have reached a plateau. The conventional methods to achieve motive of “grow more from less for more” or “per drop more crop” needs to be complemented within conventional modern technology.

Plant Breeding has a long history of integrating the latest innovation in biology and genetics to enhance crop improvement. The advances in our understanding of plant biology, the analysis and induction of genetic variation, cytogenetics, quantitative genetics molecular biology, biotechnology, nanotechnology and most recently genomics have been successively applied to further increase the scientific base and its application.

The National Symposium on “Transgenic Crops in India: Progress and Challenges” being organized will provide a platform to discuss the progress made in transgenic crops but will also discuss about the new challenges before this technology, because of this technology as it is observed that either there is a breakdown of resistance in transgenic crops or pests of minor importance have now become the major pests threatening the sustainability of the crops.

I hope, the scientists will come out with new novel ideas to thwart such fears/threats to ensure the nutritional and food security to the ever increasing population of this world.

I wish the National Symposium a great success.

(S. S. Siwach)

**CHAUDHARY CHARAN SINGH HARYANA AGRICULTURAL UNIVERSITY HISAR, HARYANA
(INDIA)**



Dr. Rajinder Kumar Jain
Dean, College of Basic Sciences & Humanities
Director, Centre of Food Science and Technology

It is a matter of great happiness that Department of Molecular Biology, Biotechnology and Bioinformatics is going to organize a National Symposium on “Transgenic Crops in India: Progress and Challenges” on March 16 and 17, 2016 at CCS Haryana Agricultural University, Hisar under the aegis of Society of Plant Biochemistry and Biotechnology, New Delhi. Application of plant breeding and allied agricultural sciences has led to ‘Green Revolution’ with a five-fold increase in food productivity in the last sixty years to feed the growing population. Scientists are now working hard for a green ‘Gene Revolution’ for sustainable food production using conventional breeding, biotechnological and genomic approaches in combination. It should be noted that we not only improve the crop productivity but also the nutritional quality, industrial utility of crop species and improve the farmers’ income.

A transgenic plant is one that contains a gene or genes which have been introduced artificially into the plant's genetic makeup using a set of several biotechnology techniques collectively known as Recombinant DNA Technology. A rapid progress has been made towards the development of transgenics in many crop species (cotton, maize, soybean, potato, brinjal, etc.). Such Biotech crops have helped not only in improving the crop productivity but also prevent significant ecological damage with fewer herbicide and insecticide sprays. In India, the first transgenic crop, Bt cotton was approved for commercial growing in 2002. In 2014, the adoption of Bt cotton in India increased to record 11.6 of 12.25 million hectares total cotton area and cotton production has been tripled from 13 million bales in 2002 to over 40 million bales in 2014. Needless to say, we must follow all the biosafety rules and regulations and deliver the right information about the biotech crops to the public, media and policy makers. There is also a need to remove the unnecessary fear and prejudices on the use of GM crops, which are as safe as their traditional counterparts.

I am sure this symposium will facilitate the active interaction among scientists, students, entrepreneurs and industrialists. Consequently, fruitful recommendations and strategies shall be worked out for future research to address the problems of sustainable agriculture.

(R.K. Jain)



Dr. R.C. Yadav
Prof. & Head
Department of Molecular Biology,
Biotechnology & Bioinformatics
COBS&H, CCS HAU Hisar

Preface

Following the rediscovery of Mendel's Laws in 1900, selective plant breeding has made dramatic progress leading to the green revolution. The global population is estimated to reach 9.6 billion by 2050, a more than five-fold rise since 1900. Conventional breeding approaches alone will not be sufficient to feed such a large populations yield plateau has reached in some of the crops like wheat and rice. Biotechnology can supplement the conventional methods to meet the global food demand. Transgenic technology offers a directed approach to target specific traits and allows that trait to be introduced in the crops. Some of the benefits of the technology include improving yield, resistance to various biotic as well as abiotic stresses, adaptation to the climatic changes and enhanced nutritional quality.

Since 1996, when GE crops were first grown commercially, their global cultivation had seen an approximately 100-fold jump making GE crops the most accepted crop technology of today. In India Bt cotton was approved for commercial growing in 2002. Since then, adoption rate of Bt cotton by the farmers in India has been increasing every year and recently it has crossed 95%. This has resulted in a very significant increase in cotton production in the country. Successful cultivation of large number of GE crops across the world indicates the farmer's acceptance. The overall trend of insecticide usage to control bollworm decreased drastically resulting in saving of insecticide sprays to control cotton bollworm. There has been a substantial increase in net income of farmers by adoption of Bt cotton.

Since the commercialization of Bt cotton in India, a number of private and public institutions have been focusing on development of transgenic crops. Utilizing the plant genomic information, genome editing has advanced the transgenic technology and can generate many new crop varieties having traits for commercialization. The present symposium is a timely platform to discuss the status of transgenic crops in India and challenges involved. The safety concerns and regulatory aspects of transgenic crops will also be discussed in this symposium

I am extremely grateful to our worthy Vice Chancellor, Dr. K. S. Khokhar, for granting permission to hold this symposium at the campus. He has always been a source of inspiration to us. The programme is being organized in collaboration of Society for Plant Biochemistry and Biotechnology as part of its Silver Jubilee Celebrations. I am thankful to the President of this society Dr. S.L.Mehta and the executive committee of the society for extending financial support to hold this symposium. I extend my heartfelt gratitude to Director of Research, Dr. S.S.Siwach and Dean, COBS&H, Dr. R.K.Jain for their constant encouragement, guidance and support in organizing this symposium. I am thankful to all the contributors for their cooperation.

I thank the members of various committees, friends, faculty, staff and students of college for making the symposium a grand success in the field of transgenic technology.

R.C. Yadav



National Symposium on *Transgenic Crops in India: Progress and Challenges*



PROGRAMME SCHEDULE

Wednesday 16th, March, 2016	
8:00 AM	Registration (Venue: Auditorium, College of Basic Sciences and Humanities)
Inaugural Function	
10:00 AM	Arrival of Chief Guest
10:05 AM	Lighting of Lamp and Saraswati Vandana
10:10 AM	Welcome Address by Director of Research
10:20 AM	About Symposium (Organizing Secretary)
10:30 AM	Release of Symposium CD
10:35 AM	Felicitation of Guests
10:40 AM	Address by Dr. N.K. Singh, (Guest of Honor), NRCPB, IARI, New Delhi
10:50 AM	Presidential address by Dr. S. L. Mehta, President SPBB
11:00 AM	Address by Dr. K S Khokhar, Vice Chancellor, CCS HAU Hisar
11:15 AM	Vote of Thanks (Dean COBS&H)
11:20 AM	Tea
March 16th, 2016	
Technical Session I	
Chairman: Dr. S.S. Siwach	Rapporteur: Dr. Pushpa Kharb
11:30 AM - 12:00N	Dr. Rakesh Tuli (UIET)-Shifting paradigms in improving crop plants
12:00N -12:30 PM	Dr. K.K. Sharma (ICRISAT)-Transgenics for legume crop improvement: from technology development to deployment
12:30 PM –1:00 PM	Dr. N.K. Singh (NRCPB) – Regulating GM crops: Is there a ban on the commercialization of GM food crop plants in India?
1:00 PM – 1:30 PM	Dr. K.C. Bansal (NBPGR) –Use of agro-biodiversity for searching novel genes for climate resilience in agriculture
1:30 PM – 2:15 PM	Lunch (Faculty Club)
Technical Session II	
Chairman: Dr. R.K. Tuli	Rapporteur: Dr. Veena Jain
2:15 PM – 2:45 PM	Dr. P.K. Jaiwal (MDU) - Development of cowpea transgenic plants resistant to MYMV through <i>RNAi</i> technology
2:45 PM – 3:15 PM	Dr. P.K. Jain (NRCPB)- Genetic engineering for RNAi mediated resistance against nematodes
3:15 PM – 3:30 PM	Mr. Nishat Pasrichaa (ICGEB) – Lectin Receptor like kinase: A key element of salinity stress tolerance
3:30 PM - 3:45 PM	Tea
Technical Session III	
Chairman: Dr. N.K. Singh	Rapporteur: Dr. Neelam R. Yadav
3:45 PM - 4:15 PM	Dr. R.C. Bhattacharya (NRCPB)- Engineering plant defense response against insect pests
4:15 PM -4:45 PM	Dr. Vibha Ahuja (BCIL) - GM crops and biosafety
4:45 PM - 5:00 PM	Dr. Surender Khatodia (Amity, Manesar) - CRISPR/Cas9 mediated virus interference in plants

Poster Session IV

Chairman: Dr. K R Koundal

5:00 PM - 6:30 PM	Poster presentation & Evaluation
6.30 PM -7.30 PM	Cultural Evening (COBS&H Auditorium)
8.00 PM	Dinner (Faculty Club)

Thursday 17th, March, 2016

Technical Session V

Chairman: Dr. K.K. Sharma Rapporteur: Dr I S Yadav

9:00 AM - 9:30 AM	Dr. K.R. Koundal (NRCPB)-Advantages and constraints of application of plant biotechnology for developing and commercialization of GM crops for increasing productivity
9:30 AM -10:00 AM	Dr. Yogesh Kumar (Monsanto)- Regulatory process for commercialization of GM crops in India
10:00 AM -10:30 AM	Dr. Ashok Chaudhury (GJU) – Generation of transgenic rice for studying Regulations of circadian clock associated genes.
10:30 AM -11:00 AM	Dr. Nand Kumar (Beejsheetal Research)Public private partnership role in deployment of GM crops in India: Call of the day
11:00 AM -11:15 AM	Tea

Technical Session VI

Chairman: Dr. Nand Kumar Rapporteur: Dr. Sunita Jain

11:15 AM-12:15 PM	Dr. Pushpa Kharb (HAU) Development of Bt chickpea and Bt pigeonpea resistant to <i>Helicoverpa armigera</i> (Huber)
12: 15 PM -12:30 PM	Dr. Raman Saini (KUK) G M Crops: Need of the hour and their safety assessment
12:30 PM -12:45 PM	Mr. Rajneesh Sharma DCRUST)
1:00 PM - 1:30 PM	Lunch (Faculty Club)

Plenary -Brain Storming Session (2:00 PM - 3:30 PM)

Chairman: Dr. G.K Garg

Rapporteur: Dr. Virendra K. Sikka

Panelists:

Dr S.L. Mehta
Dr.R.P. Sharma,
Dr. K.R. Koundal,
Dr. H.S. Nainawatee
Dr. C. Srinivasan,
Dr. T.R. Sharma,
Dr. V.K. Chowdhury
Dr. K.K. Sharma,
Dr. Nand Kumar
Dr Yogesh Kumar, Monsanto

3:30 PM

Tea Break

Oral presentations

Generation of Transgenic Rice for Studying Regulation of Circadian Clock Associated Genes

Ashok Chaudhury

Department of Bio and Nano Technology, Bio & Nano Technology Centre, Guru Jambheshwar University of Science and Technology, Hisar-125001, Haryana
Email: ashokchaudhury@hotmail.com

Circadian rhythm is an instrumental biological process that displays an endogenous, entrainable oscillation of about 24 h. These rhythms are driven by a circadian clock, which have been widely observed in plants, animals, fungi and Cyanobacterium. Plant circadian rhythms control all the major biological activities of the plant and govern subset of events such as flowering time, best time of pollination, leaf movements, germination, growth, gas/stomatal exchange, enzyme activity, photosynthetic activity, fragrance emission. Circadian rhythms occur as biological rhythms with incidence of light generated endogenously, self-sustaining and are constant over a range of ambient temperature but are subject to environmental cues. A better understanding of plant circadian rhythm in cereals, particularly, rice has tremendous applications in agriculture such as helping the farmers stagger crop cultivation and harvest thus extending crop availability and to secure against massive losses due to extreme and fluctuating weather conditions as well as other abiotic and biotic stress such as increasing salinity, drought, heat, cold and disease incidence. Rice is an important cereal crop and main food source for billions of people worldwide. Transformation of rice to introduce agronomically important genes is predicted to significantly strengthen rice breeding programs qualitatively and quantitatively which is need of the present time in view of exponentially increasing world population and limited food resources. In *Arabidopsis thaliana* Circadian Clock Associated 1 (CCA1) and its redundant homolog Late Elongated Hypocotyl (LHY) transcription factors containing single MYB domain and five Pseudo-response regulators (PRRs) have been reported by Alabadi *et al.* (2002); Mizuno and Nakamichi (2005); Nakamichi *et al.* (2005); Murakami *et al.* (2007). Constitutive expression of the CCA1 gene disrupts circadian rhythms and suppressing its own expression has been reported by Wang and Tobin (1998). It has been proposed that Timing of CAB Expression 1 (TOC1) together with CCA1 form an auto-regulatory negative-positive feedback loop at the transcription level leading to circadian rhythms by Ma *et al.* (2003). The CCA1 has been shown to directly modulate TOC1 and other downstream genes in circadian clock, photosynthesis and starch metabolism as well as for increased biomass and growth vigor by Ni *et al.* (2009) in *Arabidopsis thaliana*.

The circadian clock in plants is comprised of functionally coupled morning and evening oscillators by Jiao *et al.* (2007). In *Arabidopsis*, the two MYB transcription factors, CIRCADIAN CLOCK ACCOCIATED 1 (CCA1) and LATE ELONGATED HYPOCOTYL (LHY) together with the PRR 5, 7, 9 jointly function as a Morning Element Loop. It interlocks with an Evening Element Loop comprising of TIMING OF CAB EXPRESSION 1 (TOC1) or PRR1, GIGANTEA (GI). The Evening Element Loop also contains EARLY FLOWERING 3 & 4 (EFF3 & ELF4) as well as LUX ARRHYTHMO (LUX). The regulation of TOC1 rhythmic expression is essential for proper functioning of the circadian clock in plants. In *Arabidopsis* the oscillator coupling mainly relies on a core loop in which the evening oscillator component TOC1 is proposed to activate subset of morning-expressed oscillator genes. However, recently,

Huang *et al.* (2012) have shown that TOC1 does not function as an activator but rather functions as a general repressor of oscillator gene expression by using the chromatin immuno-precipitation followed by deep sequencing (Chip-Seq) through high resolution map of TOC1 chromatin occupancy on a genome wide scale. The repression occurs through TOC1 rhythmic binding to the circadian oscillator gene promoters. Furthermore, they have shown hormone-dependent induction of TOC1 using RNA interference plants, TOC1 prevents the activation of morning-expressed genes at night. Their study has overturned the prevailing model of *Arabidopsis* circadian clock, thereby, showing the morning & evening oscillator loops are connected through the repressing activity of TOC1. Circadian clock transcription regulation is still quite complicated and more research work is required to unfold the existing multiple feedback loops and gene expression for morning and evening oscillators in cereal crop plant species such as rice. As evidenced above the CCA1 has been shown to directly modulate TOC1 and other downstream genes in circadian clock, photosynthesis and starch metabolism as well as for increased biomass and growth vigor by Ni *et al.* (2009) in *Arabidopsis thaliana*. Keeping this in view transgenic rice plants have been generated by introducing *CCA1* gene under the control of TOC1 promoter using *Agrobacterium*-mediated genetic transformation of *Japonica* rice variety Taipei 309 in both up regulated and down regulated manner for studying regulation of CCA1 gene.

Use of agro-biodiversity for searching novel genes for climate resilience in agriculture

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National Bureau of Plant Genetic Resources is home to the 2nd largest gene bank in the world with about 4.25 lakh accessions conserved *ex situ*. However, effective utilisation of this large genetic wealth for searching novel genes for food security and climate resilient agriculture is a major concern today. In the past few years, ICAR-NBPGR has taken necessary steps to promote utilisation of germplasm. Efforts have been initiated to systematically characterise and evaluate the entire germplasm collections conserved in the National Gene Bank. In the crop season 2011-12, the entire wheat germplasm collection comprising over 20,000 accessions was characterised and evaluated for heat tolerance. Similarly, ~18,000 accessions of chickpea were characterised in 2011-12. Thus, generating suitable material for cloning of genes/QTLs for developing climate resilient crops through genetic engineering, markers assisted breeding and/or genome editing approaches. We successfully cloned several candidate genes and promoters, and functionally validated them for abiotic stress tolerance by using plant genetic resources such as wild species of *Brassica*, and land races of wheat, rice and sorghum. We cloned and validated genes coding for transcription factors (*DREB1s/CBFs*, Zinc finger proteins), signalling components (*ERA1*), effector genes (*osmotin*, *LEA4*, *APX*), and genes for osmolyte biosynthesis, and a few stress-inducible promoters (*LEA1*). Use of these genes and promoters is currently underway in various laboratories in the country for developing climate resilient transgenic crops. Further, efforts have been made using genomic approach for delineation of candidate genes regulating agro-morphological traits in chickpea, particularly pod number for improving yield. Relevance of PGRs and their utilisation for improving crop plants with regard to climate resilience and food security will be highlighted.

Transgenics for legume crop improvement: from technology development to deployment

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Grain legumes including chickpea, pigeonpea and peanut being major food crops grown in the drylands are important constituents in the diets of a large number of people in the developing countries. The growing climatic adversities have led to an overall increase in the disease and pest pressure, besides harsher abiotic stresses. Since most of these crops have a narrow genetic base, and levels of resistance to some biotic and abiotic constraints are low, their improvement becomes an overarching research-for-development challenge for maximizing the benefits to smallholder farmers. Running against the headwinds, modern genetic engineering tools and approaches have immensely benefitted to evolve sustainable solutions for various pest and diseases, besides potentials against complex problems of drought to develop future climate-ready crops for difficult environments. ICRISAT combines multiple platforms to test new approaches for developing transgenic grain legumes, specifically for constraints for which no genetic traits are available for conventional plant breeding. Our pipelines encompass a broad range of biotechnology solutions that are progressively merging to deliver effective solutions to reflect a holistic approach for our mandate legumes.

As with new emerging technologies, there are perceived potential risks that highlight the need for utmost compliance in terms of biosafety, regulatory, intellectual property, etc. during technology development. With this view, a Platform for Translational Research on Transgenic Crops (PTTC) with support from the Department of Biotechnology, Government of India, has been established that facilitates a collaborative and coordinated approach for the translation of existing genetic engineering technologies to the development of transgenic crop varieties. Governed by the regulatory policies, ICRISAT adheres to the highest international standards and follows stringent regulations and conducts research in a socially responsible way. A comprehensive appraisal of different transgenic interventions for grain legumes, highlighting the pitfalls and possible solutions through integrated approaches will be discussed.

Advantages and constraints of application of plant biotechnology for developing and commercialization of GM crops for increasing productivity

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Agricultural production is becoming more and more dependent on agrochemicals, thereby increasing input costs and causing significant damage to the environment and human health. Both farmers and consumers are at the receiving end, farmers by exposure to agrochemicals and consumers due to residues of agrochemicals in the consumed food. The challenges of malnutrition, enhanced productivity and crop diversification can be met by better resource management and by breeding more productive, more nutritious and at the same time less resource input demanding crops. Conventional technologies of agriculture are inadequate to meet the formidable challenges. Advances in modern biology, especially biotechnology, offer many advantages over traditional techniques of plant breeding. The biotechnological approach offers possible solutions to many problems and difficult targets such as biotic and abiotic stress tolerance. The genes from varied sources like bacteria, viruses and higher plants and animals have been transferred to plants to develop transgenic crops. The genomes of rice and *Arabidopsis* have been unraveled which will provide better opportunities in future to manipulate crops for developing resistance of both biotic and abiotic stresses. The development of transgenic crops expressing a variety of novel traits such as insect resistance, disease resistance, herbicide tolerance, hybrid production, improved oil quality and post harvest technology etc. have led to large scale cultivation of GM (genetically modified) crops globally. In recent years, development of transgenic crops has led to large scale cultivation of GM (genetically modified) crops which currently occupy 181.5 million hectares (2014) on a global scale in 28 countries. Thus, the application of biotechnology will go a long way in improving the genetic potential of crop plants for enhancing the crop productivity.

However, there are several constraints and apprehensions regarding genetically modified food crops. These include toxicity, allergenicity, carcinogenicity, use of antibiotic resistance genes and nutritional value. The most of the environmental concerns about transgenic crops have derived from the possibilities of gene flow to close relatives creating super weeds or causing gene pollution among other crops. There is a need of thorough risk assessment of the issue of public concern on safety especially with respect to animal and human health and environment. Biosafety regulations cover assessment of risks and the policies and procedures adopted to ensure environmentally safe applications of biotechnology. These rules and regulations cover the areas of research as well as large scale applications of GMOs and products made there from throughout India. The field trials for agronomic evaluation should be made against the best possible alternatives for the problem for which GM technology is used and after assessing the socio economic and ecological impact of the technology. The Supreme Court has suggested moratorium of at least 10 years on commercial release of all GM crops till all the systems are in place for independent research and regulation. There is a need to streamline and harmonize the regulatory requirements for deployment of genetically engineered plants and farmers should be free to choose what kind of technologies they want to adopt for sustainable crop production.

We must ensure that society will continue to benefit from the vital contribution that plant breeding offers, using both conventional and biotechnological tools. The GM technology coupled with tissue culture approaches should be used to enhance the crop productivity and farmer income.

Regulating GM crops: is there a ban on the commercialization of GM food crop plants in India?

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Dilemma over acceptance of GM food crops continues as GEAC once again last month deferred a decision on allowing commercial cultivation of GM Mustard DMH-11 developed by Centre for Genetic Manipulation of Crop Plants at Delhi University. It revived the memories of Bt-brinjal, whose commercial approval is on hold since 2009. As of now Bt-cotton is the only GM crop released for cultivation by the Indian farmers. As such there is no ban on the research and development of GM crops in the country but there is strong opposition by some sections of the society and public awareness on the subject is very low. A PIL has been filed in the honorable Supreme Court of India seeking moratorium on the field-testing of GM crops but the case is not decided as yet. The technical expert committee (TEC) constituted by the court was also divided in opinion and submitted two separate contradictory reports, hence there is need to reconcile the recommendations of the two reports before the court can reach a decision. In addition, parliamentary standing committee on agriculture has also submitted a report, which is very critical of the GM crops. On the other hand the science academies of India including NAAS have advocated the deployment of GM technology for enhancing and sustaining crop production. A bill on setting up of 'Biotechnology Regulatory Authority of India', a transparent independent single window regulator for the GM crops was introduced in the Indian parliament but it has expired and needs to be reintroduced. Meanwhile, with the existing regulation it is possible for GEAC and Ministry of Environment, Forest and Climate Change to take decision on a case-by-case basis. This presentation will provide a brief review of these developments and present status of regulation of GM crop commercialization in India.

Public private partnership role in deployment of GM crops in India: Call of the day

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Innovative, complementary and synergistic public-private sector partnerships are becoming an increasingly important way for developing countries to move a step closer to exploiting the benefits of modern biotechnology. Genetically engineered crops initially were developed to benefit large scale commercial outfits. More than 50% of the global area under cultivation is in developing countries. The uptake of genetically modified (GM) crops by farmers in both developed and developing countries has been one of the most spectacular examples of adoption of a new technology in recent global agriculture. Many critics of biotechnology argue correctly that the root cause of hunger is poverty and not the non-availability of food. To overcome the problem of poverty in developing countries where large numbers of people rely either directly or indirectly on agriculture, the productivity of small scale needs to be increased as large numbers of agrarian societies are concentrated in rural sector and their livelihoods are based on agriculture. Deploying new technologies like biotechnology would be crucial for poverty reduction; this can also enhance the growth and employment in rural non-farm sector.

India cannot totally depend on the public sector for development of genetically modified (GM) products to feed its large population; the private companies have to play a role. Public-private partnership for joint development and ownership of GM food crop products is necessary to introduce the biotechnology based products for the benefit of small scale farmers. "The government needs to take a long term vision for a practical solution. Big questions will be asked if private players come into the picture but participation of indigenous private sector is needed to encourage the GM crop regime in the country," Since GM products are almost entirely from multinational companies because Indian seed companies are smaller in terms of their R&D expenditure, this also affects public confidence, particularly among farmers.

Initiative taken in the direction of public private partnership BeejSheetal Research Pvt. Ltd. (Earlier known as Bejo Sheetal Seeds Pvt. Ltd.) has licensed Bt Brinjal event 142 from Indian Agricultural Research Institute, Pusa New Delhi and transferred the Bt gene in two Brinjal hybrids Janak and BSS793. As per the regulatory authorities, Govt. of India guidelines, Biosafety studies have been completed including two times Biosafety Research Level-I (BRL-I) field trials. Recently GEAC has given permission for BRL-II trials. However although technology is developed by public sector & products are developed by private sector, the involvement and support for regulatory clearance from public sector remains limited. Models need to be worked out for Public private partnership for successful deployment of the technology for farm level application. More rigorous efforts are needed from both public and private sector to speed up the regulatory clearances, so as to make available the products in pipeline. Many novel technologies are ready developed by both public and private sector at research level. These technologies can change the scenario of Indian agriculture if they are made available for farmers after proper evaluation for Environment & food safety.

Lectin receptor like kinase: A key element of salinity stress tolerance

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All-important biochemical reaction in a cell is regulated by kinases. Kinases are the major player in the cell signalling pathways. Lectin receptor-like kinases (*LecRLKs*) family proteins are the important candidates those are involved in various signalling pathways and majorly control the cell physiology. Plant lectin receptor like kinases (*LecRLKs*) genes are majorly intron-less with few exceptions. LecRLK proteins are transmembrane proteins which are divided into three domains- amino-terminal extracellular lectin domain, hydrophobic transmembrane domain and carboxyl-terminal intracellular Ser/Thr kinase domain. Till date various reports have been claimed that LecRLK genes are involved in biotic and abiotic stress tolerance, still in crop plants such as rice it is poorly studied family. In this study, we report the importance of LecRLK genes in salinity stress tolerance. Salinity is a major abiotic stress to crop production that will result into 30% land loss within next 25 years and up to 50% land loss by the middle of 21st century. Microarray analysis revealed the involvement of LecRLK genes in salinity stress. Expression analysis of selective putative LecRLK genes by real-time PCR also confirmed their possible role in salinity tolerance. Microarray and expression analysis by real-time helped in selection of *OsLecRLK* gene from *Oryza sativa* which may have played key role in salinity tolerance in rice plant. In this study, overexpression and downregulation lines of *OsLecRLK* gene were used for a comparative analysis under the salinity stress conditions. Confocal microscopic study of root for the localization of sodium and calcium ion of these lines give a comparative result which strongly suggest that *OsLecRLK* overexpression decrease the sequestration of sodium and calcium ion in roots and provide salinity tolerance. Other physiological tests such as leaf disc assay and chlorophyll estimation under different stresses suggest the key role of *OsLecRLK* in abiotic stress tolerance. We are also trying to suggest the mechanism pathway for *OsLecRLK* gene with the help of its interaction partner study by yeast-2-hybrid method. The function of all the identified interacting partners and their role in tolerance is yet to be predict. This study will lead us to dissect the molecular mechanism of LecRLK genes in stress tolerance and detailed study of this gene may provide us a strong player in salinity stress tolerance in crop plants.

OP 08

Genetic engineering for RNAi mediated resistance against nematodes

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OP 09

Development of cowpea transgenic plants resistant to MYMV through RNAi technology

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Molecular characterization and resistance evaluation of *Bt* chickpea and *Bt* pigeon pea plants against the major pest, *Helicoverpa armigera* (Hübner)

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Transgenic plants of the chickpea (*Cicer arietinum* L.) cv. C-235 expressing *cryIAa₃* gene were generated by tissue culture independent, rapid and efficient transformation method (Patent No. 252590) using *Agrobacterium tumefaciens* strain EHA105 harboring pBIN-35*ScryIAa₃*. Transgenic plants of the chickpea cvs. C-235 and HC-1 expressing *cryIAc* gene were also generated by the same method using *Agrobacterium tumefaciens* strain LBA4404, harboring pBIN-35*ScryIAc*. Transgenic pigeon pea [*Cajanus cajan* (L) Millsp.] plants of variety Manak (H 77-216) expressing *cryIAc* gene were generated by the same method using *Agrobacterium tumefaciens* strain LBA4404, harboring pBin-35*ScryIAc*. The transgene integration and expression was tested by PCR and ELISA, respectively. Southern analysis revealed integration of a single copy of the transgene in both chickpea and pigeon pea transgenic plants. The single copy integration was also confirmed by Real Time PCR assay with SYBER green chemistry. Quantitative assessment of Bt Cry toxins by ELISA in transgenic pigeon pea and chickpea plants showed variable level of expression of the inserted *cry* gene. Bioassays against major pests of the chickpea and pigeon pea, *Helicoverpa armigera* revealed T₄ plants perform well against the larvae with larval mortality up to 87 per cent.

Regulatory framework and commercialization prospecting for food production in India

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The basic technical terminology defines the term 'transgenics', as an organism/living entity that has been modified with genetic material from another organism. The means for accomplishing this genetic modification are involve insertion of DNA into an embryo with the assistance of a virus, plasmid or a gene gun. This embryo in the course of its development, allows the expression of the DNA that has been inserted into its genome. The most common reason for an emerging interest in the development of a transgenic organism is its potential exploitation in agriculture, where for instance the development of genetically modified crops has led to a number of advancements with respect to the manner in which agriculture is practised world over. A FAO report of 2009 estimates that by 2050 the world would be requiring more than 60 % more food, feed and biomass from the same amount of land. The pressure of rising population, income, dietary preferences and urbanization in developing countries such as India would require extraordinary steps to meet such huge demands by substantially increasing the crop yield and optimizing the use of input resources. It is here that the transgenics and their commercialization hold the key. There is some controversy over the practice of genetic modification. Some advocates are concerned that interbreeding between transgenic and wild organisms could have unforeseen consequences, and others worry that consuming things like transgenic organisms could be dangerous. However, at the same time on account of ethical considerations despite the solution being within our reach, the picture does not appear to be too good. It is here that the need for a regulatory framework that strikes a right balance becomes significant. The present study aims at analyzing the need for same.

Shifting paradigms in improving crop plants

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Through the last about 30 years, the knowledge of genes and their functions has increased at an unprecedented pace. The progress has led to understanding how a very large number of genes function in a complex network of pathways to determine the traits that define field performance of crop plants. This has also revealed to molecular biologists the overwhelming gaps in knowledge that need to be filled before any genetic perturbations can be designed to successfully achieve the targeted changes in crop plants. Such complexity results in the limitations faced in developing crop plants with higher agronomic performance and safety.

A higher order of creativity is required of our scientists to evolve game changing technologies themselves, rather than perennially catch up with the leads, tools and approaches developed and patented elsewhere. The science departments are required to take clearer decisions to identify selected missions for developing breakthrough technologies, and their applications for enhancing food and nutritional security. Once identified, the government needs to evolve clearer policies and ensure timely and sufficient support to the mission teams, rather than watch them find a place for themselves in unlevelled playing field, amidst multinational technology developers and global seed companies. While India is still struggling to position itself with respect to the regulatory issues related to GM crops, recent developments in genome editing are already suggestive of a new framework that may get facilitated for rapid crop improvement. We need to catch up with the shifting of the era of "transgenic or genetically modified crops" to "genetically edited" crops, which are expected to be safer and more assuring than RNA interference or even marker assisted selection

The above would be illustrated by two examples of researches from my laboratory. One is the case of developing Bt-crops, specially Bt-cotton by our group at National Botanical Research Institute at Lucknow. Despite very innovative research on plant promoters and the development of the first indigenous gene against a herbivorous pest, the novel technology could not succeed in market. Of the 6 events of Bt-cotton released in India since 2002, by private or public sectors, all have failed, except the Bollgard II by Monsanto, which now covers 96% of cotton cultivated in India. This is a model example for the government and other stakeholders to introspect and make sure that we do not fail our researchers again. Some details of an exemplary technology developed recently by us against sucking pests will be presented. This is the next opportunity at threshold to succeed or fail, depending upon how strategically our ecosystems manage indigenous innovations.

The second research failure, already becoming visible in the country, is in the area of genome editing for crop improvement. This tool has been applied to eukaryotes only in the last 4 years. It received "Breakthrough of the Year Award, 2015" by the AAAS journal Science. The glaring example is our widely cited contribution of the application of CRISPR-Cas technology in 2013. Ours was one of the first seven global publications in this area: all published in 2013. The development could have been adopted by India, as a game changer mission for future crop improvement. We reported

from National Agri-Food Biotechnology Institute, Mohali, how sequence- specific editing of genes can be achieved in wheat and tobacco (GGG, 2013). As of now, no other publication from India has reported any original contribution to this area in plants or animals. No mission mode application of the technology to crop plants has been supported in the country. The technology has meanwhile been protected by nearly 300 patents globally and has received funding of several hundred million dollars during the last less than 4 years! Success with rice, tomato, sorghum, maize and sweet orange has been reported by several labs elsewhere. The paradigms to crop improvement are shifting; we need to take decisions faster.

GM crops – Need of the hour and their safety assessment

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The present paper focuses on need of GM crops and various studies related to safety and risk assessment of GM crops by taking examples of BT- cotton and Brinjal. The target of enhanced yield of food grains has been achieved somehow in first “Green revolution”. But with the passage of time this growth has associated with several problems such as environmental pollution, health hazards, loss of biodiversity and decline in nutritional security as well as has reached at its almost peak with stagnancy in production. The problem of food security along with malnutrition is still stood as such in front of us. Today around 800 million people cannot afford two course of meals and about 30,000 people, half of them children, die *every day* due to hunger and malnutrition. The food demand is lagging behind the production due to several of the factors, one of the important among them is continuously over increasing population. Today, the only way to combat the scarcity of food is the involvement of biotechnology as a major partner to other strategies of crop improvement. Today, GM crops are growing with their great success over their non GM counterparts and without much chemical inputs in sustainable manner. Till to date, a large number of transgenic plants for quantitative and qualitative growth in food have been formed and some are under field trials. Even having several advantages GM crops are still not widely accepted, the major concern in it is their safety. But in reality, there are safety and risk assessment guidelines for GM plants and can be stated with confidence that no other food technology has gone through such rigorous safety testing procedures as biotech derived crops. Traditionally hardly anything what we eat today has been assessed for food safety so much. In conclusion, we must have to think again about GM crops prior to say NO.

OP 14

Engineering plant defense response against insect-pests

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The CRISPR/Cas9 mediated virus interference in plants

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The Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) associated Cas9 nuclease and guide RNA system is adopted as a novel targeted genome-editing technique. Cas9 protein is an RNA guided endonuclease which creates double stranded DNA breaks targeted by a 20bp guide RNA sequence to confer recognition of the target sequence. The CRISPR/Cas system of genome editing termed as RNA-guided engineered nucleases (RGENs), which were used as sequence specific nucleases for precise genetic modifications. Recently CRISPR/Cas9 has been transferred to plants for a fundamentally different approach to engineering resistance against geminiviruses, a damaging family of DNA viruses. Geminiviruses has double-stranded viral DNA phase which could be targeted by the single guide RNAs to Cas9 endonuclease to inhibit viral infection. These gRNAs could be engineered for targeting open reading frames encoding the viral Rep and coat CP proteins as well as the conserved non-coding intergenic region, the origin of replication. CRISPR–Cas system has great potential for conferring plant immunity, as it can be used to simultaneously target several sites at viral genomes and/or different viruses in the same plant. An approach to develop a platform based on CRISPR system for genome editing technology in mungbean is described. Genome editing can be used to allow resistance to Mungbean Yellow Mosaic virus infections by development of a Genome Editing (GE) system using CRISPR-Cas9 system in mungbean and to test the system in inducing genetic resistance to MYM virus infections. This system can be evaluated for the phenotypic resistance to viruses and to gain insight into gene regulatory networks of plant resistance. The simplicity and robustness of this new innovative technique has made it a routine method for simplistic genetic manipulations, which could facilitate the adaption of this platform to the improvement of important traits in other specialty crops.

GM crops and biosafety

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Genetically modified (GM) crops also referred as genetically engineered (GE) crops or transgenic crops have been developed to incorporate various traits such as insect pest resistance, herbicide tolerance, disease resistance, altered nutritional profile, enhanced storage life etc. The benefits of their use include increased crop yields, reduction in farm costs and thereby increase in farm profit as well as protection of the environment. The range of crops being targeted for genetic improvement include several commercially important crops such as maize, soybean, tomato, cotton, potato, mustard, rice etc.

Though more and more GM crops are being released for field-testing and commercialization, concerns have been expressed regarding the risks arising from their use due to potential risks to both human health and environment. These apprehensions arise because GM technology crosses the species barrier as compared to classical selection techniques, thereby permitting the gene transfer among microorganisms, plants and animals. There is no evidence that any unique hazards exist in the development of GM crops, because of novel combinations of genes. It is also not true that all GM crops are toxic or are likely to proliferate in the environment. However, specific crops may be harmful by virtue of novel combinations of traits they possess. This means that the concerns associated with use of GMOs can differ greatly depending on the particular gene-organism combination and therefore a case-by-case approach is required for assessment of safety concerns.

Safety concerns have led to the development of regulatory regimes in various countries for research, testing, safe use and handling of GM crops and their products. In India, the Ministry of Environment, Forest and Climate Change (MoEFCC) has notified the rules and procedures for the manufacture, import, use, research and release of GMOs as well as products made by the use of such organisms in 1989 under the Environment (Protection) Act, 1986. These rules and regulations, commonly referred as Rules 1989 cover areas of research as well as large scale applications of GMOs and its products. These Rules and Guidelines are implemented by the MoEF&CC and the Department of Biotechnology DBT, Government of India.

These rules also defined the competent authorities and composition of such authorities for handling of various aspects of the rules. Presently there are six Competent Authorities as per the rules as indicated below:

- (i) Recombinant DNA Advisory Committee (RDAC)
- (ii) Institutional Biosafety Committee (IBSC)
- (iii) Review Committee on Genetic Manipulation (RCGM)
- (iv) Genetic Engineering Appraisal Committee (GEAC)
- (v) State Biotechnology Coordination Committee (SBCC)

(vi) District Level Committee (DLC)

RDAC has an advisory role, IBSC, RCGM and GEAC are involved in approval process of LMOs/GMOs and SBCC and DLC have monitoring functions.

India is also a signatory to the Cartagena Protocol on Biosafety, an international agreement under the Convention on Biology Diversity (CBD) for ensuring the safe transfer, handling and use of GMOs subject to transboundary movement.

Commercial GM crop production is the final stage of a four step process. The first step begins in government and private sector laboratories and greenhouses, where scientists investigate potential biotech traits and undertake genetic transformations. If these lab results are successful, the plant may advance to the second step i.e. open field trials, where breeding and testing continue in a real life environment. The third step to commercialization is securing regulatory approval in a country where the plant will be grown, and/or consumed by humans or animals. The fourth and final step is market acceptance and widespread production.

Safety assessment of a GM crop is the most important step in this development process. Extensive testing and a long approval process accompany every GM crop introduction. The approval process includes comprehensive risk analysis to ensure food, feed and environmental safety before entering the market place.

Several public and private sector institutions are involved in the research and development of GE plants in India. More than 20 plants with varying traits such as hybrid seed production, insect resistance, herbicide tolerance etc. are under various stages of field trials and safety assessment.

The presentation will provide an overview of transgenic crops and biosafety requirements in India

Regulatory process for commercialization of GM crops in India

Yogesh Kumar

Monsanto India Limited

Poster Presentations

High throughput phenotyping has a central role for engineering abiotic stress tolerance

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Crop plants are affected by a variety of abiotic stresses which are the primary cause of yield losses worldwide. Transgenic technologies are generating large numbers of GM crops for stress tolerance. For future work on generating transgenic plants with higher levels of tolerance, the new knowledge may be used via guided genetic engineering of multiple genes to create high yielding stress tolerant varieties. The genotyping of transgenic populations is now highly efficient. However, due to non-distinct and complex mechanisms involved in the responses of plants to abiotic stresses, including salinity, drought, extreme temperatures, and nutrient deficiencies, phenotyping these populations is highly laborious, time consuming and often a big challenge. The increasing power of digital imaging and computational technologies offers the opportunity to relieve this phenotyping bottleneck via High Throughput Phenotyping (HTP). HTP is a key to deliver a strong identification of one or more positive candidates extracted from a pool of 10^3 to 10^8 possible candidates based on specific criteria, hence provides -omic-scale phenotyping of large populations of plants. It integrates technologies such as photonics, biology, computers, and robotics to receive the full benefit of the available genomic information. These reliable, high-throughput phenotyping tools will give plant scientists new insights into the information encoded in the plant genome. The application of this technology in transgenic crops provides opportunities to significantly increase abiotic stress tolerance.

Genetically Engineered Rice with high levels of Iron and Zinc

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According to the World Health Organization, Micronutrient deficiencies or “hidden hunger” affect about 38% of pregnant women and 43% of preschool children worldwide. Fe deficiency is the most extensive form of malnutrition and a leading cause of anemia in women and children. Zn deficiency causes stunting and has serious consequences for health, particularly during childhood. Polished rice grains generally contain only about 2 micrograms of Fe and 16 micrograms of Zn per gram. With limited variation in grain Fe content across the rice gene pool, conventional breeding efforts have fallen short of reaching 13 micrograms of Fe and 28 micrograms of Zn per gram of polished rice to fulfill 30% of the estimated average requirement (EAR) in humans. The genetically engineered rice has significantly increased levels of Fe (up to 15 micrograms) and Zn (up to 45.7 micrograms) per gram of polished rice that human cells can potentially absorb. Genes used—nicotianamine synthase from rice and ferritin from soybean—that together produce high-micronutrient grains. Introduced the genes to a rice variety, IR64, and bred these into other popular indica varieties, the world’s most widely grown type of rice from South and Southeast Asia where Fe and Zn deficiencies are prevalent. This significant increase in Fe and Zn levels was achieved through rigorous gene optimization, large-scale plant transformation.

Morphological Analysis of T1 Transgenic Progeny Plants Harboring *CCA1* Gene under Green House

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In higher plants, circadian rhythms regulate various biological processes including metabolism, photosynthesis, onset of flowering, seed setting, water uptake, hormones, light and dark response to environmental cues. Studies in *Arabidopsis thaliana* have found that suppression of Circadian Clock Associated 1 (*CCA1*) gene and altered circadian clock rhythms may be a source of increased biomass and growth vigour. Independent lines derived from *Agrobacterium*-mediated genetically transformed Japonica rice variety Taipei 309 T0 seeds harbouring circadian clock gene constructs A, B & C for up-regulation and down-regulation of *CCA1* genes were germinated in Transgenic Green House and maintained at 25-30 degrees Celsius under standard agronomic management practices in plastic pots having sand, soil, peat and vermiculite and maintained in trays on standing water in rice growing season. Out of the three gene constructs, construct A is for over expression and other two i.e. B and C are RNAi constructs for down regulation of *CCA1* gene. In present study attempts were made to record data of morphological traits such as number of tillers per plant, number of panicles per plant, plant height, seeds per panicles in T1 progeny of transgenic rice variety Taipei 309 so as to monitor the morphology and growth characteristics. Progeny plants derived from construct B and C showed better seed yield, increased grain weight, increased plant height (66.05 cm) and number of full grain/panicle (90 seeds) as compared to construct A. Construct A shows lowest grain yield, number of panicles, full grain/panicles. PCR analysis of progeny plants showed presence 324bp band for *hyg* gene. Further analysis of transgenic plants and regulation of *CCA1* gene is under progress.

Improving salt tolerance in wheat (*Triticum aestivum* L.) by transgenic approach

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Salinity is one of the important environmental stresses influencing the crop productivity and quality of economically important crops. Molecular genetics and functional genomics provide a new opportunity to synthesize molecular and physiological knowledge to improve the salinity tolerance of plants relevant to food production and environmental sustainability. Development of salt tolerant transgenic plants through the use of bio-molecular techniques to selectively introduce desired genes may provide alternative ways to achieve salinity tolerance. These techniques will benefit the development of salinity-tolerant cultivars based on specific traits. Over-expression of the vacuolar Na⁺/H⁺ antiporter compartmentalise Na⁺ in the vacuole, where Na⁺ has little chance of toxic effect on metabolism, or to be transported to younger leaves. Studies indicate great potential for transgenic methodology. An important consequence of salt stress in plants is the excessive generation of reactive oxygen species (ROS) such as superoxide anion (O^{•-}), hydrogen peroxide (H₂O₂) and hydroxyl radicals (OH[•]) by a number of metabolic pathways. Plants possess a number of antioxidant enzymes like superoxide dismutase (SOD), ascorbate peroxidase (APX), glutathione reductase (GR), and peroxidase (POX) for protection against the damaging effects of ROS. An experiment was conducted to evaluate the effect of salt stress on two wheat varieties viz. HD 3086 and KRL 210 grown under different salinity levels (8 dS/m and 12dS/m). The leaf samples were collected and analysed for various antioxidative enzymes and non-enzymatic metabolites at 0, 10 and 20 days. HD 3086 suffered greater damage to cellular membrane due to lipid peroxidation as indicated by higher accumulation of MDA and greater leakage of electrolytes than KRL 210.

Genetically modified crops in India with reference to Bt cotton: Pros and cons

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Since time immemorial, human beings have modified nature's organisms for usage in agriculture. Novel technologies have enhanced this trend: recombinant DNA technology allows biotechnology firms to insert DNAs into plant genomes, thereby creating plants that express the desired traits. Use of such genetically modified organisms (GMOs) has prompted controversy, especially for its role in ensuring food security. Since health concerns in case of genetically modified crops remains a major issue, therefore, several surveys were conducted to study their impact on environment, health and the soil. It was revealed that Bt cotton gives higher yield than conventional varieties of cotton and led to improvement of economic condition of farmers. On contrary Bt cotton does have an adverse effect on health, environment and soil. However, most of the farmers are satisfied with Bt cotton and are ready to accept other GM crops. Research agenda needs to address constraints associated with Bt cotton hybrids considering the near and medium term futuristic needs of the region including development of technology to maintain proper plant stand of Bt hybrids. Maximum benefit will be derived if robust plant breeding and crop management programmes have ready access to all the modern crop biotechniques, both transgenic and non-transgenic, to address food security issues.

Optimization of genetic transformation of finger millet (*Eleusine coracana*) using *Agrobacterium* containing *gus* gene construct

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Finger millet (*Eleusine coracana*) is the primary food source for millions of people in tropical dry land regions of the world. It has nutritional qualities superior to that of rice and is on par with that of wheat. Development of efficient tissue regeneration and transformation system is an essential prerequisite for successful production of transgenic plants. In the present study mature seeds of *Eleusine coracana* GE-3885 were used for callus induction and plant regeneration and transformation. For callus induction Murashige and Skoog (MS) medium supplemented with different concentrations of NAA alone and in combination with BAP, 2, 4-D and Kinetin were used. The *Agrobacterium* strain LBA4404 harbouring binary vector Pcambia1301, which contained hygromycin phosphotransferase (*hptII*) as selectable marker gene and β -glucuronidase (GUS) as reporter gene, was used for optimization of transformation conditions in embryogenic callus. The optimal conditions for the *Agrobacterium*-mediated transformation of finger millet were found to be the co-cultivation of embryogenic calli on exposure for 30 min to agrobacterial inoculums supplemented with 100 μ M acetosyringone. After 4 days of agrobacterial infection, antibiotic hygromycin resistant embryogenic calli were obtained on the selection medium. The results of the GUS histochemical assays indicated that the GUS gene was expressed in the *Agrobacterium* infected embryogenic calluses. Different concentration of cefotaxime and hygromycin were used for the selection of transformed cells. This study underpins the introduction of numerous agronomically important genes into the genome of finger millet in the future.

Potential of Quantitative Trait Loci for biofortification in wheat

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The rapid development of DNA marker technology provides great opportunities to enhance nutritive values of traditionally cultivated crops and grains. Molecular markers augment conventional plant breeding for efficient and precise identification or selection of a trait of interest linked to them. They are used in assessment of genetic variability and characterization of germplasm; estimation of genetic distances between populations, inbreds and breeding materials; genetic mapping; detection of monogenic and quantitative trait loci (QTLs); marker assisted selection; increase the precision of backcrossing to introgress desirable traits from distantly related varieties to elite germplasm; identification of sequences of useful candidate genes, etc. Depending on the traits and the site, the QTL accounted for 12–81% of the genetic variation. A major locus GPC-B1 (250 kb-locus), mapped as a simple Mendelian locus associated with increased protein, Zn, and Fe from *T. dicoccoides*, encodes a NAC transcription factor (NAM-B1) that accelerates senescence and increases nutrient remobilization from leaves to grain. 6B and 5B chromosome substitution lines were higher in content (total amount) of Zn and Fe per seed, respectively. In a study, eighty two QTLs were mapped for 10 different minerals with most of the positive alleles contributed by a cross between wild emmer x durum wheat and many QTLs mapped to homoeologous positions, reflecting synteny between the A and B genomes. These QTLs can be exploited for increasing nutrient efficiency in our elite germplasm.

Salinity stress alleviated by the addition of supplemental Ca^{2+} on different physiological parameters of tomato (*Solanum lycopersicum*)

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Salinity is a major abiotic stress that adversely affects plant growth and productivity. In arid and semi arid lands the plants are subjected throughout their life cycle to different stresses. Some of these plants can tolerate these stresses in different ways depending upon plant species and type of stress. Salt stress affects some major processes such as germination, root/shoot dry weight and Na^+/K^+ ratio in root and shoot. Among most of the vegetable crops tomato are the most important horticultural crop worldwide and grown on over 4 million hectares of land area. Tomato is cultivated for its fleshy fruits and it is called as protective food because of its special nutritive value and its wide spread production. Salinity is one of the most severe abiotic factors in agricultural production. Calcium is an essential plant nutrient and has a role in metabolic activities, like stabilization of membranes, signal transduction through second messenger and control of enzyme activity. Calcium ion is not only an essential structural element that strengthens plant cell walls and membranes but also, is a well-known secondary messenger in cell signaling processes. The adverse effects of salinity on plants might be alleviated by the addition of supplemental Ca^{2+} in the growth medium. This is due to the ability of Ca^{2+} in decreasing the influx of Na^+ and the efflux of K^+ through the inhibition of non-selective cations and outward rectifying K^+ channels. Calcium has a role in building salt tolerance in plants. Adequate levels of calcium are necessary for the membrane to function normally. Supplementary Ca has been shown to significantly ameliorate the negative effects of salinity on plant growth and fruit yield. Concentrations of Calcium in these plants will be increased by increasing the external Calcium supply under saline conditions.

Enhancing water stress tolerance in wheat by arbuscular mycorrhizal symbiosis: an approach supplementing transgenics

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Drought, one of the most frequent and severe abiotic stress factors, limits plant growth and crop productivity. Molecular genetics and functional genomics provide a new opportunity to synthesize molecular and physiological knowledge to improve the drought tolerance of plants relevant to food production and environmental sustainability. Development of drought tolerant transgenic plants through the use of bio-molecular techniques to selectively introduce desired genes may provide alternative ways to achieve drought tolerance. These techniques will benefit the development of drought-tolerant cultivars based on specific traits. A pot experiment was conducted to examine the effects of arbuscular mycorrhizal fungi on antioxidative system of two varieties of wheat (*Triticum aestivum* L.) i.e. WH 1025 and WH 1105. The plants were grown under well water and water stress conditions. The seeds were treated with and without arbuscular mycorrhizal (AM) fungi before sowing and water stress was created by withholding irrigation at jointing and heading stage. The antioxidant enzymes were estimated in roots of water stressed and control plants. The activities of antioxidative enzymes viz superoxide dismutase (SOD), ascorbate peroxidase (APX) and glutathione reductase (GR) in tolerant and susceptible variety under water stress of mycorrhizal wheat plants increased significantly compared to non-mycorrhizal ones. It was found that drought tolerant and drought susceptible varieties showed the different response under drought conditions. WH 1105 suffered greater damage to cellular membrane due to high level of reactive oxygen species (ROS) as indicated by hydrogen peroxide (H₂O₂), superoxide radical (O⁻) and malondialdehyde (MDA) content during stress conditions. But it was observed that the level of ROS was found less in susceptible genotype WH 1105 when treated with AM fungi but it was more than tolerant variety WH 1025 treated with AM fungi. Basal level of antioxidative system was found higher at heading stage under all conditions. Results showed that under water stress conditions, mycorrhizal inoculation significantly decreased the H₂O₂, superoxide radical and MDA content and enhance the activities of antioxidative enzymes viz. SOD, APX and GR and metabolites ascorbate and proline both in tolerant and susceptible varieties at both growth stages of plant. Activity was higher in tolerant variety than susceptible variety under water stress. Hence, overall results suggest that mycorrhizal symbiosis play a vital role in combating drought stress by enhancing the activities of antioxidative enzymes and decreased the ROS content thus may be an approach to transgenics for drought tolerance.

Floral Micro Injection: A high efficiency *in-planta* transformation method for *Brassicajuncea*

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For the development of value added transgenic crops, plant transformation procedure is a critical step. Various chemical, physical and biological methods of plant transformation are employed to get optimal transformation efficiency. *Agrobacterium* mediated *In-planta* transformation methods are widely appreciated for being simple, less laborious and have high transformation efficiency in comparison to plant tissue culture based methods. However the applicability of these *in-planta* methods is rather restricted, with maximal successful attempts being reported in *Arabidopsisthaliana*. Hence, there is a need to extend the application of *in-planta* transformation method to other crops. In the present study, we report an *Agrobacterium* mediated *in-planta* transformation method for transformation of *Brassicajuncea*. We have successfully transferred monodehydroascorbate reductase (*mdar*) gene in *Brassica juncea* using this high efficiency protocol. Monodehydroascorbate reductase gene, coding for an antioxidant enzyme, was isolated from a drought tolerant variety of *Eleusinecoracana*, and is used to enhance the stress tolerance potential of *Brassicajuncea*. *Agrobacterium* suspension containing 5% sucrose and 0.005% silwet L-77 was injected into the young floral buds of *Brassicajuncea*, before the opening of the floral bud. Putative transgenic seedlings obtained from the seeds germinated in presence of BASTA were confirmed through PCR using transgene gene specific primers. A transformation efficiency of 17.7 % was recorded in the current protocol.

Biotechnological approaches for the production of secondary metabolites from medicinal plants

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The medicinal and aromatic plants are sources of a large number of active principles of herbal and modern medicine. Herbal medicines being natural, non-narcotic, having no side effects, cost effective, preventive and curative therapies could be useful in achieving the goal of "Health for all" in a cost effective manner. There are estimated to be around 25000 effective plant based formulations available in the indigenous medical texts. Medicines in common use, such as aspirin and digitalis, are derived from plants. Biotechnological approaches, specifically, plant tissue culture and genetic transformation, are found to have potential as a supplement to traditional agriculture for multiplication and genetic enhancement of the medicinal plants and also for industrial production of bioactive plant metabolites. Plant tissue culture techniques offer an integrated approach for the production of standardized quality phyto-pharmaceuticals through mass-production of consistent plant material. The biosynthetic activity of cultured cells can be enhanced by regulating environmental factors, as well as by artificial selection or by induction of variant clones. A wide variety of pharmaceutical compounds like alkaloids, terpenoids, steroids, saponins, phenolics, flavanoids and amino acids are being produced using cell cultures. Gene transfer technology promises a significant impact on *in vitro* production of secondary metabolites. New transgenic varieties can be created for production of pharmaceuticals, vaccines as well as for anticancer drugs. Genetic transformation of more than 120 species of at least 35 families, including the major economic crops, vegetables, ornamental, medicinal, fruit, tree and pasture plants has been reported using *Agrobacterium*-mediated and direct transformation methods. Transformed hairy roots induced by *A. rhizogenes* are characterized by high growth rate, genetic stability and growth in hormone free media. They mimic the biochemical machinery active in the normal roots and can produce levels of secondary metabolites comparable to that of intact plants. Hairy root cultures offer promise for production of valuable secondary metabolites.

Optimization for *Agrobacterium*-mediated transformation in Indian mustard

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For crop improvement, the important step is development of an efficient plant regeneration system. The work was done to produce a regeneration protocol for *B. juncea* genotypes RH 406 and RH 555 using seedling as explants for optimization for transformation. Explants used were hypocotyls and cotyledons. These explants were excised from *in vitro* grown 5-day old seedlings and were cultured on MS medium supplemented with different concentrations and combinations of growth regulators. Highest per cent shoot formation was observed on MS medium with 2.5 mg/l BAP (64.8 ± 1.4) from cotyledon explants in genotype RH 406. The regenerated plants were transferred to pots. Survival percentage was high in genotype RH 406 (81.8%) as compared to RH 555 (67%). *Agrobacterium tumefaciens* strain EHA 105, harboring pCAMBIA 2301 vector was used for transformation. This binary vector contains *gus* as reporter gene. Transformation protocol was developed in genotype RH 406 as hypocotyls and cotyledons explants of this genotype showed GUS assay test positive. GUS expression in the explants was determined through histochemical X-gluc staining. When the time of agro-infection was increased from 10 minutes to 30 minutes, the per cent *gus* expression was observed higher (80%). Cotyledon explants showed 75% while hypocotyl explants showed 80% GUS expression. Hence, regeneration and *Agrobacterium*-mediated transformation conditions were developed.

Transgenics: a novel way for crop improvement

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Biotechnological applications, especially transgenic plants, probably hold the most promise in augmenting agricultural production as plants are a treasure trove of interesting and valuable compounds and have evolved most impressive panoply of products to thrive in ever-changing environments. Food production will have to be doubled or preferably tripled by the year 2050 to meet the needs of the expected 11 billion people, of whom ninety percent will reside in the developing world. Genetic engineering has opened up new opportunities for plant breeders by enabling them to incorporate genes isolated from organisms outside the gene pools to which they usually have access. This broadens the possibilities they have for overcoming a number of biotic and abiotic stresses. With the tools now being harnessed through the many “omics” and “informatics” fields, there is the potential to identify genes of value across species, phyla, and kingdoms. Through advances in proteomics and glycomics, quantification of many individual proteins and the post translational alternations in their metabolic pathways can be done. Transgenic plants of over 20 plant species that are resistant to more than 30 different viral diseases have been produced by using different variations of the pathogen-derived resistance strategy. Insect-resistant plant varieties, using the δ -endotoxin of *Bacillus thuringiensis*, have been produced for several important plant species including tobacco, tomato, potato, cotton, walnut, maize, sugarcane, and rice. Of these, maize, potato, and cotton are already under commercial production. *TaWRKY44*-a wheat gene was found to exhibit multiple abiotic stress tolerance in transgenic tobacco. The study related to transgenic tall fescue with value added traits evidence that outcrossing grass plants generated through transgenic approaches are suitable materials for incorporation into breeding programs. Hence, emerging genetic engineering techniques are providing breeders with the never-before-seen capability to create novel plants by combining genetic material from a wide array of sources. Although not without controversy, the options seem limitless and, with the proper oversight and understanding, should provide extremely powerful options to develop durable and highly productive plant varieties for almost any production environment.

Single nucleotide polymorphism genotyping for defining agriculturally important traits and improvement of Finger millet (*Eleusine coracana*)

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Finger millet (*Eleusine coracana*), commonly known as Madua (ragi) is an ethnic cereal crop, which harbours many important nutritional traits such as high amount of minerals, quality grain proteins that are good source of essential amino acids with anti-diabetic properties as its phytochemicals help in slowing digestion process and this helps in controlling blood sugar level. Hence, these properties of finger millet could be effectively deployed to enhance the value of agri-food products. Markers have been utilized extensively for marker assisted selection, based on their association with genes/QTLs controlling grain calcium and protein content trait. It is of paramount importance to characterize and genotype its diverse gene pool of 113 genotypes through single nucleotide polymorphism (SNPs) genotyping by sequencing to identify the valuable SNPs. This resulted in a genome wide set of >23000 SNPs segregating across the entire collection and several thousand SNPs segregating within every accession. A model based population structure analysis reveals the presence of three subpopulations among the *Eleusine coracana* accessions which are in parallel with the results of phylogenetic analysis. A total of 1128 gene ontology terms were assigned to SNP carrying genes for three main categories-biological process, cellular component and molecular function. On the basis of population structure, we are trying to associate these SNPs with precise phenotyping of nutritionally important traits such as calcium, iron and zinc and protein content. It will provide a better opportunity to dissect complex traits and identification of superior alleles at genes contributing to the target trait.

Transgenics for value addition: A case for biofuel feedstocks

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Food production will have to be increased in the future, and increased use of agrochemicals and mechanised agriculture will contribute further to environmental degradation and loss of biodiversity. If transgenic crops in any way reduce these adverse effects, without themselves causing additional problems, they represent a technical and ethical advance. In addition to food production challenges, the limiting availability of energy sources may assume threatening dimensions. Therefore, limitations in the current availability of bioenergy feedstocks are a major problem in next-generation biofuels. There are global economic, political and environmental pressures to increase biofuel production and utilization, to offset petrol and diesel fuel use, especially in the transportation sector. In fact, there are several commercially available first-generation transgenic **biofuel feedstocks**, including maize grown for ethanol and canola/rapeseed grown for biodiesel, that are cultivated internationally on a multimillion hectare scale. In both cases, these crops can also be used for food and feed; these are annual crops. For instance, for meeting the goals of the US Department of Energy (DOE) billion ton annual supply of biomass translates into 5% of the nation's power, 20% of the nation's transportation fuels and 25% of the nation's chemicals by 2030. This goal is equivalent to 30% of current petroleum consumption. Similarly, India must take initiatives to increase efforts to produce transgenic biofuel feedstocks. Engineering efforts are being made to utilize transgenic cotton sticks as biofuel feedstocks. Similarly, efforts are being made to utilize paddy crop residues to produce biofuel. Laccase-Mediator Systems (LMS) presents a technological challenge and opportunity that needs further development and optimization.

Molecular mechanism of Drought and Salinity tolerance in transgenic plants

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Salinity and Drought are among the major stresses, which adversely affect plants growth and productivity; hence it is important to develop stress tolerant crops (Transgenics). In general, salinity and drought exerts its malicious effect mainly by disrupting the ionic and osmotic equilibrium of the cell. In plants, Abscisic acid (ABA) is accumulated under osmotic stress conditions caused by drought, and has a key role in stress responses and tolerance. Comprehensive molecular analyses have shown that ABA regulates the expression of many genes under osmotic stress conditions. Major plant transcription factor families such as *bZIP*, *NAC*, *AP2/ERF*, and *MYB* orchestrate regulatory networks underlying abiotic stress tolerance. Salinity is a major abiotic stress limiting growth and productivity of plants in many areas of the world due to increasing use of poor quality of water for irrigation and soil salinization. Plant adaptation or tolerance to salinity stress involves complex physiological traits, metabolic pathways, and molecular or gene networks. Development and production of Abiotic stress tolerant varieties through genetic engineering is to be of great significance in ensuring food security, shortage of water resources, protecting environment and increasing income.

Can genetically modified crops feed the world?

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The growing demand for food poses major challenges to growers and researchers. We have to safeguard both biodiversity and arable land for future agricultural food production. It is predicted that the human population will reach nine billion within coming decades, and this is of urgent concern. To keep pace with population growth, food production must increase greatly with the limited availability of arable land, water, and fossil fuels, exacerbated by climate change. To increase food production in order to meet the demand from an expanding world population while being faced with anticipated climate changes will definitely entail increased access to genetic resources. Proponents of genetically engineered (GE; also called genetically modified or GM) crops claim that we need this technology to address the current global hunger crisis, and to feed a growing global population. The biotechnology industry also tells us that GM crops are better for the environment, and will provide the tools that farmers need in a time of climate chaos and provide higher yields and higher incomes for farmers. The hard truth is that in the case of GM crops, it makes farmers dependent on the products of a few large companies whose primary objective is to maximize their profits. As such, we will not promote their widespread use until more research has been done on long term health effects, GMO seeds are available outside of corporate agriculture control, the biological effects of gene insertion are better understood, and research confirms that the presence of GMOs will not harm the native species in an ecosystem.

Expression of Bt cry toxins in cotton (*Gossypium hirsutum* L.) cultivars

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Extent of cry proteins in leaves is crucial to the protection expected against insects feeding on cotton leaves. The present study was carried out on eighteen cotton genotypes to quantify Bt toxin levels at two growth stages *viz.*, vegetative and reproductive stage and in different plant parts (apical leaves, bottom leaves and squares). The results showed that quantitative levels of Bt toxin in cotton genotypes analysed were variable and in general were higher during vegetative stage (90 Days After Sowing) and decreased as the reproductive stage approached (120 Days After Sowing). However, the expression across the diverse plant parts remained comparable. Further it was observed that Cry 1Ac levels were higher than that of Cry 2A throughout the vegetative and reproductive phases. Therefore, it was observed that cry toxin level decreases as the plant attains maturity. The genotypes showing high expression during late stage like MRC 6304, Bunt 2113-2, Platinum 605, MRC 7017 and RCH 134 may be more promising to fight out insect attack.

A robust screening approach: Southern-by-sequencing for molecular evaluation of transgenic plants

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Molecular depiction of events in genetically modified (GM) crops by polymerase chain reaction (PCR) and Southern blot analyses has become past now. We have a brand new approach which can provide sequence-level detail, Southern by-Sequencing (SbS). This involves blending of NGS with sequence capturing where hybridization of Indexed and pooled whole-genome DNA libraries from GM plants to biotinylated probes is done. Probes are premeditated to target the sequence of transformation plasmids, which are used to generate events within the pool. This sequence capture process embellishes the sequence data obtained for DNA adjacent to the targeted bases and helps in detecting plasmid-to-genome and plasmid-to-plasmid junctions introduced during insertion into the plant genome. Analysis of these junction sequences provides sequence-level information related to the number of insertion loci including detection of unlinked, independently segregating, small DNA fragments; copy number; rearrangements, truncations, or deletions of the intended insertion DNA; and the presence of transformation plasmid backbone sequences. This molecular portrayal from SbS analysis is used to deduce and select GM plants gratifying optimal molecular characterization criteria. On the other hand, Southern blot analysis can be time-consuming and comparatively expensive and does not provide sequence-level detail. Thus, SbS technology has proven to be a robust event screening tool for high throughput molecular characterization to replace Southern blot analysis/PCR.

Mitigating ROS: A tool for establishment of plant stress tolerance

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The agricultural productivity is constantly challenged by changing environment, which is characterized by high soil salinity, temperature fluctuation, drought stress and UV radiations. Abiotic stresses resulting from these lead to the reduction in photosynthesis, transpiration and other biochemical processes associated with plant growth, development and crop yield. Due to exposure to these stresses, plants tend to accumulate reactive oxygen species (ROS) in their cells affecting the plant growth and production. Depending on the nature of the ROS species, some are highly toxic and rapidly detoxified by various cellular enzymatic and nonenzymatic mechanisms. Whereas plants are surfeited with mechanisms to combat increased ROS levels during abiotic stress conditions, in other circumstances plants appear to purposefully generate ROS as signaling molecules to control various processes including pathogen defense, programmed cell death, and stomatal behavior. Synchronous action of superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), monodehydro ascorbate reductase (MDHAR), dehydroascorbatereductase (DHAR) and glutathionereductase (GR) is part of the antioxidative system, which protects the plants against ROS. Transgenic plants exhibiting higher level of expression of this antioxidant enzyme are more tolerant to high salt, drought, chilling, oxidative stresses, by less severe cell death, lower ROS production, malonaldehyde (MDA) content, relative electrolyte leakage (REL) and stomatal conductance, and prevent chlorophyll loss and the production of scavenger reactive oxygen species under different biotic and abiotic stress.

Prospects to Rice Transgenics for abiotic stress and its impact on cereal production

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World population is escalating day by day and by 2050 it is expected to reach 9.1 billion, but agricultural production is not rising with a parallel pace. Agriculture production has been declined mainly due to biotic and abiotic stresses. Major abiotic stresses worldwide posing risks to food security are high salinity, drought, submergence and cold. All of these negatively impact the crop growth and productivity world-wide. Among these, drought affects on plant are more pronounced than any other stress factor both physiologically and anatomically. It reduces plant growth, yield, membrane integrity, pigment content, osmotic adjustments, water relations and photosynthetic activity. Salinity is the second most prevalent soil problem in rice-growing countries after drought over world diaspora. To improve the yield under drought and salt stress condition, countless breeding programs have been initiated. Although conventional breeding programmes such as hybridization, hybrid breeding, wide hybridization and ideotype breeding have resulted in development of some salt-and drought tolerant rice varieties but the success rate of conventional breeding is not promising to present need. Drought and salinity tolerance in rice are polygenic traits with complex intergenic interplay and huge environmental interaction resulting low heritability. Therefore, development of genetically engineered plants with enhanced tolerance to drought and salinity is a challenge in rice biotechnology research. Considering rice as a model of cereal genomics, rice transformation is the major goal in cereal biotechnology. Genetic engineering has been used as a prominent tool for rice improvement. Although gene transformation in rice is performed, still the hurdles like the number of copies of a gene(s) inserted and uncontrollable chromosomal locations of the integrated genes as well as their expression varies among individual transformants. Therefore, a large number of transgenic plants must be developed using advanced methods and precision technology in order to select desirable transformants. Since the last two decades, a large number of salinity tolerance genes were isolated and cloned which are involved in signal transduction, transcription regulation, ion transporters and metabolic pathways, there is still a long way to harvest the research potential on rice transgenics.

Genetic improvement of *Camelina sativa* for biofuel and industrial bio-products

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Environmental concerns related to available fuel resources and ever-increasing demand has forced to look for sustainable and green alternatives. Biofuels have been identified as an important component of our future energy supply because they are renewable, efficient and clean burning fuels. Various edible vegetable oil crops like soybean, palm, rape seed, groundnut, sunflower are used for biodiesel production. However, these feed stocks compete with food crop for high quality arable land and water. Therefore, oilseed crops used for biofuel production should be from non-human food sources to sustain food production. Camelina is a short life cycle, low input, non-food oil seed crop which can be easily transformed and has been emerged as an attractive platform for genetic/metabolic engineering. Use of camelina diesel reduces green house gases (GHG) emission up to 40-60% in comparison to petroleum diesel. Till recently, camelina improvement has largely remained unexploited due to the limited availability of genetic and genome tools. However, upsurge of 'omics' data for camelina has enhanced the probability of genetic manipulation of lipid biosynthesis pathway(s) for improvement of traits like seed yield, oil content and composition and resistance to abiotic and biotic stresses. Fatty acid manipulations like medium-chain fatty acids, long-chain polyunsaturated fatty acids, accumulation of omega-7 monounsaturated fatty acids, hydroxylated fatty acids or high-oleic oils have been exploited mainly for biofuel or other industrial by products. One or two genes or several pathway genes stacked together have been introduced in camelina to improve its performance under adverse conditions, seed yield, and to alter major seed lipid composition, including high nervonic acid (6-12%), high Omega-3 LC-PUFA (>12%), high palmitic acid (41%), high oleic acid (70%), high omega-7 monounsaturated acid (60-65%) and acetyl-TAG (85%). Further, Camelina has also been used for the production of high-value industrial products such as polymer (polyhydroxybutyrate), wax esters and terpenes.

Optimization of various parameters for efficient regeneration of Chickpea (*Cicer arietinum* L.)

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Chickpea (*Cicer arietinum* L.) is one of the most important crop which plays a significant role in the nutrition of rural and urban population of developing and developed countries. This crop have high protein content and rich source of amino acids like methionine, threonine and lysine. The present investigation describes an efficient in vitro regeneration from 4-d old cotyledonary node explants. PUSA547 was found to be the best cultivar for multiple shooting. 4-d-old donor plant responded better as compared to others. Two type of explants cotyledon and cotyledonary node were used for multiple shooting. Cotyledonary node was best for in vitro regeneration as there were 100% regeneration frequency with 9.4 multiple shoots. The multiple shoots formation were maximum i.e 30 shoots per explant from cotyledon but the regeneration frequency was very low (25%). Three different cytokinins, BAP, kinetin and zeatin were used for shooting but out of three, kinetin was the best cytokinin with a concentration of 0.25 mg/l for multiple shooting because the shoot regenerated in the presence of kinetin were healthy, green and robust. The inhibiting concentration of a selective agent (kanamycin) was also standarised. The percent regeneration of the explants decreases with the increase in the concentration of kanamycin. However, there is still a scope to further improve the regeneration protocol from cotyledon explants which should give high frequency of regeneration to develop an efficient regeneration system which will be compatible for development of transgenic plants against, the biotic as well as abiotic stresses.

A comparative study of *in vitro* regenerative potential of cotyledonary node and primary leaf (with petiole) explants of a recalcitrant grain legume mungbean (*Vigna radiata* L.wilczek)

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Legumes, also referred as pulses, are the major source of protein in Asia and constitute an important supplement to the predominantly cereal-based diet. Green Revolution tremendously increased production of cereals in Asia; however it compelled legumes, including mungbean, to periphery. To meet the demand of overgrowing population, yield of food crops including legumes is to be enhanced and stabilized in their traditional growing areas. Their improvement through conventional breeding is low or often not possible due to the lack of sufficient and satisfactory level of genetic variability within the germplasm. So, biotechnological approaches are required to widen the gene pool beyond sexual boundaries and transfer of alien genes of proven value offer possible viable option for crop improvement. Therefore, the development of genetically engineered plants by the introduction and/or over expression of selected genes seems to be a viable option to enhance the tolerance to various stresses and hence stabilize yield. Availability of efficient *in vitro* regeneration system is a pre-requisite for effective genetic transformation. In case of direct shoot regeneration, chances of somaclonal variations are low. Hence, the factors affecting *in vitro* plant regeneration via direct shoot organogenesis from cotyledonary node and primary leaf petiole explants have been compared in the present study. The genotype, age and size of the explant, type and concentration of cytokinins and combinations of cytokinins with auxins influenced the frequency of shoot formation and the number of shoots per explant. The age of explant also affects the multiple shoot forming response. The cotyledonary node and primary leaf explants excised from 3-d and 4-d-old seedlings respectively developed a maximum of 5.5 and 8 shoots per explant in 88% and 92% of the cultures respectively, on medium containing 2.5µM BAP as a sole growth regulator. Out of various cultivars checked, K-851 produced the maximum number of shoots from both the explants. The shoots regenerated from both explants were rooted on medium containing IBA (2.5 µM). Shoots with well-developed roots (plantlets) were established in soil and developed into morphological normal plants which subsequently produced flowers and pods with viable seeds. Because, the primary leaf petiole explants produced the maximum number of shoots per explant, the regeneration site (cut end of petiole) was completely exposed and hence may be easily accessible to *Agrobacterium* and an extra wounding treatment is not required as in cotyledonary node explants, this plant regeneration protocol is amenable for the production of transgenic plants.

Phylogenetic and Recombination analysis of AC1 gene of various isolates of Mungbean Yellow Mosaic Virus

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Mungbean yellow mosaic virus belongs to the family *Geminiviridae* possessing bipartite genome. It is a menace in legume production being responsible for yellow mosaic disease transmitted by the whitefly *Bemisia tabaci*. The DNA A component encodes for the information related to viral DNA replication, transcription, and encapsidation, whereas DNA B encodes for movement proteins which are responsible for virus translocation. As AC1 gene codes for a replication- associated protein (Rep) which is essential for replication of virus, it is an important target for various strategies to develop virus resistance in legumes. Evolutionary relationship among AC1 genes of different MYMV isolates was studied using Molecular Evolutionary Genetics Analysis (MEGA6). Phylogenetic trees obtained from different methods gave different results revealing a pattern in diversity among these isolates. Regions of recombination in the Rep gene of different MYMV isolates were detected using Recombination Detection Program (RDP4). There are greater chances for a recombinant virus to emerge by increasing host range and to provide sources of variation having unpredictable effects on the viral pathogenicity.

Spermine modulates the antioxidant defense system in wheat (*Triticum aestivum* L.) under salt stress

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Significance of naturally occurring intracellular polyamines (PAs) in relation to the mechanism and adaptation to combat abiotic stress has been well established in plants. The recent approaches in genetics, transcriptomic, proteomic and metabolomic approaches have unraveled many significant functions of different PAs in the regulation of plant abiotic stress tolerance. Recently it has been shown PAs inhibit tumor growth and hence have health benefits. Therefore, the development of transgenic food plants with high or low PA levels may increase their nutritional and health values. The present study reports on the role of spermine in modulating the antioxidative defense system in wheat under salt stress. An experiment was conducted to evaluate the potential effects of foliar application of spermine (with two concentration 0.5mM and 1.0mM) on four wheat (*Triticum aestivum* L.) varieties viz. DBW 88, HD 3086, Karchia 65 and KRL 210 grown under salinity (with 8 dS/m and 12dS/m). The leaf samples were collected and analysed for various antioxidative enzymes and non-enzymatic metabolites at 0, 5, 10 and 15 days after foliar treatment of spermine. Exogenous application of spermine showed increase in the enzymatic activity of superoxide dismutase (SOD), catalase (CAT), Ascorbate peroxidase (APX) and differential response in glutathione reductase (GR) activity. Salt-induced oxidative stress (Superoxide radicle, hydrogen peroxide) and lipid membrane damage malondialdehyde (MDA) was more pronounced in DBW 88 and HD 3086 and it was mitigated by exogenous spermine application. It was found that exogenous spermine could combat the oxidative stress by modulating the antioxidant system of the wheat varieties under salinity and significantly improved tolerance in wheat.

Method for Genetic Transformation of *Arnebia hispidissima*

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Arnebia hispidissima, wild plant species from Boraginaceae family, accumulating Shikonin fail to produce a sufficient raw material for commercial production of Shikonin. Shikonin possesses several medicinal properties. Shikonin is used as remedy for various skin ailments and as a dye for silk and cosmetic industry. Most of the species of Boraginaceae yield a red pigment 'Ratanjot' which has wide application in food industry. Biotechnology can be of immense help to circumvent these problems, especially the micropropagation and Hairy Root Culture serves as an alternative approach for production of secondary metabolites *in vitro*. A method for *Agrobacterium rhizogenes*-mediated genetic transformation of *Arnebia hispidissima* for Hairy Root Cultures was optimized for enhancing the Shikonin production to meet the ever increasing demand for pharmaceutical industry. The etiolated explants viz. shoot tip, nodal, leaf and internodal segments were co-cultivated with *Agrobacterium rhizogenes* for induction of Hairy Root Culture. Among the various explants employed, leaf explant showed maximum 70.7% response followed by shoot tip 52%, nodal segment 38.7% and internodal segment 9.3%. The presence of *Ri* plasmid *rolB* gene in the transformed Hairy Root Cultures was confirmed by PCR analysis using forward (*FrolB*) and reverse (*RrolB*) primers of *rolB* gene resulted in the amplification of ~0.8 kb fragments. Medium composition have been optimized for *in vitro* induction of Shikonin in Hairy Root Cultures of *Arnebia hispidissima*. The formation of Hairy Root on hormone free MS medium showed red spots in the older part of the tissue. However, these reddish root turned white after a second subculture to the hormone free MS medium. Whereas, Hairy Roots cultured on RC medium showed faster growth and produce large amount of Shikonin. The Shikonin content in transformed Hairy Root was estimated spectrophotometrically by making standard curve using authentic sample of Shikonin. The results will help to bridge the gap between ever increasing demand and supply of raw products necessary for pharmaceuticals, medicinal, food, cosmetic and dyeing industry.

Genetic transformation of MPK3 gene in *Brassica juncea* cv. Varuna to enhance disease resistance against *Alternaria* blight

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Indian mustard [*Brassica juncea* (L.) Czern & Coss.] is an important *Rabi* oilseeds crop. India is one of the major mustard growing countries of the world. According to USDA report, the total production of Rapeseed mustard in 2015-2016 is decreased by 6.35% in the World and 4.91% in India as compare to previous year 2014-2015. Slightly decrease oilseed production is due to biotic as well as abiotic factors. *Alternaria* blight is one of the most important diseases of mustard that leads to major yield losses as well as deterioration in quality of oilseeds. Various reports indicate that signaling protein, Mitogen activated protein kinase (MAPK) of signal transduction pathway plays an important role in plant defense. Earlier studies of our lab show that MPK3 might be potential candidate gene for plant defense. Expression of *mpk3* gene was affected in *Alternaria brassicae* inoculated plant and an increased activity of MPK3 protein in the cell might be inducing defense against *Alternaria brassicae* in Brassica. Agrobacterium mediated floral bud transformation method was used to CaMv35S-MPK3 gene introgression in Brassica genotype. 3rd generation stable MAPK3 selection lines were studied for disease index as well as host pathogen interaction. There was a significant change in disease index in transgenic Brassica. The host pathogen interaction studied showed that the low growth pattern of fungal hyphae and less penetration of *A. brassicae* spores were seen in transgenic *Brassica juncea* as compare with *Brassica juncea* cv. Varuna. Together, these results suggest that *Bj*MPK3 might be involved in defense pathway and provide resistance against *Alternaria* blight.

Transgenic development for drought tolerance in indian mustard (*Brassica juncea* l. Czern & coss.)

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Biotechnology is emerging as one of the most innovative achievements in the life sciences and influencing almost every aspect of human life. Abiotic stress is known as the most harmful factor concerning the growth and productivity of crops, posing a substantial challenge in the face of an ever increasing world population. Undoubtedly, drought is one of the prime abiotic stresses in the world. *Brassica juncea* commonly known as Indian mustard is economically very important crop because of its high oil content and superior oil quality. Isolation of a novel DREB gene, named BjDREB1B, from high salt-treated *Brassica juncea* and its expression pattern, DRE-binding ability, and transcription activation activity were investigated. Characterization of DREB genes from *Brassica juncea* would help us understand the molecular mechanisms of stress resistance in order to improve tolerance to adverse environments in oil seed crops using transgenic technology. Besides DREB, full length cDNA for a gene encoding an annexin protein was isolated and characterized from *Brassica juncea* (AnnBj1). The transgenic tobacco plants expressing annexin showed significant tolerance to dehydration, salt, heavy metal and oxidative stress at the seedling stage. Identification of the BjDREB1B downstream target genes and annexins would help us further clarify its roles in stress tolerance and *Brassica* improvement.

Recent Advances in genetic engineering against Abiotic Stress

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Abiotic stress conditions pose serious limitations to modern agriculture. Although scientists have succeeded in identifying many genes associated with plant responses to abiotic stresses and used to generate stress tolerant plants, but the success in producing stress-tolerant crops is limited. The classical approach to engineer plants for enhanced tolerance to abiotic stress consists in strengthening the endogenous systems by intervening at different levels of the response, from sensors and signalling/regulatory elements (e.g. kinases, transcription factors), to direct- action genes or effectors (e.g. antioxidant enzymes, heat-shock proteins, enzymes for the synthesis of osmoprotectants). A better and more comprehensive knowledge of the complex mechanisms involved in the stress responses provided by the various 'omics' metabolomics, transplastomic, proteomic, transcriptomic has allowed to identify novel points of intervention, dealing in particular with a new layer of control or fine tuning of the main response scheme. Synthetic biology applies basic engineering principles to the rational design and development of new biological modules from natural existing components, thus facilitating the de novo engineering of genetic switches and circuits, and the manipulation of signalling processes. Synthetic biology approaches in plants include the development of synthetic regulatory elements (promoters and other cis-elements, synthetic small RNAs, among others) and switches for the spatiotemporal manipulation of gene-expression and engineering of signalling networks. To realize the goal of producing abiotic stress tolerant plants newer and more effective methods of genetic transformation are needed, which can be achieved by using nanotechnology tools. By applying nanotechnology tools, species which are not amenable to genetic transformation can be transformed too.

Transgenic vegetable breeding: An emerging approach to enhance vegetable production, nutritional quality and health benefits

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Vegetables are the protective foods. It plays an important role in human diet because they provide vitamins, minerals, dietary fibre and phytochemical. By the 2020, global population is expected to reach 9 billion. Conventional breeding methods alone cannot feed growing population. About 3.5 billion populace on the planet are malnourished due to imbalanced diets. Vegetables effectively contribute to the avoidance of undernourishment disorders. Genetic engineering enables vegetable breeders to incorporate desired trans-genes into elite cultivars, thereby improving their value considerably. Genetic engineering offers unique chance for improving nutritional value and bringing other health benefits by eating more nutritious transgenic vegetables. Transgenic vegetable breeding could make important contributions towards sustainable vegetable production, higher yield, disease and pest resistance and increased shelf life. Many vegetable till today genetically modified to improve traits such as higher nutritional status, better flavour with inbuilt edible vaccination. Consumers could benefit further from eating more nutritious transgenic vegetables through genetic engineering. Genetically engineering vegetables can also be enhanced through transgenic approaches to contain increased level of food safety and health benefits by reducing bitterness or anti-nutritional factors from vegetables. Biotechnology derived vegetable crops have high advantages to both farmers and consumers. Among all vegetable crops potato, tomato, eggplant, cucumber, capsicum, Cole crops and root crops has received the maximum attention particularly in the areas relating to insect pests, disease herbicide resistance and quality, shelf life, yield improvement. There has been a drastic increase in the total cultivable area under transgenic crops globally.

***In vitro* regeneration and *Agrobacterium tumefaciens* mediated genetic transformation of mungbean using cotyledon explants**

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Mungbean is an important grain legume. Besides its utility for human consumption, it also serves as a nutritive fodder and green manure. Production of overall legumes and in particular of mungbean is low due to several constraints. Improvement in resistance by classical breeding is limited due to the lack of sufficient and satisfactory levels of genetic variability within cultivated mungbean germplasm. Hence plant biotechnology is an effective alternate method. Mungbean is notoriously recalcitrant to *in vitro* regeneration and genetic transformation. The present study reports optimization of various parameters affecting *in vitro* regeneration and development of transgenic plants of mungbean using cotyledon explants. The cotyledon explants excised from 16-hr-old seedlings were cultured for regeneration on MS salts and B₅ Vitamin medium containing BAP. These explants developed multiple shoots (6-7) in 100% of the cultures within 24 days. *Agrobacterium tumefaciens* strain EHA105 harbouring pCAMBIA 2301 carrying *nptII* and *gusA* marker genes was used for transformation studies. The explants co-cultivated with *A. tumefaciens* up to 3 days were cultured on shoot regeneration medium containing kanamycin 75 mg/l and cefotaxime 500 mg/l. For the second round of selection the shoots regenerated on selection medium were rooted in the presence of kanamycin (10mg/l). The transient and stable histo-chemical GUS activity was observed in explants and plant parts. The presence of transgenes was confirmed by PCR. Southern hybridization for the integration of transgene is in progress.

Effect of chitosan and selenium on oxidative stress and antioxidant system for delayed ripening in guava (*Psidium guajava* L.) fruit

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Guava fruit of variety Hisar surkha (shelf- life 3-5 days) harvested at physiological maturity were treated with two concentrations of selenium (0.02 and 0.05 ppm) with and without chitosan (1.5%) for 5 minutes and stored at room temperature. Samples were taken at three days interval until complete decay and analyzed for various components of oxidative stress and of enzymatic and non-enzymatic antioxidative system. Hydrogen peroxide and malondialdehyde content increased significantly during storage while the activities of antioxidative enzymes *viz.* superoxide dismutase, catalase, ascorbate peroxidase and glutathione reductase were invariably low at the later stages during storage. Fruits treated with chitosan showed increased activities of antioxidative enzymes suggesting that these chemicals have the ability to scavenge free radicals. This may be due to modulation of antioxidative enzyme activities and/or expression of genes involved in the membrane deterioration, lipid peroxidation and senescence as compared to control during storage. Additionally, consumption of selenium treated fruits will improve its level in human body which is required for proper functioning of immune system, production of active thyroid hormone etc.

***In vitro* propagation of *Tinospora cordifolia* through nodal explants**

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Tinospora cordifolia is a genetically diverse divine herb of immense medicinal importance. The plant has also been listed as a highly prioritized medicinal plants required for high volume trade. As a result of high demand, plant has been overexploited by pharmaceutical companies and folk people which led to the acute scarcity of *Tinospora cordifolia*. Although, *T. cordifolia* can be propagated through traditional methods like vegetative and seed propagation but, there are number of limitations for propagation and conservation through conventional approaches such as low percentage of seed set, poor seed germination ability and total dependence of vegetative cutting on environmental conditions. Therefore, we need *in vitro* propagation protocol of *T. cordifolia* for its rapid and efficient multiplication.

In the present investigation a method has been standardized for rapid clonal propagation using mature nodal explants cultured on MS medium along with various combinations and concentrations of cytokinins (BAP and Kinetin) and auxin (IAA). Shoots were initiated and further elongated on MS supplemented with Kin and BAP (3mg/l). Among the cytokinins tested, BAP was more effective than Kin for axillary shoot proliferation. Kin was superior to BAP in terms of shoot elongation. Elongated shoots were rooted in half-strength MS medium supplemented with 2mg/l indole-3-acetic acid (IAA). Rooted plantlets were successfully transferred to soil and established with 80% survival.

Transgenics and genome editing

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Transgenic technology allows genetic manipulation in which an exogenous gene called as transgene inserted into a living organism to exhibit a new property and transmit that property to its offspring. But transgene integration is limited by the random nature of the insertion of the new sequence into the genome and may lead to inactivate or disturb the functioning of other genes or even cause severe unwanted effects and offer no degree of reproducibility. By contrast, genome-editing is an advanced genetic engineering tool that can more directly modify a gene within a plant genome by using nucleases (molecular scissors). There are currently three families of engineered nucleases being used: Zinc finger nucleases (ZFNs), Transcription Activator-Like Effector-based Nucleases (TALEN), and the CRISPR-Cas system. Recent reports regarding genome-editing of major crops, including barley, maize, rice, soybean, sweet orange, and wheat have demonstrated a high efficiency of indels in addition to the introduction of exogenous DNA in a targeted locus. Some reports have demonstrated that Non-homologous end joining mediated indels can lead to disease resistance without the need to use a transgene. For example three homeoalleles of *TaMLO* were simultaneously edited in hexaploid bread wheat, resulting in heritable resistance to powdery mildew.

Transgenic approaches for biotic stress tolerance in plants

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Biotic stress drastically affects the capacity of crop plants to produce their maximum yields. With the advent of genetic transformation techniques, it has become possible to analyse the function, clone, insert and pyramiding of desired genes into the crop plants to confer resistance to different biotic stresses. Reinforcement of resistance against insect-pests and pathogens attack using genetic engineering has proven to be an effective strategy to develop resistant crop plants and could offer a remedy, allowing more precise targeting of pest and disease management. Interactions between plants and pathogens the so-called “gene-for-gene” relationship, induce a series of plant defense responses by observing calcium and other ion fluxes, and protein kinases are activated. Production of signaling compounds such as reactive oxygen intermediates, salicylic acid, nitric oxide, ethylene and jasmonic acid causes activation of many downstream responses. Strategies for developing transgenics have been based upon the use of single genes and manipulation of signal-transduction pathways. The recent development of microarray-based expression profiling methods, together with the availability of genomic and/or EST sequence data for some plant species, has allowed significant progress in the characterization of plant PR responses. The technologies open up tremendous opportunities to identify new PR genes, to identify co-regulated genes and the associated regulatory systems, and to reveal interactions between different signaling pathways. This exciting technology allows access to an unlimited gene pool without the constraint of sexual compatibility. Crop plants engineered showed spontaneous activation of defence mechanism and have been suggested to represent an improvement in crop production.

Transgenics as an efficient biomass for biofuel production

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The world energy demand is increasing steadily. The current predominant energy source is fossil fuel. It is needed to complement fossil fuel with renewable energy sources. The plant is most prominent species of living organisms which serves as raw material for many human purposes like food, paper, fiber, biofuel etc. The composition of the cell wall decides the fate of plant biomass. The composition varies one in several ways among species. The main components of cell wall include cellulose, pectin, hemicellulose, lignin and protiens. There may be some other components also present in some species like suberin, waxes, cutin, etc. Plants are highly efficient energy generator as it converts light energy into simple sugars through photosynthesis and CO₂ fixation. Cell wall polysaccharides use feedstock for biofuel production after being broken down into simple sugars (saccharification). The limitation in production of biofuels are cellulose crystallinity, reduced accessibility of cellulose microfibrils due to degradation-resistant phenolic polymer lignin, adsorption of hydrolytic enzymes to the lignin polymers and costs of pretreating lignocellulosic matter. To overcome such problems, development of transgenics with altered cell wall composition serve as efficient biomass for biofuel production. The composition can be altered by overexpressing the genes, downregulation of the genes, heterologous expression of the gene or expressing efficient enzymes over native enzymes in the biosynthetic pathways of cellulose, lignin, pectin, etc.

Genetically modified food: Associated controversies

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The controversies linked with genetically modified food are disputes on the utilization of foods and other edible products derived from genetically modified food rather than conventional crops and other uses of genetic engineering in the production of food. The dispute involves consumers, farmers, biotechnology companies, governmental regulators, non-governmental organizations and scientists. The key areas of controversy related to genetically modified food (known as GM food or GMO food) are whether such food should be labeled, the role of government regulators, the objectivity of scientific research and publication, the effect of genetically modified crops on health and the environment, the effect on pesticide resistance, the impact of such crops for farmers and the role of the crops in feeding the world population. There is a concern among the public that eating genetically modified food may be harmful. Specific concerns include mixing of genetically modified and non-genetically modified products in the food supply, effects of GMOs on the environment, the rigor of the regulatory process and consolidation of control of the food supply in companies that make and sell GMOs. The safety assessment of genetically engineered food products by regulatory bodies starts with an evaluation of whether or not the food is substantially equivalent to non-genetically engineered counterparts that are already deemed fit for human consumption. No reports of ill effects have been documented in the human population from genetically modified food. There is general scientific agreement that food on the market derived from these crops poses no greater risk to human health than conventional food, but should be tested on a case-by-case basis. Scientists tend to be more concerned about the potential for genetically modified organisms to cause ecological damage.

Chemical treatments improve the keeping quality of fruits: An alternative approach to genetic engineering

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India, the world's second largest producer of fruits, loses 35-40 % of fresh produce due to excessive softening and damage during shipping and handling processes. Fruit softening occurs due to enzymatic hydrolysis of cell wall polysaccharides like cellulose, hemicellulose, calcium pectate, polyuronides and glycoproteins. The membrane deterioration leads to cellular decompartmentation and loss of tissue structure due to over accumulation of reactive oxygen species. Biotechnological approaches, in particular genetic engineering, have the potential to engineer fruits with delayed ripening characters. Fruits with improved keeping quality can be developed either by modification of ethylene metabolism or by manipulating cell wall metabolism using certain chemicals. The guava and ber fruits treated with chitosan, selenium, gibberellic acid and calcium chloride resulted in higher fruit firmness, decreased total soluble solids and physiological loss in weight during storage at room temperature. These treatments could decrease the activities of cell wall degrading enzymes *viz.* pectin methyl esterase, polygalacturonase and cellulase as compared to control during storage, thus slowing down its degradation process and hence improving their keeping quality. Also, consumption of fruits treated with selenium and calcium improves their levels in human body which further helps in proper functioning of immune system, bone development etc.

Review and perspectives of transgenic rice research

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The world population is on rise and will reach 9.5 billion mark by year 2050 as per current statistics. Global food production will need to increase by 70%, which means global rice and maize production must be two fold from same available resources. Rice being the staple food crop globally, it is needed to give immense importance for its improvement. The rice genome sequence has served as a catalyst to accelerate efforts on the functional analysis of genes/QTLs by reverse and forward genetics in India. Rice transformation via particle bombardment succeeded in 1991, which later became one of the most common methods of rice transformation. Multigene transformation has played an important role in rice transformation. Golden rice is a famous example, in which a novel β -carotenoid biosynthesis pathway is established in rice endosperm by introducing two foreign genes into transgenic rice. Recently, scientists have accomplished the proof of concept on attaining Fe/Zn nutritional targets under flooded field conditions to fulfil 30% of EAR in the human diet in a well-characterized GM event of the widely consumed indica rice cultivar without a yield penalty. So, transgenics coupled with MAS and conventional breeding programmes can exert their advantages to develop more and better rice varieties.

Prominent issues concerning public acceptance of transgenics

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In the modern times it is a well-documented fact, that the corpus of 'food and feed' that is generally derived from both plants and animal sources that have been grown, maintained and bred by humans ever since pre-historical times. It is also a commonly drawn inference, that with passage of time these plants and animals have undergone a vast amount of significant genetic change since it was always a case of those with good genetic traits being accorded precedence over the others. With the help of modern gene transfer technologies of today, it has become relatively easy to modify the genetic material of an organism. This has the potential to address one of the key problems confronting mankind, that is, of meeting the demand for food and feed in the times to come. However, this is loaded with some riders on account of certain ethical and safety concerns due to which the public acceptance of transgenics has become a highly debatable issue. The present study aims at analyzing the need for same with help of some models and case studies.

Uncovering new properties of kaic gene in Cyanobacteria using bioinformatics tools

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The circadian clock protein KaiC, is expressed in the kaiABC operon that controls circadian rhythms in cyanobacteria. Circadian clocks are internal biological timers that control several processes at regular intervals round the clock. They subsist in different organisms, however, cyanobacteria are the simplest organisms known to maintain a clock. KaiC have been broadly studied and are widely spread among Cyanobacteria. This article is mainly focus on bioinformatics analysis of KaiC protein in *Prochlorococcus marinus*. According to the conserved motifs obtained by MEME and MAST tools, two motifs were common in all proteins. The structural and functional analyses of KaiC proteins were investigated by ProtParam, SOPMA, SignalP 4.1, TMHMM 2.0 and ProDom tools in ExPASy database. The protein sequences were aligned with ClustalW algorithm by MEGA 6.06 software and phylogenetic tree was constructed using the Neighbor-joining (NJ) method. In protein–protein interactions analysis by STRING 9.1 tool, 10 significant protein interaction groups were identified in KaiC. Phylogenetic tree showed a great relation between evolutionary process of KaiC and the living environment of strains because of the gene transfer and recombination. KaiC participates in various regulations of cyanobacterial circadian rhythms.

Developing transgenic plants for abiotic stress tolerance: manipulation of stress tolerance mechanisms

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The major abiotic stresses, such as drought, extreme temperatures and salinity are responsible for huge losses in agricultural production. These abiotic stresses are the major problems for agriculture because these adverse environmental factors prevent plants from realizing their full genetic potential. These abiotic stresses induce various biochemical, molecular and physiological responses in plants. Thus understanding the physiological and molecular aspects of plants under stress and thereby targeting the regulatory machinery like receptors, transducers, transcription factors, and metabolites is desirable rather than focusing on the single gene transfer approach. Use of modern molecular biology tools like gene discovery and functional genomics projects for elucidating the control mechanisms of abiotic stress tolerance have revealed multitudinous mechanisms and gene families, which confer improved productivity and adaptation to abiotic stresses. These gene families can be manipulated into novel combinations, expressed ectopically, or transferred to species in which they do not naturally occur or vary. Hence, the ability to transform the major crop species with genes from any biological source is an extremely powerful tool for molecular plant biotechnology. Transgenic plants can be used as sources of new cultivars and they are also extremely useful as tools to dissect and characterize the activity and interplay of gene networks for abiotic stress resistance. Hence, genetic engineering for developing stress tolerant plants, based on the introgression of genes that are known to be involved in stress response and putative tolerance, might prove to be a faster track towards improving crop varieties.

Micro RNAs: The new frontier in crop engineering

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The global population is estimated to reach 9.6 billion by 2050, a more than five-fold rise since 1900. Since 1996, when GE crops were first grown commercially, their global cultivation had seen an approximately 100-fold jump making GE crops the most accepted crop technology of today. In the past two decades, scientists have innovated biotechnological tools for improving crop yield and quality. Among these, microRNA (miRNA) is becoming an emerging target for engineering new crop cultivars with high yield and quality as well tolerance to environmental abiotic and biotic stresses. miRNAs are an extensive class of small regulatory RNA molecules with about 20-24 nt in length, which are newly discovered in almost all eukaryotes, including both plants and animals. miRNAs is an extensive class of gene regulators, which negatively regulate gene expression at the post-transcriptional levels and many miRNAs are evolutionarily conserved from species to species. Zhang and colleagues (2013) found that overexpressing miR397 increased the overall grain yield of up to 25% in rice; this is the first study for directly improving crop yield using miRNA-based biotechnology. miRNAs are becoming new targets for engineering new plant species not only for improving crop yield, quality and tolerance to both biotic and abiotic stress as well for improving plant metabolism pathway for producing compounds for agricultural and biomedical purpose.

Manipulation of ethylene biosynthesis to extend the shelf-life of guava fruit

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Ethylene is known as 'ripening hormone' and it affects the transcription and translation of many ripening-related genes. Rate of ethylene production in fresh fruits and vegetables is considered good index for the determination of storage life. Efforts have been made to increase the shelf-life of fruits using antisense RNA technology to inactivate the expression of ripening related proteins such as ethylene biosynthetic enzymes *viz.*, ACC synthase and ACC oxidase. Silencing of specific genes in transgenic plants has made it possible to genetically modify fruit ripening process to achieve the desired objective to delay ripening. However, exogenous application of chemicals is, still being used to retard the physiological changes of the produce and to increase their shelf-life which is more acceptable to general public. Two varieties of guava *viz.*, L-49 and Hisar Surkha differing in their shelf lives were treated with different concentrations of CaCl₂ and GA₃ at their mature green stage and then analyzed for ethylene evolution and ACC oxidase activity. The GA₃ treatment did not show any significant effect on ethylene evolution while CaCl₂ significantly inhibited ethylene evolution. CaCl₂ treatment might slow down the process of ripening by retarding the pre climacteric respiration rate and subsequently ethylene production. Pretreatment of fruits with different chemicals had no significant effect on ACC oxidase activity and hence, it suggested that calcium may be preventing ethylene production, by means of inhibiting ACC availability for ACO. In addition, calcium is one of the most important minerals for the human body which is required for vascular contraction and vasodilatation, muscle function, nerve transmission, blood clotting, intracellular signaling and hormonal secretion. Proper levels of calcium over a lifetime can help to prevent osteoporosis.

Transgenics in *Brassica juncea* for salinity tolerance

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The various biotic and abiotic stresses affect the crop productivity. Salinity is one of the abiotic stresses governed by multigenes and is quantitative in nature. Salinity imposes a variety of stresses on plant tissues. Two of these are osmotic stress, which results from the relatively high soil solute concentrations, and ioncytotoxicity. With the application of genetic transformation in various *Brassica* genotypes, there is a scope to improve salinity tolerance. Genetic engineering approaches have been shown to be a relatively fast and precise means of achieving improved stress tolerance. In this direction, the complete genome sequence of related wild plants such as *Arabidopsis* can also serve as a platform for identification of “candidate genes”. Myb genes which have role in early signaling for drought and salt tolerance in *Brassica juncea* have been cloned and sequenced, osmotin gene and *codA* gene for biosynthesis of glycinebetaine have been used for transforming Indian mustard through *Agrobacterium* mediated transformation. Fortunately, Indian mustard is also amenable to *in planta* transformation. Therefore, the potential of these genes can be utilized for developing salinity tolerant plants in this crop which is one of the challenges under climate change.

Drought stress: A major challenge for rice breeders

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Rice is the staple food for almost half of the world's population and rice farming is the most sustainable and productive cropping system. Rice is semi aquatic and thrives well in water logged soils and its production system relies on ample water supply. Draught or water stress is one of the major factors negatively affecting the crop growth and productivity all over the world. A reasonable number of cultivars can be generated through conventional breeding program effectively utilizing draught tolerance traits. But there is a lack of appropriate screening techniques as drought stress can occur at any time of the developmental stage; starting from sowing/transplanting to grain maturity. Generally it is widely accepted that stress at reproductive phase is most critical and requires attention. However, genotypic response that contributes to drought stress tolerance depends on the genetic mechanism of tolerance in that environment. With the advancement in biotechnological tool, the genetic basis of drought tolerance has received considerable attention. Gene governing quantitative traits can be identified using a variety of molecular markers and their loci controlling drought tolerance is mapped on the chromosomal regions. However, transgenic and functional genomics approaches have offered a reliable promise in identifying stress responsive genes, pathways and understanding the mechanism of stress tolerance. Further it enabled to solve several essential key questions associated with stress tolerance through gene expression profiling and engineering of tolerant traits. Further, blending of conventional plant physiology, genetics, biochemical and biotechnological approaches and by adapting comprehensive screening technique, the challenge in stabilizing high yield under drought condition could be achieved in near future.

Effect of chitosan and calcium chloride treatments on postharvest quality and cell wall degrading enzymes of guava (*Psidium guajava*L.) fruits during storage

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The present investigation was undertaken to assess the effect of chitosan and calcium chloride (CaCl₂) treatment on quality attributes and cell wall degrading enzymes of guava fruit variety 'Hisar Surkha'. Fruits were harvested at mature green stage and were given postharvest treatment of 1.5% chitosan, 1.5% CaCl₂, 1.5% chitosan+1.5% CaCl₂ and water (control) for five minutes followed by storage at room temperature. Samples were analyzed at three day interval until complete decay for loss in weight, firmness, total soluble solids and titratable acidity. Treatment with 1.5% chitosan+1.5% CaCl₂ provided an effective control in reducing weight loss, maintained firmness, delayed changes in total soluble solids during storage. The titratable acidity declined throughout the storage period, though at a slower rate in 1.5% chitosan+1.5% CaCl₂ coated fruits as compared to the control and other treatments. The activities of cell wall degrading enzymes, pectinmethylesterase (PE), polygalacturonase (PG) and cellulase were also assayed. PG and cellulase exhibited progressive increase in activity during storage but increase was much less in 1.5% chitosan+1.5% CaCl₂ treated fruits. PE activity increased upto 6th day and then decreased. Similarly, 1.5% chitosan+1.5% CaCl₂ treated fruits exhibited slow increase in PE activity. These findings suggest that chitosan in combination of CaCl₂ is more effective in prolonging the shelf-life and maintaining keeping quality of guava fruits.

Role of transgenic plants in basic studies

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Transformation methods have allowed precise, fast and breakage of boundaries of sexual incompatibility in genetic modification resulting in transgenic organisms. These transgenics are known as pragmatic solution to global challenges like greater stress on food production. Norman Borlaug also had view that genetically engineered plants hold great potential to increase production under different environmental pressures. Besides these implications, transgenic plants are also proving to be powerful tools to study various aspects of plant sciences. For detailed functional characterization of genes and regulatory elements, establishment of a true link between genes and their biological function is required. The purpose of functional characterization makes use of a variety of approaches based on forward and reverse genetic studies. The emerging scientific revolution sparked by genomics based technologies is producing enormous amounts of DNA sequence information that, together with plant transformation methodology, is opening up new experimental opportunities. These include gene and regulatory element's function prediction using T-DNA and transposon tagging based insertional mutagenesis in various plant species. Sequence indexed insertional mutants are particularly valuable tool in this context because mutations in a given gene can be identified by simply searching a database. Thus, the large, sequence-indexed T-DNA and transposon-tagged mutant collections are available and continuously being generated and characterized as an invaluable resource for such forward genetic studies. Other applications include overexpression of nearby genes for investigating essential genes by using Activation tagging vectors, tissue-specific expression patterns of promoter with gene trapping vectors, introduction of deletions to ensure gene knockouts, designing of a single inverted repeat transgene to silence multiple, closely related genes, development of novel promoters and their functional analysis, to find out transcription factors associated with metabolism pathways in plants and application of transgenic methods to improve the efficacy of the Sterile Insect Technique(SIT) in agriculturally important crops. Thus transgenics coupled with functional genomics provide an efficient tool for identification of genes and their expression patterns.

Transgenic crops: Present status and future prospects

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More than seventeen years since the first transgenic plants were generated experimentally. During this period, there have been dramatic advances in our understanding in both basic and applied aspects of plant biology. Transgenic plant research depends on the availability of procedures for plant transformation. Agrobacterium can be used to transform a wide range of plants. Using these procedures, a number of transgenic crops have been developed experimentally and field-tested, while few of them are currently cultivated worldwide, predominately in industrial countries. In India, Global area under different GM crops has shown a sustained increase over the last few years. Bt cotton grown over 90% of total area under cotton production. Flavr Savr and Golden Rice transgenic varieties of tomato and rice respectively have been improved in their quality by prolonged period of ripening in tomato and increased beta-carotene content in rice. Now a day's many transgenic crops have been developed to improve human health and they are to be used as beneficial drug for the treatment of different viral and bacterial diseases. There is need to increase the food production to meet out the over increasing demand and to benefit the small farmers especially in the developing countries. Up to some extent, this can be achieved by adopting GM crops. In India, many private and government research and development organizations have considered this and many field trails are going on for the successful transformation. Finally, the implication of advancement in this relatively new technology especially in the area of biosafety, production patterns, biodiversity, intellectual property rights and other critical factors should be sufficiently addressed and understood.

Enhancing C₃ photosynthesis: A feasible intervention for crop improvement

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Despite of collective measures taken to eradicate hunger at World Food Summits, food security remains one of the biggest issues that we are faced with. Thus, there is an urgent need to increase crop productivity beyond existing yield potentials to address the challenge of food security. The targets considered for possible intervention include RuBisCO, RuBisCO activase, Calvin Benson cycle enzymes, CO₂ and carbohydrate transport, and light reactions among many others. In addition, other areas which promise scope for improvement of C₃ photosynthesis, such as mining natural genetic variations, mathematical modelling for identifying new targets, installing efficient carbon fixation and carbon concentrating mechanisms. The substitution of RuBisCO in C₃ plants with more efficient forms from different species like *Griffithsia monilis* (a red alga) and C₄ plants like *Amaranthus edulis* and *Zea mays* could significantly increase carbon gain as demonstrated in literature. Reports provide evidence that ectopic expression of the small subunit of RuBisCO with a high kcat (carboxylation reaction rate) from the C₄ plant *Sorghum bicolor* in rice resulted in enhanced kcat of the chimeric RuBisCO. Similarly, Overexpression of fructose-1,6- bisphosphatase and/or sedoheptulose- 1,7-bisphosphatase (FBPase/SBPase) in C₃ plants has been shown to enhance photosynthesis and plant growth significantly. Transgenic tobacco plants overexpressing *Arabidopsis thaliana* SBPase exhibited higher photosynthetic rates than wild type when grown under FACE (free air CO₂ enrichment) conditions. These examples provide evidence of the efficacy of genetic engineering technique for improving in C₃ photosynthesis to address the challenges of food security.

Role of GM crops in Integrated Pest Management

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Twenty years after their introduction, a large majority of the area planted to genetically modified (GM) crops still remains in just 28 countries. Total world's area under GM crops has increased dramatically, from 3 million hectares in 1996 to nearly 181.48 million hectares under total 9 crops in 2015. Four crops account for 99% of worldwide GM crop hectareage. These are soybean, corn, cotton and canola. Out of the total area, India occupies 11.6 million hectares under cotton alone. Protecting our food from pests has been an ongoing battle ever since humans began cultivating food. IPM involves careful consideration of all available pest control techniques, by integrating appropriate measures to reduce development of pest populations. With the advent of genetic engineering, new highly targeted strategies for pest management have become available in the form of transgenic plants. Major Bt crops which have been released like Bt cotton play a major role in IPM systems of crops with benefits to farmers and the environment as, it helps in reducing the pesticide sprays which nowadays of social concern regarding their adverse effects on human health. Several Bt toxins have been inserted into crop plants to provide protection against different groups of pest insects. GM crops will become more and more important to developing countries due to their ease of production, decreased production cost, and increased yields. To avoid resistance problems to Bt toxins, follow the strategies like use of multiple toxins, rotation of toxin genes, spatial and temporal refugia and gene pyramiding.

Identification of microRNA and their use for engineering agronomically important traits in crops

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MicroRNAs (miRNAs) are small non-coding regulatory RNA molecules that regulate gene expression post transcriptionally. These are 22 nucleotides in length, evolutionarily conserved and are generated by cleavage from larger precursor molecules (pre-miRNA). The plant miRNA were first identified in *Arabidopsis* and rice in 2002, since then a great interest has been shown in using miRNAs for different applications. Both computational and experimental approaches have been widely employed to identify large number of miRNA and their targets in plants using homologue-based comparative genome approach and high throughput deep sequencing and several databases and computational tools have been developed. MicroRNAs negatively regulate the expression of specific target messenger RNAs (mRNAs). Therefore, miRNAs can be used as a potential tool for studying plant development and improving tolerance to abiotic and biotic stresses. Artificial miRNAs have also been developed and found effective in gene silencing and serves a good strategy for functional study and crop improvement. Transgenic approach can be used for over-expression or suppression of one or more specific miRNAs, depending upon whether the target gene(s) has a desirable or an undesirable effect. The over-expression of miRNAs can be achieved through artificial miRNAs associated with high-expression promoters. Similarly, suppression of miRNA activity can be achieved using microRNA inhibitors. MicroRNA-profiling in cancer therapy is widely known and the idea that miRNA can be used in therapy of plant stress is certain. Future conventional plant breeding will be aided integrating miRNA approaches in engineering stress tolerance and Marker Assisted Selection for crop improvement.

Molecular Characterization of Transgenic Tomato (*Lycopersicon esculentum* Mill.) for fruit borer [*Helicoverpa armigera* Hübner] resistance

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Tomato (*Lycopersicon esculentum* M.) is the most imperative vegetable crop cultivated across the globe. In India, the tomato productivity is stumpy as one of the major yield constraint is the tomato fruit borer (*Helicoverpa armigera* Hübner). Therefore, Bt transgenic technology provides a secure and consistent means for management of this pest. Transgenic tomato (cv. Hisar Arun) expressing *cry1Ac* gene coding for an insecticidal crystal protein (ICP) of *Bacillus thuringiensis* (*Bt*) was developed through *Agrobacterium*-mediated transformation method. Molecular and biochemical analysis was carried out to confirm the integration and expression of the transgene into tomato genome in T₁, T₂ and T₃ generations. PCR and RT-PCR analysis showed amplification of expected fragments of 533 bp for *cry1Ac* gene and 1050 bp for *npt II* gene which confirmed presence of both the genes at DNA and mRNA level. Double-antibody sandwich ELISA analysis revealed high levels of *Bt* ICP expression in the leaves of transgenic plants. Evaluation of Cry1Ac was judged by the mortality of fruit borer *Helicoverpa armigera* (Hübner) when fed on *Bt* tomato. The results indicate a significant amount of Bt protein was present in most of the transgenic lines and that plants expressing *cry1Ac* gene could be used for management of the target lepidopteran insect pests. At the end of the analysis in the T₃ generation, five plants from twelve events were selected as promising on the basis of expression and bioefficacy analysis. Therefore, the study proved not only the amenability of the tomato to the transformation protocol but also showed that stability of the introduced transgenes was throughout all the three generations for the gene integration, expression and good bioefficacy against the insects, *H. armigera*.

Possibility of using ISSR Marker for the development of drought tolerant genotypes in chickpea

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Chickpea (*Cicer arietinum* Linnaeus), a member of family Fabaceae, is an ancient self pollinated leguminous crop. It is ranked 3rd after common bean (*Phaseolus vulgaris* L.) and pea (*Pisum sativum* L.) and is mainly concentrated in semi-arid environments. Drought is the most important constraint to yield in chickpea, accounting for about 50% yield reduction globally. Therefore, it is essential to develop varieties which can make use of available water resources and produce maximum yield. Most of the physiological traits associated with drought tolerance are quantitative in nature. An important research strategy that has been widely used over the past two decades to deal with such complexity is to use molecular markers to identify quantitative trait loci (Qtls) and their applications in marker-assisted selection (MAS) programmes. The present study on chickpea was conducted in the dryland area of CCS Haryana Agricultural University, Hisar for two consecutive years to screen 21 genotypes for rainfed conditions. In addition to morphological and physiological traits recorded on these genotypes, their ISSR DNA profile was also analyzed. Lots of genetic diversity was recorded at DNA level among all the genotypes. NTSYS pc2.0 was used to cluster them. One of the ISSR markers UBS841 was found to be associated with deeper root length in chickpea. Morphological, physiological and molecular observations in combination can be used to identify high yielding plants in chickpea for rainfed conditions (drought). The ISSR marker thus identified can also be successfully employed in MAS programmes for drought tolerance.

Herbicide resistant plants by biotechnological approach

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Plants can be made resistant to herbicides by a number of mechanisms. The molecular target site of the herbicide can be modified so that it no longer binds it and is thereby resistant. One or more herbicide-inactivating or herbicide-degrading enzymes can be introduced to or increased in a plant. The plant can be altered to have a mechanism that prevents the herbicide from reaching the molecular target site (increased sequestration, or decreased uptake or translocation). All three mechanisms have been described in weeds that have evolved resistance to herbicides. Metabolic inactivation or degradation is the principle mechanism in most cases of natural crop resistance to selective herbicides. The first two approaches have been useful in producing commercial HRCs.

Glyphosate's molecular target site is an enzyme of the aromatic amino acid pathway (the shikimate pathway). This enzyme, 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS), is highly sensitive to glyphosate, and there are apparently no other good inhibitors of the enzyme. All plant EPSPS is sensitive to glyphosate, making it a nonselective herbicide that can be used to kill almost all weed species. Some fungi and bacteria also have EPSPS, and there are bacterial versions of the enzyme that are very resistant to glyphosate. A gene encoding *Agrobacterium* sp. EPSPS, the CP4 gene, has been used as a transgene for almost all GR crops.

Transgenics: savior of farmers

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There is too much work to invest for a field of failed crops. Lots of farmers face this kind of problem and many of them turning to new technology. If you grow any crop like maize, corn, wheat, rice the amount of money a farmer can make is depend on many factors like the weather, the choice of fertilizer, the quality of seed. One of the choices that a farmer can make is to use a seed which is not harmed by insect, pest, weed and also give high yield. Genetically modified plants help in eliminating the problem of insect, weeds and reduce the use of chemical pesticides and directly minimize the cost of growing crop. There are also evidences of improving nutritional quality (Golden Rice) in developing world to fight malnutrition. Edible vaccine production through transgenics is another milestone in healthcare for masses. A Meta-Analysis of impacts of genetically modified crops in which researchers used data from 147 separate studies to analyze the outcomes for farmers using the genetically modified seed and non-genetically modified seeds. A statistical study revealed that farmers who used GMO they used 37% less pesticide to grow more crops, and they made large profit (58%). They also found that using insecticide- resistant crop led to 25% greater profit while using herbicide-tolerant crop led to only 9% greater profit. Farmers in developing countries saw 60% greater profit than farmers in developed countries. Using transgenic crops is therefore in the favour of farmers.

Bt brinjal: should we cultivate it or not

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Brinjal (*Solanum melongena*) also known as egg plant and important solanaceous crop of tropics and subtropics. In term of both yield and area cultivate first genetically modified crop which is created out of inserting a synthetic gene (*Cry IAb*) from the soil bacterium *Bacillus thuringiensis* in brinjal. However the yield of brinjal could be much higher would it not decimated by the lepidopteran pest like brinjal shoot and fruit borer, (*Leucinoide arbonalis*), brinjal stem borer, (*Euzophera perticella*) and *Lepinotarsa decemlineata* (carrying *Cry 3b*) which are most destructive insect pest in South and South- East Asia. Bt Brinjal is being developed in India by the Maharashtra Hybrid Seeds Company (Mahyco). Genetically modified brinjal has the potential to increase the agricultural productivity in India and other countries. On Oct 30, 2013 with approval from the Ministry of Environment and Forest and MOA, BARI releases the permission of varieties of Bt brinjal namely; Bt Uttra, Bt Kajla, Bt Nayantara and Bt ISD006. Recently, the GEAC has cleared Bt Brinjal—country's first genetically modified food—for commercial use. In India, Karnatka has 40 varieties, out of them Mattu Gulla a special brinjal endemic Udupi in Karnatka. Here provide a brief review Bt brinjal should we cultivate it because Bt brinjal will reduce the pesticide cultivation 80%.it summarise the result of research undertaken on the environment safety of Bt brinjal could a significant contribution in agriculture sector and more broadly of country economy, living standard of poor people (farmer), ingredient in ayurvedic medicine and in treatment of diabetes. In Punjab and Haryana, a number of farmers mortalities happen due exposure of pesticides during spraying operation. If Bt brinjal can reduce pesticide consumption with no environmental pollution, health hazard and safe to naural enemies and pollinators, we should cultivate Bt brinjal.

Benefits of genetically modified corn

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Corn is one of humankind's earliest innovations. As needs change, so does plant breeding. Today, while biotech super-giants manipulate corn genetics to satisfy farmer desires and a global market, indigenous Mexican farmers do so to fulfill individual needs. Although the tools differ, the goal remains the same to cultivate desirable traits. With the goal of ever-widening the pool of genetic diversity, conventional plant breeding has gotten more technologically savvy in recent years. For example, realizing that natural mutants often introduce valuable traits, scientists turned to chemicals and irradiation to speed the creation of mutants. Bt corn considered safe to humans, mammals and most insects. *Bacillus thuringiensis* produces toxins which destroy the gut of the larval caterpillars. Bt corn reduces the need for pesticides, and while the primary benefit comes largely during a heavy corn-borer infestation, an unpredictable event, a secondary effect is that beneficial insects fare much better under these conditions. Surprise benefits have also occurred. According to the recent International Council for Science (ICSU) review of GM crops, disease-resistant corn crops may have lower levels of mycotoxins, potentially carcinogenic compounds to humans. They result from fungal activity in insect-infested corn crops. With fewer insect holes in plant tissue, associated fungi are not able to invade and produce toxins.

Market value of GM crops

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Genetically modified crops are plants used in agriculture, the DNA of which has been modified using genetic engineering techniques. In most cases, the aim is to introduce a new trait to the plant which does not occur naturally in the species. Farmers have widely adopted GM technology. Use of GM crops expanded rapidly in developing countries, with about 18 million farmers growing 54% of worldwide GM crops by 2013. A 2014 meta-analysis concluded that GM technology adoption had reduced chemical pesticide use by 37%, increased crop yields by 22%, and increased farmer profits by 68%. Yield and profit gains are higher in developing countries than in developed countries. There is general scientific agreement that food on the market derived from GM crops poses no greater risk to human health than conventional food, but should be tested on a case-by-case basis. The market for transgenic seeds has a significant impact on agriculture production. Transgenic seeds are gaining better response compared to other pest control agents. The driving factors for this market are the increasing global population and food requirements, rise in cost of fertilizers & pesticides, and easier residue management. Transgenic seeds gained popularity due to various factors, including increasing cost of seeds, production of high-quality agricultural produce, increasing rates of crop rotation, and raising awareness among farmers with respect to the increasing toxicity of the agrochemicals. Additionally, increased strive for yield maximization across the globe is encouraging the farmers to adopt transgenic seeds.

Wheat Transgenics: Need and Prospects under Future Climatic Conditions

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In Proceedings of the 3rd International Workshop of the Wheat Yield Consortium 2013, it was discussed that climate changes rather than lack in genetic progress have been considered to be responsible for decrease in wheat yield all over the world in different agro-climatic regions. At present wheat production rate increases only by 0.5 % per year whilst 1.4% is necessary to cope up with the growing population.

Forthcoming global warming impairs dry matter production and reduces grain weight in all major cereals, which imposes the challenges to understand and improve heat tolerance in crop plants. Heat stress has multiple effects on plant development, typically involving shortening of developmental phases and affecting physiology and growth of plants, especially of seeds. Yield development in wheat is dependent on accumulated resources. In cereal crops, heat stress can promote grain abortion and decrease synthesis of starch and storage proteins. Grain development is highly sensitive to elevated temperatures. During caryopsis development heat stress down regulates genes related to storage compound biosynthesis and cell growth. Various metabolic changes are rapidly induced by heat stress. Therefore, it is the need of time to study about sucrose transport and starch biosynthesis in normal as well as heat stress conditions. Differential gene expression between these environmental conditions may reveal transcription factors, phytohormone biosynthesis/signaling, calcium and sugar signal, ribosome proteins, primary and secondary metabolites and diverse stress related proteins. A state of art research along with cutting edge technology has all the potential to look for the results to satisfy the need of time to come forward.

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