Book of Abstracts - National Seminar on GM Crops: Prospects and Issues, 2014 March 17-18, Kerala Agricultural...

Book · March 2014

DOI: 10.13140/2.1.3377.9521

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Abstracts

National Seminar

GM Crops:
Prospects and Issues

17-18 March 2014

Contributory Papers

Sponsored by
Kerala State Planning Board
Govt. of Kerala

Organized by
Centre for Plant Biotechnology and Molecular Biology
College of Horticulture
Kerala Agricultural University
Vellanikkara, Thrissur, Kerala – 680 656, India

National Seminar
On

GM Crops: Prospects and Issues

17-18 March, 2014

Abstracts

Of

Contributory Papers

Centre for Plant Biotechnology and Molecular Biology
College of Horticulture
Kerala Agricultural University
Vellanikkara, Thrissur, Kerala – 680 656, India

Editors

Deepu Mathew
Nazeem P. A.

March, 2014

Centre for Plant Biotechnology and Molecular Biology
College of Horticulture
Kerala Agricultural University
Vellanikkara, Thrissur, Kerala – 680 656, India

Published in connection with the National Seminar on GM Crops : Prospects and issues sponsored by Kerala State Planning Board.

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FOREWORD

A genetically modified (GM) crop is the plant used for agricultural purposes into which one or several genes coding for desirable traits have been inserted through the process of genetic engineering. The first GM crops became commercially available in the mid-1990s. Three categories of GM traits can be distinguished: First-generation GM crops gave trust for improvement in agronomic traits, such as better resistance to pests and diseases. Second-generation GM crops concentrated on enhanced quality traits, such as better nutrient contents of food products. Third-generation GM crops were designed to produce special substances for pharmaceutical or industrial purposes. The potentials of GM crops are manifold. Against the background of a dwindling natural resource base, productivity increases in global agriculture are important to ensure sufficient availability of food and other raw materials for a growing population. GM crops can also bring about environmental benefits. Furthermore, new seed technologies have played an important role for rural income growth and poverty alleviation in developing countries. In spite of these potentials, the development and use of GM crops have aroused significant opposition. Though, it is widely claimed that biotechnology, particularly genetically engineered food offers dramatic promise for meeting the 21st century's greatest challenges; like all new technologies, it also poses certain apprehensions and risks, both known and unknown. It is, therefore, highly relevant this context, to know the basic processes involved in genetic modification for proper appreciation of the related issues and challenges.

I am happy that the Centre for Plant Biotechnology and Molecular Biology, COH Kerala Agricultural University is organizing a National Seminar on "GM Crops: Prospects and Issues" during March 17 -18 2014 with the financial assistance of Kerala State Planning Board, Govt. of Kerala to review the facts and myths in this field. The proceedings containing the abstracts of the contributed papers and posters is a summing up of the research accomplishments in different areas of genetic engineering in various crops.

The valuable contributions made by the various authors from all over India in making the seminar highly successful are greatly appreciated. I also profusely congratulate the editors for their sincere and dedicated efforts in brining out this publication. I am confident that the publication would provide valuable information on the different aspects of GM technology.

I congratulate Dr. Deepu Mathew, Organizing Secretary of the National Seminar, Dr. P.A. Vasala, Head, Centre for Plant Biotechnology and Molecular Biology [CPBMB], and Dr. Vaalsalakumari, Associate Dean, College of Horticulture the initiative taken in this regard.

PREFACE

The plant science and its exploitation can profoundly change our approach to the world's food, medical, and environmental problems. The introduction of the first transgenic plant 30 years ago heralded the promise for a second green revolution, providing food to the starving, profits to farmers and environmental benefits. Many GM crops fulfilled the promise. But their success has been mired in controversy with arguments on their safety, their profitability and their green credentials. A polarized debate has left little room for consensus. National Seminar on "GM Crops: Prospects and Issues" is organized with this background at KAU main campus, Vellanikkara during March 17 -18 2014 to discuss the current scenario for the scientific discussions on the potential risks and benefits of transgenic crops.

This publication consists of the abstracts of the various papers/posters to be presented in the various technical sessions of the seminar and the abstracts have been arranged under five different sessions. The sessions are i) Transegenics in food and non-food crops. ii) Clean gene technology. iii) Biosafety and IPR in GM crops. iv) Techniques for detection of GM crops and products and v) Computational biology for transgenics.

The support provided by Kerala State Planning Board for the conduct of this seminar is greatly acknowledged.

Editors

SESSION I

Transgenics in Food and Non- Food Crops

Oral

Abstract No I.O1	Session I Transgenics in Food and Non- Food Crops

Herbicide tolerant genetically modified crops- status, claims and apprehensions: Indian perspective

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Extensive reliance on herbicides for weed control in various cropping systems across the world has resulted in the evolution of resistance to herbicides. Interestingly, such problem of weed resistance to herbicides has provided an invaluable opportunity for scientists to develop crops resistant to non-selective herbicides. Researchers explored ways to develop crops resistant to herbicides with the goals of improving herbicide selectivity, expanding weed control spectrum, as well as minimizing crop injury. Herbicide-tolerant (HT) crops are plants modified to tolerate exposure to a specific herbicide. A larger share of world's genetically modified (GM) crops is from HT technology. HT crops are gaining acceptance because of several advantages over present day weed management practices. However, there are several apprehensions about the use of HT crops and their subsequent impact. In this context, several deliberations were held in India over the introduction of HT crops such as Roundup ready soybean and corn. Some scientists and anti-GM groups warned that HT crops may hasten the evolution of herbicide resistance in many weeds by virtue of overuse of a single herbicide. Number of glyphosate-resistant weed species has been identified since introduction of glyphosate-tolerant crops in 1996. But herbicide resistance is a problem for farmers regardless they grow HT crops or not and even before the advent of HT crops. Problem of weedy rice, which became a threat to rice production in many parts of India including Kerala, and left 'almost no option' to farmers for management of this weed, can be tackled with this technology. However, one should take utmost care in selection of appropriate approach for generating of HT crops keeping in mind all possible adversaries. Effort will be made to highlight current global status, claims and apprehensions regarding the herbicide tolerant technology keeping in mind Indian perspectives.

Abstract No I.O2	Session I Transgenics in Food and Non- Food Crops

Development of transgenic plants of *Hevea brasiliensis* integrated with osmotin gene

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Hevea brasiliensis Muell. Arg, (Para rubber tree) is a perennial out breeding species and is the major source of commercial natural rubber (cis-1,4-polyisoprene). Increasing demand for natural rubber has necessitated the development of high yielding *Hevea* clones tolerant to adverse climatic conditions. The prolonged gestation period, high heterozygosity, brief and seasonal nature of flowering, poor seed set etc. pose hindrance to the development of new clones in this perennial crop through conventional breeding. In this context, *Agrobacterium* mediated transformation of *Hevea* was attempted with the gene coding for osmotin protein in order to impart abiotic stress tolerance. *Agrobacterium* strain *GV 2260* harboring the plasmid osm/BinAR with osmotin gene under the control of CaMV35S promoter was employed for genetic transformation. Neomycine phosphotransferase gene (*npt*II) imparting kanamycin resistance was used as the selectable marker. The target explant used in this study was embryogenic calli induced from immature zygotic embryo following *half ovulo* embryo culture technique. A transformation frequency of about 44.8% could be achieved. Putative transgenic lines were proliferated and the gene integration was confirmed by PCR analysis. The sequence of the cloned PCR product showed 100% similarity with the inserted gene sequence. Successful embryo induction (43.5%), maturation (46.0%) and plant regeneration (23.6 %) were achieved. The regenerated plants were acclimatized in growth chamber and maintained in the containment facility in big polybags. Evaluation of stress tolerance on transgenic callus showed positive indications towards drought and salinity tolerance. Transgene integration and expression have been confirmed by PCR, Southern blot and RT-PCR. These osmotin transgenic plants are expected to perform well under stressful environments.

Key words: *Agrobacterium, G*enetic transformation, *Hevea brasiliensis,* osmotin, transgenic plants

.

Abstract No I.O3	Session I Transgenics in Food and Non- Food Crops

Genetic modification for designer starch from cassava

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Starch is a basic raw material for many food and non food industries ranging from frozen foods to dextrin and adhesives, biodegradable plastics, gypsum binders and so on. Additionally modified starches are used as fillers, emulsion stabilizers, consistency modifiers etc. Starch contains two chemical compounds amylose and amylopectin. Varying the proportion of these will result in modified starch with enhanced suitability for specific use in various industries. Such modified starch, in this context, is referred to as 'designer starch'. Chemical modification is the usual procedure for making starch suitable for industrial use, which is less eco-friendly and cost generating. Through modifying genes in starch metabolism it is easier to get a stable supply of modified starch. For example waxy cassava is one such source of designer starch.

Among the 5 major sources of starch viz., rice, maize, wheat, potato and cassava, cassava is the largest source in the tropical region. CIAT has developed waxy cassava using antisense RNA technology. Efforts for making waxy starch from cassava are undertaken at various parts of the world including Netherlands, China, Thailand and also India. Waxy starch from cassava has improved paste clarity, low retro-gradation and better freeze thaw stability. There are reports saying that the pasting temperature of waxy starch from cassava is better than cereal derived waxy starch.

Genetic modification through silencing the RNA is the method adopted in all the cases and by far Netherlands have attained the field trials of GM waxy cassava. This paper aims at explaining the method of developing waxy starch from cassava.

Abstract No I.O4	Session I Transgenics in Food and Non- Food Crops

Genetic transformation in *Artemesia annua* L. for hairy root induction and enhancement of secondary metabolites

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Artemesia annua L. is one among the top ten pharmaceutical crops and is a source of powerful antimalarial drug, artemisinin. Hairy root induction was attempted for enhancing the secondary metabolite production in this crop. Genetic transformation was carried out in *Artemesia* using three different *A. rhizogenes* strains like A4, ATCC 15834 and MTCC 2364. Leaf segments, shoot tips and nodal segments were used as explants. Influences of explants, type of bacterial inoculum, stimulents, and co-cultivation periods on transformation frequencies were evaluated. Among the three strains, ATCC 15834 recorded the highest transformation efficiency. The hairy root cultured in hormone free basal media showed high lateral branching. B_5 liquid media with 3.0 per cent sucrose was found to be superior in promoting hairy root growth. The transformation was confirmed through opine detection and PCR analysis. A Thin Layer Chromatographic method was employed for artemisinin estimation. The pink spot corresponding to Artemisinin was observed by immersing the plates in a pool of freshly prepared glacial acetic acid: conc. sulphuric acid: anisaldehyde (50:1:0.5) followed by drying in a chromatographic oven at 110˚C for 15 min. Artemisinin content in normal young roots, *in vitro* derived roots and hairy roots were below the detectable limits. However, the biotic elicitor *Aspergillus* homogenate had a positive influence on the biosynthesis of artemisinin in the hairy root cultures.

Abstract No I.O5	Session I Transgenics in Food and Non- Food Crops

Agrobacterium tumefaciens mediated transfer of exogenous hydroxy methyl glutaryl CoA (HMG CoA) reductase gene to *Centella asiatica* L.

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Centella asiatica, commonly known as Hydrocotyl or Asiatic pennywort or Vallarai and described by Charaka as an anti-aging plant, is used in many ayurvedic formulations. It contains a blend of secondary metabolites including triterpenes which are responsible for its medicinal properties. Among them, asiaticoside possesses remarkable pharmaceutical value due to its anti-inflammatory, antitumour, neuroprotective, skin care and toning effects. For commercial extraction the asiaticoside content should be more than three per cent but in Indian ecotypes the content is less than one per cent and so this cannot meet the industrial demands. In this study, a protocol for genetic transformation of *Centella asiatica* using *Agrobacterium tumefaciens* has been standardized that can aid in the improvement of the plant through metabolite engineering. *Agrobacterium tumefaciens* strain EHA 105 harbouring the plasmid pBE2113 containing *nptll* and *hmgr* gene was used for transformation. *hmgr* gene codes for Hydroxy methyl glutaryl coenzyme A reductase (HMGR) an important enzyme which acts at the upstream of the mevalonate pathway producing mevalonic acid, further downstream produce Iso Pentenyl Pyrophosphate (IPP), squalene, β-amyrin and finally asiaticoside. Transformation was confirmed by PCR analysis with *nptll* gene specific primer. Protocol for callus induction and regeneration were also standardized. Callus initiation was faster (23 days) from node compared to leaf (25 days), with 100 and 92.85 per cent induction respectively on Murashige and Skoog (MS) medium supplemented with Kinetin (Kn) 4 mg l^{-1} and Naphthalene Acetic Acid (NAA) 2 mg l^{-1}. The highest regeneration (0.052%) was obtained on MS medium supplemented with Kn 4 mg l^{-1} and NAA 2 mg l^{-1}.

Keywords: *Agrobacterium tumefaciens*, Asiaticoside, *Centella asiatica*, HMGR gene, Metabolite engineering, Plasmid pBE 2113.

Abstract No I.O6	Session I Transgenics in Food and Non- Food Crops

Genetic transformation of *Hevea brasiliensis* using intact explants as target tissues for *Agrobacterium* infection

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Genetic transformation is a promising tool for incorporation of agronomically important genes leading to genetic improvement of the natural rubber producing tree, *Hevea brasiliensis. Agrobacterium* mediated genetic transformation in *H.brasiliensis* was achieved earlier using callus as target tissue and transgenic plants were regenerated. In the present work, the feasibility of using explants as such, as target tissue for *Agrobacterium* infection, was assessed. Three target tissues such as leaf explants collected from glass house and pre-cultured in modified MS medium for one week, leaf and root explants from *in vitro* developed somatic plants were used for infection with *A. tumefaciens* harbouring the binary vector carrying superoxide dismutase (MnSOD) gene for abiotic stress tolerance, GUS as reporter gene and *npt* II gene for antibiotic selection. The effect of explant pretreatment on transformation efficiency was also studied. Different pre-treatments such as air drying the explants in laminar air flow hood, soaking in sterile water, sterile water containing acetosyringone (40 mg l^{-1}) and sterile water containing acetosyringone (40 mg l^{-1}) and picloram (2.0 mg l^{-1}) for 20 minutes were given prior to infection with *Agrobacterium*. Both precultured leaf and *in vitro* root explants soaked in sterile water containing acetosyringone (40 mg l^{-1}) and picloram (2.0 mg l^{-1}) responded well to bacterial infection with i*n vitro* root explants giving maximum transformation efficiency (67%). The co-cultured explants were transferred to selection medium after three days. The optimum concentration of kanamycin required for selection of infected explants was 50 mg l^{-1}. Callus induction was obtained from the infected explants after three weeks. Newly formed callus from infected root explants were proliferated and when subjected to GUS histochemical assay gave positive results. The genomic DNA isolated from randomly selected putatively transgenic callus lines were used for PCR analysis with GUS, *npt* II and MnSOD gene specific primers. Gene amplification could be obtained using GUS (650 bp), *npt* II (804 bp) and MnSOD (702 bp) gene specific primers. This is the first report on using intact explants as target tissues for bacterial infection in *Agrobacterium* mediated genetic transformation of *Hevea*.

Key words: *Agrobacterium*, explant, genetic transformation, pre-treatment, target tissue

Abstract No I.O7	Session I Transgenics in Food and Non- Food Crops

Genetic transformation in *Aswagandha* (*Withania somnifera* (L.) Dunal) for hairy root induction and enhancement of secondary metabolites

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Withania somnifera (L.) Dunal commonly known as *Ashwagandha* or Indian ginseng is a high valued medicinal plant. Genetic transformation was carried out in *W. somnifera* using three different *A. rhizogenes* strains for inducing hairy roots. The explants such as hypocotyls, cotyldonary segments, leaf segments, shoot tips and nodal segments were used for transformation. The influence of parameters such as type of explants, bacterial inoculum, co-cultivation periods were assessed. A4 and ATCC 15834 strains induced successful transformation. A4 strain produced transformation by direct inoculation of bacteria from single cell colonies as well as in the suspension form but ATCC 15834 induced transformation only in the suspension form. The hairy root cultures established on MS + 250 mg l^{-1} cefotaxime showed phenotypic variations in growth habit. Half MS liquid media was found to be superior in promoting hairy root growth. The transformation was confirmed by PCR and dot blot analysis. A Thin Layer Chromatographic method was employed for withanolide estimation. The spot corresponding to Withaferin A was observed under UV at 254 nm. Normal roots possessed more withaferin followed by hairy roots and *in vitro* roots contained the least. Enhancement of secondary metabolite production was also attempted. The biotic elicitor *Aspergillus* homogenate (250 and 500 µl /125 ml) had a positive influence in the enhancement of secondary metabolites in hairy roots.

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Abstract No I.O8	Session I Transgenics in Food and Non- Food Crops

Isolation and characterization of a defense gene for early blight of tomato caused by *Alternaria solani* (Ellis & Martin)

Abida,P.S., Krishnaveni, S., Madhura,J.S., Sajeevan,R.S and Murugan,M

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Tomato seeds of 32 accessions (wild and cultivated) were screened for early blight resistance. Thirty five days old cultivated and wild genotypes of resistant and susceptible tomato plants were spray inoculated with early blight pathogen, *Alternaria solani* and plants sprayed with double distilled water served as control plants. Total RNA was extracted from the leaf tissues collected at 6h and 24h post inoculation from the control and treated plants by TRI-reagent method. DD-RT-PCR was performed to identify the differentially expressed cDNAs during different treatments. Differentially expressed transcript derived fragments were cloned in pTZ57R/T vector (MBI Fermentas) and sequenced by Automated sequencer. The sequence information was annotated to identify the putative function of the differentially expressed genes against sequences deposited in NCBI and TIGR databases and *In silico* structure –function analysis was also done with the sequences obtained from resistant genotype LE 996. The target sequences was subjected to NCBI-BLASTp to get the template sequences. The sequences were further analysed using Expasy tool-proteomic tools. Homology modeling was done for the target sequence from a resistant genotype LE 996 by submitting to swiss model server. The protein molecule was visualized through molecular graphics program Rasmol. The structure was further validated by Ramachandrans plot and force field calculation.. The energy computation value was –3222.620. The protein was classified as stable structure. The physico-chemical properties were studied using PROTPARAM and the secondary structure details was studied using SOPMA. Analysis of primary structure revealed the number of amino acids to be 134 and theoretical pI to be 9.6. The amino acid arginine has highest content of 11.9%. Analysis of secondary structure revealed highest random coil content of 45.52%. Further functional analysis was done using Pfam and prosite.

Keywords: DDRT-PCR, defense gene, swiss model and Expasy-proteomic tool

Abstract No:I.O9	Session I Transgenics in Food and Non- Food Crops

Molecular assessment of Sexual forms in *Myristica fragrans*

Divyasree, P., Lokesh, K. A., Lidiya John., Tintu Joy., Teena Ramachandran, V., Anu Thomas Muttakulath., Manila, T.M., Sandhya Rajan, V.T.K and Deepu Mathew

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A modified Doyle and Doyle protocol for the extraction of quality DNA from polyphenolic rich *Myristica* was developed. Successful amplification using RAPD primers indicated that the DNA is of good quality. Initially, RAPD primers were screened to obtain the polymorphism among male and female plants. The male plants were polymorphic for the presence of specific bands at 400 bp, 1200 bp and 1400 bp amplified by OPK 1, OPF 5 and OPE 11 primers, respectively. A characteristic band at 200bp amplified by OPA 27 was observed in female samples. The bands generated using OPK 1, OPE 11 and OPA 27were further eluted and cloned. The sequence was generated and being analysed to design SCAR primers for differentiating the sex forms.

Abstract No I.O10	Session I Transgenics in Food and Non- Food Crops

*atp*D gene expression of probiotic *L plantarum* 91 under acidic environment

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Lactobacillus plantarum is a flexible and versatile microorganism that inhabits a wide variety of environmental niches, including the human gastrointestinal (GI) tract. Strains of *L. plantarum* have proven ability to survive gastric transit and can colonize the intestinal tract of humans and other mammals. The ability of these microorganisms to grow in harsh environment prevailing in is linked to their ability to resist acidic conditions in the stomach of healthy humans. Changes in pH in the environment have been reported to influence the expression of many genes and atp operon is chiefly involved in the acid tolerance of probiotic lactobacilli in the gut. The *atp* genes are included in the category of housekeeping genes. However, the regulation of this pH-inducible phenotype has not been clearly established at the molecular level. In this study the influence of low pH on inducible gene expression in *Lactobacillus plantarum* was investigated both *in vitro* and *in vivo*. Logarithmic phase cultures were exposed to pH 2.5, 2.0 and 1.5 for various time intervals and cultured for monitoring survivability. The cultures were able to survive at pH 1.5 to an appreciable level even after 1-3 hours. *In vivo* study was carried out by feeding *L.plantarum* cultures to mice followed by isolation of bacterial RNA from stomach at different time intervals. The isolated RNA was reverse transcribed and the resultant cDNAs were subjected to RT-qPCR and the products were resolved by electrophoresis. The *atp*D gene was significantly up-regulated to 1.48, 2.04 and 3.05 folds after 15, 30 and 60 min transit in the stomach of mice. This result clearly demonstrates that *atp*D gene expression is essential for survival of probiotic bacteria under acidic environment prevailing in the stomach.

Abstract No I.O11	Session I Transgenics in Food and Non- Food Crops

Molecular analyses of coconut palms (*Cocos nucifera* L.) with mid whorl yellowing

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Kerala the 'Land of coconut' occupies first position in area and production of coconut in India, but the productivity is below the national average. This is because of unproductive and senile palms, lack of adaptation of recommended cultivation practises and serious incidence of pests and diseases. Mid whorl yellowing a type of yellowing in coconut characterised by shedding of immature nuts, drying of inflorescence and serious reduction in nut yield is rapidly spreading throughout Kerala. Association of phytoplasma was detected in palms with mid whorl yellowing using nested PCR. The phytoplasmal DNA from infected samples was amplified by nested PCR using the universal primers derived from conserved regions of the 16S ribosomal sequence. Nested primer pairs P1/ P7 -R16F2n/ R16R2; R16mF2 /R16mR1- R16F2n/ R16R2; and semi nested primer pairs 1F7/7R3- 1F7/7R2 were used. Amplification of phytoplasma specific universal primers in palms with mid whorl yellowing indicates that phytoplasma has got a role in development of the mid whorl yellowing symptom.

Key words: Coconut, mid whorl yellowing, phytoplasma

Abstract No I.O12	Session I Transgenics in Food and Non- Food Crops

Transgenic Plants for adverse environmental zones of Kerala-An Ecological alternative

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The planet is heating up, weather is getting weird, and the human population is growing. Plants are engineered to tolerate non-biological stresses like drought, frost, high soil salinity and nitrogen deficiency. When food systems are being disrupted by droughts, extreme heat, bitter cold, and other unpredictable weather events, genetically modified (GM) crops currently in development produce higher yields with less water. Drought tolerance technology, which allows crops to withstand prolonged periods of low soil moisture, is anticipated to be commercialized within five years. Nitrogen use efficiency technology is also under development, which can reduce run-off of nitrogen fertilizer. The present paper discusses the impact of GM crops as a suitable alternative when introduced into problem zones of Kerala where future land usage for agricultural purposes is facing stalemate with the already existing conventional varieties of crops and methods of cultivation. The drought tolerance technology has particular relevance for dry areas of northern Kerala, where drought is a common occurrence and access to irrigation is limited. Salt tolerance addresses the increasing problem of saltwater encroachment on freshwater resources. Introduction of saline tolerant GM crop varieties to saline affected coastal regions of Pokkali and Kaipad tract can go a long way in saving fragile ecosystems of these areas where land is being depleted and diverted to non-agricultural purposes.

Keywords: GM crops, environmental zones, drought tolerance, salt tolerance.

SESSION I

Transgenics in Food and Non- Food Crops

Poster

Abstract No I.P1	Session I Transgenics in Food and Non- Food Crops

Evergreen revolution through biotechnological interventions

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Green revolution has long sustained the world's masses with an abundance of food and it was the first major scientific and technological intervention that agriculture witnessed in its history. The term Green revolution was coined by William S. Gaud in 1968. In scientific terms, it implies enhanced crop production through higher productivity per units of land and water .Green revolution technologies help in harnessing the photosynthetic pathway of development, which makes agriculture the largest solar energy harnessing enterprise in the world. (Swaminathan, 1996).Biotechnological interventions for an ever green revolution include genetic engineering, accelerated crop improvement through molecular tools, commercial plant tissue culture, biofertilizers, biofuels, and biopharming. Socio-economic surveys confirms that the celebrated product, Bt cotton continues to deliver significant and multiple agronomic, economic, environmental and welfare benefits to farmers and society (James, 2009). In future, biotechnology can contribute much more for enhancing crop productivity, food quality, environmental benefits, and pharmaceutical production, which ultimately results in yet another revolution. Biotech crops being developed by the public sector include brinjal, cotton, groundnut, mustard, papaya, and potato, while the private sector partaking through brinjal, cabbage, cauliflower, cotton, maize and okra. There are 16 biotech crops in field trials in India including Bt maize. HT maize (James,2009). The joint contribution of the two sectors is of critical importance, given that national food security is the strategy. To meet the present day agricultural challenges in India, emphasis should be given to the applications of biotechnology in a second Green Revolution which will be an ever green revolution that hopefully could eradicate the hunger and poverty of the world.
Keywords: Genetic engineering, ever green revolution, food security

Abstract No I.P2	Session I Transgenics in Food and Non- Food Crops

Transgenic rice for salt tolerance

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As rice is staple food, there is an urgent need to understand the response of this important crop towards the environmental abuses, the rapid growth in population and the deteriorating soil and water quality around the globe. With the ultimate goal to raise rice plant with better suitability towards rapidly changing environmental inputs, intensive efforts are on worldwide employing physiological, biochemical and molecular tools to perform this task. In this regard, efforts of plant breeders need to be duly recognized several salinity tolerant varieties have reached the farmers field. Parallel efforts from molecular biologists have yielded relevant knowledge related to perturbations in gene expression and proteins during stress. Employing transgenic technology, functional validation of various target genes involved in diverse processes such as signaling, transcription, ion homeostasis, antioxidant defense etc for enhanced salinity stress tolerance has been attempted in various model systems and some of them have been extended to crop plant rice too. However, the fact remains that these transgenic plants showing improved performance towards salinity stress are yet to move from 'lab to the land'. Pondering this, future efforts should be channelized more towards multigene engineering that may domesticate this multigene controlled trait. Recent technological achievements such as the whole genome sequencing of rice is leading to a shift from single gene based studies to genome wide analysis that may prove to be a boon in re-defining salt stress responsive targets.

Abstract No I.P3	Session I Transgenics in Food and Non- Food Crops

Strategies for antiviral resistance in transgenic plants

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Viruses are significant threats to agricultural crops worldwide and the limited sources of natural resistance warrant the development of novel resistance sources. Several methods of transgenic protection have been successfully applied, including protein- and RNA-mediated approaches. Increased understanding of the molecular biology of virus infection is starting to bear fruit, enabling specific strategies to be designed for virus resistance in crops.

Genetic engineering offers a means of incorporating new virus resistance traits into existing desirable plant cultivars. The initial attempts to create transgenes conferring virus resistance were based on the pathogen-derived resistance concept. The expression of the viral coat protein gene in transgenic plants was shown to induce protective effects similar to classical cross protection, and was therefore distinguished as 'coat-protein-mediated' protection. Since then, a large variety of viral sequences encoding structural and non-structural proteins were shown to confer resistance. Subsequently, non-coding viral RNA was shown to be a potential trigger for virus resistance in transgenic plants, which led to the discovery of a novel innate resistance in plants, RNA silencing. Apart from the majority of pathogen-derived resistance strategies, alternative strategies involving virus-specific antibodies have been successfully applied. In a separate section, efforts to combat viroids in transgenic plants. The potential risks involved in the introduction of transgenic crops and the specifics of the approaches will be important for producing virus resistant transgenic crops in feature.

Abstract No I.P4	Session I Transgenics in Food and Non- Food Crops

In vitro propagation of the rose species- *'Rosa damascena'*

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Tissue culture has always been a better alternative to traditional propagation methods,when the conventional breeding methods become a time consuming and expensive procedure. The main advantage of tissue culture is that, it can yield large number of self-rootedplantlets/plantsin a very short time. Today, the*invitro* propagation protocols of a large number of plants have been standardized through tissue culture techniques.Roses are the important ornamental commercial crop and arecommonlyknown as "Queen of flowers". A protocol was developed for the *invitro* propagation of the Rose species- *Rosa damascena*, commonly known as "Panineer Rose". Tender nodal segments and young leaves were used as explants. The explants were surface sterilized with 0.1% mercuric chloridesolution. The disinfected explants were inoculated aseptically on culture medium. Agar- gelled Murashige-Skoog (MS) basal media supplemented with 2mg/L benzyl adenine(BA) was used for culturing the explants. *In vitro* shooting was observed in both the nodal segment and young leaf explants, after 2 weeks of inoculation. Sub-culturing of the shooted clusters is maintained for further induction of roots.

Abstract No I.P5	Session I Transgenics in Food and Non- Food Crops

Edible Vaccines

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Vaccines have been revolutionary for the prevention of infectious diseases despite of this major reasons for failure are attributed constraints on vaccine production, distribution and delivery.

As Hippocrates said, "Let food be thy medicine and medicine be thy food", Edible vaccines are the antigenic proteins that are genetically engineered into a consumable crop. The plant will be engineered with the gene for the antigenic protein from the disease causing pathogen so that the protein will be expressed in the consumable portion, on consumption antigenic proteins will enter into the digestive system in a bio-encapsulated form i.e., encapsulated within the tough plant cell wall, which protects them from gastric secretions. On consumption these vaccines triggers immunity.

Transformation to generate these transgenic plants could be through direct biolistic method or indirect *Agrobacterium* method. Confirmation of successful transformation and consistent expression could be through marker genes, ELISA and other established protocols and further verified by animal studies. Edible vaccines are produced for various diseases. For hepatitis-B, AIDS, Cervical cancer, Small pox, Cholera, Diarrhoea and also for various animal diseases like foot and mouth disease, Newcastle disease and avian flu. Edible vaccines are proven promising for the autoimmune diseases such as Diabetes. Many transgenic crops expressing the antigenic proteins have been developed and are currently under clinical trials.

The major limitations such as inconsistent dosage, probable instability and level expression of the foreign protein in the plant system, absence of criteria for selection of best plant remain unresolved. Further aversion against GM crops is the biggest hurdle for these vaccines in their entry into the open markets.

Abstract No I.P6	Session I Transgenics in Food and Non- Food Crops

GM rice: Progress towards abiotic stress tolerance

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Rice is the most important food crop and primary source of food for more than half of the world's population. Drought and salinity are the two major abiotic stresses which severely affect the productivity of rice. Genetic manipulation is considered as one of the approaches to overcome major bottlenecks in breeding. A wide array of genes and transcription factors are involved in combating major abiotic stresses. Research efforts on GM rice is in progress in USA (Duke University), China (Yangtze University, Huazhong Agricultural University, Chinese Academy of Sciences and Hebei Normal University), Japan (National Institutes of Agrobiological Sciences), Korea (Myongji University) and India (NBPGR and ICGEB, New Delhi; DRR, Hyderabad) for abiotic stress tolerance. Studies on drought tolerance showed the action of AtDREB1A gene in proline accumulation, chlorophyll maintainance and low ion leakage, C4 photosynthesis enzymes in higher photosynthetic rate and DEEPER ROOTING 1 gene for increased root tip elongation and deep rooting. Salt stress response studies showed the role of OsMKK6 gene in increased root/shoot length and weight, less chlorophyll bleaching and higher MAPK activity, PDH45 gene imparting better physiological and yield performances including endogenous nutrient contents N, P, K, Na and AtEm6 gene showing increased expression of calcium dependent protein kinase. Genes and transcription factors like SNAC1, Os LEA-3-1, CBF3/ DREB 1A, HvCBF4, OsDREB2A, TaSTRG and OsSDIR1 also showed better drought and salinity tolerance without hampering normal yield. Also rice microRNAs and barley gamma glutamyl cysteine synthase gene conferred resistance against most of the abiotic stresses in rice. Abiotic stress being a serious constraint in rice production could be mitigated using effective genes through genetic manipulation.

Abstract No I.P7	Session I Transgenics in Food and Non- Food Crops

Investigations on genetic transformation of black pepper (*Piper nigrum* L.)

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Genetic transformation of black pepper *Piper nigrum L* was undertaken with three black pepper varieties Panniyur 1, 4 and 6 and four *Agrobacterium tumefaciens* strains EHA 105, AGL 1.1303, GV 2260 and LBA4404. Axenic cultures of selected varieties were raised from nodal segments as well as ripe seeds for embryogenesis and transformation studies. Multiple shoot induction from nodal explants and zygotic embryo explants were observed in all the varieties. Among the varieties average number of multiple shoot/explant was high for variety P6 in ½ MS medium with BA and IAA 1.0 mg/L . Sensitivity studies of black pepper tissues to various antibiotics resulted in selecting the optimum threshold level of antibiotic to be used in the screening medium. Kanamycin 25 mg/L, 50 mg/L and 100 mg/L were selected as the cut off level for the selection of transformants from zygotic embryo, leaf segments and nodal segments respectively. Genetic transformation was standardized with *Agrobacterium* strain EHA 105 using leaf disc and zygotic embryo explants. Tentative protocol for transformation include *Agrobacterium* inoculum density 0.9, infection time 10 min and co-cultivation period of 48 hr.Transient gus assay revealed faint blue staining on infected leaf explants. Leaf segments, cotyledonary node and zygotic embryo were used for transformation with *Agrobacterium* strains AGL.1.1303, GV2260 and LBA 4404. There was explant specificity for the different *Agrobacterium* strains used. Direct gene transfer method using gene gun (PDS/1000Hc) was also attempted with P^{BZ100} (Glucanase and chitinase) and cotyledonary node/ adventitious bud explants. Bonabarded cotyledonary node explants survived in the screening medium with Kanamycin, however PCR analysis for stable transformation was negative.

Abstract No I.P8	Session I Transgenics in Food and Non- Food Crops

Progressive adventure of genetic modification in Horticultural crops

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The genetically modified (GM) crops are produced by transferred genes from other species into that particular plant through various natural or artificial insertion techniques. Genetic modification however has contributed tremendously in the horticultural crop productivity and quality; such as extended shelf life, slow ripening and preventing discoloration in flowers, fruits and vegetables, anti sense RNA technology with Flavr Savr transgenic in tomato during 1994. Pest resistance by the use of *Bacillus thuringieneses* (Bt) toxins producing cry proteins, protease, lectins and virus resistance by incorporating virus coat proteins with RNAi technology, by the use of several biotechnological tools, fungal and bacterial disease resistance by incorporating pathogenesis related proteins and phytoalexins. While, abiotic stress resistant such as herbicide resistant, drought resistant, soil salinity resistant and freeze resistant gene for anti-freezing from flounder fish etc were extensively used in tomato, potato and cowpea etc. Transgenic plants with improved nutrition have been engineered for human health improvement potato with increased protein and methionine levels, canola with cis-Stearates-lowering the risk of heart diseases, sugar beet with fructans-low calorie alternatives to sucrose. Also, transgenic have been engineered for allergen absence. Most importantly, transgenic plants as bioreactors have created boom in commercial aspect for manufacturing hemoglobin, monoclonal antibodies, interferons, serum albumin, pro-insulin, edible vaccines, etc. Despite of these advantages GM crops much controversial from ethical point of view. Nevertheless, GM crops have been potentially engineered for eco-friendly way causing improvement to human health and environmental safety.

Abstract No I.P9	Session I Transgenics in Food and Non- Food Crops

GM crops: A solution for world food crisis?

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Food security has been a major and fast-growing concern worldwide. Following the 2008 food price crisis, which caused social and political unrest in several developing nations; there is a renewed sense of urgency and commitment among political and scientific communities toward increasing food production and meeting the challenges of an ever-increasing world population set to reach the 9.3 billion mark by 2050. In the current scenario, maximizing crop productivity on existing farmlands is the only logical way to address food security concerns, as the amount of arable land is unlikely to increase in the future. Genetically modified (GM) crops offer massive benefits to the third world, if the ethical obligations are considered in developing the GM crops to combat the world food crisis.

GM is not the quick fix it is often purported to be. The promises of higher yields, drought and salt resistance are currently unfulfilled by the GM crops, where as conventional crops quietly solve these problems with barely. Even if a GM crop did appear that gave higher yields than non-GM crops, this would not impact the problem of hunger. This is because the root cause of hunger is not a lack of food, but a lack of access to food. GM is not a solution to the world's hungry, but it is has the potential in certain circumstances to solve problems that can't be done in any other way.

Abstract No I.P10	Session I Transgenics in Food and Non- Food Crops

Seasonal variation in UV-B absorbing pigments - a photoprotective mechanism under changing ambient UV-B levels.

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Depletion of stratospheric ozone leads to an increase in the amount of UV-B (280-320 nm) radiation reaching on earth's surface and influence plant growth and metabolism. The photosynthetic apparatus is considered as one of the principle target of UV-B radiation. Plant species vary greatly in their response to UV-B radiation. The sensitivity of plants to this was partially explained by their ability to respond to UV-B by increasing the level of photo protective pigment molecule flavanoids. So the present study was conducted to evaluate the seasonal variation in UV-B absorbing flavonoid contentin different crop plants like rice variety-jyothi, weedyrice ,*Echinocloacrusgalli* (a rice field weed),flowering plant *Tageteserecta*(African marigold) and flowering tree *Cassia fistula* under two seasons-September-November 2013 andFebruary- April,2013.The UV-B radiation measured found to vary from 0.71 –1.02 WM^{-2} during September-November 2013 and 1.56 to 2.978 WM^{-2} during February-April, 2013.The flavanoid content recorded high value for all the plants at February – April, 2013 during which the ambient UV-B radiation was in the range of 1.56WM^{-2} to 2.978WM^{-2} . The maximum flavanoid content (64.200A_{300} g^{-1}fresh weight) was observed in *Tageteserecta* followed by *Cassia fistula* (56.665A_{300} g^{-1}fresh weight).The lowest value (15.170A_{300} g^{-1} fresh weight) was recorded in cultivated rice variety "Jyothi".The flavanoid content in weedy rice and C_4 weed *Echinocloacrusgalli* was 18.772 and 20.484A_{300} g^{-1} fresh weight respectively. All the observed plants recorded lower value for flavanoid content in the same pattern during September-November 2013.The maximum value was for *Tagetes erecta* followed by *Cassia fistula.*The difference in seasonal variation of this UV-B absorbing flavanoid pigment among different plants may be related with the genetic characters and adaptation to UV-B stress condition.

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Abstract No I.P11	Session I Transgenics in Food and Non- Food Crops

Agrobacterium mediated multiple gene integration in *Hevea brasiliensis*

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Hevea brasiliensis (Para rubber tree) belonging to the family *Euphorbiaceae*is the major source of commercial natural rubber and itaccounts for 99 percent of the world natural rubber. Susceptibility of *Hevea brasiliensis* to various biotic and abiotic stresses under various agro-climatic conditions and incidence of tapping panel dryness are thetwo major constraints for crop production. Conventionally, genetic transformation involves the integration of a single gene associated with a specific trait of interest. The advancement of gene stacking permits the integration of multiple genes for complex traits such as crop yield, stress tolerance, disease resistance, etc. In the present study, gene stacking was attempted by the integration of two genes viz.manganese superoxide dismutase (MnSOD)for abiotic stress tolerance and3-hydroxy-3-methyl-glutaryl-CoA reductase(*hmgr*I) gene for enhanced latex yield by repeated genetic transformation.Initially *Agrobacterium* mediated transformation by vacuum infiltration was carried out with the binary vector harboring MnSOD gene and*npt*IIas the selectable marker gene usingembryogenic callus derived from immature zygotic embryo as the target tissue.High frequency (40%) transformation was obtained and was detected by GUS histochemical staining and PCR using MnSOD gene specific primer.This MnSOD transgenic callus was used as the target tissue for the integration of *hmgr*I gene containing*hpt*as the selectable marker gene. PCR using *hpt* gene specific primer was performed for the detection of *hmgr*I transgene integration. Somatic embryo induction (32%)was achieved from the multiple gene integrated callus lines in modified MS medium fortified with BA and Kin (0.3 mg L^{-1} each) and GA_3(0.5 mg L^{-1}). Fifteen percent of the embryos were matured and were further cultured forplant regeneration. This is the first report on multiple gene integrationin *Hevea brasiliensis*.

Key words: *Agrobacterium*,*Hevea brasiliensis*,gene stacking,MnSOD and*hmgr*I genes

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Abstract No I.P12	Session I Transgenics in Food and Non- Food Crops

Biotechnological interventions for food security

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Agriculture in developing countries will be confronted with three major challenges in the decades to come: to increase the availability of nutritious food to an increasing population; to use natural ecosystems more efficiently and to make a contribution to economic development. It is not conceivable that agriculture can deliver the expected outputs without modern technologies such as genetic engineering and biotechnology. The potential benefits from the application of biotechnology in food crops in developing countries ranges from diagnostic aids for plant diseases, to gene mapping, which enables speedier identification of interesting genetic material for every kind of plant usable in agriculture. The main objective of biotechnological research for food security is to find improved seed varieties of high yields, lower tillage costs through qualities such as resistance to plant diseases, animal pests and stress factors like climatic variation, poor soil quality, crop rotation practices, and others. Equally important objectives are the transfer of genes with nitrogen-fixing capacity on to grains, and the improvement of food quality by manipulating levels of amino acids, fats, vitamins, minerals, carbohydrates and fiber quality as well as decreasing levels of undesirable components in major feed crops. These varieties should prove valuable in countries where millions of people suffer from dietary deficiencies and have difficulties in accessing vaccines and medicines. Major issues and safety concerns on the biosafety of foods derived from GM plants can be addressed with proper assessment before entering the marketplace, using guidelines issued by several international scientific agencies. The cluster of techniques that comprise biotechnology can, if effectively harnessed and applied, radically transform farming systems.

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Abstract No I.P13	Session I Transgenics in Food and Non- Food Crops

An overview of developments in transgenic food crops

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The crop, which has got alien genes into its cells by recombination DNA technology is called transgenic crop. These crops are used for many purposes like improve the quality of the agricultural or horticultural products such as tolerance to cold, frost or drought, resistance to insects and diseases. Improving resistance to these diseases and insects also reduces the need of herbicides and pesticides. This makes the plants safer for the environment. In the future, transgenic crops may play the pivotal role for developing country with respect to food and nutritional security. Now, this is the only alternative available to cope up with the burgeoning population. The GM crops offer both challenges and opportunities for growth and development of mankind. These technologies should be used to complement the traditional methods for enhancing productivity and quality rather than to replace the conventional methods. Globally, GM crops still occupy only 3.4 percent of the total farmland and are adopted by a minuscule minority of farmers (17.3 million out of the total 513 million farmers around the world). India is at the crossroads as far as the use of genetically engineered crops in agriculture is concerned. Since 1948, population more than doubling in the sixty years, there seems to be a compelling need for the country to find alternate ways to feed India's 1.2 billion people, thus there comes a role to play by GM crops. In India, 10.8 million hectares of GM crops were sown in 2012, out of a total of 182 million hectares of irrigated farmland in the country, Bt cotton, a patented product of Monsanto, remains the first and only GM crop to be commercially cultivated in India, starting in 2002. Following Bt cotton, the Indian government tried to introduce Bt brinjal towards the end of 2009. It was the first GM food crop to be commercialized in India. However, after serious concerns were raised on its safety by scientists, ecologists, farmers and consumers, an indefinite moratorium was put on it in 2010. The biggest challenge for the government will now be to come up with a good regulatory system and communication mechanism on GM foods, which can help allay fears regarding the safety of such crops, while also ensuring higher and sustainable agricultural productivity and remuneration to farmers.

Key words: GM crops, recombination DNA technology

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Abstract No I.P14	Session I Transgenics in Food and Non- Food Crops

Candidate genes for bioprospection in medicinal plants – Need for transgenesis and overexpression

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Medicinal plants containing alkaloids, anthocyanins, carotenoids, flavonoids, isoflavones, lignans, monophenols, monoterpenes, organosulfides, phenolic acids, phytosterols, saponins, triterpenoids etc. act as the best sources of health promoting phytochemicals. Natural products have been the basis for the formation of traditional medicines and for the discoveries of many modern drugs. The flora and fauna of our planet provide at least 50% of all pharmaceuticals and almost 85% of the world's population depends on traditional medicines for their primary health care needs, which will continue forever. The chemical constituents present in them are a part of the physiological functions of living flora and hence they are believed to have better compatibility with the human body. Many of these now been synthesized and the synthetic drugs are used, to this category belongs, ephedrine, morphine, quinine, reserpine, digitalis, vincristine etc. Phytochemicals with antioxidant capacity naturally present in food are of great interest due to their beneficial effects on human health as they offer protection against oxidative deterioration. During the last twenty years, bioprospecting has grown rapidly, fuelled by advances in technology in the field of biotechnology, pharmaceutical, agriculture and other areas of importance. A large number of medicinal plants sourced from the wild needs to be domesticated. India should adopt organized cultivation of medicinal plants that have export potential and import substitutions. For this purpose, the varieties which got the capability to produce the chemical of interest at a higher rate has to be developed. Since most of the medicinal plants belong to the perennial category, conventional breeding for this end will take long time. Thus, the crop transgenesis for overexpression of specific genes coding for the secondary metabolite will be the best option.

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Abstract No I.P15	Session I Transgenics in Food and Non- Food Crops

Differential Expression of SOS pathway genes in water stress mediated by *Pseudomonas fluorescens* in rice and its validation by electronic Northern

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Rice is the most important food crop and largest irrigated crop in the world. More than 75 per cent of the world's rice supply comes from 79 million ha of irrigated land in Asia. A popular rice variety of Kerala, Mattatriveni (PTB-45) susceptible to drought was selected for the present study. Plants were raised in earthen pots under controlled condition in sterile paddy soil under submergence. Plants were given three treatments. Water was withheld 30DAS(days after sowing) for forty-eight hours in second treatment and also in third treatment in which *Pseudomonas fluorescens* (*Pf*) was given to plants as three applications seed treatment, soil application and foliar spray. Plants without *pf* treatment i.e. treatment first served as absolute control. Transcriptome was analyzed by DD-RT-PCR in above treatments. Differentially expressed transcript derived fragments were cloned and sequenced. The sequences were annotated to biological databases like NCBI, BAR (Botany array resources). The sequence showed homology to SOS (Salt Overlay Sensitive) protein in BLASTx analysis with Nucleotide redundant database. BLASTx analysis with *Arabidopsis thaliana* database showed homology to UDP-glucosyl transferase, putative glucosyl transfrase and alpha-beta-hydrolasase. These proteins are further analyzed by eNorthern analysis. The eNorthern analysis gave a score of more than three for these proteins which indicates that these proteins are highly expressive in plants treated with *Pf* under water stress condition.

Keywords: DDRTPCR, Salt Overlay Sensitive, *Pseudomonas fluorescens*, e-Northern

SESSION II

Clean Gene Technology

Oral

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Abstract No II.O1	Session II Clean Gene Technology

Pyramiding of three resistance genes of bacterial leaf blight in Ptb 39 (Jyothy) an rice (*Oryza sativa* L.) cultivar of Kerala through Marker Assisted Selection in rice

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Bacterial blight caused by *Xanthomonas oryzae* pv *oryzae* is a recurrent and devastating disease in Kerala. Severe incidence of this disease and yield loss has been reported every year especially in elite varities Ptb 9 (Jyothy) and Mo 16 (Uma). The exploitation of host resistance has been shown to be a reliable method to control the disease across the globe. An attempt was made to transfer three resistance genes i.e. *xa5*, *xa13* and *Xa21* from the essentially derived variety Improved Samba Mahsuri into Ptb 9 (Jyothy) through marker-assisted backcross breeding. Several two gene and three gene pyramided lines have been recovered in the backcross generations. The precise transfer was aided through effective foreground selection using STS markers RG 556, RG 136 and pTA248 linked to the three target genes, respectively. Background selection was based on morphological and grain quality traits and SSR markers. Performance of backcross genotypes with durable resistance to bacterial blight is to be evaluated through multi location trials.

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Abstract No II.O2	Session II Clean Gene Technology

Cisgenic Approach for Crop Improvement and its Biosafety Issues

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A new method of genetically engineered (GE) crop plants known as cisgenics. A cisgenic plant is a plant that has been genetically modified using genes and regulatory elements exclusively from plants to which it can be crossed by normal breeding (Schaart, 2004). Because of the similarity of the introduced genes to those of the host plant, such improvements may be complitted efficiently through intragenic modification, a new approach to genetic engineering that transforms plants with native genetic elements only

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Abstract No II.O3	Session II Clean Gene Technology

Validation of Molecular Marker for Tagging Bacterial Wilt and ToLCV Resistance Genes in Tomato

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Molecular markers have been used for identification and mapping of genes and QTLs for numerous agriculturally important traits in tomato including resistance/tolerance to biotic and abiotic stresses and fruit and flower-related characteristics.Tomato leaf curl virus (ToLCV) disease caused by begomovirus and bacterial wilt, caused by *Ralstoniasolanacearum* are the serious production constraints in tomato. Breeding for disease resistance has been an important objective in tomato improvement worldwide. However, the extent to which markers have been utilized in tomato breeding programs has not been clearly determined. The utility of molecular markers for use in tomato breeding programs is limited and yet the markers have not been, validated specific to BW and ToLCV. Manymarkers are not validated across tomato genotypes orare not polymorphic within tomato breeding populations.In this study, we examined the validity ofavailable markers specific for bacterial wilt and ToLCV resistancetraits in tomato by testing them with F_3population produced by crossing to available genotypes- Sakthi (BW resistant) and IIHR 2196 (ToLCV resistant). Most of them needed PCRoptimizationfor successful amplifications as found were notinformative in the genotypes studied. Out of the eight markers examined two were analysed as specific markers for resistance to bacterial wilt and ToLCV. It appearsthat many of the available markers may need to befurther refined or examined for trait association andpresence of polymorphism in breeding lines andpopulations. However, with recent advances in tomatosequencing, it is becoming increasingly possible todevelop more informativemarkers to accelerate theuse of MAS and tagging the gene of interest in tomato breeding or transgenic crop development.

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Abstract No II.O4	Session II Clean Gene Technology

Basis of anthracnose [*Colletotrichum lindemuthianum* (Sacc. &Magn.) Br. and Cav.] resistance in the newly identified trailing-type vegetable cowpea [*Vigna unguiculata* ssp. *sesquipedalis* (L.) Verdcourt] cultivar Arimbra Local

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Anthracnose caused by *Colletotrichum lindemuthianum* is the most destructive universal disease in cowpea, leading to complete crop loss with no recommended management strategy. Few resistance sources previously recommended belong to the bush type grain cowpea (ssp. *cylindrica*) and usage of these lines in resistance breeding invites extensive backcrossing. Recently, from farmers' fields of Malappuram district of Kerala state in India, a trailing-type vegetable cowpea (ssp. *sesquipedalis*), Arimbra Local has been reported to possess resistance to this disease. The present study has confirmed the resistance through controlled artificial inoculation and the basis of resistance was analyzed through protein profiling and defence enzyme analyses. Protein profiling has revealed that the resistant varieties are capable to maintain a high level of RuBisCo, the main photosynthetic enzyme, consistently whereas; the susceptible varieties had shown a drastic fall in the level of this enzyme, soon after the infection. Further, the defence enzymes such as Peroxidase and Phenylalanine Ammonia Lyase were also found proportional to the level of resistance. Hence, Arimbra Local is recommended for the crop improvement programmes in trailing-type vegetable cowpea, intended for enhancing the resistance to the anthracnose disease.

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Abstract No II.O5	Session II Clean Gene Technology

Evaluation of thermosensitive genic male sterile donor lines suitable to Kerala through Marker Assisted Selection

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Rice is one of the most important food crops in the world. The food grain production in India is dwindling at a faster rate due to limited geographical area, plateauing yield of crops, urbanisation and other socio-economic reasons. Scientists found hybrid rice technology as the best way to tackle this crisis. Cytoplasmic male sterility system (CMS), the widely used approach in India is cumbersome. So, a new type of environment sensitive genic male sterility (EGMS) known as thermosensitive genic male sterility (TGMS) was found best method to develop hybrid red rice in tropical region like Kerala, where significant variation in temperature exists between seasons and altitudes. Kerala is lacking with rice hybrids. As people of Kerala prefer red rice, our objective was to develop a stable red rice parent for hybrid rice production through marker assisted selection. The present investigation studies the morphological, agronomic, floral traits and marker analysis of two TGMS lines from IRRI**.** From the present study, the sterility inducing temperature observed for TGMS line EC720903 was 30.3^0C /21.5^0C during the sensitive period of 22 days before heading. The TGMS line, EC720903 was found to have a shorter stature, with more number of productive tillers, early flowering when compared to the line, EC720904. Phenotypic acceptability of EC720903 was better than EC720904. SSR marker (marker ID-R7) was found polymorphic to the *tms* gene present in both the TGMS lines. The present study suggests that the TGMS line EC720903 is highly suitable to Kerala and used for further marker validation.

Keywords: Thermo-sensitive GMS, hybrid rice, marker, critical sterility

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Abstract No II.O6	Session II Clean Gene Technology

Intragenesis - An alternative to transgenesis

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The concern over the wide acceptance of transgenic crops relates to the chances of mixing of genetic materials between unrelated species and further escape to the fellow species members. New transformation concepts cisgenesis and intragenesis were developed as alternatives to transgenesis. The concept intragenesis was introduced by Rommens.

Transgenesis can be differentiated from cisgenesis and intragenesis as it makes use of foreign DNA from other species, may be microbes. Intragenesis is much similar to cisgenesis as the same gene pool is exploited but the difference is that intragenesis allows creation of novel combinations of DNA fragments by combining functional genetic elements and the recombinant DNA can be inserted into existing varieties, allows the use of antisense or RNA interference (RNAi) to silence the gene. In cisgenesis end products could be same as produced by conventional breeding whereas the resultant phenotype of the intragenic plant will be different. In a cisgenic plant, the cisgene is present as an extra copy in the recipient genome. In case of intragenesis, the inserted genes are new combinations of functional genetic elements having same native origin. In cisgenesis, T-DNA borders from Ti Plasmid and in intragenesis T-DNA border from plant derived P-borders are used.

Intragenic potatoes with good processing qualities have been obtained by silencing polyphenol (Ppo) or water dikinnase (R1) or amyloplast targeted phosporylaseL (PhL) or aspargine biosynthesis StAs1 and StAs2 genes, independently. Intragenic strawberry with overexpression of polygalactouranose produced for resistance to grey mould. Similarly, increased forage quality was achieved in alfalfa by silencing caffeic acid o-methyltransferase gene (Comt).

Intragenic modifications improve the agronomic trait or nutritional characteristics of crops but do not introduce traits that are new to the sexual compatibility group, lack new unknown DNA that might comprise genes associated with the production of toxins, allergens or antinutritional compounds selectable marker genes, powerful insecticidal genes or any other foreign genes that are new to agriculture or the food stream.

SESSION II

Clean Gene Technology

Poster

National Seminar on GM Crops: Prospects and Issues
17-18 March, 2014; Kerala Agricultural University, Thrissur, Kerala

Abstract No II.P1	Session II Clean Gene Technology

Plastid Biotechnology: An Environmentally Friendly Methodology for Transgenic Crop

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Plant cells carry DNA in their nucleus, plastids and mitochondria. Though nuclear DNA is the major player, DNA in plastids as well as mitochondria have definite roles in character expression. Any kind manipulation exercised on the plastid DNA falls under the purview of plastid biotechnology. The contamination of genetic resources through the dispersal of transgene through pollen being the major objection in adoption of GM crops, the genetic engineering of plastid DNA is highly relevant since, they are maternally inherited. Maternal inheritance excludes plastid genes and transgenes from pollen transmission. Therefore, plastid transformation is considered a superb tool for ensuring transgene containment and improving the biosafety of transgenic plants. Additionally, the inserted gene in a plastid DNA is found to offer a high level expression against the insertion in the nuclear DNA because of high copy number and there compartmentalization of toxic transgene products. Further, in a plastid DNA, it is possible to express multiple genes using a single promoter.

Plastid genetic engineering have several applications in development of crops. Herbicide resistant plants with 250 fold higher expression over nuclear transformation was developed through plastid transformation. High level resistance to velvet bean caterpillar was achieved in soybean by introducing Bt *Cry1Ab* gene in the plastid DNA. High levels expression of antimicrobial protein in chloroplast transformed plant, to provide inhibition against pathogens. The commercial production of proteins including the human therapeutic proteins is also achieved through plastid engineering.

Even with all these advantages over nuclear transformation, chloroplast transformation suffers from the limitation such as prolonged selection procedures for transformed cells and lesser efficiency in gene delivery method.

It is evident that plastid biotechnology holds great promise for the improvement of crop productivity. Efforts should also be directed to explore the possibilities of this technique in improvement of agronomically and physiologically important areas such as nitrogen fixation and metabolome modifications.

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Abstract No II.P2	Session II Clean Gene Technology

β- 1, 3- glucanase – a candidate gene for inducing foot rot resistance in black pepper (*Piper nigrum* L.) through cisgenesis

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Most cultivated black pepper (*Piper nigrum*) varieties in India are susceptible to quick wilt disease caused by *Phytophthora capsici* and results in yield losses of upto 50 per cent. Since the conventional breeding programs for disease resistance in this crop have not been successful, an attempt was made to find a solution through molecular interventions to increase the tolerance of pepper to *phytophthora* rot disease. The expression pattern of the gene coding for the pathogenesis related *β*- 1, 3- glucanase (β-glu) enzyme in a resistant (*P. colubrinum*) and a highly susceptible (*P. nigrum* var. Panniyur I) genotype of black pepper was examined, following infection with *Phytophthora capsici*. Infected leaf samples were collected at different times after inoculation, and RNA was extracted and subjected to Northern blot hybridization and Real- time polymerase chain reaction. On hybridization with a 700bp probe, Northern blots showed a marked increase in β-glu transcript levels in *P. colubrinum* at 6h after infection. The resistant genotype had a higher rate of increase and a more prolonged induction for β-glu transcript levels. Similar results were obtained in real-time PCR experiments with the housekeeping GAPDH gene as an internal control. Thus, although induction of the β-glu gene occurred in both tolerant and susceptible genotypes, the predominant difference between them was in the intensity and duration of the response. The tolerance of resistant genotype *P.colubrinum* can be associated with the high copy number and prolonged expression of the gene following infection. The antifungal activity of these hydrolase enzymes makes them rational candidates for over expression by cisgenesis to produce disease resistant black pepper. Since the source of gene is from breeder's gene pool, biosafety issues can be better addressed in this important spice crop

Keywords: *Piper nigrum, Piper colubrinum, Phytophthora* rot, β-1,3-glucanase, real-time PCR, cisgenesis

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Abstract No II.P3	Session II Clean Gene Technology

Cisgenesis: a new extension to transgenesis for production of environment friendly GM crops

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Wild relatives of cultivable crops always contain desirable genes which can be introduced in the later for crop improvement by conventional breeding. Biotechnological tool such as transgenics has allowed transfer of foreign gene into plants from any living source. Introduction of foreign genes has enabled farmers to escape many pathogens and herbicides and even increase in Vitamin A content to delaying of ripening. But the common public is blindly protesting over the consumption of genetically modified crops. So we have to think of some crop improvement technology which will satisfy peoples' needs and even do better crop improvement than traditional method.

In this regard, 'cisgenesis' can be a good option. It means transfer of native genes derived from compatible group. The product will be called as 'cisgenic'. A cisgene is a natural gene, coding for an agricultural trait, from the crop plant itself or from a sexually compatible donor plant that can be used in conventional breeding. The gene belongs to the conventional breeder's gene pool. It contains its native promoter and terminator. Cisgenics are developed through marker free transformation. It is a better than traditional breeding because of absence of linkage drag and reduced number of steps. Traditional breeding takes excess time in back crossing for fixing of desired traits and also it incorporates undesirable traits. In cisgenesis, desired traits can be incorporated in plant under a single promoter by gene stacking. Success stories in cisgenics include late blight disease resistance in potato, scab resistance in apple and many more yet to come.

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Abstract No II.P4	Session II Clean Gene Technology

Multigene engineering in plants

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In recent years there has been a rapid increase in research methodologies designed to improve plants through genetic engineering. In most of the applications, a single functional gene has been transferred along with a selectable marker gene. However, it is highly essential to manipulate polygenes, as vast majority of agronomic traits are controlled polygenetically. Various strategies have been employed in multigene manipulation, including iterative methods, co-transformation, multigene-linking, polycistronic techniques etc. Iterative strategies are more conventional and include cross fertilization and re-transformation approaches. Specifically, a transgenic plant is crossed with another transgenic plant harbouring the genes of interest. In co-transformation approaches, multiple transgenes positioned on various T-DNAs are introduced simultaneously into plants via either direct (biolistics) or indirect (*Agrobacterium*-mediated) transformation methods. The linking of multiple expression cassettes into a single binary vector has strong advantages over other approaches. Co-transformation, re-transformation and sexual crosses can be applied for the delivery of multiple genes in plant cells. Therefore, efficient systems like MultiRound Gateway have been developed, in order to assemble multiple genes and are being successfully adopted in several plant species. Golden rice, purple tomatoes and red corn developed through multigene engineering demonstrate the bright prospects for multigene transformation in plants.

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Abstract No II.P5	Session II Clean Gene Technology

Marker Assisted Selection in Aromatic Rice (*Oryza sativa* L.)

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Aromatic rice, an important commodity for international trade, has encouraged the interest of rice breeders around the globe to identify the genetic control of rice aroma. **T**he sensory qualities such as aroma, taste, and texture are the most important criteria for distinguishing Basmati rice from non-Basmati types.

Genetic analysis of traditional and evolved Basmati and Non-Basmati rice varieties using fluorescence-based ISSR-PCR and SSR markers revealed a number of markers, which could unambiguously distinguish the Traditional Basmati varieties from the Evolved Basmati and Non Basmati rice varieties (Nagaraju *et al*., 2002). A single panel of eight microsatellite markers could be used to differentiate the premium traditional Basmati, cross-bred Basmati, and non-Basmati rice varieties having different commercial value (Jain *et al*., 2004). To map the gene(s) controlling aroma, bulked segregant analysis (BSA) using Random Amplified Polymorphic DNA (RAPD) markers was applied in an F2/F3 population of Basmati 370 (aromatic) and IR36 (non-aromatic). Out of the RAPD markers AG8-AR, AN1-AR1, and AN1-AR2 linked to the gene for aroma, AG8-AR was mapped on chromosome 8 indicating the location of aroma gene in Basmati 370 (Nematzadeh *et al*., 2004).

Genetic diversity among the nineteen rice genotypes of Pakistan including three commercial Basmati cultivars *viz*., Basmati 370, Basmati 385 and Super Basmati was assessed using 40 random decamer primers in which OPI 7 generated maximum number of bands (Arif *et al*., 2005). In a molecular marker based genetic diversity analysis of aromatic rice genotypes using SSR and RAPD markers, the SSR primer RM223 showed the highest polymorphism (66.67%), whereas OPA02 and 67AB10G7 RAPD primers exhibited 100% polymorphism (Kibria *et al*., 2009).

In a study involving 22 aromatic rice genotypes, three SSR primers *viz*. RM223, RM515 and RM342 identified fifteen rice genotypes having *fgr* gene locus. Five genotypes (Indian Ndingo, NamSagui19, IR77542-127-1-1-1-1-2, Basmati 370 and Si-Feng 43) were selected having strong aroma with good agronomic performances, which could be used in breeding programme to develop new varieties (Mia *et al*., 2010). During the genetic analysis of 26 aromatic rice genotypes, the SSR primer RM223 could identify aromatic and non-aromatic genes (Jewel *et al*., 2011). Among the 24 SSR markers used across twelve elite aromatic rice genotypes for their characterisation and discrimination, RM163 was the best (Sajib *et al*., 2012).

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Abstract No II.P6	Session II Clean Gene Technology

Transplastomics in vegetable crops: Environment friendly approach for minimizing outcrossing of transgenic pollen

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Biosafety and the stability of expression of the transgenes in engineering the nuclear genome of vegetables is a major challenge. Genetically modified/transgenic vegetables must be safe for the environment and suitable for coexistence with conventional and organic vegetable crops. For achieving such safety, a major obstacle is posed by the potential outcrossing of the transgenes via pollen movement which otherwise known as gene escape. Plastid transformation, which yields transplastomic plants in which the pollen does not contain the transgene, not only increases biosafety, but also facilitates the coexistence of genetically modified, conventional and organic agriculture. As plastid DNA, in the pollen of most crops is unlikely to be inherited via the pollen to the next generation and hence transplastomic technologies are advantageous for ecological reasons. By providing transgene containment, transplastomic approaches ensure higher ecological safety than classical transgenic technologies which involves nuclear genome manipulations. Plastid transformation is achieved by homologous recombination between the transformation vector and the plastid genome, resulting in integration of the gene(s) of interest at a predictable, pre-determined site which otherwise are uncertain in nuclear gene transformations. Plastid genomes are very rarely transmitted in pollen. Since vegetables are the protective and productive foods ensuring the availability of ever increasing food demand mostly in developing countries can be utilized for transplastomic approaches. Vegetables being succulent in nature are attacked by various biotic and abiotic stresses. To overcome these barriers in vegetables, nuclear transformation is not feasible due to unstability of transgenes and gene-escape in diversified environments of their origin. Hence in many vegetables, transpastomic approaches are used and stable transformations are achieved (*e.g.*, Tomato, Potato, Carrot, Lettuce, Cauliflower, Cabbage, Brinjal etc.). Other advantages of these approaches include high levels of foreign protein accumulation in chloroplasts, ideal expression factories for high-yield protein production, more stable and uniform transgene expression among transgenic lines, nil influence of position effects and epigenetic gene-silencing mechanisms, potential of developing genetically and phenotypically identical lines, sufficiency of single transplastomic plant per construct and the simultaneous expression of multiple transgenes (“transgene stacking"). Clearly, these applications of the transplastomic technology do not represent more than a promising beginning in vegetables but also proven to be highly environment friendly and for extending the crop range. This progress remains to be

made but with the current intensification of vegetables research in this area, substantial strides forward can be expected in the near future.

Keywords: Transplastomics, transgenics, chloroplast transformation, outcrossing, biosafety, gene escape, vegetables

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Abstract No II.P7	Session I Transgenics in Food and Non- Food Crops

TILLING moves beyond functional genomics into crop improvement

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TILLING is Targeting Induced Local Lesions in Genomes. It is a method in molecular biology that allows directed identification of mutations in a specific gene TILLING does not involve transgenic modifications; it is attractive not only for functional genomics but also for crop improvement. It includes traditional chemical mutagenesis combined with high-throughput screening for point mutations. It can be directly used in gene function studies and also for discovery of induced point mutations or induced gene disruption or knockout.

For TILLING first step is Mutagenesis, for mutagenesis mutagen causing small lesions is preferred next to it a non-chimeric population is developed. Heteroduplexes were incubated with the plant endonuclease CEL I which cleaves heteroduplex mismatched sites Mungbean nuclease/S1 nuclease are also useful and the resulting products are size-fractionated using denaturing polyacrylamide gel electrophoresis and visualized by fluorescence detection. The migration of cleaved products indicates the approximate location of nucleotide polymorphisms. DHPLC can also be used where the presence of a heteroduplex in a pool is detected as an extra peak in the chromatogram. Finally sequencing of the mutant PCR product is done to locate exact nucleotide change.

For TILLING, softwares such as CODDLe (Codons Optimized to Detect Deleterious Lesions) are commonly used. Several TILLING centres exists all over the world for rice, soybean, maize and wheat (USA). In India, TILLING centre is located at - University of Hyderabad for tomato. It is widely used for creation and detection of mutated population.

Keywords: TILLING, functional genomics, mutation.

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Abstract No II.P8	Session I Transgenics in Food and Non- Food Crops

RNA interference for genetic manipulation in plants

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RNA interference is a natural process, which silences specific genes before translation by degrading mRNA which was referred in late 1980s as co-suppression. However, the underlying mechanism of the phenomenon was clear only after elaborate studies conducted in the model organism *Caenorhabditis elegans* and the process is now know as RNA interference (RNAi). RNAi operates by triggering the action of dsRNA intermediates, which are processed into RNA duplexes of 21-24 nucleotides by an enzyme Dicer. The short interfering RNAs (siRNAs) are incorporated into RNA induced silencing complex (RISC). The siRNA acts as a guide to target the degradation of complementary mRNAs. The host genome coding for small RNAs, called miRNAs are responsible for endogenous gene silencing. The dsRNAs triggering gene silencing can originate from several sources such as endogenous genes or viral RNA during replication. Delivery of dsRNA or siRNA into cells or tissues are done by either direct (biolistics, agroinfiltration) or indirect (*Agrobacterium*-mediated) transformation methods. Currently, RNA silencing is an area of intense investigations leading to exciting new discoveries in crop improvement programmes such as induction of resistance to pests and diseases, improving nutritional qualities, improving flower colour in ornamental plants and inducing male sterility. RNAi technology holds the key for future biotechnological applications in agriculture.

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Abstract No II.P9	Session I Transgenics in Food and Non- Food Crops

Epigenetic approaches towards transgenesis

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Epigenetics is defined as 'the study of heritable changes in genome function that occur without a change in DNA sequence'. Epigenetic mechanisms such as histone modifications, DNA methylation and RNA interference (RNAi), induce changes in the chromatin and give rise to epigenomes, which add diversity and complexity to the genomes of organisms. Epigenetic modifications play a role in genome imprinting, defense against transposon proliferation and regulation of gene expression. Specific combinations of histone N-tail modifications can be considered as histone codes, which determine chromatin structure and thus regulates transcription. Biotic and abiotic stresses induce epigenetic changes, which leads to alter gene expression and recombination. The methods to study the DNA methylation are bisulfite sequencing and methylation sensitive amplification polymorphism markers. For studying the histone modifications, chromatin immunoprecipitation (ChIP) was employed whereas deep sequencing is used for RNAi. Cytosine methylation categorically plays role in regulating various biotic and abiotic stresses such as low temperature, drought, bacterial blight, γ-irradiation, heavy metals and tissue culture. The major applications of epigenetics are better understanding on the physiological mechanisms such as stress tolerance and yield, phylogenetic studies, epigenetic QTL mapping and better insight into the transient expression of transgene. Epigenetic alterations in genome is generally understood to happen in the early phase of embryo development, with the reported late DNA methylation in tissue cultured plants as an exception. RNAi is being successfully employed in GM crop development. Deeper studies on epigenetics can enhance our understanding of gene expression, plant development and stress tolerance.

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Abstract No II.P10	Session I Transgenics in Food and Non- Food Crops

Manipulation of R genes for disease resistance in plants

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Plant disease is of great economic significance in global food supply. Chemical controls are often beyond the means of farmers in developing nations and in many cases agro chemicals are not sufficient to control the disease. For these reasons, much effort has been carried out towards understanding innate resistance mechanisms in plants. Plant cells autonomously maintain constant vigilance against pathogens by expressing large arrays of 'R genes' (R, resistance).

Resistance genes (R-genes) are genes in plant genomes that convey plant disease resistance against pathogens by producing R proteins. Disease resistance (R) genes in plants often determine the recognition of specific pathogens that express a corresponding avirulence (avr) gene. The main classes of R-genes consist of a nucleotide binding domain (NB) and a leucine rich repeat (LRR) domain(s) and are often referred to as (NB-LRR) R-genes. Over 20 R genes with recognition-specificity for defined *avr* genes have been isolated from several plant species, including both monocots and dicots. These genes are effective against bacterial, viral, and fungal pathogens. Resistance can be conveyed through a number of mechanisms including, interaction of R protein directly with a*vr* gene product of pathogen, guarding another protein that is degraded by *avr* gene, sensing host sensors known as pattern recognition receptors which triggers an array of defence responses, encoding enzyme that degrades a toxin produced by a pathogen.

The R gene *Ph-3* from tomato confers resistance to *Phytophthora infestans*, *mlo* gene from barley shows resistance to *Erysiphe graminis f. sp. Hordei*, rice *pib* gene shows resistance to blast disesase.Once the R protein detects the presence of a pathogen, the plant can mount a defence against the pathogen. There are prospects for transgenic use of single R gene that has previously been proven stable. Manipulation of R genes and their signaling pathways by transgenic expression is a promising strategy to improve disease resistance in plants.

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Abstract No II.P11	Session I Transgenics in Food and Non- Food Crops

Validation of apomictic genes in black pepper (*Piper nigrum* L.) for the development of lines with enhanced berry set

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Pepper is a remunerative, easy to cultivate crop and there is high demand for quality planting material of high yielding varieties. The pepper plant (*Piper nigrum* L.) a species of family Piperaceae is known as 'King of Spices' is the most important and widely used spice in the world. India is a leading producer, consumer and exporter of black pepper in the world. Black pepper is cultivated to a large extent in Kerala and to a limited extent in Tamil Nadu and other states. Constant decrease in the number of pepper growers from year to year is a phenomenon that causes concern as this would seriously hamper pepper industry. High cost of production and lesser yield are the main factors that discourage farmer to plant pepper. Thus, there is a need to breed for high yielding pepper varieties in order to sustain the industry. The low berry set due to dependency on hydrophily is a major bottleneck for higher yield. Hybridization ensures wide variability exists among the cultivars of pepper for quality attributes and yield. Possible occurrence of apomixis to enlighten the practicability of carrying out either intervarietal hybridization or interspecific hybridization has also to be checked.

The demand is not met through conventional vine cutting propagation and tissue culture planting materials. Apomixis is an asexual mode of reproduction by seed. In homozygous cultivars apomictic seedlings will true to type of mother plant. The present experiment was taken up to validate apomixis in black pepper so as to explore the possibility of using apomictic seedlings as planting material.

An essential prerequisite in this experiment is use of a reliable method which could prevent self-pollination and natural pollination. Panniyur-1 variety is used to study apomixis in pepper. Spikes were bagged prior to anthesis and pollination had been prevented through emasculation. To achieve this, spikes were bagged to prevent pollination. In this experiment, the indicator for occurrence of apomixis is the fruit setting study in which the inflorescence was bagged and the anther emasculated. Berry development was observed without pollination, which confirmed the presence of apomixis. Successful fruit set would indicate possible occurrence of apomixis while failure of fruit set would indicate absence of apomixis. The study will help to confirm apomixis in black pepper. The results thus obtained could be utilized for cloning of hybrids and heterozygous genotypes through seed and to improve the understanding of the molecular mechanism of apomictic reproduction in plants. It can also open the possibility for transfer of apomixis to sexual plants enabling cloning of crops through seeds.

Further mRNA will be isolated from young apomictic and non apomictic berries and cDNA will be synthesized. Differential expression shall be detected through cDNA SSR and cDNA ISSR. Once the genes expressed during apomixis idenfied in black pepper, it could be utilized for cloning of hybrids and for transgenics/cisgenics.

SESSION III

Biosafety and IPR in GM Crops

Oral

National Seminar on GM Crops: Prospects and Issues
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Abstract No III.O1	Session III Biosafety and IPR in GM Crops

Bio safety and IPR in GM Crops – an exploratory analysis with respect to developing countries

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As the area under GM Crops is increasing at an increasing rate world-wide, the concern on them also multiplies in geometric proportions. The proponents of the GM Crops even glorify it as a means to increase the food production and thus capable of arresting poverty especially in the developing countries of the world. Somebody even go beyond to coin the proliferation of GM Crops [GMC] as the Second Green revolution.

While the Scientists in both public and private sectors, on one hand clearly regard genetic modification as a major new set of tools, the industry, on the other hand treat GMOs as an opportunity for increased profits. Yet the public in many countries distrusts GMOs. As a result, there is no consensus in most countries on how biotechnology and GM crops in particular, can address key challenges in the food and agricultural sector.

This study is an attempt to have an exploratory analysis of the situation especially with respect to the developing countries in contrast to developed countries of the world. The analysis reveals that majority of the governments in the developing nations often lack coherent policies on GMOs, and has not yet developed and implemented adequate regulatory instruments and infrastructures. This serious back drop is in the midst of the some other important revelations made by experts globally. The small number of GM technologies currently in use points to a real danger that the scale of the investment may lead to selective concentration on species and problems of global importance, and concomitant capital inertia as developing of transgenic crops implies massive investments, and hence, the need for massive returns. At the same time, there is a growing use of "hard" intellectual property rights over seeds and planting material and the tools of genetic engineering. This changes the relationship between the public and private sectors, to the detriment of the former. Similarly, the perceived profit potential of GMOs has already changed the direction of investment in research and development, in both the public and private sectors, away from systems-based approaches to pest management, and towards a greater reliance on monocultures.

The study also endorse the view if the IPRs Commission, 2002 that, "the immediate impact of intellectual property protection is to benefit financially those who have knowledge and power, and to increase the cost of access to those without".

With due recognition to the great potential and the complications of these new technologies the study attempt to invite attention of all the stakeholders that, we have to move carefully with a full understanding of all factors involved, with respect to GMO's. In particular, we need to assess GMOs in terms of their impact on food security, poverty, bio safety and the sustainability of agriculture. GMOs cannot be seen in isolation, simply as technical achievements.

SESSION III

Biosafety and IPR in GM Crops

Poster

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Abstract No III.P1	Session III Biosafety and IPR in GM Crops

Unintentional genetic engineering by transgrafting for crop improvement

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Unintentionally, farmers have been genetically modifying their plants, for centuries, without even knowing it because in grafting parts of two plants are fused together, causes the two halves to exchange genes with each other. The vessels of the two halves eventually merge but people have long believed that they keep their genetic material to *themselves but it turns out that* they were wrong. While merging two strains of genetically engineered tobacco plant via grafting of a Samsun NN strain whose main genome was loaded with a gene that produced a glowing yellow protein and resistant to the antibiotic kanamycin, and second Petit Havana strain which was engineered to produce a glowing green protein, and was resistant to spectinomycin, researchers found that the point of fusion was widespread with cells that produced both glowing proteins and shrugged off both antibiotics and when they cut slices from the plant and grew them in liquid that contained both kanamycin and spectinomycin for a month, many of those from the graft site thrived, even producing fresh shoots.

Grafting scions to vigorous, disease-resistant rootstocks is an alternative to chemical control methods, a particularly appealing feature for organic cultivation of crops. Currently, almost every commercial vegetable crops and perennial horticultural crops (fruits or nuts) use grafting to increase yields or avoid disease. Disease resistance and environmental tolerance are highly beneficial traits that can be provided through use of grafting, although the mechanisms, in particular for resistance, have frequently been unknown. As information emerges that describes plant disease resistance mechanisms, the proteins and nucleic acids that play a critical role in disease management can be expressed in genetically engineered (GE) plant lines. Utilizing transgrafting, the combination of a GE rootstock with a wild type (WT) scion or the reverse, has the potential to provide pest and pathogen resistance, impart biotic and abiotic stress tolerance, or increase plant vigor and productivity.The main importance to these potential benefits is the question of to what extent nucleic acids and proteins are transmitted across a graft junction and whether the movement of these molecules will affect the efficacy of the transgrafting approach. Attention will be specifically drawn to the use of small RNAs and gene silencing within transgrafted plants, with a particular focus on pathogen resistance. The use of GE rootstocks or scions has the potential to extend the horticultural utility of grafting by combining this ancient technique with the molecular strategies of the modern era.

Keywords: Genetically engineered protein, Transgrafting antibiotics

National Seminar on GM Crops: Prospects and Issues
17-18 March, 2014; Kerala Agricultural University, Thrissur, Kerala

Abstract No III.P2	Session III Biosafety and IPR in GM Crops

New Pest Management strategy through Genetically modified insects

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The burden of agricultural pests is ever present while the number of control approaches is shrinking in the face of insecticide resistance and de-registration of existing chemical treatments. To survive and prosper, Indian farming will need to evaluate and embrace new solutions and new technologies which are effective, sustainable and safe. Annual crop loss by pest is 50000 crores in India. Non judicious application of chemical pesticides are killing predators and natural enemies which inturn has resulted in pest resurgence.British scientists have developed genetically modified insects as an alternative to chemical pesticides. Large number of GM olive flies will be used to kill off wild pests that damage the crop. GM male olive flies which are genetically sterile naturally mate with the females, resulting in the death of female offspring at the larvae or maggot stage. This results in reduction in the olive fly population, which would allow the trees to produce fruit without the need for chemical sprays. GM insects are harmless to other species. Commercialization of GM insects would result in the release of GM insects onto field crops like olives, tomatoes, citrus fruits, cabbages and cotton. Millions of GMO mosquitoes have already been released on experimental basis intended to reduce transmission of the tropical disease dengue fever. The release of GM insects are covered by laws and regulations that cover the release of genetically modified organisms (GMOs), however, there is no specific regulatory process for GM insects anywhere in the world.

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Abstract No III.P4	Session III Biosafety and IPR in GM Crops

Growth stimulatory effect of Inulin on two probiotic strains of *Bifidobacterium*

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The alarming rise in antibiotic resistance and the emergence of many multi-drug resistant strains has increased the demand for probiotics and prebiotics as alternative approaches for the treatment of gastrointestinal disorders. Prebiotics are often preferred over probiotics for therapeutic purposes due to biosafety issues associated with the latter. Prebiotics are expected to impart their health benefits by selectively stimulating the growth of beneficial bacteria. It is worthwhile to assess the growth stimulatory effect of prebiotics while designing symbiotic products. Considering this a the growth stimulatory effect of the prebiotic inulin on two commercially available probiotics, *Bifidobacterium animalis* subsp. *lactis* B420 (B-420, Danisco, Germany) and *Bifidobacterium lactis* Bb-12 (Chr. Hansen, Denmark) was assessed. For this, 24 h old active cultures of bifidobacteria at 2 percent levels were inoculated to semi-liquid (agar 0.1%, w/w) minimal media containing 0 (control), 0.5, 1, 3 and 5 percent (w/v) of inulin ((Orafti, Belgium) and incubated anaerobically at 37°C for 24 h. The bifidobacterial counts were taken at 0 h and 24 h of incubation. Mean generation time was determined in all the tubes and expressed as percentage reduction in comparison with control. For both the tested cultures, inulin supplementation was effective in the reducing the mean generation time. Supplementation at the rate of 0.5 percent did not show a significant decrease in generation time whereas the stimulatory effect observed with 1, 3 and 5% inulin concentrations (($P<0.05$) was significantly higher than that of the control. As the prebiotics impart the beneficial effects through modulation of resident intestinal microflora, safety evaluation is a pre-requisite before commercialization.

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Abstract No III.P4	Session III Biosafety and IPR in GM Crops

Cultivation of Bt GM crops – impact on environment and other pests

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Genetically modified insect-resistant crops that express proteins from the bacteria *Bacillus thuringiensis* (Bt) have been widely adopted against cotton bollworms and corn borers. Strains of Bt are also effective against Colorado potato beetles which is a major pest of potato. Bt toxins are not harmful to humans, other mammals, birds, fish or beneficial insects but toxic to various herbivorous insects. When it is consumed by insects, the protein is converted to its active, toxic form (delta endotoxin) which in turn decelerates the digestive process. More than 100 different variations of Bt toxin have been identified in diverse strains of *Bacillus thuringiensis*. The different variations have different target insect specificity. The genes classified under *Cry1A* group target Lepidoptera while toxins in the *Cry3* group are effective against beetles.

Cultivation of GM crops has reduced pesticide spraying by 474 million kg (-8.9%) and, as a result, decreased the environmental impact associated with herbicide and insecticide use on these crops [as measured by the indicator the Environment Impact Quotient] by 18.1%. The technology has also facilitated a significant reduction in the release of greenhouse gas emissions from this cropping area. Bt has notably reduced the incidence of acute pesticide poisoning among cotton growers. In China, it was proven that the population of an occasional cotton pest, mirid bugs (Heteroptera: Miridae), increased following the introduction of genetically engineered cotton plants. The effectiveness of the control of *H. armigera* by cultivating Bt cotton has resulted in a decrease in the amount of insecticides used on Bt cotton compared to conventional cotton. This has led to a lack of control of mirids on Bt cotton due to the reduction in broad-spectrum insecticide use and consequently to a transformation of a minor pest to a major one.

SESSION IV

Recent Techniques for Detection of GM Crops and Products

Poster

National Seminar on GM Crops: Prospects and Issues
17-18 March, 2014; Kerala Agricultural University, Thrissur, Kerala

Abstract No IV.P1	Session IV Recent Techniques for Detection of GM Crops and Products

Matrix-assisted laser desorption/ionisation mass spectrometry imaging (MALDI-MSI) for studying biomolecules in plants

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Matrix-Assisted Laser Desorption/Ionisation mass spectrometry imaging (MALDI-MSI) has developed as a promising tool to investigate the spatial distribution of biomolecules in intact tissue specimens. The differentiation of leaf, stem, root and floral architecture from the germinating seed provides an excellent example of the changes in distribution of proteins and metabolic processes in plant system. MALDI-MSI has the potential to provide new insights into the molecular analysis of plants by providing fast, high spatial resolution information about biomolecules and potentially quantitative changes during plant development or those induced by diseases and environmental variations.

MALDI-MSI has been reported to image the distribution of a wide range of compounds in plants. The localisation of primary metabolites such as sucrose, glucose-6-phosphate and arginine in wheat seed and potatoes has been mapped by MALDI-MSI. Surface molecules such as epicuticular lipids, waxes and secondary metabolites like flavonoids and alkanes were also measured by MALDI-MSI in *Arabidopsis thaliana* flowers, leaves and roots. Atmosperic pressure infrared MALDI-MSI has found useful in imaging a large number of lipids and metabolites in a number of plant organs and species like banana, strawberry, potato garlic etc. In soyabean, MALDI-MSI was used for determining the residues of agrochemical compounds. Spatial distribution of gamma-aminobutyric acid (GABA) was identified in brinjal seeds by MALDI-MSI and the same was compared with synthetic standard. Direct tissue analysis using MALDI-MSI differentiated the abundance of metabolites in different tissue regions including cortex, xylem, phloem and pith. Histopathalogical studies conducted using MALDI-MSI in grapevine revealed specific locations of *Plasmopara viticola* pathogen infection in leaves. Analysis using high resolution standard MS of proteolytic digests could detect a single protein pro3 in the peel of peach fruit using this technique. MALDI-MSI serves as a tool for studying the differences between plant samples associated with tissue types, development, disease infection and genetic differences following genetic manipulations.

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Abstract No IV.P2	Session III Biosafety and IPR in GM Crops

Molecular identification of a *Lactobacillus* isolated from green chilly (*Capsicum chinense)*

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A tradition of using green chilly to initiate fermentation for preparing curd is followed in some parts of our state. To scientifically validate this practice, an attempt was made to isolate the innate lactose fermenters present in green chilly. Stalk of green chilly (*Capsicum chinense)* was transferred to 25ml sterilized skim milk and incubated at 37 °C/48h. From this, a loopful was transferred into MRS broth and incubated at 37 °C for 48 hours. Appropriate dilutions of this turbid broth were plated in MRS agar to get discreet colonies. The isolate so obtained was subjected to primary characterization. The results were suggestive of the isolate to belong to genus *Lactobacillus.* To confirm the identity of the isolate at the molecular level, DNA was extracted by boiling method. The extract showed a DNA concentration of 985.5ng/µl when analyzed in NanoDrop. PCR reactions were performed in a total volume of 50 µl containing 4 µl DNA extract in a thermal cycler (BioRad) using genus specific (16srRNA) primer. After the initial denaturation step at 94 °C for 10 min, 35 cycles comprising of 94 °C for 30sec (denaturation), 58 °C for 30sec (annealing)and 72 °C for 45 sec (extension) were carried out. This was followed by a final extension step at 72 °C for 10 min. *Lactobacillus bulgaricus* NCDC 304 was used as the positive control. Electrophoresis of the PCR product in 1% Agarose gel, revealed a band in the same position as that of positive control, thereby confirming the identity of the isolate as *Lactobacillus*.

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Abstract No IV.P3	Session I Transgenics in Food and Non- Food Crops

Detection of GMOs in foods and assessing food safety

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The increase in adoption of GMOs (genetically modified organisms) has led to specific legislation world-wide to regulate the presence and/or the amount of GMOs in food, food ingredients, feeds and agricultural commodities. Regulatory demands of labeling and traceability of GMOs in the food chain need suitable sampling protocols and analytical methods. GM crops and their products can be identified by detecting either the inserted genetic material at DNA level or at the resulting protein level. The release of GM crops and products in the markets worldwide has increased the regulatory need to monitor and verify the presence and the amount of GMOs in crops and products. Labelling legislation and trade requirements differ from one country to another, leading to the necessity for the development of reliable and sensitive analytical methods for detection, identification and quantification of GMOs in crops and their products**.**

In this article, DNA and protein based methods are discussed. The use of DNA based approaches like Southern blots, qualitative and quantitative PCR, real-time PCR, microarray and protein based approaches like ELISA (Enzyme Linked Immuno Sorbent Assay), lateral flow strips, western blots for detection of GMOs in various crops and products are reviewed. Different systems of biosensors viz. electrochemical, piezoelectric and optical are also employed for detection of GMOs. Where information on modified gene sequences is not available, new approach such as near-infrared spectrometry is used for detection of non-approved genetically modified foods. The efficiency of screening, identification and confirmation strategies should be examined with respect to false-positive rates, disappearance of marker genes, increased use of specific regulator sequences and the increasing number of GM foods.

SESSION V

Computational Biology for Transgenics

Oral

National Seminar on GM Crops: Prospects and Issues
17-18 March, 2014; Kerala Agricultural University, Thrissur, Kerala

Abstract No V.O1	Session V Computational Biology for Transgenics

Methodologies for computation and SAS based analysis of numerical output from *Agrobacterium* mediated transformation system

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Modern biotechnological experiments aimed at developing transgenic crops employs various gene transfer techniques. *Agrobacterium tumefaciens* mediated gene trasnfer is widely used for gene tranfer because of its natural genetic engineering mechanism.explants used for transfer of gene are co-cultivated with *Agrobacterium* which has gene of interest.Once the tranfer of gene is confirmed, the expalnt has to undergo tissue culture regeneration step. Regeneration of explants with transgene is challenging job for biotechnologist's as it requires thourogh tissue culture skills. The experimenter has to fix the treatments for regeneration and collect data for further analysis. Usually people use treatment with highest mean as the best suited treatment for regeneration which is statistically not correct. But these experiments requires use of simple factorial CRD (completely randomised design) as plant tissue culture experiments are conducted *in vitro* conditions. Researcher's use many statistical tools to analyze the data generated from these *in vitro* studies. There are number of software's that come in hand to help & simplify the process in analyzing the data. When the data is less/small in size, one can use simple tools available in excel or any free software's on statistical data analysis available on net. But when the data is too large in size one has to use most sophisticated software like SAS (statistical analysis system). SAS software analyses the large size data in less time and gives correct results for accurate interpretations of the study. A sample SAS program for simple factorial CRD is as follows;

```
DATA ANOVA; /*first code begins with statement called Data, anova is file name, one can use any name   */
INPUT c g  r characters; /*  Input statement will describe features of data c=class g=genotype r= replication */
CARDS; /*Cards or datalines are used to denote the begining of data*/
Data is pasted here
; /* Semicolon denotes end of the data or statement */
PROC ANOVA; /*  Proc statement is used describe procedure statements of analysis, */
CLASS c  g  r; /* Class statement denotes the classification or data arrangement  */
MODEL characters=r g; /* model statement is used denote dependent and independent variables */
By c; /* By statement is used run analysis using what we want */
RUN; /*  End of each program Run statment is used to denote the end of the program*/
PROC CORR PEARSON; /*  corr or for correlation */
VAR characters;
BY c;
RUN;
PROC MEANS MEAN  STDERR MIN  MAX  RANGE STD  VAR  CV; /*various statistical parameters can be obtained using proc means*/
VAR characters;
BY C;
RUN;
```

SESSION V

Computational Biology for Transgenics

Poster

National Seminar on GM Crops: Prospects and Issues
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Abstract No V.P1	Session I Transgenics in Food and Non- Food Crops

Aluminium chelates of curcuminoids and their antitumour studies

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Curcuminoids (1,7-diarylheptanoids) extracted from the rhizomes of the traditional Indian medicinal plant turmeric (*Curcuma Longa* Linn) have been reported to possess anti-inflammatory, anti-oxidant, anti-arthritic and anti-tumour activities[1-6]. Chemo preventive efficiency of synthetic curcuminoids has also been established [7-9]. It has been revealed that the biological significance, especially medicinal importance of many plant chemicals is enhanced by complex formation with various inorganic species such as metal ions. Considering the presence of α,β-unsaturated 1,3-diketo moiety in curcuminoids, they are expected to form large number of coordination compounds.

Structurally curcuminoids are 1,7-diaryl-1,6-heptadiene-3,5-diones (1,7-diarylheptanoids) and are known to form metal complexes similar to other 1,3-diketones [7]. It has been reported that metal complexation of these α,β-unsaturated 1,3-diketones lead to dramatic changes in their biochemical activities [8,9,10,11] including antitumour activity. As a part of our studies [7,12] on chemical and biochemical properties of synthetic analogues of active chemical compounds of such indigenous valuable green medicines and their metal complexes, the present study reports the synthesis, structural characterization, the cytotoxic and antitumour activities of four new curcuminoid analogues **1a-d** and their aluminium complexes.

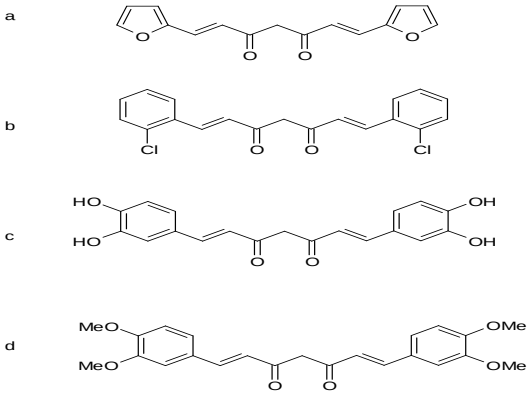

Fig 1. Structure of curcuminoid analogues.

1a (HL^1) 1,7-di(2-furyl)-1,6-heptadiene-3,5-dione,
1b (HL^2) 1,7-bis(2-chlorophenyl)-1,6-heptadiene-3,5-dione,
1c (HL^3) 1,7-bis(3,4-dihydroxyphenyl)-1,6-heptadiene-3,5-dione,

1d (HL^4) 1,7-bis(3,4-dimethoxy phenyl)-1,6-heptadiene-3,5-dione,

Aluminium(III) complexes of four curcuminoid analogues, [1,7-di(2-furyl)-1,6-heptadiene-3,5-dione,**1a**; 1,7-bis(2-chlorophenyl)-1,6-heptadiene-3,5-dione,**1b**; 1,7-bis(3,4-dihydroxyphenyl)-1,6-heptadiene-3,5-dione,**1c**; 1,7-bis(3,4-dimethoxyphenyl)-1,6-heptadiene-3,5-dione,**1d**;] of ML_3 stoichiometry were synthesized and characterized by UV, IR, ^1H NMR and mass spectral data. The *invitro* and *invivo* antitumour activity of these 1,7-diarylheptanoids and their Aluminium(III) complexes showed remarkable enhancement in cytotoxic and antitumour activities. Aluminium chelates of **1c** which posses two hydroxyl groups was found to be most active towards cultured L929 cells. Aluminium chelates of all curcuminoid analogues showed a significant reduction ($P<.001$) of solid tumour volume in mice.