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Society of Green World for Sustainable Environment (SGWSE)

(Registered Under Societies Registration Act 1860)

Aims and Objectives

The Society (SGWSE) has been functioning with following aims and objectives:

- To constitute a forum at international and national level for bringing together individuals and organizationinvolved in agriculture and biological science activities.
- To develop international research/development linkages and disseminate up-to-date technologies in the field of agriculture and biological science
- To promote and undertake research and development and extension service in the field of agriculture and biological science.
- To explore new areas in agriculture, biological research, biotechnology crop cultivation technologies, evelopment activity and logistics management.
- To propagate utilization on non- conventional and renewable sources in agriculture and biological research.
- To develop purely organic package for growing and cultivation of crops.
- To develop technology of in vivo propagation of important crops.
- To offer recognition and awards to professional groups and individual for attainment of excellence in the field of agriculture, biological science and Biotechnology.
- To interact with government agencies, scientific organization and NGOs to promote and protect interest of agriculture and biological science researcher.
- To organize symposia, seminar, and workshops and bring out timely publication(s) to meet the objectives of the society.

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NATIONAL AND INTERNATIONAL CONFERENCES

2020

1) **Sep 08-09, 2020**
ICO 2020: Oncogenesis Conference, Singapore

2) **(Sep 08-09, 2020)**
ICOCB 2020: Oncogenesis and Cancer Biology Conference, Singapore

3) **Sep 08-09, 2020**
ICOCM 2020: Oncogenesis and Cancer Metabolism Conference, Singapore

4) **Sep 08-09, 2020**
ICOCO 2020: Oncogenesis and Cellular Oncogenes Conference, Singapore

5) **Sep 08-09, 2020**
ICOCR 2020: Oncogenesis and Cancer Research Conference, Singapore

6) **Sep 08-09, 2020**
ICOMO 2020: Oncogenesis and Molecular Oncology Conference, Singapore

7) **Sep 16-17, 2020**
ICBLS 2020: Biology and Life Sciences Conference, Lisbon

8) **Sep 16-17, 2020**
ICLT 2020: Life Technologies Conference, Zurich

9) **Oct 01-02, 2020**
ICM 2020: Microscopy Conference, Baku

10) **Oct 01-02, 2020**
ICPB 2020: Physical Biosciences Conference,Dubrovnik

11) **Oct 07-08, 2020**
ICPS 2020: Photobiological Sciences Conference,Beijing

12) **Oct 21-22, 2020**
ICBSET 2020: Biological Science, Engineering and Technology Conference, Barcelona

13) **Oct 29-30, 2020**
ICCMLS 2020: Computational Models for Life Sciences Conference, Paris

14) **Nov 02-03, 2020**
ICAP 2020: Advances in Photobiology Conference, San Francisco

15) **Nov 02-03, 2020**
ICLSS 2020: Life Sciences and Sustainability Conference, San Francisco

16) **Dec 10-11, 2020**
ICDISLS 2020: Data Integration Systems in the Life Sciences Conference, Rome

17) **Dec 28-29, 2020**
ICLSE 2020: Life Science and Engineering Conference, Paris

18) Dec 28-29, 2020
ICMMT 2020: Microscience Microscopy and Technology Conference, Paris

2021

19) **Jan 14-15, 2021**
ICRAP 2021: Recent Advances in Photobiology Conference, Zurich

20) **Jan 28-29, 2021**
ICPPP 2021: Photobiology, Photochemistry and Photophysics Conference, Istanbul

21) **Feb 15-16, 2021**
ICBCLS 2021: Biology, Ecology and Life Sciences Conference, Dubai

22) **Feb 15-16, 2021**
ICPPP 2021: Photobiology, Photophysics and Photochemistry Conference, Dubai

23) **Feb 18-19, 2021**
ICPP 2021: Photobiology and Photochemistry Conference, Rome

24) **Feb 25-26, 2021**
ICLBS 2021: Life and Biomedical Sciences Conference, Tokyo

25) **Mar 22-23, 2021**
ICLSBBE 2021: Life Science, Biomedical and Biological Engineering Conference, Dubai

26) **Apr 12-13, 2021**
ICBLS 2021: Biological and Life Sciences Conference, Venice

27) **Apr 22-23, 2021**
ICLSBR 2021: Life Sciences and Biomedical Research Conference, London

28) **May 13-14, 2021**
ICPP 2021: Photobiology and Photomedicine Conference, Amsterdam

29) **May 17-18, 2021**
ICCB 2021: Chemical Biosciences Conference, Sydney

30) **May 20-21, 2021**
ICELSE 2021: Environmental Life Sciences Engineering Conference, Vancouver

31) **Sep 09-10, 2021**
ICVALS 2021: Veterinary, Agricultural and Life Sciences Conference, Singapore

32) Feb 15-16, 2021
ICBMD 2021: Biomarkers for Metabolic Disorders Conference, London

33) **Feb 15-16, 2021**
ICNMD 2021: Nutrients and Muscle Diseases Conference, London

34) **Feb 25-26, 2021**
ICBMD 2021: Biomarkers of Metabolic Disorders Conference, Sydney

35) **Feb 25-26, 2021**
ICCSPGNM 2021: Computation and Statistics for Proteomic and Genomic Networks Modeling Conference, Tokyo

36) **Mar 22-23, 2021**
ICLSBBE 2021: Life Science, Biomedical and Biological Engineering Conference, Dubai

37) **Apr 12-13, 2021**
ICEGR 2021: Environmental Genomics and Research Conference, Venice

38) **Apr 15-16, 2021**
ICEG 2021: Ecological Genomics Conference, Cape Town

39) **Apr 26-27, 2021**
ICNMD 2021: Nutrients and Muscle Disease Conference, Istanbul

40) **Jun 07-08, 2021**
ICFBCD 2021: Food Bioactive Compounds in Disease Conference, San Francisco

41) **Jun 10-11, 2021**
ICEGE 2021: Ecological Genomics and Epidemiology Conference, Tokyo

42) **Jun 10-11, 2021**
ICEGFG 2021: Ecological Genomics and Functional Genetics Conference, Tokyo

43) **Jun 10-11, 2021**
ICEGGA 2021: Ecological Genomics and Genome Analysis Conference, Tokyo

44) **Jun 10-11, 2021**
ICEGMA 2021: Ecological Genomics and Molecular Adaptation Conference, Tokyo

45) **Jun 15-16, 2021**
ICARVR 2021: Advances in Respiratory Virus Research Conference, Toronto

46) **Jun 15-16, 2021**
ICATDRB 2021: Advances of Techniques in Deep Regional Blocks Conference, Toronto

47) **Jun 21-22, 2021**
ICECM 2021: Epidemiological and Clinical Metabonomics Conference, Venice

48) **Jul 15-16, 2021**
ICCBMD 2021: Clinical Biomarkers in Metabolic Disorders Conference, Bali

49) **Jul 15-16, 2021**
ICCTADS 2021: Current Trends in Ancient Diseases Studies Conference, Bali

50) **Jul 15-16, 2021**
ICEVHA 2021: Enteric Viruses of Humans and Animals Conference, Bali

51) **Jul 15-16, 2021**
ICFBCRD 2021: Food Bioactive Compounds and their Role in Diseases Conference, Bali

52) **Jul 15-16, 2021**
ICOVI 2021: Oncolytic Viruses and Immunotherapy Conference, Bali

53) **Jul 15-16, 2021**
ICRVR 2021: Respiratory Virus Research Conference, Stockholm

54) **Jul 15-16, 2021**
ICVMIE 2021: Viral Mechanisms of Immune Evasion Conference, Bali

55) **Jul 19-20, 2021**
ICAPPC 2021: Anesthesia for Postoperative Pain Control Conference, Copenhagen

56) **Jul 19-20, 2021**
ICFBCD 2021: Food Bioactive Compounds against Diseases Conference, Copenhagen

57) **(Jul 19-20, 2021**
ICOVV 2021: Oncolytic Viruses and Virotherapy Conference, Copenhagen

58) **Jul 19-20, 2021**
ICVSIE 2021: Viral Strategies of Immune Evasion Conference, Copenhagen

59) **Jul 22-23, 2021**
ICBMD 2021: Biomarkers in Metabolic Disorders Conference, Berlin

60) **Jul 22-23, 2021**
ICCBMD 2021: Clinical Biomarkers of Metabolic Disorders Conference, Berlin

61) **Jul 22-23, 2021**
ICEGA 2021: Environmental Genomics and Adaptation Conference, Rome

62) **Jul 22-23, 2021**
ICEGCG 2021: Ecological Genomics and Conservation Genetics Conference, Rome

63) **Jul 22-23, 2021**
ICMEGI 2021: Metagenomics, Environmental Genomics and Informatics Conference, Rome

64) **Jul 22-23, 2021**
ICNMD 2021: Nutrients for Muscle Diseases Conference, Berlin

65) **Jul 22-23, 2021**
ICOVI 2021: Oncolytic Viruses and Immunity Conference, Berlin

66) **Jul 29-30, 2021**
ICEGB 2021: Environmental Genomics and Bioinformatics Conference, Zurich

67) **Jul 29-30, 2021**
ICEGM 2021: Environmental Genomics and Metagenomes Conference, Istanbul

68) **Jul 29-30, 2021**
ICRTADS 2021: Recent Trends in Ancient Diseases Studies Conference, Zurich

69) **Aug 05-06, 2021**
ICCEM 2021: Clinical and Epidemiological Metabonomics Conference, Montreal

70) **Nov 11-12, 2021**
ICDRB 2021: Deep Regional Blocks Conference, Tokyo

71) **Dec 09-10, 2021**
ICEVAH 2021: Enteric Viruses of Animals and Humans Conference, London

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Polymerase Chain Reaction (PCR) based cloning of Mitogen Activated Protein Kinase (MAPK3) in expression vector for isolation and purification of MAPK3 protein

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Abstract

Alternaria blight incited by a fungus *Alternaria brassicae* causes significant economic losses in *Brassica* crops depending upon the severity of the disease incidence. MAPK3 which is one of the important components of MAP Kinase signaling pathway is known to be involved in plant defense in *Arabidopsis thaliana* which has interstingly been shown as a host for Alternaria blight disease. Hence, this MAP kinase was used as target for raising antibodies against it for it's immunological detection during progression of Alternaria blight disease.Full length cDNA clone for MAPK3, was amplified from *Arabidopsis* thaliana plants, eluted from gel and cloned into pGEX4T2 expression vector carrying the glutathione-S transferase (GST) tag. Now the recombinant pGEX4T2 plasmid was introduced into Bl21 strain of *E. coli* for efficient synthesis of MAP Kinase3 protein. Then, IPTG induced synthesis of GST-MAP Kinase fusion protein was monitored through analysis of total bacterial protein by SDS-PAGE. After purification, these polyclonal antibodies can be used to purify respective MAP Kinase 3 from *Brassica* and to study regulation of MAP Kinases at translational level.

Keywords: Protein kinase,Cloning, Vector,MAPK3 Protein

Introduction

The main cause of low productivity of rapeseed and mustard in India, is the disease, Alternaria Blight, caused by the major pathogen, *Alternaria brassicae*. In recent years molecular investigation in to mechanism of pathogenesis, in our lab, revealed that *Alternaria brassicae*, major pathogen of disease produces chlorotic, necrotic toxins and growth regulators to produce chlorosis, necrosis and green island like disease symptoms. Chlorotic toxin was identified as destruxin B which activates a signal transduction pathway leading to perturbations in cell cycle and expression of cell death proteins like p53 and caspases (Khandelwal *et al.*, 2002). However precise molecular target of pathogen /disease could not be delineted. It is being realized that identification of complete signaltransduction pathway activated by pathogen in the event of pathogenesis will help in devising biotechnological strategies to combat the disease. In recent years , it was known that plants use MAPK signaling cascade to mediate cellular responses to plethora of environmental and developemental signals .

MAP kinase cascade is composed of three families of kinases, MAP kinase kinase kinase (MAPKKK or MAP3K), MAP kinase kinase (MAPKK or MAP2K) and MAP kinase (MAPK), which are sequentially phosphorylated during transmission of signal from receptor to downstream targets. On the basis of genomic information, 20 MAPKs, 10 MAP2Ks and 80 MAP3Ks have been identified in *Arabidopsis*, which can engage themselves in form of various modules to regulate plant responses to different environmental and developmental signals. (Colcombet and Hirt, 2008). Some of the components of MAP Kinase pathway such as MAPK3, MAPK4 and MAPK6 are known to be involved in plant defense in *Arabidopsis thaliana*. Keeping evolutionary conserved nature of MAP Kinase pathway,I t is quite possible that A*lternaria brassicae* toxin also affects some of candidate MAP Kinases during pathogenesis. It was realized that *Arabidopsis thaliana* could successfully be utilized as model plant to reveal the information on key defense related MAP Kinases involved during pathogenesis of *Alternaria* blight in *Brassica*. Utilising *Arabidopsis thaliana* as the host plant for the disease, it was observed in our lab that MAPK3, MAPK4, MAPK6 and MAPK29 play significant role in pathogenesis/defense of Alternaria blight (Kannan *et.al.*, 2012; Mondal *et.al.*, 2016; Mishra *et.al.*, 2015). Bioinformatics analysis has also shown that MAPK3 interacts with some members of WRKY family, which play important role in triggering defense (Taj *et.al.*, 2011). Realizing the importance of above MAPK3 in defense, it was used as a target for raising antibodies against it so as to use them for it's isolation and purification from *Brassica* or *Arabidopsis* by following immunoprecipitation and to study the regulation of the MAPK3 at translational level during pathogenesis process of Alternaria blight.

Materials And Methods

Source of *Arabidopsis* cDNA

The full length cDNA clones of *Arabidopsis* were obtained from ABRC (Arabidopsis biological resource centre) USA in plasmid vector pENTR/SD/D/TOPO.

PCR based cloning of MAPK3 gene

The complete sequence of cds for MAPK3 along with pENTR/SD/D-TOPO (2601bp) were retrieved from TAIR database and website of Invitrogen Lifetech. The primers were designed against putative MAPK3 gene using CLC-DNA

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Bench work primer designing software. The sequence of primer were 5'GGCCGCCTTGTTTAACTT3' (18 bases) forward and 5'TACAAGAAAGCTGGGTC3' (17 bases) reverse. PCR amplified MAPK3 gene was eluted by using Genei DNA extraction kit and cloned in pGEMTeasy vector.A ligation reaction as per the instruction provided by the manufacturer were set at 4°c for overnight and subsequently subjected to transformation using *E.coli* strain DH5α by $CaCl_2$ method of transformation. Ligated product was transformed in DH5α as a competent cell because it lacks endA1, α-portion on LacZ and F^-.

Directional cloning of MAPK3 gene in expression vector

After PCR based cloning of MAPK3, efforts were made to clone the MAPK3 gene in pGEX4T2 expression vector for the purpose of synthesis of MAPK3 protein in the form of GST-MAPK3 fusion protein. In order to ensure that reading frame for both GST and MAPK3 genes are not disrupted, directional cloning of MAPK3 gene was attempted in pGEX4T2 vector.In order to do so, MAPK3 gene specific primers were designed by using Invitrogen online primer designing tools and sequence information of MAPK3 gene. For directional cloning, *EcoRI* site was added in forward primer and *SmaI* site added in reverse primer of MAPK3 gene. The sequences of primers used were 5'GAATTCCCTTCACCATGAACACCGGC3' forward and 5'CCCGGGCTAACCGTATGTTGGATTGAGTG3' reverse primer.After PCR amplification,restriction digestion was done with *Eco* RI and *Sma* I enzymes to generate sticky and blunt end of amplified MAPK3 gene and the vector pGEX4T2 was also digested with same enzymes. Then the digested MAPK3 gene was ligated to restricted pGEX4T2 expression vector by performing blunt end and sticky end ligation. First, blunt end ligation was done using blunt end buffer with insert (GOI) (60ng), vector (60ng), T4 DNA ligase, and MB grade water followed by incubation at 16°c for 6-7 hours. After blunt end ligation, sticky end ligation was carried out using MB grade water, T4 DNA ligase, and sticky end buffer followed by incubation at 16°c for overnight for ligation. Then the recombinant vector containing MAPK3 gene was introduced in to BL21 strain of *E. coli*.

Isolation and analysis of GST- MAPK3 fusion protein by SDS PAGE

Synthesis of GST-MAPK3 fusion protein was induced by growing transformed BL21 cells in LB medium containing 0.1Mm IPTG. For this, there was inoculated a transformed colony in to 5 ml of LB broth with ampicillin and incubated at 37°C with 130 rpm for overnight and 2ml of overnight culture was incubated in 100 ml of ampicillin (100µg / ml) containing LB broth. It was allowed until the OD reaches 0.5 at 600 nm from this 5ml of culture was labeled as Before Induction (BI) and the remaining 95ml of culture was mixed with 0.1mM IPTG and marked as After Induction (AI) sample. Cultures were incubated at 30°C at 220 rpm for overnight. The incubated culture samples were lysed by boiling for quick checking of the fusion protein. 5ml of both before induction (BI) and after induction (AI) culture were centrifuged at 8000 rpm for 10 minutes and the supernatant was discarded. The pellet was resuspended in 100-150µl of cell lysis extraction buffer and boiled at 70-100°C for 20 minutes in serological water bath The concentration of protein, as estimated by Bradford method was found to be 12.9 µg /µl and 25µl sample was directly loaded on 12% PAGE gel.

Results and Discussion

Molecular cloning of MAPK3 gene in pGEMTeasy vector

After PCR amplification of *Arabidops* cDNA, an amplicon of 1,200bp was observed which was equal to expected size of full length MAPK3 (**Fig.1**). PCR amplified MAPK3 gene was eluted by using Genei DNA extraction kit as per manufacturer's protocol and the eluted MAPK3 gene was ligated to pGEMTeasy vector is commonly used for cloning of PCR products. PCR products contain 3'A overhangs which can easily be annealed with the 5' T overhangs of plasmid cut with any of restriction endonuclease within MCS. The plasmid contains T7 and SP6 RNA polymerase promoters flanking a multiple cloning region within the α-peptide coding region of the enzyme β-galactosidase. Insertional inactivation of the α-peptide due to insertion of PCR product allows recombinant clones to be directly identified by method used for identification of recombinant vector. The recombinant pGEMTeasy vector containing MAPK3 gene was introduced in to DH5α strain of *E. coli* by $CaCl_2$ mediated transformation protocol. A 10 µl of ligation mixture was subjected to transformation. Overnight incubation of LB agar plates containing ampicillin (50µg/ml) with IPTG (1mM) and X-Gal (200 µg/ml) resulted in formation of white and blue colonies in plate for ligated products in pGEMTeasy vector **(Fig.2)**

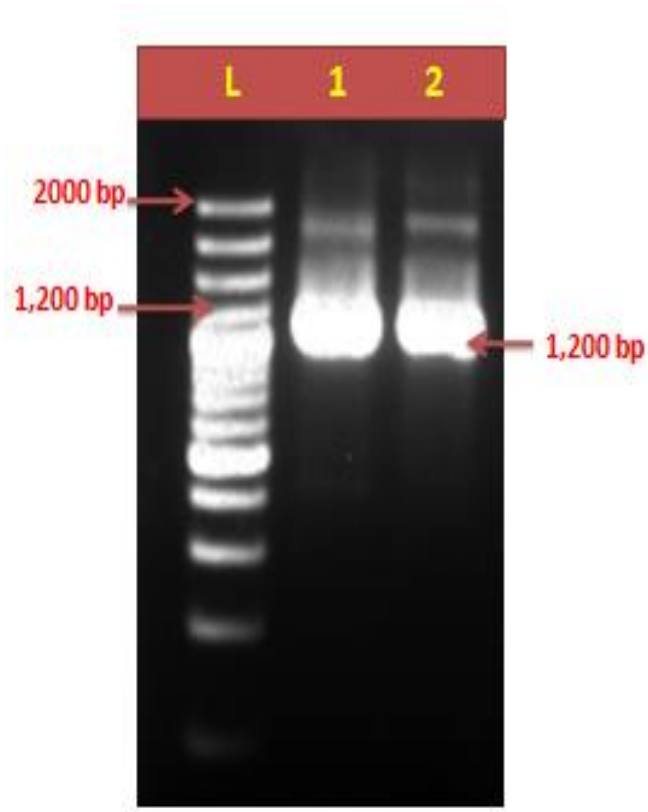

Fig.1. PCR amplification of MAPK3 gene by using gene specific primer (L= 200bp ladder, 1 and 2 = MAPK3 amplicon)

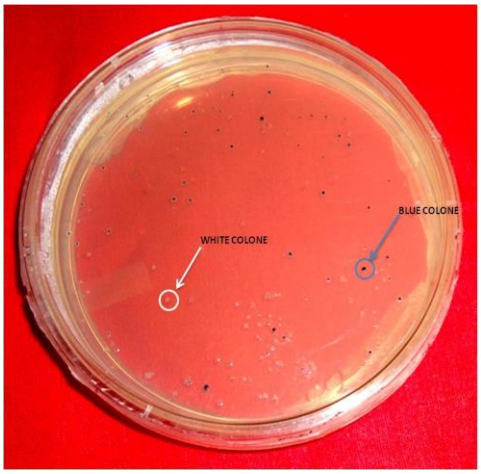

Fig.2. *E. coli* DH5α cells transformed with recombinant pGEMTeasy vector growing in Luria Agar containing 50µg/ml Ampicillin (White Arrow show Transformed colony, Blue arrow show non-transformed colony)

The recombinant white colonies obtained were confirmed for the presence of the expected size amplicon in the cloning vector by isolation of plasmid DNA from white colonies followed by restriction digestion of plasmid DNA with *E.coRI* restriction endonucleases which resulted in fall out of MAPK3 gene insert of 1,200bp (**Fig.3.)**

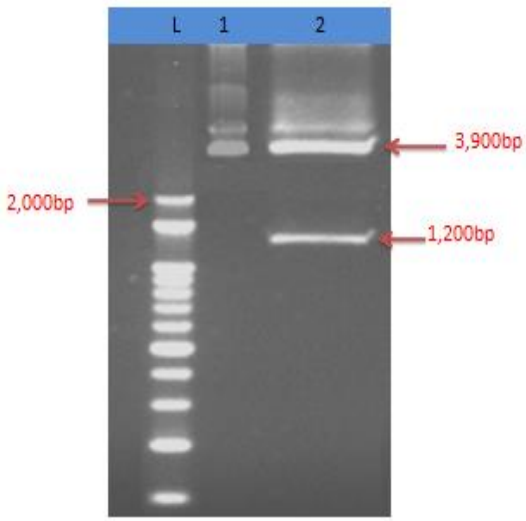

Fig.3. Restriction digestion of pGEMTeasy vector containing MAPK3 gene.

Sequencing and *In-silico* analysis of MAP Kinase 3 Sequence

Bidirectional sequencing of the cloned product was carried out. After getting the sequencing results, the vector sequences were separated from cloned gene sequences using Vec-screen online software tool. The two stands of the cloned gene were combined together to obtain double stranded sequences of the cloned gene. The sequence of MAP Kinase3 gene was searched for homology with other sequence present at the NCBI database (http://www.ncbi.nlm.nih.gov) using BLASTn tool. The sequence showed maximum identity of 99% with *Arabidopsis thaliana* MAP Kinase 3 (ATMPK3) mRNA complete cds on single hit**(Figure 4.)**. This confirmed that the amplified and cloned gene gene is MAP Kinase 3 from *Arabidopsis thaliana*.

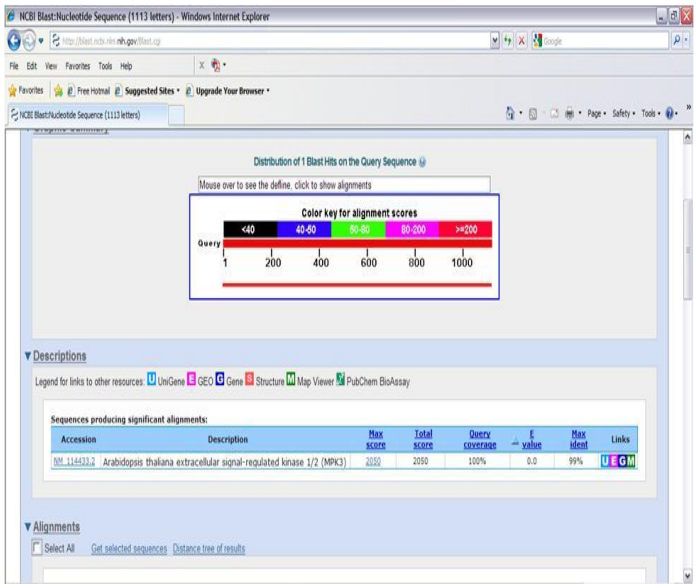

Fig .4. Homology search results for MAP Kinase 3 at NCBI – BLAST

Directional Cloning of MAPK3 gene in pGEX4T2 expression vector

After PCR based cloning of MAPK3, efforts were made to clone the MAPK3 gene in pGEX4T2 expression vector for the purpose of synthesis of MAPK3 protein in the form of GST-MAPK3 fusion protein. It was realized that MAPK3 protein can be purified by Glutathione affinity chromatography. Synthesis of GST-MAPK3 fusion protein requires the gene for GST in correct reading frame. In order to ensure that reading frame for both genes is not disrupted, directional cloning of MAPK3 gene was attempted in pGEX4T2 vector.The full length ORF of AtMAPK3 was obtained by PCR, and ligated with vector sequence from start codon of GST (ATG) from stop codon of MAPK3 and resulted protein product was analyzed for the presence of characteristic domain. The protein sequence was analyzed using SMART online tool. Two domain corresponding to GST (N→C terminal) and S-T_{KS} were found, confirming the fact that the primer pair can amplify the functional *At*MAPK3 gene along with GST tags. On translating the fused MAPK3 and GST nucleotide sequence in ExPasy and checking with SMART domain finding tool. It was found that both the proteins are fused and are in the same reading frame (**Fig.5.)**

The recombinant pGEMTeasy vector containing MAPK3 gene was PCR amplified by primers containing *Eco* R I and *Sma* I site whose sequences are given in Materials and Methods section.After PCR amplification, amplified MAPK3 gene was eluted with Genei DNA extraction kit. After PCR amplification, restriction site of *EcoRI* and *SmaI* were supposed to be present at 5' and 3' end of amplicon respectively. Therefore restriction digestion was done to generate sticky and blunt end of amplified MAPK3 gene, so as to facilitate the ligation reaction.The restriction digested product were subjected to ethanol precipitation so as to

remove restricted nucleotide bases from gene and to purify restricted MAPK3 gene with sticky end at one side and blunt end at another side of the gene.

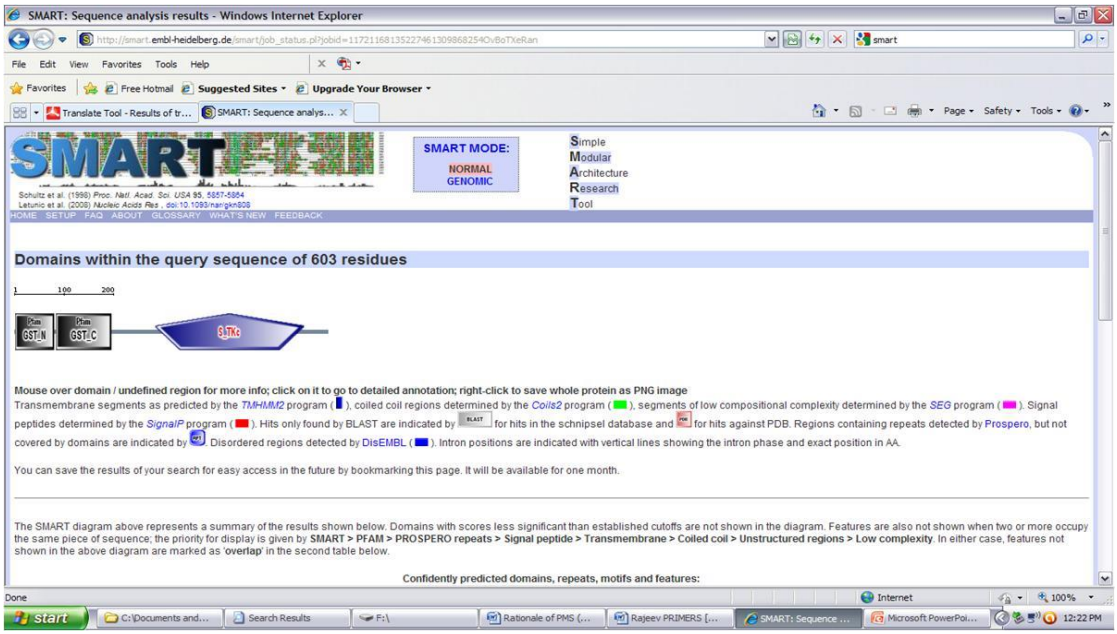

Fig 5. Domain Analysis with translated MAP Kinase 3 protein by SMART Translate tool

For directional cloning, the expression vector, plasmid pGEX4T2 vector was also digested with *EcoRI* and *SmaI* restriction endonucleases so as to generate *EcoRI* sticky end and *SmaI* blunt ends. After digestion, restricted plasmid vector fragments were ligated to digested MAPK3 gene through blunt and sticky end ligation.

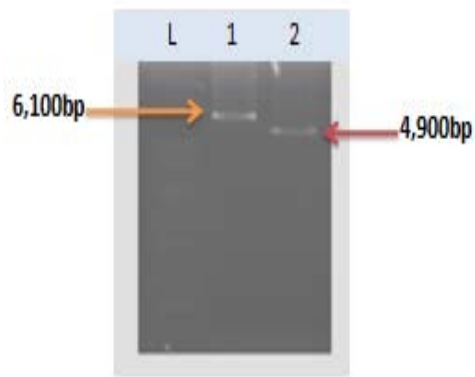

Fig: 6. Ligation of MAPK3 gene to the pGEX4T2 vector (Lane L - 100bp DNA ladder Lane 1 - Ligated MAPK3 to the pGEX4T2 vector Lane 2 - Control pGEX4T2 vector (4.9 kb)

Ligation of MAPK3 gene to the vector was checked by running it on agarose gel which showed the presence of 1,2 kb ligated MAPK3 gene **(Fig:6)**

Competant cells of Bl-21 strain of *E.coli* which has been reported to be optimum host for GST tagged fusion protein. were transformed with the recombinant pGEX4T2 vector containing MAPK3 gene and transformed cells were selected Luria agar medium containing 50 µg/ml ampicillin. There was observed single colony in the selection medium perhaps due to poor ligation of MAPK3 gene with vector.(**Fig.7**)

Fig.7. Transformed E.coli BL21 cells containing MAPK3 gene in pGEX4T- 2 expression vector

Isolation and confirmation of pGEX4T2 recombinant plasmid in transformed cells

In order to verify the presence of recombinant plasmid pGEX4T2 in transformed cells, the plasmid was isolated from overnight grown culture developed from single colony growing in selection medium. In order to confirm the presence of MAPK3 gene insert into pGEX4T2 vector, the isolated and purified recombinant plasmid pGEX4T2 was digested with EcoRI SmaI. Upon double digestion, MAPK3 gene fall out insert of 1,200bp was observed when restricted plasmid was run on 1.5% agarose gel along with molecular marker and undigested pGEX4T2 vector. This confirmed the presence of MAPK3 gene in the pGEX4T2 plasmid vector (**Fig. 8)**

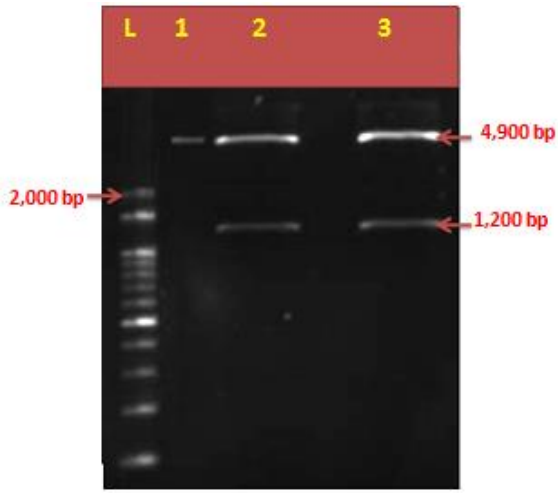

Fig.8. Restriction digestion of pGEX4T2 vector containing MAPK3 gene(Lane L - 100bp Ladder , Lane 1 – Control pGEX4T2 , Lane 2 and 3 - double digested products)

Isolation and analysis of GST- MAPK3 fusion protein by SDS PAGE

Synthesis of GST-MAPK3 fusion protein was induced by growing transformed BL21 cells in LB medium containing 0.1Mm IPTG. After boiling, lysate was centrifuged by ultracentrifugation. Supernatants (proteins) were quantified by spectrophotometer and proteins were checked by SDS PAGE.

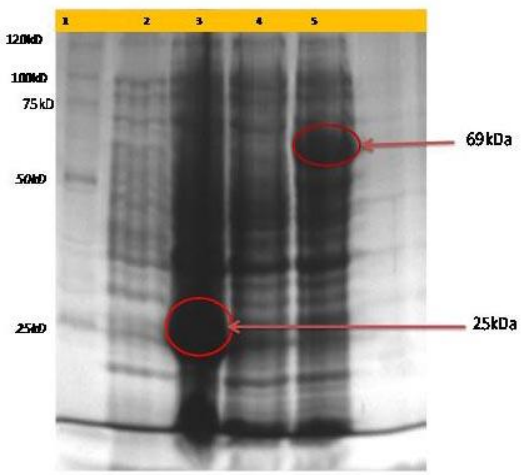

Fig 9. Analysis of GST- MAPK3 fusion protein by SDS PAGEin figure5.2. shows :

Lane 1 : Protein pre-stained molecular weight marker.
Lane 2 : BI (Before induction) pGEX4T-2 sample which has no band without IPTG induction
Lane 3 : Shows AI (After induction) pGEX4T2 sample with thick band indicating that IPTG has induced GST and is about 25 kd
Lane 4 : equilibrated sample of MAPK3 with out IPTG, no bands were seen, which indicates the absence of GST
Lane 5 : MAPK3 protein with IPTG induction , which shows sharp thick band measuring about 69KDa

Upon SDS PAGE analysis a band of 25kda equivalent to molecular weight of GST was observed from the protein isolated from BL21 cells containing nonrecombinant pGEX4T2 vector .The protein from transformed BL21 cell lysate revealed the absence of 25kD GST specific band but a new band of 69 kD appeared. The size of new band were found to be equal to sum of size of MAPK3 protein (44 kD) and GST (25kD) and hence considered to be the band for GST –MAPK3 fusion protein as shown in **Fig: 9.**

Conclusion

In conclusion, the present study describes the successful PCR based cloning of MAPK3 in form of MAPK3-GST fusion protein in pGEX4T2 vector in Bl21 strain of *E.coli*. Subsequently, MAPK3 protein can purified by GST-affinity chromatography and purified protein can be injected into rabbit for raising antibodies against it. These antibodies can be utilized to purify respective MAPK3 protein from *Brassica*by immunoprecipitation and to study the regulation of MAPK3 at translational level during pathogenesis of Alternaria blight.

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RESEARCH ARTICLE

Prediction and validation of protein-protein interaction using protein 3D structure and physicochemical properties by the aid of support vector machine.

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Abstract

Protein–protein interactions are interactions between proteins that take place due to certain chemical reactions and electrostatic forces. Predicting these interactions plays an important role in reducing the time and cost of wet lab experiments. Firstly, identifying the interaction sites is needed to predict the protein–protein interactions. Using SVM these interacting sites were predicted. The prediction was done using the structural information plus features like hydrphobicity, x-coordinates, y-coordinates, z-coordinates, surface tension, charge, alpha helix, β helix, turn, van der waals, molecular weight, solubility.

Keywords: *sklearn, hydrphobicity, SVM*

Introduction

The biological function performed by proteins is a result of interaction between them or with RNA, DNA and other compounds. In order to understand the interactions we need to know the properties of the interfacial sites that are involved in the interaction process. Number of methods of predicting these interaction sites have been reported including non-automated (Fariselli *et.al*., 2002) and automated methods (Gallat *et.al.*, 2000) based on the hydropathy of the structural surface. The surface is divided into patches and the chemical and physical characteristics of the patches, such as hydrophobicity, flatness, protrusion index, and accessible surface area, are calculated. The interfacial patch is predicted using the sum of these values (Jones and Thornton, 1997b).

A prediction method using sequence profiles, with/without the accessible surface area, and neural networks for machine learning has also been reported(korn and Burnett, 1991; Ofran and Rost, 2003b; Young *et.al*., 1994). Another study reported that interfacial sites can be predicted using the hydrophobic moment and averaged hydrophobicity, although the application of this method is limited (Zhou and San, 2001).

These interfacial prediction has been found to be possible to some extent. However, many of the results reported have been obtained using limited, manually selected data or preliminary predictions without recall and precision or without consideration of unpredictable protein types.

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It is therefore unclear which types of protein are predictable or unpredictable and how precisely their interaction sites can be predicted.

A particular protein's function is dependent on the other proteins with which it interacts. Hence it indicates that the residues determine the level of specificity of interaction between proteins. The residues of one protein when interacting with the residues of other protein, these proteins undergo structural changes which enables changes in positions of the structure to enable better bonding. Certain times these changes are not reversible while in some other cases its irreversible. Some times there are also changes in chemical properties of the interacting proteins.

In this study, interaction sites were predicted from a diverse database of known pprotein-protein interactions using the structural information of proteins, plus additional informations like hydrphobicity, x-coordinates, y-coordinates, z-coordinates, surface tension, charge, alpha helix, β helix, turn, van der waals, molecular weight, solubility. Support vector machines (SVMs) were used in this prediction because they are known to be a powerful technique for making binary decisions. Using the model created by SVM from the training data , a graphical user interface was developed that can predict whether or not two given proteins are interacting.

Implementation

The protein-protein interaction database was downloaded from BioGrid database [http://thebiogrid.org/]. The data files downloaded contained uniprot ids for all the proteins. To fetch the respective pdbs, an automated script was created to map uniprot ids with pdb ids and to download the respective pdbs. Once all the pdb files were downloaded, BioPython [http://biopython.org] was used for retrieving the coordinates from pdb file for every residues. The summary of the work flow can be seen in figure 1.

A database was created to add the pdbs and their information. Using a database wrapper the coordinate data along with additional features like hydrophobicity, surface tension, charge, alpha helix, beta strands, turns, van der walls volume, molecular weight and solubility were inserted into the database table.

Inorder to create negative controls in the dataset, the FASTA sequence of every pdb as retrieved using pdb o fasta tool. That equence as shuffled using sequence manipulation tool

[http://imed.med.ucm.es/Tools/SMS/shuffle_prot.html] and adb model is obtained from he shuffled seqence using swiss mode [http://swissmodel.expasy.org/]. Using this methodology, negative control was achieved and added into the table. If there is interaction between pdb's, the interaction column in the database table is set to 1 otherwise 0.

The complete data from database is exported into a comma separated file (.csv) format. The data is divided into training dataset and test dataset. Training dataset is used for training the svm model. Sklearn scikit is used for training the svm on the training [http://scikit-learn.org/stable/]. The dataset is converted into 2 python [http://www.python.org/] multidimensional lists, one list contains the interaction (x) and other multidimensional list (y) contains the coordinates and other features. Initially the linear classifier is run on the training dataset with 4 number of folds. Classifier is used to fit the both x and y lists using fit() function. The object from fit is used to predict the interactions for test dataset.

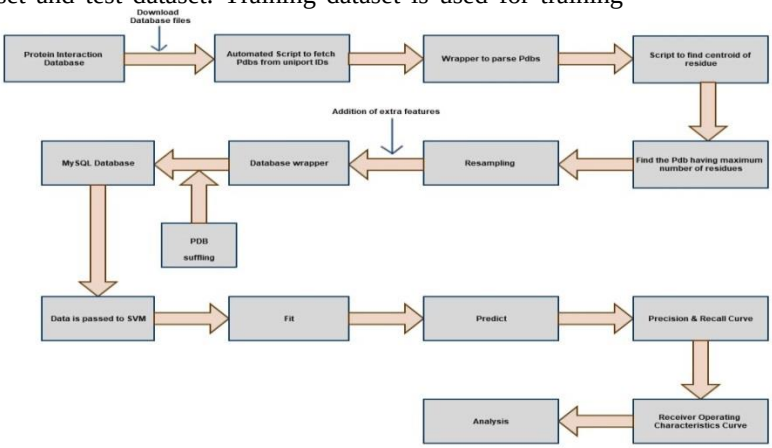

Figure 1 : Over view of the methodology to train SVM and get ROC and PR curve

After the object is created from fit() function, a confusion matrix having true positive (TP), true negative (TN), false positive (FP) and false negative (FN) values is generated. Using the matrix, the Receiver Operating Characteristic curve (ROC) is computed using roc_curve() function. Area under curve is calculated and ROC curve plotted on graph (Figure 1). Similarly, Precision-Recall curve is obtained using precision_recall_curve() function and plotted on the graph (figure 2).

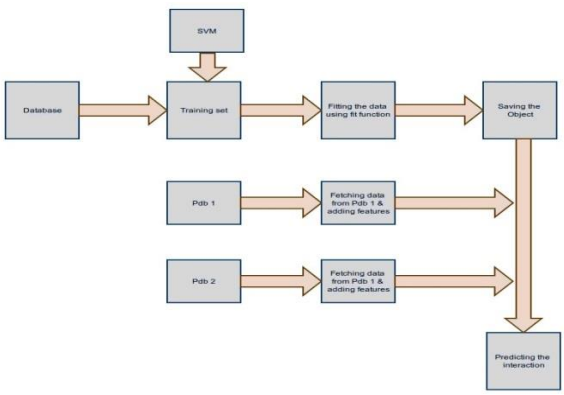

Figure 2 : Over view of the methodology for creating graphical user interface

The object obtained from classifier.fit() is saved using pickled object in python [http://docs.python.org/2/library/pickle.html]. Graphical User Interface is created using PyQt [http://www.riverbankcomputing.com/software/pyqt/intro]. Operations in the GUI is maintained using threads in python so that application will not freeze while performing the operation. Two pdb's are taken as input in the graphical user interface and using the classifier object saved, prediction of interaction among those two pdbs are displayed. The summary of the workflow for creating the graphical user interface can be seen in figure 2.

Results and Discussion

For each statistical background comprising *k*-let orders 1–2, about 88% potential protein interactions are correctly estimated by the system. It bears reiteration here that only 3D structure and features data have been used to train the SVM.

To draw an ROC curve, only the true positive rate (TPR) and false positive rate (FPR) that are calculated using the confusion matrix are needed

TPR = TP/(TP +FN)

FPR = FP/(FP+TN)

In figure 3, the ROC curve shows the area under the curve for two folds with fold 0 area = 0.85, fold 1 area = 0.84, and mean ROC area = 0.84. An area of 1 represents a perfect test; an area of .5 represents a worthless test. Hence the result of ROC is classified as being "good" . In figure 4, the precision-recall curve shows an area under curve of 0.95 .

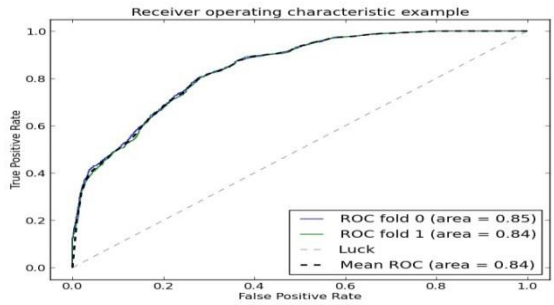

Figure 3 : ROC curve showing the area under the curve for two folds.

Fold 0 area = 0.85 ; Fold 1 area = 0.84 ; Mean ROC area = 0.84

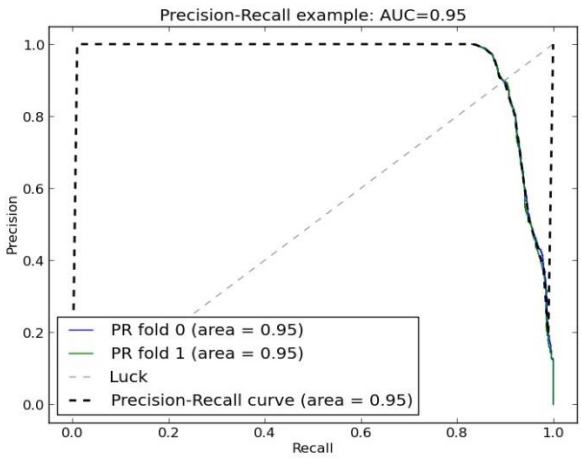

Figure 4: Precision-Recall with AUC being 0.95

In a Receiver Operating Characteristic (ROC) curve the true positive rate (Sensitivity) is plotted in function of the false positive rate (100-Specificity) for different cut-off points. ROC curve = 0.84 for the dataset. The precision-recall (PR) curve = 0.95 for the same set of data. sklearn SVM is used to fit the data , the object from SVM is saved as pickled object in python and the same object is used for predicting the PPIs in the GUI created using PyQt. The prediction accuracy is found to be 88.07% .

Conclusion

There have been methods created to predict the interaction between proteins. Earlier only primary sequence along with several properties were used to predict the interactions. But by using the 3D structural information of proteins along with other properties such as hydrphobicity, x-coordinates, y-coordinates, z-coordinates,surface tension, charge, alpha helix, β helix, turn, van der waals, molecular weight, solubility the percentage of correct prediction is more than in methods that use primary sequence. The GUI serves as a easy way of prediction of interaction. This GUI can be used by anyone and do no require the knowledge of any particular programming language or the use of internet.The prediction can be more accurate if limited family of proteins are used and the prediction also depends on the interaction site ratio. The future scope of work can be that pdbs can be automatically fetched by the application and the application can be made available on web server.

Using this standalone application that is based on SVM model the interactions between two given proteins can be predicted easily without the aid of internet. It also reduces the time and cost in wet lab experiments for protein–protein interactions predictions

Availability and requirements

Project name: Prediction and validation of Protein - Protein interaction using protein 3D structures and physicochemical properties by the aid of support vector machine

Operating system: Windows 8

Programming languages: Python 2.7

Modules : numpy 1.8, scipy-0.13.3, matplotlib 1.3.1, pyLab, pandas, sklearn 0.14, MySQL-python, 1.2.5, mechanize 2.7, BeautifulSoup 4.2, PyInstaller 2.1

Database : MySQL 5.5

GUI Designer : PyQT Designer

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

Veena Rajan has developed all codes, worked on database and created the Graphical User Interface. The DC Mishra has provided guidance and ideas for the entire work, Neeraj Budhlakoti has helped in writing the code, Sundeep Kumar has provided biological insight.

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RESEARCH ARTICLE

Physiological Basis of Crop Response to Climate Change

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Abstract

The elevation of atmospheric CO_2 and temperature is a result of carbon emissions mainly arising from anthropogenic sources. Recently, there has been an increase in the researches carried out worldwide to better help us understand the interactions of climate change with cultivation of rice and how climate change affects the productivity of rice influencing the global food security. The increase in temperatures due to rising atmospheric CO_2 concentrations have a direct effect on the physiology of plant, viz., respiration, photosynthetic carbon uptake, grain filling etc. Response of rice to carbon dioxide is not linear and there are variations among species and cultivars. The potential increase in photosynthetic rates to elevated CO_2 is negated by higher temperature and acclimation or down regulation is observed over time. Rising temperature can increase floral sterility and ultimately reduce yield. Recent projected levels of CO_2 are likely to reduce or alter nutritional quality of crops. This paper illustrates the recent evidences and potential effect of global climate change on the quality and productivity of crops.

Key words: Elevated CO_2, temperature, photosynthesis, spikelet fertility, quality, rice

Introduction

The global atmospheric CO_2 is gradually enhancing due to burning of fossil fuel, rapid advancement in industrialization and deforestation (IPCC, 2001). Atmospheric CO_2 concentrations have increased from 280 ppm to 410 ppm since the Industrial Revolution (Ciais *et al.,* 2013). The global circulation models projected that the concentration of atmospheric CO_2 will increase from the current 410 ppm to 550 and by the end of the century it will be 1000 ppm, which will lead to another 1-3.7^{0}C increase in global mean air temperature (Ciais *et al.,* 2013). One of the important sectors that might be affected by the increasing atmospheric CO_2 and temperature is agricultural crop production system. This can have a negative effect on the world food security through its effect on photosynthetic rates and productivity. Myers *et. al*., 2015 predicted content of essential elements like protein, iron and zinc to decline in grains of C_3 plants and legumes due to increased concentrations of atmospheric CO_2 which will pose a threat to the nutrition of billions of populations in the next 50 years.

Global rice production has been greatly affected due to increased air temperatures resulting from increase in concentration of atmospheric CO_2. Rice is considered as one of the most important sources of nutritional and caloric requirement especially among the low and middle-income Asian countries (Kennedy *et.al*., 2002). Therefore, the concurrent rise in atmospheric CO_2 and temperatures will affect rice growth and quality which will threaten food security of 138 million to 1.4 billion people (Zhu *et. al.*, 2017).In this review, we would highlight some of the key impacts of elevated CO_2 on the physiology of some plant life processes at the same time, trying to understand the overall impact of the changing environmental scenario on plant growth and development.

Growth And Development

Large intra-specific and inter-specific variation on plant growth has been reported by many researchers as a result of elevated CO_2 concentration (Bowes, 1993; Rogers *et al.* 1997). Elevated CO_2 is generally shown to enhance crop growth in C_3 crops like rice. Ainsworth *et al*., (2004) has reported an increase in photosynthetic rate by 30–50% in C_3 plants due to doubling of atmospheric CO_2 concentration (keeping other factor constant). High CO_2 concentrations enhanced photosynthesis by enhancing the availability of CO_2 substrate for Rubisco whereas at the same time suppressing photorespiration (Drake *et al.*, 1997). Increase in the production of biomass due to elevated CO_2 may theoretically enhance yield, considering that environmental factors like drought stress or high temperature stress do not disrupt various critical stages. Ziska *et al.*, (1996) in their glasshouse experiments reported, an increase in biomass of 22 and 70 percent at elevated CO_2 treatment at37/29 ^{0}C and 29/21^{0}C respectively, while 17 contrasting cultivars recorded grain yield of <1% filled spikelets. Ziska and Manalo, (1996) also reported potentially significant increase in root/shoot ratio due to increased allocation of biomass to roots. Ziska and McClung , (2008) from their study on six cultivated and six weedy biotypes of rice (*Oryza sativa* L.) reported greater growth response in terms of both total biomass and leaf area among the wild relative as compared to cultivated rice (*Oryza sativa* L.) to recent increase in CO_2 (300 to 400 ppm) (Fig.1) which suggested that increased fitness relative to the crop

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might have been contributed by rapid evolution of weedy biotypes. The response of climate change on some developmental processes of hot chilli (*Capsicum chinense*Jacq.) has been studied (Das *et al*., 2020). They reported the effect of elevated CO_2 and temperature on some developmental processes *viz.* Phyllochron index and days to anthesis. A higher leaf emission rate and accelerated days to anthesis have been reported at elevated CO_2 and temperature.

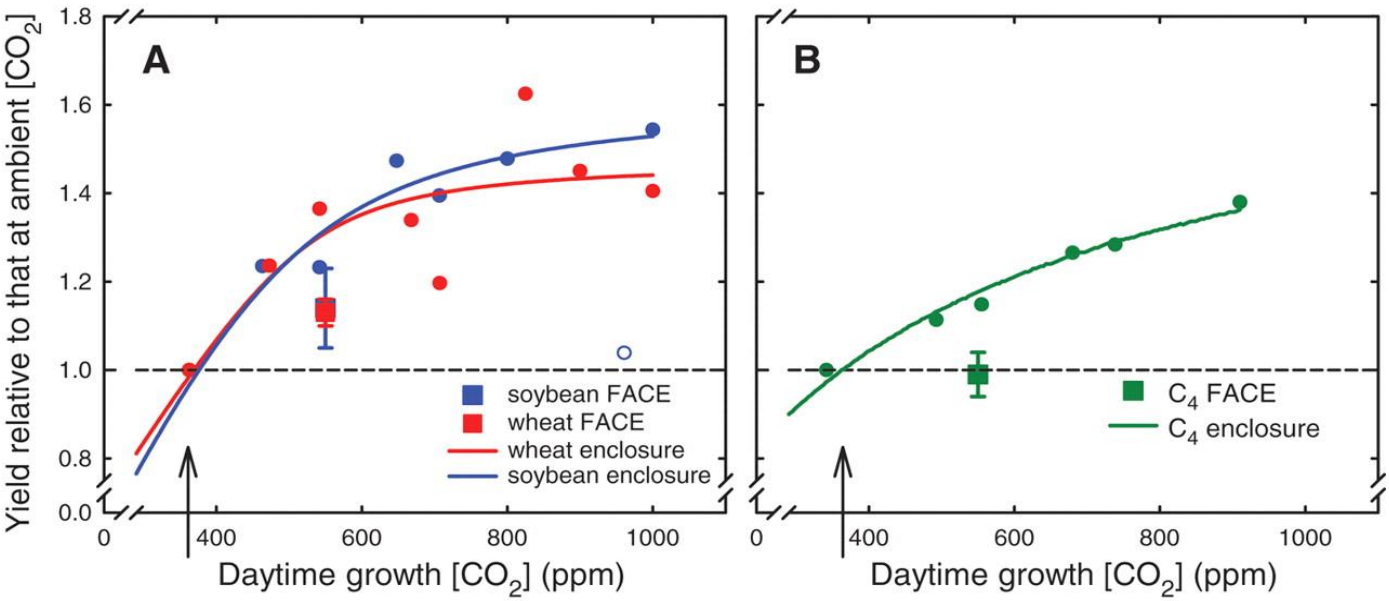

Fig. 1: Effects of elevated [CO_2] on crop yield. Data are yields at elevated [CO_2] relative to those at ambient [CO_2] (arrow) for (A) soybeans in chambers (solid blue circles) and FACE (blue square, hidden behind red square) and wheat in chambers (red circles) and FACE (red square); and (B) C_4 crops (maize and sorghum combined) in chambers (green circles) and FACE studies (green square). (Long *et al*., 2006)

Photosynthesis, Photorespiration And Respiration

According to Goudriaan *et al.*, 1990 and Drake *et al.*, 1997 enhanced level of atmospheric CO_2 concentration increases the photosynthetic rate particularly in C_3 plants since catalyzing enzyme rubisco has single site for both CO_2 and O_2 and under higher CO_2 condition rubisco tend to react more with CO_2. However, in C_4 plants the mechanism of enhancing concentration CO_2 permits CO_2 to contend additional successfully with O_2 for reacting with rubisco (Allen *et al.*, 1994). Thus, as compared to C_3, the photosynthetic apparatus in C_4 plants are more efficient (at temperatures higher than 25 ^{0}C) in the present scenario of CO_2 concentrations and cannot increase their photosynthetic efficiency further as C_3 plants in response to enhanced CO_2 concentration(Ziska and Bunce 1997 ; Ziska, *et. al*., 1999).

The photosynthesis stimulation in plants full-grown at higher CO_2 concentration is adequately defined (Ainsworth and Rogers, 2007). Net photosynthesis is stimulated by higher CO_2 concentrations by increasing availability of CO_2 for reacting with the enzyme Rubisco and concurrently minimizing photorespiration (Drake *et al.*, 1997). Increased leaf photosynthetic rates are therefore expected from elevated CO_2 concentrations, but the extent to which this will actually occur is not clear as photosynthetic enhancement by CO_2 depends on temperature of leaf, moisture and nutrient availability (Zhu *et al.*, 2017). This stimulation is also not regular over time, as it decreases with increase in duration of exposure to enhance CO_2 condition (Wang *et al.*, 2012). Eventually, the carbon assimilation in response to high CO_2 will lead to decreased Rubisco concentrations, resulting in feedback inhibition on photosynthesis (Moore *et al.*, 1992). This down regulation will limit the extent of photosynthetic stimulation in plants full- grown at increased CO_2 concentrations as compared to a short term exposure to elevated CO_2. Kaiser *et al.*, (2017) reported an increase in the relative carbon by 12% at 800 µ bar as compared with CO_2 at 400 µ bar after increasing irradiance, a decreased loss of photosynthetic induction by 14% and an increased photosynthetic dynamics during sine waves by 17% (Fig. 2).

Photorespiration is suppressed by high CO_2 and it is regarded as a wasteful process of Rubisco. However, there are evidences that suggest photorespiration enhance tolerance to stress in plants (Voss *et al.*, 2013). Takahashi & Badger (2011) reported that during drought or high light stress, photorespiration can be beneficial, both of which reduces the capability of Calvin cycle to use the NADPH and ATP produced during electron transfer process of photosynthesis. It is also linked to increased nitrogen uptake particularly nitrate, therefore questioning whether plant nitrogen uptake may be reduced when nitrate is the main source of nitrogen available under elevated CO_2 (Rachmilevitch *et al.*, 2004).

According to Crawford & Glass, (1998),since nitrate is the main source of nitrogen in soil for most crops in cultivated soils, this effect is chiefly relevant for crop yield; thus by limiting assimilation of nitrate, elevatedCO_2 concentrations will jeopardized the nutrient content of food by reducing protein content crop (Carlisle *et al.*, 2012).

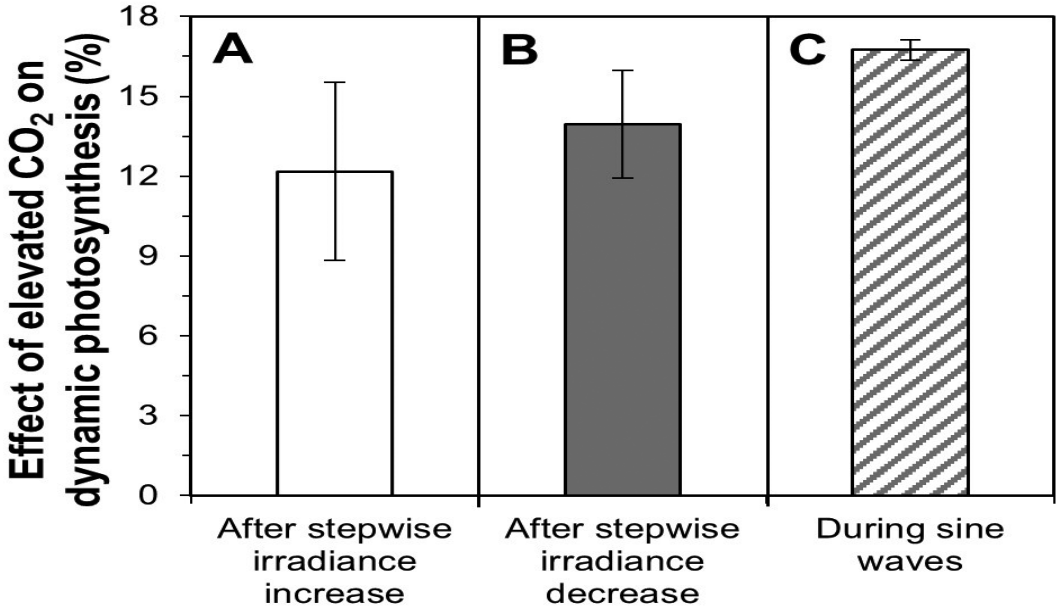

Fig. 2: Effects of elevated CO_2 partial pressure (800 µbar, comparedwith 400 µbar) on the dynamic response of *A* to changes in irradiance.(A) Effects on rate of relative increase in photosynthesis rate until15 min after stepwise increase in irradiance. (B) Effects on loss of photosynthetic induction until 15 min after stepwise decrease to background irradiance. (A) and (B) show averages ±SEM, averaged across background irradiances (*n*=4). (C) Average ±SEM of dynamic responses at1 min and 3 min periods, relative to dynamic response at 5 min period(*n*=2).(Kaiser *et al.,* 2017)

The primary source of energy is plant respiration, which helps in the plant growth and maintenance plants, but it is also a metabolic wastage that restricts availability of carbon for productivity of crop. Increased respiration from global warming is of serious concern as a larger portion of total assimilates can be consumed by respiratory processes (Paembonan *et al.,* 1992). Higher plant respiration rate in response to temperature is associated with greater carbohydrate consumption which suggests a role in the demand for ATP or other respiratory products (Turnbull *et al.,* 2001). Respiration rates were found to be directly comparative to the overall nitrogen concentration of rice plant grown in CO_2 ranging from 160 to 900 ppm (Baker *et al.,* 1992). High temperatures also enhance the dark respiration rates in plant regardless of CO_2 concentration. Furthermore, enhanced temperature decreased the dissolvable of CO_2 relative to O_2 in the cytosol which in turn decreased photosynthetic efficiency, but this effect on photosynthesis is usually more detrimental in higher CO_2 than in current CO_2.

Elevated CO_2 in general affect plants by modifying the biochemistry of Rubisco and stomatal opening. High temperatures affect nearly all the biochemical process in plants, such as ontogenesis, composition and membrane lipid fluidity and cambial activity (Quint *et al.,* 2016). Therefore, whereas the impact of temperature on photosynthesis, respiration and photorespiration can be studied independently, the board range effects of high temperatures on other biological processes are clearly going to down regulate the metabolism of carbon. Furthermore, with increased leaf temperatures, photorespiration rates will increase quicker than photosynthetic rates (Long, 1991). This can be illustrated by the fact that selectivity of Rubisco for CO_2 to O_2 decrease at an increase temperature, allowing oxygenation reaction prone to occur. Moreover, with rise in temperature, the solubility of O_2 also reduces less fast than dissolvable of CO_2 (Ku and Edwards, 1977), as in higher temperature, there is comparatively more O_2 available to react.

Formation Of Reactive Oxygen Species

The interactive effect of moisture stress and elevated CO_2 have been reported in Brassica species using Free Air CO_2 Enrichment (Das and Uprety , 2006). Increased accumulation of H_2O_2 and oxyradicals have been reported under moisture stress treatment. According to them, reduction in active oxyradical (H_2O_2 and MDA) accumulation and increase in activity of antioxidant enzymes under elevated CO_2 which ameliorated the drought-induced oxidative stress effects (Table1). Similar response was obtained in broccoli where lipoxygenase enzyme activity that is responsible for hydro-peroxide (MDA) formation which plays role in inducing membrane deterioration. LOX mediated lipid peroxidation is related to chlorophyll degradation (Zhuang *et al*., 1994). Elevated CO_2 could inhibit the effect of oxy-compounds and lipoxygenase activity thereby delaying leaf senescence. This delayed senescence in *Brassica* leaves due to sustenance of chlorophyll pigments activity for a longer period could be due to the LOX activity and their intermediates depression (Das and Uprety, 2006). Under moisture stress condition, they also reported that elevated CO_2 could bring about a decrease in relative stress injury and an increase in membrane stability index and antioxidant enzyme activity (Superoxide dismutase, Peroxidase, Glutathion reductase, Ascorbate oxidase).

Table 1.Interactive effect of elevated CO_2 and moisture stress on H_2O_2 TBARS content and RSI of leaves

Treatments	H_2O_2 content (µg/g/dry wt.)		Lipid peroxidation (nmol TBARS /g^{-1} dry wt.)		Relative stress injury (RSI)	
	Pusa Gold	RH-30	Pusa Gold	RH-30	Pusa Gold	RH-30
FACE irrigated	2.83	2.33	1.18	0.93	2.78	2.00
FACE moisture stress	3.02	2.53	1.24	0.98	2.92	2.17
Ambient irrigated	3.05	2.58	1.22	0.97	2.91	2.16
Ambient moisture stress	4.00	3.36	1.55	1.15	3.77	2.62
CV.	0.263		0.122		0.238	
CO_2	0.342		0.848		0.113	
CV. x CO_2	0.467		0.104		0.225	
MS	0.346		0.085		0.331	
CV. x MS	0.472		0.101		0.468	
CO_2 x MS	0.626		0.111		0.662	
CV. x CO_2 x MS	NS		17.60		0.936	

Adapted from Das and Uprety 2006

Under moisture stress, the activity of these anti oxidant enzymes increased under both elevated CO_2 and ambient condition, but activity was many folds higher in elevated CO_2 (Table.-2). Role of the entire anti oxidatie enzyme system (including SOD, CAT and AOPD) in scavenging of the Reactive Oxygen Species (ROS) produced during any kind of stress was evident. A significant decrease in the activity of these enzymes was observed due to decrease in cellular ROS production (Pritchard *et al*., 2000). This reduction may reflect in oxidative stress due to CO_2 enrichment. With an increase in CO_2 : O_2 ratio within chloroplast, there may be decrease in electron leakage from PSI to O_2, or might decrease oxygenase activity of Rubisco reducing photorespiration and resultant cellular H_2O_2 production or might be due to stimulation of antioxidants activities under elevated CO_2 in moisture stress condition (Das and Uprety, 2006).

Table 2. Interactive effect of elevated CO_2 and moisture stress on SOD, catalase, AOPD, POD and Gluthione reductaseTBARS content of leaves

Treatments	**SOD activity unit^{-1}mg^{-1}protein**		**Catalase µmol H_2O_2 reduced ^{1}protein min^{-1}mg^{-}**		**Ascobate peroxidadase Activity µmolmin^{-1}g^{-1} FW**		**Gluthione reductase Δ OD min^{-1}mg^{-1} protein**	
	Pusa Gold	RH-30	Pusa Gold	RH-30	Pusa Gold	RH-30	Pusa Gold	RH-30
1 FACE irrigated	0.06	0.06	0.51	0.60	58.20	66.93	0.20	0.03
2 FACE moisture stress	0.32	0.36	0.29	0.36	166.70	247.10	0.16	0.18
	0.20	0.22	1.88	2.15	133.40	157.20	0.05	0.10
	0.24	0.29	2.33	2.85	161.20	196.90	0.12	0.13
3 Ambient irrigated Ambient moisture stress								
CV.	0.020		0.41		23.11		0.018	
CO_2	0.008		0.24		12.56		0.007	
CV. x CO_2	0.009		0.11		9.67		0.022	
MS	0.018		0.32		16.44		0.010	
CV. x MS	0.019		0.17		14.21		0.044	
CO_2 x MS	0.026		0.28		17.33		0.051	
CV. x CO_2 x MS	0.35		0.39		24.44		0.062	

Adapted from Das and Uprety 2006

Temperature And Floral Sterility

It is predicted that increased CO_2 environments might improve yield of rice in the future. However, a concomitant temperature rise during the flowering and grain filling stages offsets the positive effect of increased CO_2 on the partitioning of assimilate and sink strength in rice (Chaturvedi *et al.*, 2017). According to Yoshida *et al.*,(1981), the reproductive stage is more sensitive to heat than the vegetative stage in rice. Similarly, Satake and Yoshida, 1978; Nakagawa *et al.*, 2002 reported anthesis / flowering followed by micro gametogenesis (Fig.3) to be most sensitive processes during the reproductive stage to higher temperature. Viable pollen development, pollen shedding, pollen tube growth, and fertilization are the important processes occurring during this phase. Kazuhiro *et al.*, (2019) and reported that the elevated CO_2 may affect many traits like pollination, anther dehiscence and length which is associated to heat related sterility and at times aggravate floral unfruitful by reducing the pollen grain deposition. High temperature induced spikelet sterility becomes very severe near 40^0C and resulted in the complete loss of crop production (Yoshida and Parao, 1976). Spikelet at anthesis exposed to temperature more than 35^0C for about 5days during flowering period resulted in sterile spikelet and no seed set (Yoshida *et al.*, 1981). Similarly, a 34% decline in spikelet fertility was observed at high temperatures of 35^0C during microsporogenesis. Heat stress, even for a short period of time, can cause decrease in floral bud and flower abortion during reproduction whereas a great disparity in sensitivity within and between plant species and variety exist (Sato *et al.*, 2006). Bhattacharya, 1970, reported premature grains resulting from high temperature at ripening mainly due to the inability of spikelet to serve as a sink since the opening of the spikelet was closed. Moreover, higher day and night temperatures during panicle development of rice resulted in spikelet sterility which highly correlated with the number of engorged pollen grains per anther (Gunawardena and Fukai, 2005).

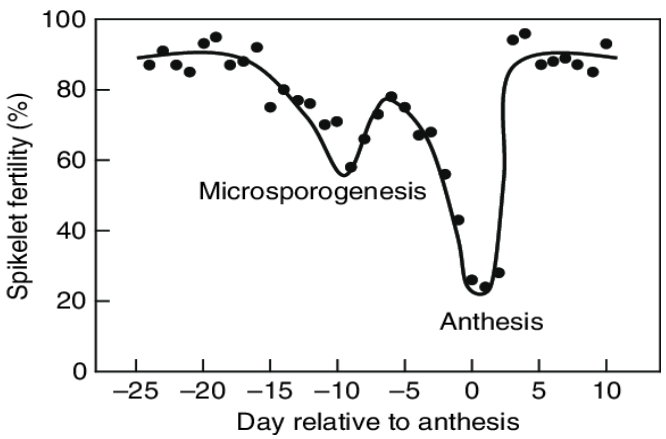

Fig. 3. Spikelet fertility of BKN6624–46–2 exposed to high temperature of 35^0C during different panicle development stages for 5 days (Yoshida *et al.*, 1981)

Yield And Quality

Increase in seed yields in general is nonlinear in respect to elevated CO_2 and the increment is not same as the enhance in net photosynthesis (Reddy and Hodges, 2000).Temperatures above the thresholds limit reduces crop growth period of rice, increases spikelet sterility (Jagadish *et al.*, 2008), reduces period of grain forming (Kim *et al.*, 2011) and enhances respiration rate (Mohammed and Tarpley, 2009) resulting in reduced yield and quality of rice grain (Fitzgerald and Resurreccion, 2009). Furthermore, a 10% reduction in rice yield for each 1^0C rise in minimum temperature above 32^0C was reported by Pathak *et al.*, (2003). The decline in grain weight in high temperature might be due to the high energy consumption to meet the seeds respiratory required (Tanaka *et al.*, 1995).Quality in rice is primarily reflected in the appearance, nutritional and edible qualities that determines the price of rice in all markets. Appearance includesrate of head rice and chalky rice while nutritional quality includes amylase content, protein content and essential elements; and edible quality includes gel consistency. Zhao and Fitzgerald (2013) reported that increase of 1% in head rice yield for every1% decrease in chalkiness, showing the dual impact of chalkiness on amount of marketable rice and its value. Zhu (2017) observed a decline in protein, zinc, iron and vitamins B1, B2, B5, and B9 but on the contrary, found an increase in vitamin E in rice (Fig 4). They reported a clear correlation between the effectsof increased CO_2 on content of vitamins based of the molecular nitrogen fraction in the vitamin. The impact of elevated CO_2 and temperature on quality parameters of hot chilli (*Capsicum chinense* Jacq.) have been reported (Das *et al.*, 2016). Higher fruit diameter and capsaicin content of hot chilli cultivar (cv Manipur) has been recorded under elevated CO_2 and temperature (550 ppm CO_2.+ 2^oC greater than ambient condition).

From quality point of view, alteration in fatty acid composition might be one of the important consequences in changed climate due to the rise in atmospheric CO_2 in near future. A significant alteration in fatty acid composition has been reported under elevated CO_2 (Das , 2003).

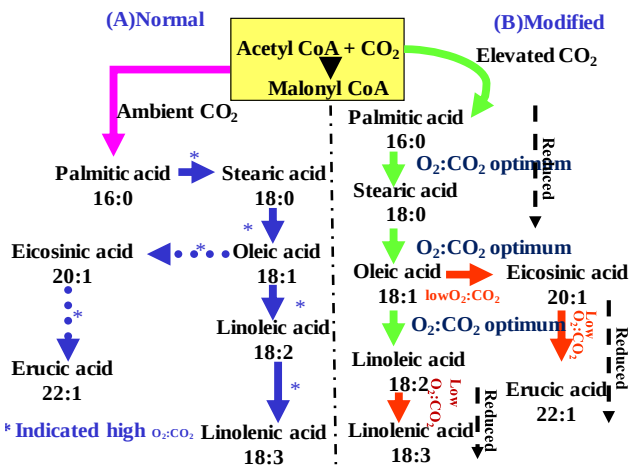

Fig 5. A= Normal pathway of fatty acid synthesis in *Brassica* seeds (Stumpf , 1983); indicated that the O_2: CO_2 ratio was high during biosynthesis of fatty acids. B = CO_2 induced modification: Optimum O_2:CO_2 ratio up to the biosynthesis of i. linoleic acid. ii. reduction in O_2:CO_2 during biosynthesis of (a) linoleic acid to linolenic acid (b) oleic acid to erucic acid modified by (Das *et al.*, 2007)

Reduction of saturated fatty acid pool and some unsaturated fatty acid like linolenic and erucic acid are important. CO_2

enrichment significantly increased the oil content in the seeds of Brassica spp. which can be attributed to additional CO_2 in stimulation of the activity of acetyl CoA enzyme (Uprety et al., 1997). They reported that under elevated CO_2, the increased activity of Acetyl CoA leading to formation of abundant malonyl CoA plays a positive role in regulating the fatty acid biosynthesis. Brassica spp. grown under elevated CO_2 brought about a reduction in the saturated fatty acids (palimitic, stearic acid) and mono-unsaturated fatty acids (oleic acid) content which indicates that most of the fatty acid undergo desaturation and produced unsaturated fatty acid due to higher CO2 effect (Fig. 5). The linolenic acid content in Brassica seeds were found to be significantly higher in seeds of CO_2 enriched plant.

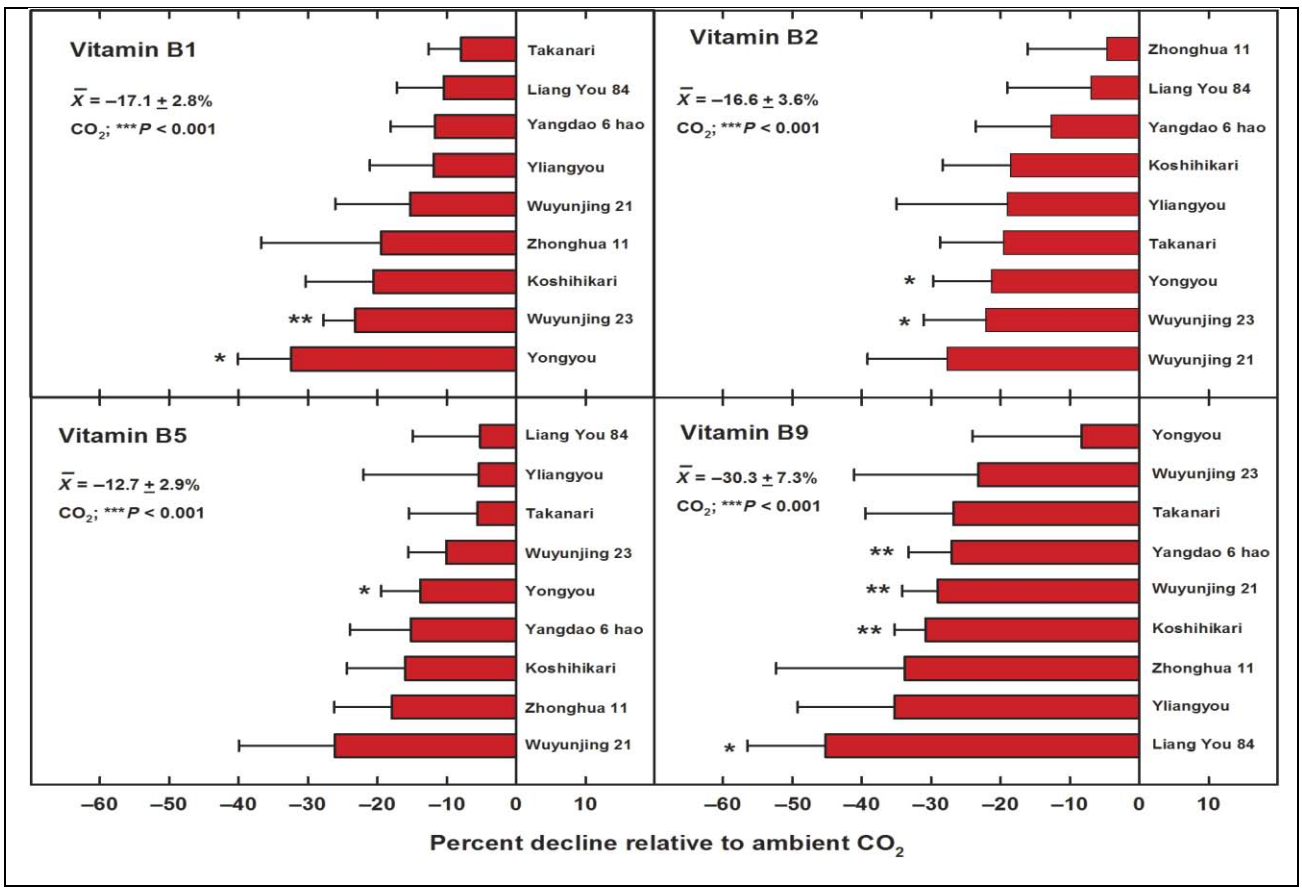

Fig. 4.CO_2induced reductions in vitamins B_1 (thiamine), B_2 (riboflavin), B_5 (pantothenic acid) and B_9 (folate) by cultivar (Zhu, 2017).

Conclusions

Innovative approaches like OTC Open-Top Chambers and FACE - Free Air CO_2 Enrichment experiments had been designed to ascertain how high CO_2 in a more natural environment affects the vegetation. Such investigations are necessary as our plant varieties are selected for the present scenario CO_2 and their reaction to the twofold CO_2 concentration are necessary to be identified for developing a plant type for future changes in CO_2 concentration. Photosynthesis and carbon assimilation increases in elevated CO_2 but their beneficial effect is negated by increasing temperature which varies among species and cultivars and growth stages. It is however important to note that assimilation does not necessarily accounts for biomass gain as carbon can be lost as volatile organic carbon and exudates in the soil. Wild lines of rice are shown to be more responsive to elevated CO_2 and temperature as compared to cultivated rice. Therefore, the genetic diversity in weedy germplasms needs to be explored more to utilize them for further crop improvement in the future. Grain yield increase under optimal temperature at elevated CO_2, however, beyond optimum temperature, particularly during the reproductive stage, the yield gets reduced significantly which is attributed to sterile spikelet, pollen abortion and poor seed set. This results in grains which are chaffy and chalky thereby reducing the overall quality of the grains. Furthermore, increased atmospheric CO_2 concentrations are expected to reduce the essential elements content in rice grains, like protein, iron and zinc which consequently will put billions of people's nutrition at risk in the future. Therefore, CO_2 and temperature interaction at vegetative as well as flowering and fruiting stages needs to be further studied to optimize the elevation of CO_2 for yield enhancement.

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RESEARCH ARTICLE

Exploring Biofuel Potential of Dominant Microalgae of North-East Region of India

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Abstract

Present communication deals with the comparative study of evaluation for potential of biofuel in the form of lipids of three strains of dominant algal genera *viz. Microchaete* Thuret ex Bornet *et* Flahault, S*pirogyra* Link and *Trentepohlia* Martius grow dominantly in North-Eastern Region of India. The biomass of these three strains *viz. Microchaete* sp., S*pirogyra* sp. and *Trentepohlia* sp. were collected from different natural habitats of Kailashahar and adjoining area of Unakoti district of Tripura, India. The biomass was evaluated for lipid profiling for using them as feedstock for biofuel production in future. On the basis of comparative study on the synthesis of lipid by all the three selected strains revealed that maximum lipid (443mg/g) was observed in *Trentepohlia* sp. and minimum lipid (236mg/g) was observed in *Spirogyra* sp.Results revealed that *Trentepohlia* sp. can synthesise 152.75% more than that of *Microchaete* sp. and 187.71% more than that of *Spirogyra* sp.From the present study, it has been concluded that *Trentepohlia* sp. can synthesis high amount of algal lipids in comparison to other naturally occurring dominant algal genera and it can be a source of algal feedstock for the production of biofuel.

Keywords: Algae, biofuel, lipid, North-East, Hotspot

INTRODUCTION

The need of energy is increasing continuously because of rapid industrialization and population explosion. The basic sources of energy are fossil fuel (petroleum, coal, natural gas), hydro, nuclear and bioenergy / Biofuel. Bioenergy is one of the substitutes of fossil fuels and most important components to mitigate greenhouse gas emissions. Bioenergy are produced from living organism including algae. Algae (both macro and microalgae) are photosynthetic autotrophs and considered as one of the substitute source of energy for biofuel production (Alam *et.al.,* 2015). Algae have chlorophyll as their primary photosynthetic pigment and lack a sterile covering of cells around their reproductive structures (Lee, 2008). Algae are diverse group of photosynthetic organisms and their thallus ranging from unicellular to multicellular forms.

They grow in wide range of habitats including North eastern region of India. North eastern region of India is considered as one of the mega hotspot of the world and kwon for its diversity richness of fauna and flora including algae. Microalgae are capable of producing liquid fuels. The high lipid content, high growth rate and ability to rapidly improve strains and produce co-products, without competing for arable land make algae an exciting addition to the sustainable fuel (Hannon *et.al.*,2010). Phototrophic microalgae require light, carbon dioxide, water, and inorganic salts and an optimum temperature between 15° and 30°C (~60-80°F) for the growth.The oil producing efficiency of algae is much higher than that of conventional and oil seed crops such as corn and soybean (Li *et.al.*, 2008). The growth medium contributes the inorganic elements that help in make up of the algal cells, such as nitrogen, phosphorus, iron, and sometimes silicon (Grobbelaar, 2004). But, starvation of key nutrients, such as nitrogen or silicon, could lead to the production of more algal lipids and concept of utilizing the lipid stores as a source of energy. However, such observation gained serious attention during the oil embargo of the early 1970s. Acetyl-CoA carboxylase (ACCase) act as a key enzyme which catalyzes the first step in the biosynthesis of fatty acids used for TAG (Tri-acyl Glycerol), was found increased under the nutrient stress conditions (Roessler, 1988). Green microalgae contain about 20%-70% lipid and exhibit extraordinary potential for cultivation of as energy crops (Xu *et.al.,* 2006; de Vries *et.al.*, 2010).

The present work is focussed on comparative study of the synthesis of algal lipid from naturally growing three strains belonging to three algal genera including one prokaryotic alga, *viz. Microchaete* sp. (Microchaetaceae, Stigonematales, Cyanophyceae) of Cyanophyta and two eukaryotic algae *viz. Spirogyra* sp. (Zygnemataceae, Zygnematales, Zygnematophyceae) and *Trentepohlia* sp. (Trentepohliaceae, Trentepohliales, Ulvophyceae) of Chlorophyta. The *Microchaete* is a filamentous, uniseriate and heteropolar blue green microalga with false branching (K.). *Spirogyra* Link is commonly known as water silk. The thallus of the *Spirogyra* is filamentous, unbranched with spiral chloroplast and numerous pyrenoids. *Spirogyra* frequently grows on aquatic habitats including flowing water, permanent ponds and temporary pools all over the globe (McCourt *et.al.*, 1986). *Spirogyra* is an important primary producer in aquatic ecosystem (Hoshaw, 1968). *Trentepohlia* Martius is a common sub-aerial green alga and usually grow on tree bark, leaves, rock and artificial substrata including drain and building walls (Printz, 1939; Chapman, 1984; Zhu *et.al.*, 2017).

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Materials And Methods

Sample Collection sites

Fresh algal biomass of clearly visible unialgal growth samples were collected from three different of three localities of Kailashahar and adjoining area of Unakoti district Tripura, Tripura, India. Collection area is situated in Latitude N 24° 18' 52.8534 and Longitude E 91° 59' 48.7387. Details of collection sites are mentioned in the Map (fig.1).

Algal habitats

Algal samples were collected from three different habitats (aquatic habitats: 02 and sub-aerial habitat: 01) from different places of Kailashahar and adjoining area of Unakoti district of Tripura. All the samples were collected in polybags from water bodies and walls and identified at the Department of Botany, Ramakrishna Mahavidyalaya, Kailashahar, Unakoti, Tripura (fig.1).

Algal strains	Habitats	Collection sites
Microchaete sp.	Aquatic	Pond- stagnant water, Kailashahar, Tripura
Spirogyra sp.	Aquatic	Monu river- Kailashahar, Tripura
Trentepohlia sp.	Subaerial	Walls of cemented drain, Kailashahar, Tripura

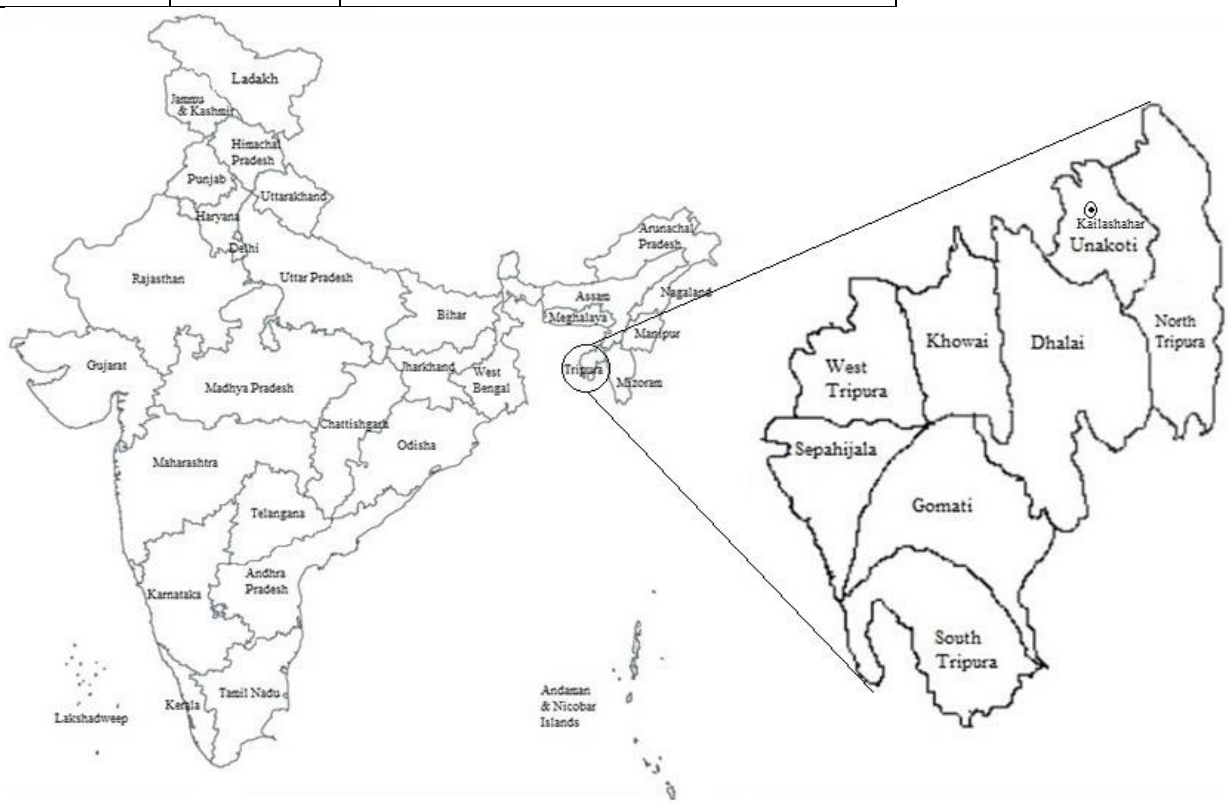


Figure 1. Location map of Kailashahar, Unakoti (Tripura)

Morphological observation

Morphological observations of *Microchaete* sp., *Spirogyra* sp., and *Trentepohlia* sp. were recorded with the help of microscope (Olympus, CH20i microscope) and digital camera (Magnus, Magcam DC 10).

Identification of Algal Strains

Collected Algal samples were identified upto the genus level with the help of morphological observations, available literatures and monographs (Printz, 1939; Randhawa, 1959; De Wildeman, 1891; Rindi, *et.al.*, 2005; Desikachary, 1959; Komárek, 2013). All the identified algal strains were confirmed by the Professor G.L. Tiwari, University of Allahabad, Allahabad (Prayagraj), U.P., India as *Microchaete* sp., *Spirogyra* sp., and *Trentepohlia* sp.

Lipid Extraction

Lipid content of the algal strains was determined by the method described by Bligh and Dyer (Bligh and Dyer, 1959). Briefly, 1g of fresh algal biomass was successively extracted with 7.5mL of chloroform, methanol (1:2) mixture, 2mL of chloroform, and 2mL of water each for 10 min in a pre-weighed vial (Borosil). The solid particulates of algae were removed by filtration through Whatman No. 1 filter paper. The filtrate was centrifuged at 1000 rpm for 5 min to obtain a biphasic system of water and organic solvents. The water phase was discarded and the organic phase was recovered for

the estimation of oil content. The oil content was calculated gravimetrically by using the formula.

Weight of lipids (gm)	=	(Weight of glass vial + algal lipid) - Weight of glass vial
Total lipids (gm)	=	Weight of lipids × Total volume of chloroform layer (ml) / Volume of Chloroform layer evaporated (ml)
% Total Lipids	=	Total lipids (gm)/ Weight of sample (gm) × 100

Experimental design

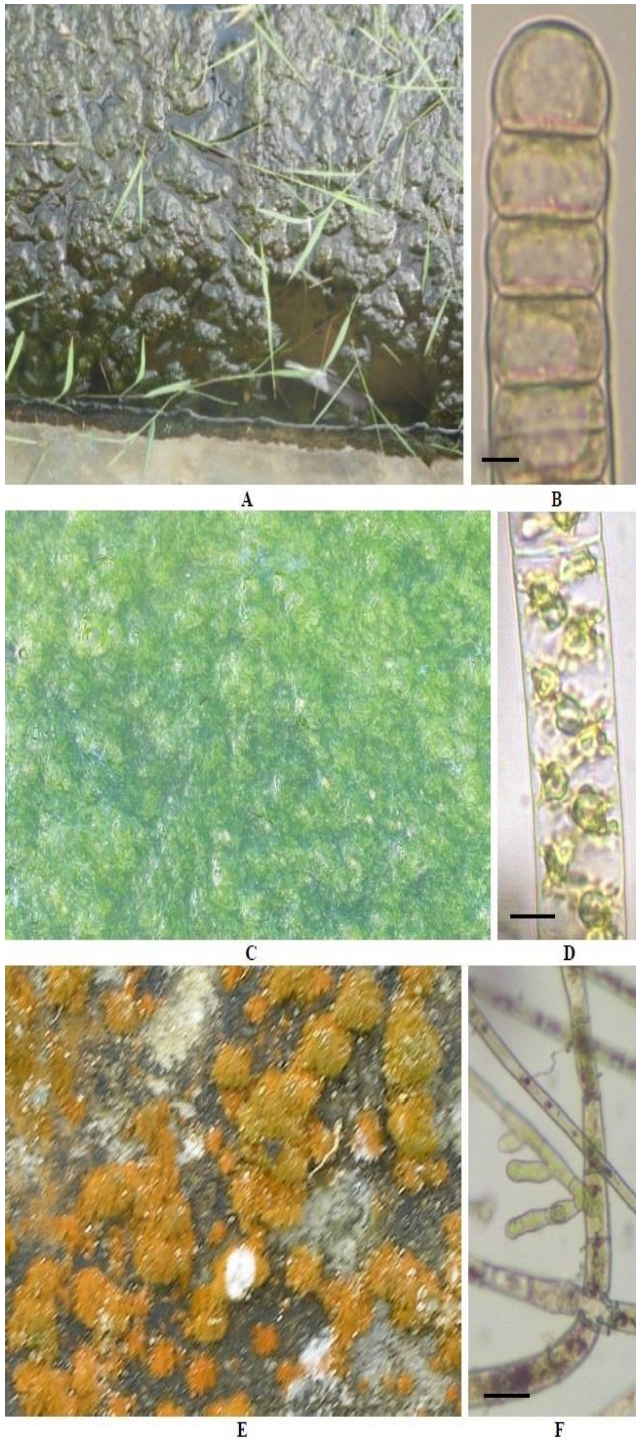
All the experiments were conducted in triplicates.

Statistical analysis

The data were subjected to analysis of variance (ANOVA) by using a completely randomized design (Steel *et.al.*, 1966). The statistical analysis was carried out in MS Office excel 2007. Each mean value was calculated from the triplicate. Standard deviation and standard error was calculated against the obtained final values.

Results And Discussion

The experimental organisms used in the present study belong to two algal groups, prokaryotic and eukaryotic algae. They belong to three algal genera *viz*. *Microchaete* Thuret ex Bornet *et*Flahault, S*pirogyra* Link and *Trentepohlia* Martius. Morphological and Taxonomical details are given in photoplate (fig.2.). These three strains of algae grow dominantly in the North Eastern states of India including Tripura. Collected fresh algal biomass was analysed for Lipid productivity and total lipid is considered as the key factor. Lipid content was extracted from all the three algal strains *Microchaete* sp., *Spirogyra* sp. and *Trentepohlia* sp. It has been found that highly bioactive lipid content found in all the algal strains. Comparative study on the productivity of algal lipid by *Microchaete* sp., *Spirogyra* sp. and *Trentepohlia* sp. collected from different places was determined by the interaction of algal species and nutritional content of that particular place, and their effects on oil content are shown in Figure 1. From the present study it has been found that *Trentepohlia* sp. grown under normal environmental condition can yield 443mg/g lipid where *Microchaete* sp. and *Spirogyra* sp. can yield 290mg/g and 236mg/g of lipid respectively. It has been found that *Trentepohlia* sp. grown under natural condition extract about 152.75% more lipid than that *Microchaete* sp. and 187.71% more lipid than that of *Spirogyra* sp. grown under natural condition. Present study has also revealed that naturally growing *Microchaete* sp. can synthesise a maximum of 46.43% of algal lipid where as *Spirogyra* sp. can synthesise a maximum of 34.47% and *Trentepohlia* sp. can synthesise a maximum amount of 70.41% of algal lipid. On comparison of results of extracted lipids from the samples revealed that maximum lipid content (443mg/g) was produced by the *Trentepohlia* sp. and minimum lipid content (236mg/g) was observed in *Spirogyra* sp. Results in details are presented by Fig. 3.



Explanation of Figure-2. Details of Algal strains for used for the lipid profiling: **A.** Growth of *Microchaete* sp.in nature in cemented tank.**B.** morphological details of *Microchaete* sp. **C.** Growth of *Spirogyra* sp. in nature in stagnant water of Manu River; **D**. morphological details of *Spirogyra* sp. **E.** Growth of *Trentepohlia* in nature on wall of cemented drain. **F.** morphological details of *Trentepohlia* sp.

(Scale bar=**B**-3 μm, **D**&**F**-10μm)

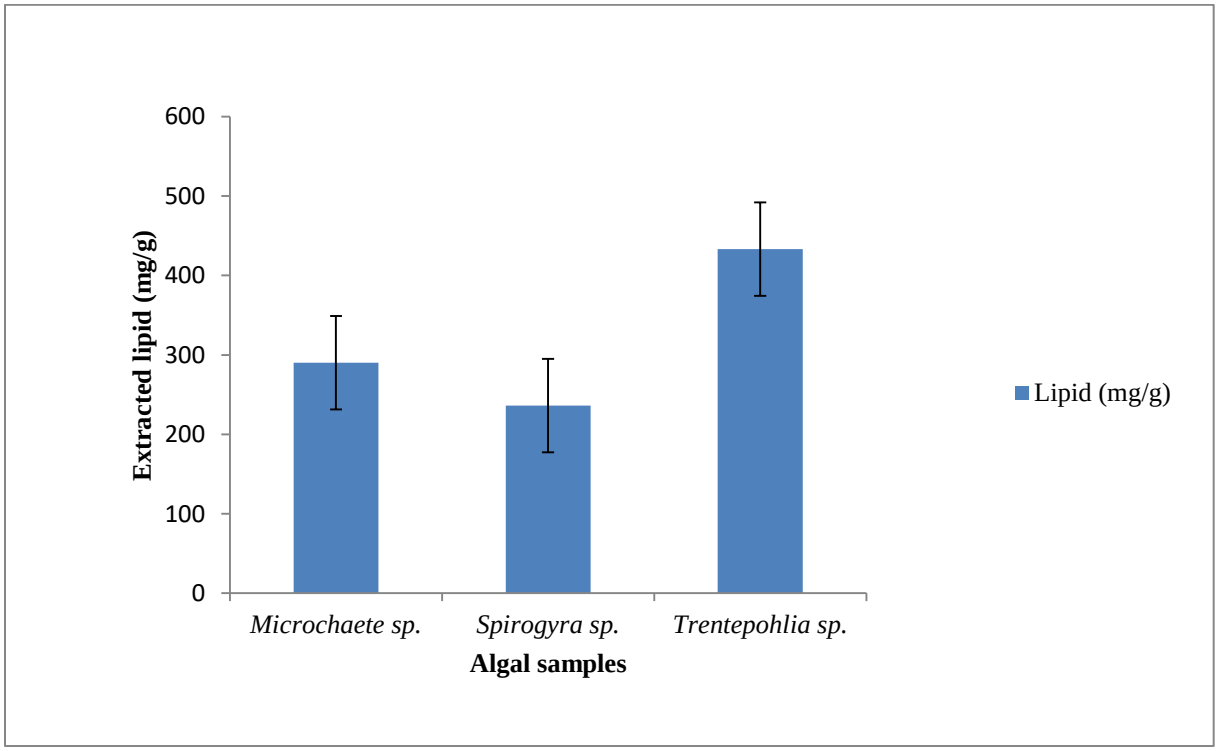


Figure 3: Showingcomparativeextracted lipid from three different algal strains

Microalgae are excellent alternative source of biofuel. Algae have higher rates of biomass and oil production than conventional crops because of their simple thallus structure (Becker, 1994). Biodiesel production from algae is technically feasible but it has not yet become economically viable (Chisti, 2008). Borowitzka, (1992) worked out cost analysis of biofuel production and he revealed that the major economic obstacles are algal productivity, followed by labour and harvesting costs. Laboratory yields are reportedly rarely reached in large-scale production of biomass and open ponds cultivations face a lot of issues including contamination, evaporation, flooding and lack of control over temperature, availability of light and many other difficulties.Due to fast growth rate and high photosynthesis efficiency, microalgae are considered as promising biofuel source for biodiesel production and these can also be produced at commercial level (Miao and Wu, 2006). Although microalgae can be cultivated industrially but, biodiesel production from microalgae is not economically feasible because fast-growing cells contain less oil and cells accumulating high oil content show slow growth potential.

Although microalgae are promising alternative source of lipid for biodiesel production but it is mainly based on one most important decision that is the selection of algal species to use for production of desirable amount of algal biomass with high lipid productivity. High lipid productivity is a key desirable characteristic of an algal species for biodiesel production. Griffiths and Harrison (2009) reviewed literature on microalgal growth rates, lipid content and lipid productivities for 55 species of microalgae, including 17 Chlorophyta, 11 Bacillariophyta and five cyanophyta as well as many other taxa.Microalgae can produce a wide variety of nutrients and secondary metabolites which are beneficial for human beings or animals. To make algae-based fuels commercially feasible with petroleum, various strategies have been discussed (Hannon *et.al.*,2010).Bioprospecting to identify algal species that have desired traits including high lipid content, growth rates, growth densities and/or the presence of valuable co-products. Microalgae can also produce a wide variety of useful valuable potential products including carotenoids such as lutein, zeaxanthin, lycopene, bixin, β-carotene and astaxanthin and polyunsaturated fatty acids (PUFAs). However, commercial production of carotenoids is mainly restricted to β-carotene and astaxanthin (Vilchez *et.al.*, 1997; Del Campo *et.al.*, 2000; Jin *et.al.*, 2003).

Conclusion

From the present study, it is concluded that there are numerous algal strains growing luxuriantly in large amount in natural condition in North Eastern Region states of India, which could be helpful to overcome from bottleneck of availability of suitable algal strains with high lipid content and high biomass production rate. In present study, all the three different algal strains grow under natural environmental conditions can produce a good quantity of lipid. But from this comparative study it has been found that *Trentepohlia* sp. is capable of producing more lipids in natural environmental condition than *Microchaete* sp. and *Spirogyra* sp.

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RESEARCH ARTICLE

Drought Tolerant of Rice (*Oryza sativa* L) Implication Through Antioxidant Defense Mechanism

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Abstract

An experiment was conducted at department of Crop Physiology, Assam Agricultural University, Jorhat under rain out shelter to assess the responses of selected *upland* rice varieties, ARC-10372, Lachit, Bandana, Maibee and Kopilee to moisture stress. Moisture stress was imposed by withholding water. Water deficit resulted in a significant increase in H_2O_2 content thereby increased lipid peroxidation in terms of malondialdehyde (MDA) accompanied by reducing the membrane stability index and consequently reduced the relative water content (RWC) compared to control. Moisture stress significantly increases the activities of antioxidative enzymes namely superoxide dismutase, catalase, ascorbate peroxidase, peroxidase, glutathione reductase in all the varieties. Antioxidatant enzymes activities were significantly increased in ARC-10372 and Maibee thus restoring the membrane integrity and enhancing the defence system and it may be suggested that the enhancement of antioxidants in the both of rice varieties of drought tolerance under stress condition.

Key words: Antioxidative enzyme activities, Chlorophyll stability index, moisture stress, Lipid peroxidation, Relative water content, Relative stress injury, Upland rice

Introduction

Upland rice is one of the important crops of Assam, the central state of North- East India. It is used as buffer stock for food grain and fodder during the lean period (during flood/after flood). Periodic drought is a problem in some pockets of upland rice growing areas, may be due to variation in onset, duration, distribution and intensity of monsoon, which greatly influence the crop growth and yield. Production of reactive oxygen species (ROS), including superoxide (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl radical (OH^-), is an inevitable consequence of life in an oxygen rich environment under stress condition (Polle *et al*., 1990). Plants have their own systems for scavenging superoxide radicals. Superxide dismutase (SOD) catalyses the disproportionate of O_2 to H_2O_2 and O_2^-. Hydrogen peroxide is decomposed by catalase or ascorbate peroxidase (APOD), the latter enzyme oxidizing ascorbate monodehydroascorbate (MDA), which is then re-reduced by NAD(P)H in a reaction catalysed by monodehydroascorbate reductase (MAR).

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MDA also undergoes spontaneous dismutation to ascorbate and dehydroascorbate (DA), which is re-reduced to ascorbate by the reduced form of glutathione (GSH) in a reaction catalysed by dehydroascorbate reductase (DAR), with resultant production of the oxidized form of glutathione (GSSG). Finally, NADPH reduces GSSG again in a reaction catalysed by glutathione reductase (GR). Thus, six antioxidative enzymes (SOD, catalase, APOD, MAR, DAR and GR) and two antioxidant substrates (ascorbate and glutathione) are involved in O_2^- detoxification in plants (Smirnoff, 1993).

Many investigators reported that the generation of reactive oxygen species under abiotic stress is accompanied by an increase in the activities of some antioxidant enzymes (Sairam *et al.*, 2005; Deneto *et al*., 2006; Noreen *et al*., 2010). Superoxide dismutase (SOD), Catalase (CAT), Ascorbate Peroxidise (APX), Phenol Peroxidase (POX) and Glutathione Reductase (GR) are the antioxidant defense enzyme system designed to scavenge superoxide and hydrogen peroxide (Peltzer *et al.*, 2002). Drought stress stimulated some antioxidant enzymes (POX, GR) and reduced CAT in leaves of bean seedlings (D'souza and Devaraj, 2011). The non-enzymatic antioxidants such as Ascorbic Acid (ASA) and Reduced Glutathione (GSH) are responsible for scavenging of H_2O_2 by the Hallwell-Asada pathway. Under drought conditions, the production of ROS exceeds the capacity of the antioxidant defense system, causing oxidative stress (Shehab *et al.*, 2010). Therefore, in the present study, relative significance of antioxidant enzyme activities and lipid peroxidation have been examined in seedlings of drought-tolerant and drought sensitive rice varieties.

Materials and methods

Seed collection

The present investigation was carried out in the Department of Crop Physiology, Assam Agricultural University, Jorhat in summer season. The experiment was conducted with five rice varieties viz. ARC-10372, Lachit, Bandana, Maibee and Kopilee, collected from the Regional Agricultural Research Station, Titabar and Diphu, Assam India.

Experimentation

The experimental site is situated at 26°47′ N latitude; 94°12′ E longitude having an elevation of 86.6 m above mean sea level. The experiment was conducted under UV sterilized polythene roof rainout shelter. Fine and sterilized field soil were used to germinate the rice seeds of different lines in the earthen pots

(28 cm height and 30 cm in diameter) filled with a mixture of soil and organic matter (50:50) The pots were fertilized with 15:15:15, N: P: K fertilizers (Pieters and Souki, 2005).

Water deficit was imposed by suspension of irrigation, in half of the plants of each variety and other half were kept irrigated throughout the crop season. In the first round of stress development, water was withheld between 30 and 45 days after sowing for 15 days as described by Kalita (1996) and then the stressed plants were re-supplied water for 2 days.

In the second rounds, the treatments of 15 days stress followed by 2 days watering were imposed on plants throughout the growing period. Soil moisture content was recorded following gravimetric method (Dastane, 1972). The average moisture content of soil ranging from 17-18 per cent under irrigated condition and between 7-8 per cent under moisture stress condition during crop growth period were maintained.

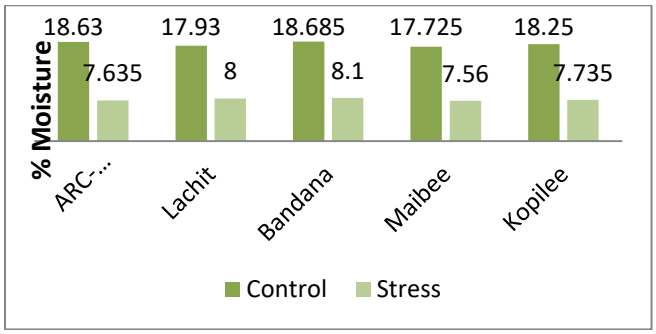

Fig. 1: Average soil moisture content during experimentation

***Assay of* antioxidative enzymes activities**

The antioxidative enzymes activities were measured in two fully expanded uppermost leaves of remaining intact plants in the pots at maximum tillering stage using various methods.

***Superoxide dismutase** (SOD; EC 1.15.1.1)*

For the superoxide dismutase (SOD; EC 1.15.1.1) leaf sample weighing 500 mg was homogenized in ice cold 50 mM phosphate buffer (pH 7.0) containing 0.5 mM EDTA in a pre chilled pestle and mortar. The homogenate was centrifuged at 4° C for 30 minutes at 30,000 g. The supernatant was transformed to another tube and used as enzyme extract. The SOD activity was determined spectrophotometrically by measuring the inhibition of blue diformazone formation in the presence of riboflavin/nitro blue tetrazolium (NBT) and light (Beauchamp and Fridovich, 1971).

***Catalase** (CAT; EC 1.11.1.6)*

Fresh leaf sample of 500 mg was macerated in 0.1 M potassium phosphate buffer (pH 7.5). The homogenate was centrifuged at 12,000 g for 20 minutes at 0° C. The supernatant was collected and used as enzyme extract. Catalase activity in leaves was measured following the method of Teranishi *et al*., (1974) with minor modification. Reaction mixture without enzyme developed maximum colour with titanium reagent. Catalase activity based on colour intensity was measured spectrophotometrically at 415 nm. The enzyme activity was assayed by estimating the residual H_2O_2 in the reaction mixture using H_2O_2 standard.

***Ascorbate Peroxidase** (APOD; EC 1.11.1.11)*

Leaf sample weighing 500 mg was homogenized in ice-cold 50 mM phosphate buffer (pH 7.0) containing 0.5 mM EDTA in a pre-chilled pestle and mortar. The homogenate was centrifuged at 30,000 g in 4° C for 30 minutes. The supernatant was transferred to another tube and used as enzyme extract. Ascorbate Peroxidase activity was measured by a modified spectophotometric procedure based on the rate of decrease in absorption of ascorbate at 290 nm during ascorbate oxidation (Dalton *et al*., 1986).

***Peroxidase** (POD; EC 1.11.1.7)*

For Peroxidase (POD; EC 1.11.1.7), fresh leaf sample of 1g was homogenized in 10ml of ice-cooled 0.1 M phosphate buffer (pH 6.0). The homogenate was centrifuged at 16,000g at 4^0C for 20 minutes. The supernatant was used as enzyme extract. The Peroxidase activity was measured spectrophotometrically based on the changes in absorption at 430 nm (Thimmaiah, 1999).

***Glutathione** (GR; EC 1.6.4.2)*

Glutathione (GR; EC 1.6.4.2)reductase activity was measured by a spectrophotometric procedure based on the rate of increase in absorbance at 412 nm in presence of leaf sample weighing 500 mg was macerated in 10 ml of 0.1 M potassium phosphate buffer (pH 7.5) containing 0.05 mM EDTA and filtered through cloth. The filtrate was centrifuged for 15 minutes at 15,000 g. Supernatant was collected and used as enzyme extract. The enzyme protein content was determined according to the method of Lowry *et al*., (1951) using BSA as standard.

Hydroxide peroxide content

Leaf sample (0.5 g) was homogenized in 10 ml of cold acetone. The homogenate was filtered through Whatman No. 1 filter paper. Four ml of titanium reagent was added to the whole extract followed by 5 ml of concentrated ammonium solution to precipitate hydrogen-peroxide-titanium complex. This complex was centrifuged for 5 minutes at 10,000 g. The supernatant was discarded and precipitate was dissolved in 1M sulphuric acid. It was re-centrifuged to remove undissolved material and absorbance was recorded at 415 nm against blank. Concentration of H_2O_2 was determined using standard curve plotted with known concentration of H_2O_2.

Hydrogen peroxide content (H_2O_2) was estimated spectrophotometrically according to the method of Mukherjee and Choudhury, (1983) using titanium reagent. The standard H_2O_2 was used for calibration. The data was expressed as H_2O_2 μg^{-1}gDW.

Lipid peroxidation assay

Leaf sample of 0.5 g was homogenized in 10 ml 0.1 per cent trichloro acetic acid (TCA). The homogenate was centrifuged at 15,000 g for 5 minutes. Two ml of aliquot of the supernatant and 4 ml of 0.5% thio-barbuteric acid (TBA) in 20 per cent of TCA was mixed. The mixture was heated at 95° C for 30 minutes and cooled in ice bath. It was centrifuged at 10,000 g for 5 minutes and the absorbance of supernatant was recorded at 532 nm. The value for non-specific absorption at

600 nm was subtracted from value of 532 nm. The MDA content was calculated using its absorption coefficient of 155 n mol^{-1} cm^{-1}.

Chlorophyll stability index (CSI) and Membrane stability index (MSI)

The chlorophyll stability index (CSI) was estimated by following the method of Chetty *et al*., (2002). Membrane stability index was calculated by using the formula of Premachandra *et al*., (1990). Both CSI and MSI were expressed in percentage (%).

Physiological parameters

Twenty discs were taken from the leaves of each treatment and weighed. Leaf discs were submerged in water for four hours. They were blotted and their saturated weight was measured. Discs were dried at 80^0C till constant weight. Relative water content (RWC) was calculated by following equation (Weatherly and Barrs, 1962) and was expressed as percentage.

$$\text{Relative leaf water content} = \frac{\text{Fresh weight - Dry weight}}{\text{Turgid weight - Dry weight}} \times 100$$

The Senescence index (SI) was calculated following the method used by following formula,

$$SI = \frac{\text{Number of senescence leaves}}{\text{Numbers of physiologically active leaves + Numbers of senescenced leaves}} \times 100$$

The statistical analysis of data was done following the method of analysis of variance (ANOVA) given by Panse and Sukhatme, (1967) adopting completely randomized design (CRD) having 2 factors with four replication. The critical difference (CD) values were calculated at 5 percent probability level.

Results

Relative leaf water content (%) at tillering stage

Averaging over all the varieties, the moisture stress resulted in significantly decreased relative leaf water content (Fig. 2). Highest leaf relative water content was observed in variety Bandana(80.12%) which was 20.83% higher as compared to the check variety. The lowest leaf relative water content was found in variety Maibee (50.24%), which was 24.23% lower, compared to variety ARC-10372.

Varieties differed significantly in their response to moisture stress in respect of leaf relative water content. Stress induced reduction was highest in Lachit (15.03%) and it was lowest in ARC-10372 (12.19%) which was followed by Maibee (12.89%).

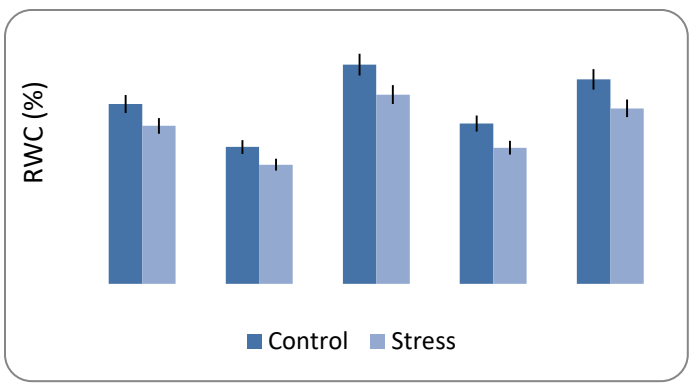

Fig.2: Effect of moisture stress on relative leaf water content at tillering stage

Chlorophyll stability index

Averaging over all the varieties, the moisture stress resulted in decreased chlorophyll stability index. The reduction was 14.88% compared to normal condition. (Table 1). There was no significant difference in the chlorophyll stability index among the varieties.The interaction between varieties and moisture stress was also found to be non- significant.

Membrane stability index

Moisture stress significantly affected the membrane stability index. The membrane stability index was reduced by 13.85% under moisture stress condition. (Table1). Varieties differed significantly in terms of membrane stability index. Highest membrane stability index was observed in Maibee (34.05%) followed by ARC-10372 (33.15%). The lowest membrane stability index was recorded in Kopilee (19.35%).The responses of the varieties to moisture stress condition in terms of membrane stability index were significantly different. Stress induced reduction in membrane stability index was highest in variety Lachit (25%) whereas the reduction was lowest in Maibee (6.53%).

Relative stress injury (%)

Moisture stress significantly increased the relative stress injury, which was 3.48% more under stress condition. (Table 1). Among the varieties relative stress injury was highest in variety Maibee (44.62%)that was 11.42% higher as compared to that of check variety ARC-10372(40.05%) and lowest in variety Kopilee that was 0.77% lower than that of check variety. Varieties differed significantly for relative stability index as affected by moisture stress. Stress induced increase in relative stress injury was highest in variety Lachit (5.29%) and the increase was lowest in variety Kopilee (2.16%) followed by variety Maibee (2.27%).

Senescence index

Moisture stress significantly increased in senescence index. On an average senescence index was increased by 74.70% under moisture stress condition. (Table 1). The varietal differences for senescence index were found to be significant. The highest senescence index was observed in Lachit (29.57%) and it was recorded to be lowest in ARC-10372 (20.29%) followed by Maibee(21.91%). The senescence index in Lachit was 45.75% higher over the check variety. The

varieties differed significantly in the response to moisture stress for senescence. Stress induced increase was highest in Lachit (91.91%) but it was lowest in Maibee(48.86%), followed by ARC-10372 (71.86%).

Table 1: Effect of moisture stress on physiological parameters

Treatments	Chlorophyll stability index (%)						Membrane stability index (%)						Senescence index (%)						Relative stress injury (%)					
	ARC-10372	Lachit	Bandana	Maibee	Kopilee	Mean	ARC-10372	Lachit	Bandana	Maibee	Kopilee	Mean	ARC-10372	Lachit	Bandana	Maibee	Kopilee	Mean	ARC-10372	Lachit	Bandana	Maibee	Kopilee	Mean
Control	0.541	0.570	0.547	0.478	0.515	0.530	35.70	27.60	33.27	35.20	21.10	30.57	14.92	20.26	20.91	17.61	20.25	18.79	39.13	42.00	40.00	44.12	39.31	40.91
Stress	0.414	0.483	0.436	0.454	0.470	0.451	30.60	20.70	29.90	32.90	17.60	26.34	5.65	38.88	36.64	26.21	36.75	32.82	40.96	44.22	41.22	45.12	40.16	42.33
Mean	0.478	0.527	0.492	0.466	0.492		33.15	24.15	31.58	34.05	19.35		20.28	29.57	28.77	21.91	28.50		40.04	43.11	40.61	44.62	39.73	
CD (0.05)																								
T	0.044						0.011						1.961						0.175					
V	NS						0.017						3.101						0.277					
TXV	NS						0.024						4.386						0.392					

Hydrogen peroxide content ($\mu g^{-1} g$ DW)

Hydrogen peroxide content was significantly increased under moisture stress condition; on an average the increment was 34.97%. (Fig.3). The variety Lachit showed the highest hydrogen peroxide content (3.77 $\mu g^{-1} g$ *DW*) which was 50.72% higher as compared to the check ARC-10372 and the latter registered the lowest hydrogen peroxide content (2.50 $\mu g^{-1} g$ *DW*). The varietal differences were significant.

Interaction between varieties and treatment in terms of H_2O_2 content was statistically significant. The stress induced enhancement was highest in the Kopilee (46.85%) and the increment was lowest in the ARC-10372 (22.22%).

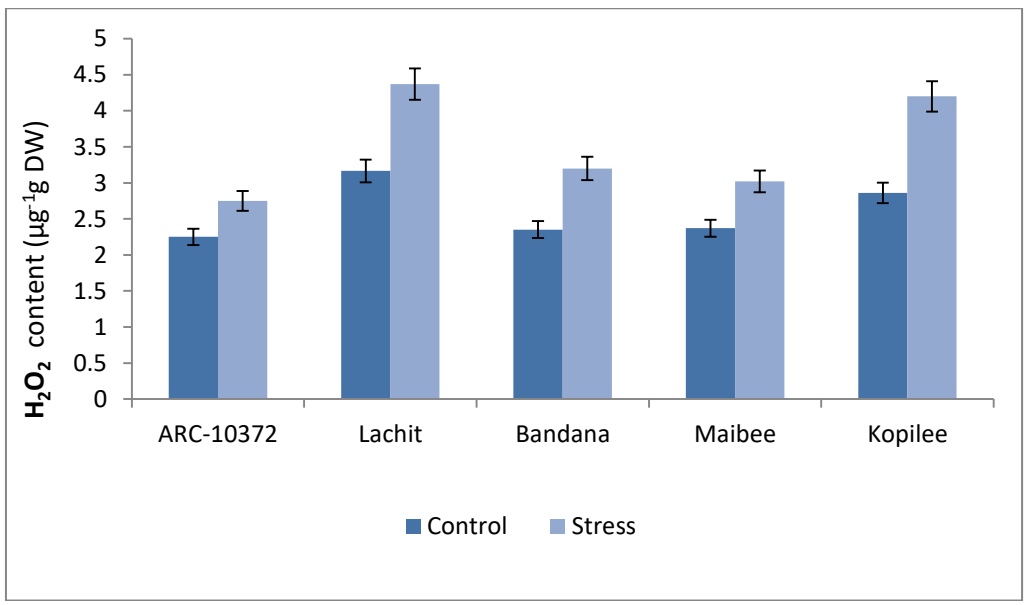

Fig. 3. Effect of moisture stress on hydrogen peroxide content

Lipid peroxidation (nmolg^{-1}FW)

Moisture stress significantly increased the lipid peroxidation by 11.56%. (Fig. 4). A significant difference among the varieties was observed for lipid peroxidation. On an average the highest lipid peroxidation was recorded in Bandana (12.44 *nmolg^{-1}FW*) that was 101.24% higher as compared to the check ARC-10372 and the latter registered the lowest lipid peroxidation (6.18 *nmolg^{-1}FW*). Moisture stress increased the lipid peroxidation in varieties by significantly different amounts. The stress induced increment in lipid peroxidation was highest in Bandana (12.84%) and least in ARC-10372 (10.17%) followed by Maibee (10.57%).

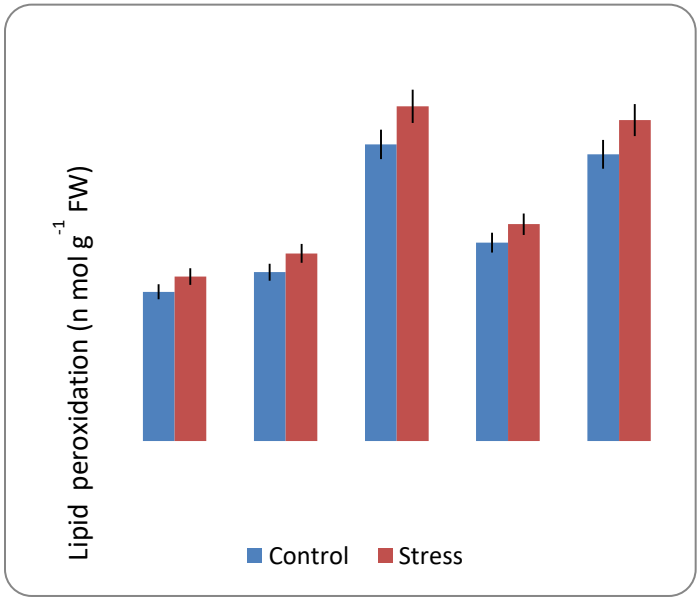

Fig. 4. Effect of moisture stress on lipid peroxidation content

Antioxidative enzymes

Superoxide dismutase activity **(mg^{-1}-protein)**

Moisturestress significantly increased the superoxide dismutase activity (SOD) up to 8.51% more under moisture stress compared to normal condition. (Table 2). There was a significant difference in SOD activity among the varieties. Highest SOD content was observed in Bandana (7.87 mg^{-1}-protein) and lowest was observed in ARC-10372 (3.11 mg^{-1}-protein).The varietal response to moisture stress was significantly different in SOD activity. The stress induced increase was highest in Maibee (13.57%) whereas the increment was lowest in Lachit(4.19%).

Catalase activity (µmol H_2O_2 min^{-1}mg^{-1} protein)

A significant increased in catalase (CAT) activity (16.14%) was observed under moisture stress condition. (Table 2). Averaging over all the treatments, the highest catalase activity was observed in variety ARC-10372(0.62 µmol H_2O_2 min^{-1}mg^{-1} protein) followed by Bandana (0.57 µmol H_2O_2 min^{-1}mg^{-1} protein) that was 9.12% lower as compared to the ARC-10372.The lowest activity was found in Maibee (0.19µmol H_2O_2 min^{-1}mg^{-1} protein). The varieties differed significantly in their response to moisture stress so far as catalase activity is concerned. Stress induced increment was highest in Maibee (22.86%) and it was lowest in ARC-10372 (8.33%).

Ascorbate peroxidase activity*(nmol min^{-1}g^{-1}FW)*

Data in Table 2 reveals that moisture stresssignificantly increased the ascorbate peroxidase activity (14.77%). Averaging over the treatments, ascorbate peroxidase activity was highest in Lachit (1.13 nmol min^{-1}g^{-1}FW) that was 98.25% higher as compared to that of check variety ARC-10372 and the latter variety was the one, which registered lowest activity (0.57 nmol min^{-1}g^{-1}FW).

The extent of increment in this enzyme activity in the varieties as influenced by moisture stress was significantly different. The increase in ascorbate peroxidase activity was highest in ARC-10372 (48.35%) followed by Maibee and Kopilee (9.65%) whereas the increment was lowest in Lachit (8.37%) under moisture stress condition.

Peroxidase activity (µmol H_2O_2 reduced mg^{-1} protein min^{-1})

The peroxidase activity increased (14.89%) under moisture stress. Maibeeshowed the highest peroxidase activity (3.49 µmol H_2O_2 reduced mg^{-1} protein min^{-1}) which was 11.15% higher as compared to that of check ARC-10372 (3.14 µmol H_2O_2 reduced mg^{-1} protein min^{-1}).The lowest peroxidase activity was recorded in Lachit (1.58 µmol H_2O_2 reduced mg^{-1} protein min^{-1}). The varietal responses to moisture stress were significantly different for peroxidase activity. Stress induced increase in peroxidase activity was highest in ARC-10372(18.06%) followed by Maibee (17.13 %) whereas it was lowest in Lachit (10%).

Glutathione reductase activity (ΔOD_{412nm} min^{-1}mg^{-1} protein)

Averaging over all the varieties, moisture stress increased the glutathione reductase activity by 12.24%. This activity under moisture stress condition was statistically higher compared to that under normal condition. (Table 2). The varieties differed significantly in terms of glutathione reductase activity. The highest activity was shown by ARC-10372 (0.28 ΔOD_{412nm} min^{-1}mg^{-1} protein) and lowest was recorded in Kopilee (0.23 ΔOD_{412nm} min^{-1}mg^{-1} protein) that was 17.56% lower as compared to ARC-10372. Varietal responses to moisture stress in terms of glutathione reductase activity were significantly different. Moisture stress caused highest increment in glutathione reductase activity in Maibee (17.5%) whereas it was least in Bandana (7.86%).

Tiller number at Panicle initiation stage

Moisture stress significantly reduced (26.14%) the number of tillers per plant during panicle initiation stage (Table 3). The varieties differed significantly in terms of number of tillers per plant. The highest tiller number was recorded in ARC-10372(17) which was 2.94% higher than that of Maibee (16.5). Stress induced reduction in tiller number was found to be highest in Kopilee (29.41%) and reduction was lowestin ARC-10372(21.1%) followedby Maibee (26.32%).

Table 2 Effect of moisture stress on antioxidative enzymes

Treatments	Superoxide dismutase (mg^{-1} protein)						Catalase (µmol H_2O_2 $min^{-1}mg^{-1}$ protein)						Ascorbate peroxidase (nmol $min^{-1}g^{-1}FW$)						Peroxidase (µmol H_2O_2 reduced mg^{-1} protein min^{-1})						Glutathione reductase (ΔOD_{412nm} $min^{-1}mg^{-1}$ protein)					
	ARC-10372	Lachit	Bandana	Maibee	Kopilee	Mean	ARC-10372	Lachit	Bandana	Maibee	Kopilee	Mean	ARC-10372	Lachit	Bandana	Maibee	Konilee	Mean	ARC-10372	Lachit	Bandana	Maibee	Konilee	Mean	ARC-10372	Lachit	Bandana	Maibee	Konilee	Mean
Control	3.04	3.58	7.59	6.41	3.94	4.91	0.60	0.43	0.52	0.17	0.33	0.41	0.46	1.08	0.96	0.87	1.04	0.88	2.88	1.5	2.19	3.21	1.98	2.35	0.27	0.23	0.23	0.24	0.22	0.24
Stress	3.18	3.73	8.15	7.28	4.31	5.33	0.65	0.51	0.62	0.21	0.38	0.48	0.68	1.17	1.04	1.04	1.14	1.01	3.40	1.65	2.5	3.76	2.21	2.70	0.29	0.27	0.25	0.28	0.24	0.27
Mean	3.11	3.65	7.87	6.84	4.12		0.63	0.47	0.57	0.19	0.35		0.57	1.13	1.00	0.96	1.09		3.14	1.58	2.35	3.49	2.10		0.28	0.25	0.24	0.26	0.23	
CD (0.05)																														
T	0.001						0.005						0.024						0.02						0.002					
V	0.01						0.008						0.038						0.04						0.003					
T×V	0.01						0.012						0.054						2.88						0.004					

Table 3: Effect of moisture stress on tiller characters

Treatments	Number of tiller at Panicle Initiation stage					
	ARC-10372	Lachit	Bandana	Maibee	Kopilee	Mean
Control	19.00	15.00	18.00	19.00	17.00	17.60
Stress	15.00	11.00	13.00	14.00	12.00	13.00
Mean	17.00	13.00	15.50	16.50	14.50	
CD (0.05)						
T	0.248					
V	0.392					
TXV	0.554					

Discussion

Drought usually leads to oxidative stress, when the production of ROS exceeds the capacity of antioxidant defense system the membranes loss their stability, increasing ion leakage (Blokhina *et al.*, 2003). The figure 2 and table 1 showed significant decrease in relative water content and increase in ion leakage in leaves of the stressed rice plant compared with control. The membrane stability index were reduced 13.85% under moisture stress condition (Table.1). Varieties were differed significantly in terms of membrane stability index. Stress induced reduction in membrane stability index was highest in Lachit (25%) whereas the reduction was lowest in Maibee (6.53%). Varieties differed significantly in their response to moisture stress in respect of leaf relative water content. Stress induced reduction was highest in variety Lachit (15.03%) and it was lowest in ARC-10372 (12.19%) and followed by Maibee (12.89%). The reduction in the relative water content respone to drought stress has been reported in a

wide variety of plants (Valentovic *et al*., 2006; Masoumi *et al*., 2010). Drought stress disturbs plant water relations (Anjum *et al*., 2011). The observed decrease in RWC was concomitant with an increase in ion leakage and loss of membrane integrity (Table 1). The membranes are subjected to changes often associated with the increases in permeability and loss of integrity under environmental stresses (Blokhina *et al*., 2003). Therefore, the ability of cell membranes to control the rate of ion exchange of the cell is used as an indicator of membrane damage. Plants under water deficit condition accumulate superoxide radical and hydrogen peroxide, which can directly attack membrane lipids (Menconi *et al*., 1995).

Averaging over all the varieties, the moisture stress resulted in decreased chlorophyll stability index (CSI). The reduction in CSI was 14.88% compared to normal condition. (Table 1).Lichtenthaler and Rinderle, (1988) reported that, under normal condition, the chlorophyll (a/b) ratio amounts to value of 2.6 to 3.1. In stressed leaves, however, the chlorophyll (a/b) ratio eventually decline to one or even less than one. They added that, high values of the ratio chlorophylls up to 5–6 indicate the full efficiency of photosynthetic apparatus. This result is accordance with the result of Emam (2012) in rice. In this study, the same result was obtained, the chlorophyll (a/b) ratio sharply decrease in stressed rice leaves compared to the well watered control. Therefore, CSI may significantly reduce under moisture stress which leads to significant increased in SI under moisture stress. One of the most important observations is that the Chlorophyll a: b ratio is significantly increased under moisture stress in tolerant Maibee variety (Fig.5)

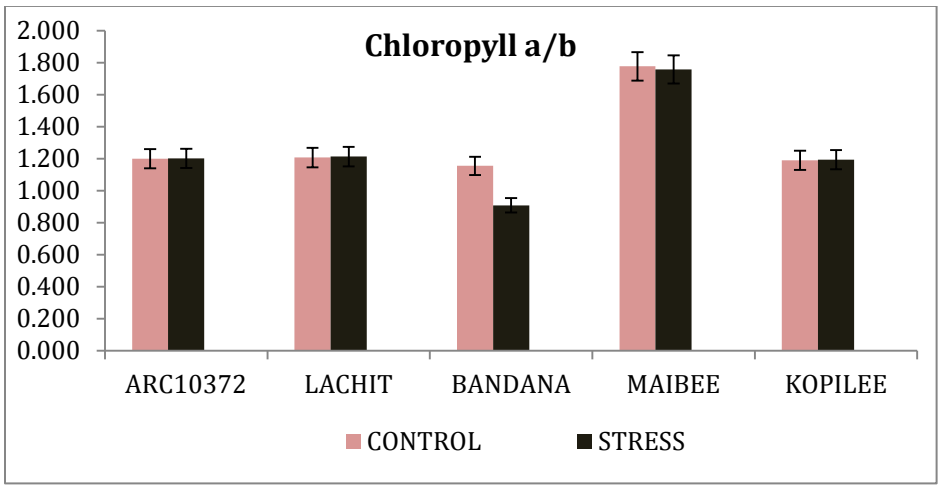

Fig.5: Effect of moisture stress in Chl a: b ratio.

Average senescence index was increased by 74.70% under moisture stress condition and the varieties differed significantly in response to moisture stress for senescence. Stress induced increase was highest in Lachit (91.91%) but it was lowest in variety Maibee (48.86%), followed by ARC-10372 (71.86%) (Table 1).

In addition, the degree of damage by Reactive oxygen species (ROS) depends on the balance between the product of ROS and its removal by this antioxidant scavenging mechanism (Azooz *et al*., 2009). The membrane of plant cells is subjectedto rapid damage with increase in water stress. This leakage of membrane is caused by an uncontrolled enhancement of free radical, which causes lipid peroxidation. Damage to fatty acids of membrane could produce small hydrocarbon fragments including Malondialdehyde (MDA) (Moussa and Aziz, 2008). MDA is the final product of plant cell membrane lipid peroxidation and is one important sign of membrane system injury (Cunhua *et al*., 2010).

Moisture stress brought about significant increased in hydrogen peroxide (H_2O_2) Fig. 3 and Malondialdehyde content (MDA)/ lipid peroxidation, Fig. 4. The higher lipid peroxidation and H_2O_2 content was observed in variety Kopilee and Lachit as compared to the other varieties. According to Emam, (2012) drought stress induced a highly significant increase in lipid peroxidation product (MDA) in both shoots and roots of stressed rice seedlings. The increase in MDA content was accompanied by an increase ion leakage resulting in enhanced membrane permeability. Similar results have been reached by Zhou *et al*., (2007).

The varietal response to moisture stress was significantly different in antioxidant enzymes activity (Table 2) The stress induced increase in SOD was highest in variety Maibee(13.57%)whereas the increment was lowest in variety Lachit(4.19%) and in case of catalase highest in variety Maibee (22.86%) and it was lowest in variety ARC-10372 (8.33%). The increase in ascorbate peroxidase activity was highest in variety ARC-10372 (48.35%) followed by varieties Maibee and Kopilee (9.65%) whereas the increment was lowest in variety Lachit (8.37%) under moisture stress condition. Similar trend was also observed in case of peroxidase activity, it was highest in variety ARC-10372 (18.06%) followed by variety Maibee (17.13 %) whereas it was lowest in variety Lachit (10%). But this trend is slightly different in case of GR, where activity is highest in variety Maibee (17.5%) whereas it was least in variety Bandana(7.86%). The lower level of GSH might be attributed to decrease of GSH biosynthesis and/or increase of its degradation (Noctor and Foyer, 1998). Moreover, such effect might be also attributed to the role of GSH in the regeneration of another potent antioxidant as ASA. In tolerant varieties it may possible that under water deficit conditions, ASA levels increased in ascorbate glutathione cycle, whereas GSH levels decrease. The decreased activities of ASO have been reflected

by the increased levels of ASA these enzymes withdraw ascorbate as substrate (Emam, 2012). ASA can directly scavenge superoxide, hydroxyl radicals and singlet oxygen and reduce hydrogen peroxide to water *via* ascorbate peroxidase reduction (Noctor and Foyer, 1998).

Moreover, POX showed higher activity in water stressed rice seedlings (Emam, 2012). POX plays an important role in the formation of suberin and lignin, thus increase the rigidity of plant cell walls under drought stress (Quiroga *et al*., 2000). An increase in the activity of antioxidant enzymes under drought stress could be indicative of an increased production of ROS and build up of protective mechanism to reduce oxidative damage triggered by drought stress (Noreen *et al*., 2010).

Water stress conditions may trigger an increased production of reactive oxygen forms, which can explain the remarkable damage to the enzymes with active sulfydrylic groups, chloroplast pigments, the membrane lipids and proteins and alteration of their structural integrity. This formation is a consequence of the Mehler reaction, which provides a pathway for the removal of excess electrochemical energy caused by drought stress (Nakano and Asada, 1981). During water depletion, O_2^- can also react non-enzymatically with H_2O_2 giving rise to products such as hydroxyl radicals and singlet oxygen, which are even more reactive than O_2^-itself.

The results obtained from the present work clearly demonstrated that the upland rice varieties displayed distinct variation in drought tolerance during PI stage. Accordingly, we can identify the drought tolerant varieties namely ARC-10372 and Maibee and the drought sensitive varieties namely Kopilee and Lachit based on the data obtained in the present investigation. All the upland rice varieties displayed significant reduction in Tiller number at the highest drought levels as compared with control (Table 3). This reduction in panicle number may be due to low RWC in leaf as well as a decrease in assimilate translocation process.

Tolerance to drought stress in higher plants correlates to levels of antioxidant systems and substrates (Athar *et al*., 2008). The effects of drought induced oxidative stress, plants develop a complex mechanism of antioxidant system. Relatively higher activities of ROS scavenging enzymes have been reported in tolerant genotypes when compared to susceptible ones, suggesting that the antioxidant system plays an important role in plant tolerance against environmental stress. Generally, all varieties exhibited lowest enzymatic activity under normal condition. This indicated plants will produce more CAT, SOD and POD under water stress conditions to remove the extra ROS in cells. In this study, CAT SOD and POD activities increased markedly in the drought tolerant varieties, while they reduced in sensitive varities. This showed that drought-tolerant varieties were efficient scavenger of H_2O_2, which may result in better protection against H_2O_2. The CAT is one of the highest turnover rates for all enzymes with the potential to directly dismutate H_2O_2 into H_2O and O_2 and is indispensible for ROS detoxification in peroxisomes during stress condition (Sairam and Srivastava, 2001). The SOD detoxifies superoxide anion free radicals (O_2^-) by forming H_2O_2, and then the H_2O_2 can be eliminated by CAT and POD (Hasheminasab *et al*., 2012). Moreover, POD also involved in various plant processes, including lignification (Hendriks *et al*., 1991), oxidation of phenolics (Largrimini, 1991) regulation of cell elongation (Mohammadkhani and Heidari, 2008) and detoxification of toxic compounds such as H_2O_2 (Chaparzadeh *et al*., 2004). The tolerance of some genotypes to environmental stresses has been associated with higher activities of antioxidant enzymes. For example, the drought tolerant species of wheat (*Triticum aestivum*) (Hasheminasab *et al*., 2012) and black gram (*Phaseolus mungo*) (Pratap and Sharma, 2010) had higher activities of SOD, POD and CAT than the drought sensitive species.

Conclusion

The upland rice varieties in this study showed differential responses for growth, pigment system and enzymatic activities. The scavenging system in drought tolerant varieties ARC-10372 and Maibee exhibited higher CAT, POD and SOD activities, than in the drought sensitive varieties like Lachit and Kopilee. Thus, the drought tolerance of these upland rice varieties seems to be linked to the activities of these antioxidant enzymes. From the results of this experiment, it can be specifically concluded that low concentration of MDA and H_2O_2 and higher antioxidant activity in drought stress conditions lead to higher water stress tolerance of ARC-10372 and Maibee varieties.

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RESEARCH ARTICLE

Phytochemical Studies and Anti-Microbial Activity of *Curcuma Longa* Linn Rhizomes

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Abstract

Turmeric is the rootstalk of a tropical plant which is known as *Curcuma longa.* It belongs to family Zingiberaceae. *C. longa*, commonly known asturmeric (Haldi). It is well-known plant whichis used as a drug in Ayurvedic and Unanisystem of medicine. The rhizomes of *Curcuma longa* contains natural medicinal properties, including antioxidants, anti-cancer and anti-bacterial pproperties. Therefore, the present study was aimed to test the phytochemical constituents associated with antimicrobial characteristics present in *Curcuma longa* linn rhizomes using two chemical methods. The percentage yield of Secondary metabolites using acetone and methanol extracts of *Curcuma longa* was recorded from 6.56 to 26.50%. Anti-bacterial studies are very crucial for the use of spices as alternative or supplementary medicine to reduce the burden of high cost allopathic medicines and have several side effects. Use of alternative medicines is also important due to increasing drug resistance against pathogens in case of allopathic medicines.

Keywords: *Curcuma longa,*secondary metabolites, extraction methods

Introduction

Turmeric is the rootstalk of a tropical plant and it is known as *Curcuma longa*. Turmericis a rhizomatous herbaceous plant grown in tropical Asia, Africa and Australia. India is the largest producer of turmeric in the world. The genus *Curcuma* contains approximately 40 species among which *C. longa* is popular and widely cultivated in India.Turmeric is a perennial herb belongs to family Zingiberaceae which reaches astature of up to one meter. It is highly branched, yellow-to-orange, cylindrical and having aromaticrhizomes. *C. longa*, commonly known asturmeric (Haldi), is popular and used as a drug in Ayurvedic and Unanisystem of medicine (Ashraf, 2012; Chauhan *et al.*, 2017).

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It is widely used as a spice and as an orange and yellow dye.The rhizomes of *Curcuma longa* considered to have natural medicinal properties, including antioxidant properties (Majumdar, 2000) anti-inflammatory (Manimegalai, 2011) wound healing (Menon and Sudheer, 2007) anti-cancer (Nair, 2005) and anti-bacterial activity (Pathak, 2010). It contains a number of monoterpenoids, sesquiterpenoids, and curcuminoids (Fang, 2003). Turmeric is also used as a natural painkiller and cox-2 inhibitor. It aid in fat metabolism and helps in weight management. Antimicrobial activities of many plants have been reported by the researchers and antimicrobial activities of medicinal plants can be attributed to be the secondary metabolites (Chauhan *et al*., 2017). Rhizomes of *Curcuma longa* shows medicinal properties as it contain phytochemical constituents (Sawant and Godghate, 2013).

Therefore, the present study was aimed to study the phytochemical constituents and antimicrobial characteristics of *Curcuma longa* rhizomes. Phytochemical analysis can be of great significance in therapeutic treatments. Such studies will be of importance for the use of spices as alternative or supplementary medicine to reduce the burden of high cost medicine.

Material and Methods

Collection, Extraction, Filtration and evaporation

Rhizomes of *Curcuma longa* (cv. culture 39 and PTS) was collected from Horticultural Research Centre, Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut- 250110. They were dried under the sunlight. After drying, it was ground into fine powder using mechanical grinder. Three grams powder of rhizomes of *Curcuma longa* was dissolve in 60 ml of different extracts i.e. acetone and methanol for 5 days with intermediate shaking at regular intervals. After appropriate mixing, the plant extract was filtered with Whatman filter paper no. 1. The residue was discarded while the filtrate is collected into a beaker. The filtrate was then kept on a hot plate at 40-60ºC for complete evaporation of the solvent. After evaporation, a gummy extract was obtained which was re-dissolved in solvents to make the concentration of 3 mg/ml. This extract was stored in a clean bottle and stored at 4ºC for further analysis of phytochemicals.The percentage yield and loss on drying was calculated using given formula:

$$\text{Percentage Yield \%} = \frac{Weight of product after evaporation}{Weight of powder used} \times 100$$

Phytochemical Tests And Antibacterial Activity

Each extract was subjected to the qualitative phytochemical screening for the presence of Carbohydrates, Alkaloids, Flavonoids,Glycosides,Phenols,Tannins,Saponins,Terpenoids, Steroids, Quinones and Proteins. Above phytochemical tests were carried out according to standards procedures (Trease and Evans 1983; Kokate *et al.*, 2002; Harbone, 1999) with slight modifications. The extracts were screened for their antibacterial activity by well diffusion method and streptomycin was kept as positive control (50 µg/ml). The lawn culture of test organism on nutrient agar media were used for well diffusion methods. The rhizome extract of dry *Curcuma longa* (50 mg/ml) was added into the well and allowdiffusing in the agar medium. The plates wereincubated at 37˚C for overnight. The antibacterialactivity of the extract was determined by measuringthe diameters of zone of inhibition. For each bacterialstrain, controls were maintained of pure solventswithout extracts.

Results and Discussion

Phytochemical Analysis

Effects of solvents on extraction of phytochemicals yield

The percentage yield of acetone and methanol extracts of *Curcuma longa* is presented in Table 1. The extraction yield of phytochemicals was observed from 6.56 to 26.50%. Both varieties showed highest percent of phytochemicals yield with 50% Aqueous Acetone extract and lowest percent yield at 100% acetone extract. This showed that the extraction yield of crude *C. longa* extracts increased as polarity of the solvent used is increased. Such results indicates that yield of secondary metabolites extract is higher using aqueous solvent of 50% compare to other concentrations of solvents. It is also evident from the results that extraction of secondary metabolites is higher compare to pure solvent extracts.

Table 1: Percentage Yield of acetone and methanol extracts of rhizome of *Curcuma longa* L.

Solvent	% yield of C-39	% yield of PTS
Acetone (100%)	5.00	6.56
Aqueous Acetone (75%)	7.33	10.43
Aqueous Acetone (50%)	19.70	26.50
Methanol (100%)	10.03	9.43
Aqueous Methanol (75%)	12.66	15.66
Aqueous Methanol (50%)	16.06	21.93

Table 2:Qualitative Phytochemical Analysis of *Curcuma longa* rhizomes extracts () in different solvents.

Sr. No.	Secondary metabolites	Name of test	Acetone	Methanol	Acetone	Methanol
			C-39		PTS	
1.	Carbohydrates	Molisch's	-	+	-	+
		Borfoed's	-	+	-	+
2.	Alkaloids	Mayer's reagent	-	+	-	+
		Wagner's reagent	-	+	-	+
3.	Flavonoids	Alkaline reagent	+	+	+	+
		NaOH	+	+	+	+
4.	Glycosides	Keller- Killiani	+	+	+	+
5.	Phenols	Ferric chloride	+	+	+	+
6.	Tannin	Ferric chloride	+	+	+	+
7.	Saponins	Foam	+	+	+	+
8.	Terpenoids	Salkowski's	-	+	-	+
9.	Steroids	Salkowski's	-	+	-	+
10.	Quinones	HCl Test	+	+	+	+
11.	Proteins	Xanthoproteic	+	+	+	+

Positive sign (+) = Present; Negative sign (-) = Absent

Phytochemical Analysis of *Curcuma longa* rhizomes extracts.

The results of phytochemical analysis of Turmeric rhizomes extracts using Acetone and Methanol are presented in Table 2.In both the varieties, it was observed thatall phytochemicals included in studies were extracted in methanolic solvent however, flavonoids, glycosides, phenols, tannins, saponins, quinones and proteins were extracted only in acetone extract (Table 2). The phyto-constituents of both varieties were similar as they contained the same secondary metabolites when using methanol extract. It indicates that methanol extract is efficient in terms of extracting secondary metabolites in *Curcuma longa.*

Antibacterial activity of different concentration of methanol extract of *Curcuma longa* against bacterial pathogen.

Methanol extract of *Curcuma longa* was evaluated for its anti-bacterial activity at 50 mg/ml concentration against *Bacillus sp., Pseudomonas sp.* and *Streptococcus sp*. by using Agar well diffusion method. The observations were recorded on zone of inhibition in growth of bacterial pathogen after 2 days as shown in Table 3. In *Bacillus sp*., 100% methanol extract of *Curcuma longa* C-39was observed with maximum zone of inhibition (18.00 mm) and for 50% methanol extract it was minimum (05.00 mm). PTS shows maximum zone of inhibition (17.00 mm) and minimum zone of inhibition (07.00 mm) at 100% and 50% methanol extract respectively. In *Pseudomonassp.*, C-39 showed maximum zone of inhibition (12.00 mm) at 100% methanol extract and showed minimum (07.00 mm) at 50% methanol extract. PTS variety with 100% methanol showed maximum zone of inhibition (11.00 mm) and minimum (07.00 mm) at 50% methanol extract.In *Streptococcus sp.*, C-39 showed maximum zone of inhibition (07.00 mm) at 100% methanol extract while it was minimum (05.00 mm) at 50% methanol extract. PTS with 100% Methanol and 50% Methanol showed no zone of inhibition.

Table 3: Zone of inhibition with different concentration of methanolic extracts of *Curcuma longa* against pathogen.

S.No.	Treatment	*Bacillus sp.* zone of inhibition		*Psuedomonas sp.* zone of inhibition		*Streptococcus sp.* zone of inhibition	
		Culture-39	PTS variety	Culture-39	PTS variety	Culture-39	PTS variety
1.	Streptomycin	22.00	20.00	22.00	20.00	22.00	20.00
2.	Methanol 100%	18.00	17.00	12.00	11.00	07.00	05.00
3.	Methanol 75%	12.00	11.00	05.00	10.00	05.00	05.00
4.	Methanol 50%	05.00	07.00	05.00	07.00	05.00	05.00

Table 4: Zone of inhibition with different concentration of acetone extracts of *Curcuma longa* against pathogen.

S.No.	Treatment	*Bacillus sp.* zone of inhibition		*Psuedomonas sp.* zone of inhibition		*Streptococcus sp.* zone of inhibition	
		Culture-39	PTS variety	Culture-39	PTS variety	Culture-39	PTS variety
1.	Streptomycin	22.00	20.00	22.00	20.00	22.00	20.00
2.	Acetone 100%	17.00	16.00	19.00	18.00	11.00	05.00
3.	Acetone 75%	15.00	13.00	11.00	10.00	07.00	05.00
4.	Acetone 50%	07.00	07.00	06.00	05.00	05.00	05.00

Antibacterial activity of different concentration of acetone extract of *Curcumalonga* against pathogen.

Acetone extract of *Curcuma longa* was evaluated for its anti-bacterial activity at 50 mg/ml concentration against *Bacillus sp., Pseudomonas sp.* and *Streptococcus sp*. by using Agar well diffusion method. The observations were recorded on zone of inhibition in growth of bacterial pathogen after 2 days as shown in Table 4. In *Bacillus sp*., 100% Acetone extract of *Curcuma longa* C-39 was observed with maximum zone of inhibition (17.00 mm) and for 50% Acetone extract it was

minimum (07.00 mm). PTS shows maximum zone of inhibition (16.00 mm) and minimum zone of inhibition (07.00 mm) at 100% and 50% Acetone extract respectively. In *Pseudomonassp.,* C-39 showed maximum zone of inhibition (19.00 mm) at 100% Acetone extract and showed minimum (06.00 mm) at 50% Acetone extract. PTS variety with 100% Acetone showed maximum zone of inhibition (18.00 mm) and minimum (05.00 mm) at 50% Acetone extract.In *Streptococcus sp.,* C-39 showed maximum zone of inhibition (11.00 mm) at 100% Acetone extract while it was minimum (05.00 mm) at 50% Acetone extract. PTS with 100% Acetone and 50% acetone showed no zone of inhibition. It is clear from the above studies that the yield of various secondary metabolites was highest with 50% Aqueous Acetone extract compare to other concentrations and chemicals. It was also observed that methanolic solvent is efficient for extraction of phytochemicals compare to other methods.

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RESEARCH ARTICLE

Bioproduction of Lactic Acid Exposed to Metronidazole

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Abstract

The efficacy of metronidazole on bioproduction of lactic acid by some lactic acid producing bacteria such as *Lactobacillus jugrti* NCIM- 2367, *Lactobacillus lactis* NCIM-2369, *Lactobacillus plantarum* NCIM-2373, *Lactobacillus brevis* NCIM-2436 and *Lactobacillus delbrueckii* NCIM-2025 has been studied. The bacterial strain *Lactobacillus delbrueckii* NCIM-2025 has been found quite effective and useful for maximum bioproduction of lactic acid, i.e,; 11.253 g/100 ml of lactic acid which is 17.096% higher in comparison to control fermentor flasks, i.e,; 9.610 g/100ml at 7.0×10^{-5} M molar concentration of metronidazole mutagene. It has been found that bioproduction of lactic acid in the presence of metronidazole at optimum molar concentration, i.e; at 7.0×10^{-5} M attains its best activity when 24% (w/v) molasses solution is allowed to ferment for 5 days of optimum incubation period, at 38^0C temperature by maintaining the pH value of the fermentation medium at 5.9 along with other nutritional ingredients required by the lactic acid bacteria.

Keywords: Lactic acid fermentation, metronidazole, molasses, *Lactobacillus delbrueckii* NCIM-2025, pH, temperature and incubation period.

Introduction

Lactic acid is used as a food preservative, curing agent, and flavoring agent. It is an ingredient in processed foods and is used as a decontaminant during meat processing. Lactic acid is produced commercially by fermentation of carbohydrates such as glucose, sucrose, or lactose, or by chemical synthesis. Lactic acid is a natural organic acid with a great variety of application in pharmaceutical, chemical, food and health care industries. (Narayanan *et.a.,* 2004). The worldwide demand for lactic acid is estimated roughly to be 130 000 to 150 000 tons per year (Randhawa *et.al.,* 2012). However, the global consumption of lactic acid is expected to increase rapidly in the near future.

Lactic acid bacteria (LABs) are recognized for their fermentative ability and thus enhancing food safety, improving organoleptic attributes, enriching nutrients and increasing health benefits (Panesar, 2011).

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A number of chemical mutagens are well known to show physiological and pharmacological property (Tiwari and Singh 1980, Singh *et.al* 1990, 1993, 1997, 1998, 2001 and Shivankari *et.al* 2017 and 2019) Information regarding their role in biological system is very much limited and still unsettled. There are large group of some mutagens which when introduced to the fermentation medium can effect the enzyme responsible for the biosynthesis of micro and macro molecules in the microbial cells as well bioconversion of raw substrate into desired products and such mutagenic compounds may be referred to as mutagenic compounds. Though biologically active mutagenic biomolecules are not essentially growth promoter for some microbes yet a few mutagenic biomolecules are utilized by some or all microbes. Although a group of workers (Rahman *et.al*, 2011; farooq *et.al*, 2012; Abdel-Rahman *et.al*, 2013; Singh *et.al*, 2010, Snigdha *et.al*, 2011) have tried to explore the effect of some mutagenic biomolecules and on microbial enzymes systems, yet there is no definite opinion regarding its influence on bioproduction of lactic acid. In view of the scarce knowledge regarding involvement of active mutagenic compounds to any fermentation processes specially lactic acid fermentation (Kumar *et.al*, 2014) the authors have made an attempt to study the effect of some active mutagenic compounds on lactic acid fermentation by some lactic acid bacteria.

Materials and Methods

Compositions of the production medium

The composition of the production medium for the bioproduction of lactic acid by *Lactobacillus delbrueckii* NCIM-2025exposed to metronidazole is as follows :

Molasses: 24% (w/v), Malt Extract : 1.60%

Yeast Extract: 1.60%, Peptone : 1.60%, $(NH_4)2HPO$ 4 : 1.60%, $CaCO_3$: 11.0%, pH : 5.9

(The pH was Adjusted by adding requisite amount of phosphate-buffer solution). Distilled water : To make up 100 ml.

Assay methods

Evaluation of lactic acid (barker and summerson, 1941) formed and molasses (Dubois *et.al.,* 1956) left unfermented was made colorimetrically.

Sterilization : The growth and production medium was sterilized in an autoclave maintained at 15 lbs steam pressure for 30 minutes.

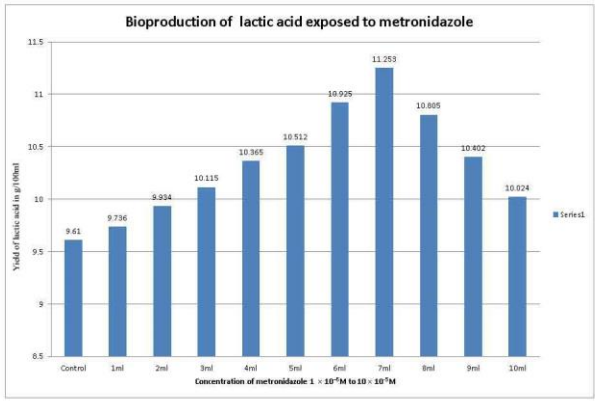
Strain : *Lactobacillus delbrueckii* NCIM-2025 has been employed in the present study. The strain was procured from NCL - Pune, India

Table – 1 Bioproduction of lactic acid exposed to metronidazole

Concentration of mutagens	Incubation period in days	Yield of lactic acid* in g/100 ml	Molasses* left unfermented in g/100 ml	% of lactic acid increased in 5 days
Control	5	9.610	2.433	-
1×10^{-5}M	5	9.736	2.278	+ 1.311
2×10^{-5}M	5	9.934	2.084	+ 3.371
3×10^{-5}M	5	10.115	1.899	+ 5.254
4×10^{-5}M	5	10.365	1.647	+ 7.856
5×10^{-5}M	5	10.512	1.505	+ 9.386
6×10^{-5}M	5	10.925	1.093	+ 13.683
7×10^{-5}M**	5	11.253***	0.769	+ 17.096
8×10^{-5}M	5	10.805	1.209	+ 12.434
9×10^{-5}M	5	10.402	1.657	+ 8.241
10×10^{-5}M	5	10.024	1.998	+ 4.308

*Each value represents mean of three trials. ** Optimum concentration of barbitalunder trial. *** Optimum yield of lactic acid in 5 days. Experimental deviation $\pm$ 1.5 – 3.0%. +ve values indicate % increase in the yield of lactic acid.

Lactobacillus delbrueckii



Age of the inoculum : 48 hours old.

Quantum of the inoculum: 0.5 ml bacterial suspension of *Lactobacillus delbrueckii* NCIM-2025.

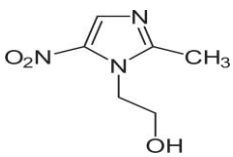
Incubation period : 4, 5 and 6 days

Concentration of metronidazole used

M/1000 solution of metronidazoleunder trial has been prepared and 1.0 x 10^{-5}M to 10 x 10^{-5} M molar concentration of metronidazole has been employed.

Results and Discussion

The influence of metronidazole on production of microbial lactic acid by *Lactobacillus delbrueckii* NCIM-2025



Metronidazole (Mutagen)

The data given in the table 1 shows that the mutagen metronidazole has been found stimulatory for production of microbial lactic acid by *Lactobacillus delbrueckii* NCIM-2025. From the data given in the table it is obvious that metronidazole influences the lactic acid fermentation process in different phases. The main characteristics of the metronidazole is as follows :

(i)Metronidazole is stimulatory at its all molar concentrations used during course of the lactic acid fermentation, i.e. from 1.0 x10^{-5}M to10.0 x 10^{-5}M.

(ii) The molar concentration 1.0x10^{-5}M, 2.0 x10^{-5}M and 3.0 x 10^{-5} of metronidazole influence the yield of lactic acid in a approximately regular doubling order after each state, i. e., 1.311%, 3.371% and 5.254%.

(iii)The molor concentration 4.0 x 10^{-5}M, 5.0 x 10^{-5}M and 6.0 x10^{-5} of metronidazole now influence the productivity of lactic acid in a regular manner enhancing the yield lactic acid. The % increase in the yield of lactic acid at respective molar concentration of metronidazole has been found to be as follows: 7.856%, 9.386 and 13.683%)

(iv) The higher molar concentrations, i.e., 7.0 x 10^{-5}M, 8.0 x 10^{-5}, 9.0 x 10^{-5} and 10 x 10^{-5}M of

metronidazole influences the yield of lactic acid without any distinction and therefore, the % difference in the yield of lactic acid has been found to be almost same as mentioned below :

17.096%, 12.434%, 8.241 and 4.308% respectively.

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RESEARCH ARTICLE

Effect of Integrated Nutrient Management on Yield Attributes of Black Carrot and Physico-chemical Properties of Soil

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Abstract

A field experiment was conducted during Rabi season of 2017-2018 to assess the effect of integrated nutrient management on yield attributes of black carrot and physico-chemical properties of soil at Experimental Farm of Division of Vegetable Science SKUAST-K Shalimar. The experiment was laid out in randomized complete block design with nine treatments replicated thrice. The nine treatments wereT_1-RFD-(control), T_2 (50%RFD+50%FYM), T_3(50% RFD +50%VC), T_4 (50%N&K+25%P+PSB+50%FYM), T_5(50%N&K+ 25%P+PSB+50%VC),T_6 (50%N&P+25%K+KSB+50% FYM), T_7 (50%N&P+25%K+KSB+50%VC) ,T_8 (50% N+ 25% P&K+PSB+KSB+50% FYM) and T_9 (50%N+ 25%P&K+PSB+KSB+ 50%VC). Results revealed that among the different treatment combinations, treatment T_9 recorded the higher values for average root weight (148.85 g), root yield per plot (23.81 kg), root yield per hectare (285.79 q). It was further revealed that treatment T_9 recorded lowest soil pH (7.04) which was statistically at par with treatment T_8 and treatment T_5. Bulk density (1.10 g cc^{-1}) was found to be lowest in treatment T_9 which wasstatistically at par with treatment T_8 . The treatment T_9 resulted in improvement of soil electrical conductivity with a value of 0.287 dSm^{-1} which was statistically at par with treatment T_8 and significantly higher than rest of the treatments. The treatment T_1 showed least increase in electrical conductivity of soil.

Keywords: Black carrot, integrated nutrient management, yield , physico- chemical properties

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Introduction

Carrot is one of the major vegetable crops grown throughout the world (Vilela, 2004) and it is considered as an important economical vegetable due to its high yield per unit area (Hassan *et al*.,2005) .

Although orange carrot varieties are more common but consumption of black carrots is increasing as well. The black carrot is rich in phenolic content, flavinols, calcium, iron zinc, vitamin A, B, C, E and selenium. It also contains calcium pectate which is a very good source of fibre. The continuous use of chemical fertilizers has resulted in the depletion of soil heath and the increasing cost of chemical fertilizers makes it impossible for marginal farmers to purchase it at such higher rate .The ill effects of chemical fertilizers on physicochemical properties of soil has resulted in depletion of soil structure, decrease in the microbial population in the soil which adversely affected the yield and quality of vegetables (Agarwal, 2003). On the other hand, organic manures are cheap source of nutrients and key factor in restoring the productivity of degraded soils as they supply the multiple nutrients and improve the organic matter content in the soil which in turn improves the physical properties, enhances the biological diversity and soil microflora, leading to sustainable vegetable production, devoid of harmful residues (Acharya and Mandal, 2002) however, it has been observed that the crop response to organic manures is not as spectacular as with the chemical fertilizers owing to the slow release of nutrients during the initial years. Biofertilizers also play an important role in maintaining the sustainability of soil as biofertilizers are ready to use live formulations of such beneficial microrganisims which on application to seed, root or soil mobilizes the availability of nutrients by their biological activity in particular and help to build up the soil micro-flora and thereby the soil health. Therefore, to maintain the soil fertility and to supply the plant nutrients in balanced proportion without compromising the yield and quality of the crop an integrated approach is to be practiced under specific agro-ecological situation through the combined use of

inorganic and organic sources along with the application of biofertilizers.

Materials and Methods

The experiment was carried out at Experimental Farm, Division of Vegetable Science, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Shalimar, Srinagar. The experimental field is situated at 34.1^{o} North latitude and 74.89^{o} East longitude with an altitude of 1587 meters above mean sea – level. The experiment was laid out in randomized block design with nine treatments and three replications. The treatment combinations were T_1 RFD-(control),T_2(50%RFD+50%FYM),T_3(50%RFD+50%VC),T_4(50%N&K+25%P+PSB+50%FYM),T_5(50%N&K+25%P+PSB+50%VC),T_6(50%N&P+25%K+KSB+50%FYM),T_7(50%N&P+25%K+KSB+ 50%VC), T_8 (50%N+25%P&K+ PSB+KSB+50% FYM), T_9 (50%N+ 25%P&K+ PSB+ KSB+ 50%VC). Twenty seven plots of 3.0 m × 2.5 m size each were prepared as per layout specifications. The seeds of Black carrot variety Local Black were sown at spacing of 30 cm × 15 cm. Recommended dose of Nitrogen, Phosphorus and Potassium (90:60:60 kg ha^{-1}) was provided through urea, diammonium phosphate and muriate of potash according to the treatment. Organic manures viz., well decomposed farmyard manure (FYM), vermicompost were incorporated as per treatment to respective plots 15 daysprior to sowing on the basis of nitrogen percentage. Biofertilizers (PSB&KSB) @ 5 l ha^{-1} were applied into the respective treatments before sowing of seed. Observations were recorded on various aspects like yield attributes of crop and physico chemical properties of soil. The experimental data was then subjected to statistical analysis as per the standard statistical procedure given by.

Results and Discussions

Perusal of data presented in (Table 1) revealed significant effect of different treatments on yield attributes of black carrot. Among all treatments, treatment T_9 (50% N+25%P&K + PSB+KSB+50% VC) recorded a maximum average root weight of 148.85 g followed by treatment T_8 (50% N+25%P & K +PSB+KSB+50%FYM) which recorded average root weight of 144.18 g while the minimum average root weight of 104.19g was observed in treatment T_1 (RFD). Among different treatments the maximum root yield per plot of 23.81 kg was recorded in treatment T_9 (50% N+25% P&K + PSB+KSB+50% VC) which was significantly superior to all other treatments .However the minimum root yield per plot (16.67 kg) was observed in treatment T_1 (RFD).The maximum root yield per hectare (285.79q) was observed in treatment T_9 (50% N+25% P & K + PSB+KSB+50% VC) which was significantly superior to all other treatments while the minimum root yield per hectare (200.05q) was observed in treatment T_1 (RFD) which received nutrients only in inorganic form. Chemical fertilizers contain higher amounts of nutrients and are sources of readily available form of nutrients, but the fertilizer use efficiency is often low due to the inherent soil characteristics, losses and low uptake. On the other hand, the integration improves nutrient availability and uptake influences the soil physical and biological properties favourably, which reflects positively on the growth and yield of crops as reported by Isaac and Verghese, (2016) and Singh and Verma, (2011). The yield promoting effect may be ascribed to improved plant nutrition due to continuous supply of nutrients by organic manures as explained by Summer (1990) and Somani *et al*., (1990). The increase in weight may also be attributed to accelerated mobility of photosynthates from source to sink as influenced by growth hormones, released or synthesized due to organic sources as well as biofertilizers which contributed to increased root yield. These findings are in conformity to the observations made by Devlin (1973), Rabindra Kumar and Srivastava, (2006).

The experimental findings presented in (Table 2) provided a detailed account on effect of integrated nutrient management on physico-chemical parameters of soil .The data presented in table 2 revealed significant effect for soil pH due to various treatments after the harvest of crop and the lowest soil pH (7.04) was recorded in treatment T_9 (50%N+25%P&K+PSB+KSB+50%VC)which was statistically at par with treatment T_8 (50%N+25%P&K+PSB+KSB+50%FYM) and T_5 (50% N&K +25%P+PSB+50%VC).The biofertilizers and organic manures have ability to reduce the pH of surrounding environment by the production of organic acids especially gluconic acid, acetic acid, citric acid, propionic acid , glycolic acid, fumaric acid , tartaric acid, humic acid etc which might have resulted in lowering of pH. These results are in close conformity with Thornsbury *et al*., (2000), Deubel *et al*., (2000), Chatoo (2006), Chen *et al* ., (2006), Song *et al*., (2008), Meena *et al* . (2014).The data presented in (Table 2) reveal that among all treatments, highest EC (0.287dSm^{-1}) was recorded in treatment T_9 (50%N+25%P&K+PSB+KSB+50%VC) after the harvest of crop which was statistically at par with treatment T_8 (50%N+25%P&K+PSB+KSB+50%FYM)and the lowest was recorded in treatment T_1(RFD).Decrease in pH due to application of organic manures and biofertilizers (PSB &KSB) induces an acidic condition in soil which results in more solubility of salts. Since the salts present in soil becomes more mobile and conducts more electric current which results in increase in electrical conductivity. These results collaborate with Thornsbury *et al*., (2000), Sharma *et al*., (2003) and Chatoo, (2006) .The data presented in the table 2 further reveal that bulk density of soil after crop harvest significantly decreased from 1.38 g cc^{-1} in treatment T_1(RFD) to 1.10 g cc^{-1} in treatment T_9 (50%N+25%P&K+PSB+KSB+50%VC).The decrease in bulk density might be due to improvement in the structural status of soil by judicious application of bulky organic manures (VC) , biofertilizers (PSB&KSB) as well as chemical fertilizers. The results are in conformity with Martens and Frankenberger (1992), Islam *et al.*, (2012).

Table 1: Effect of Integrated Nutrient Management on Average Root Weight(g), Root Yield per plot (kg) Root yield per hectare (q) of Black carrot

Treatment	**Treatment Combinations**	**Average Root Weight (g)**	**Root Yield per plot (kg)**	**Root Yield per hectare (q)**
T_1	RFD	104.19[i]	16.67[h]	200.05[i]
T_2	50% RFD+50%FYM	111.64[h]	17.86[h]	214.32[h]
T_3	50% RFD+50% VC	115.67[g]	18.50[g]	222.08[g]
T_4	50%N&K+25%P+PSB+50%FYM	132.29[d]	21.16[d]	253.99[d]
T_5	50% N&K +25%P+PSB+50%VC	137.37[c]	21.97[c]	263.75[c]
T_6	50%N&P+25%K+KSB+50%FYM	121.24[f]	19.39[f]	232.77[f]
T_7	50% N&P +25%K+KSB+50%VC	126.24[e]	20.19[e]	242.38[e]
T_8	50%N+25%P&K+PSB+KSB+50%FYM	144.18[b]	23.06[b]	276.83[b]
T_9	50%N+25%P&K+PSB+KSB+50%VC	148.85[a]	23.81[a]	285.79[a]
	C.D (p≤0.05)	3.65	0.60	6.64
	S.E (d)	1.711	0.285	3.109

Mean values with same letters don't differ significantly

Table2 : Effect of Integrated Nutrient Management on soil physico-chemical properties

Treatments	Treatment combinations	Soil pH	Soil EC (dsm-1)	Bulk density (gcc-1)
T1.	RFD	7.18a	0.144g	1.38a
T2.	50%RFD+50%FYM	7.17ab	0.158f	1.33b
T3.	50%RFD+50%VC	7.15b	0.173e	1.29c
T4.	50%N&K+25%P+PSB+50%FYM	7.08de	0.233c	1.18e
T5.	50%N&K+25%P+PSB+50%VC	7.06ef	0.25lb	1.16e
T6.	50%N&P+25%K+KSB+50%FYM	7.1lc	0.197d	1.25d
T7.	50%N&.P+25%K+KSB+50%VC	7.10cd	0.206d	1.22d
T8.	50%N+25%P&K+PSB+KSB+50% FYM	7.05f	0.278a	1.11f
T9.	50%N+25%P&K+PSB+KSB+ 50%VC	7.04f	0.287a	1.10f
	CD (p≤0.05)	0.020	0.010	0.030
	S.E (d)	0.09	0.005	0.014

Mean values with same letters don't differ significantly

Conclusion

The integrated nutrient management exhibited a significant influence on all treatments under study over sole application of chemical fertilizers (treatment T_1). The treatments (T_4, T_5, T_6, T_7, T_8 and T_9) which received biofertilizers in addition to chemical fertilizers and organic manures are more responsive in terms of improving the yield, soil properties (physical and chemical) in comparison to the treatments that receive only chemical fertilizers and organic manures (treatment T_2 and T_3).

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RESEARCH ARTICLE

Studies on Biochemical Parameters of Pomegranate (*Punica Granatum L.)* with Special Reference to Red Aril Cultivars

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Abstract

The present investigation was conducted at Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut to find out the promising cultivars of pomegranate on the basis of biochemical perameters.The maximum level of TSS was measured in cv. Ganesh (14.71^{0}brix) followed by cv. Arakta (13.84^{0}brix) and cv. G-137 (12.71^{0}brix). Of the eight cultivars studied, significantly maximum level of total sugars was found in cv. Arakta (12.98 %) followed by cv. Mridula (12.36 %) and cv. Ganesh (12.33 %). The level of titratable acidity was found to be significantly lowest in cv. Jalore Seedless (0.30 %) followed by cv. Mridula (0.31). In the present study, maximum TSS:Acid ratio was observed in cv. Ganesh (45.96) followed by cv. G-137 (41.00) and cv. Jalore Seedless (40.73). Significantly maximum Sugar:Acid ratio was recorded in cv. Mridula (39.87) closely followed by cvs. Jalore Seedless (39.40), Ganesh (38.53) and Arakta (38.17).

Key word: Pomegranate, Red Aril, Cultivars, Fruit quality.

Introduction

Pomegranate (*Punica granatum* L.) is one of the important minor fruit crops suitable for growing in arid and semi-arid regions owing to its versatile adaptability, hardy nature, low maintenance cost and high yield. Pomegranate is liked for its cool refreshing juice, nutritional and medicinal properties. There is a growing demand for good quality fruits both for fresh use and processed products (Pruthi and Saxena, 1984). This fruit crop has wide adaptability and it grows well in tropical, sub-tropical and even temperate regions. However, in Uttar Pradesh, particularly in western region of the state, the suitable cultivar of pomegranate has not been identified for commercial cultivation so far. Unlike other major fruit crops, there are few cultivars of pomegranate which are grown commercially in India. It has been observed that performance of commercial cultivars of pomegranate is location specific and various problems like unfavourable environmental conditions, improper cultural techniques, excess moisture condition etc. lead to poor yield.

In view of above facts and considering the potential of pomegranate in the western plain zone of Uttar Pradesh, the present study was carried out to evaluate the performance of different cultivars of Pomegranate under the climatic conditions of western Uttar Pradesh on the basis of biochemical parameters.

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Materials and Methods

The present investigation was conducted at Horticutural Research Centre (HRC) of theDepartment of Horticulture, Sardar Vallabhbhai Patel University of Agriculture and Technology- Meerut, during 2015-2016.The experiment was laid out on eight cultivars of pomegranate. The experiment was conducted using randomized block design (RBD) with five replication in each cultivar/treatment. The cultivars were considered as a factor and each plant under study as a replication. Eight cultivars of Pomegranate namely G -137, Arakta, Phulerakta, Ganesh, Jalore Seedless, Mridula, Muskat Red & Bhagwa were studied. The cv. Ganesh was used as a standard cultivar to find out the performance of other cultivars over this cultivar. The data were recorded on 3 year old plants of different cultivars of pomegranate on biochemical parameters viz., Total soluble solids, Acidity and Total sugars.

Results and Discussion

Among the pomegranate cultivars studied, the soluble solids in different pomegranate cultivars ranged from 8.81 ^{0}brix to 14.71 ^{0}brix. The maximum level of TSS was measured in cv. Ganesh (14.71^{0}brix) followed by cv. Arakta (13.84^{0}brix) and cv. G-137 (12.71^{0}brix), while minimum TSS was estimated in cv. Mridula (8.81 ^{0}brix) (Table & Fig.01). Of the eight cultivars studied, significantly maximum level of total sugars was found in cv. Arakta (12.98 %) followed by cv. Mridula (12.36 %) and cv. Ganesh (12.33 %). However, the lowest level of total sugars was recorded in cv. Muskat Red (11.44 %)(Table & Fig.02). The titratable acidity analyzed in eight cultivars ranged from 0.30 % to 0.34 %. The level of titratable acidity was found to be significantly lowest in cv. Jalore Seedless (0.30 %) followed by cv. Mridula (0.31), while the highest level of titratable acidity (0.34 %) was recorded in cvs. Arakta and Bhagwa (Table & Fig.03). In the present study, maximum TSS:Acid ratio was observed in cv. Ganesh (45.96) followed by cv. G-137 (41.00) and cv. Jalore Seedless (40.73), while cv. Mridula (28.41) had minimum TSS:Acid ratio (Table & Fig.04). Significantly maximum Sugar:Acid ratio was recorded in cv. Mridula (39.87) closely followed by cvs. Jalore Seedless (39.40), Ganesh (38.53) and Arakta (38.17). However, cv. Bhagwa had lowest Sugar:Acid ratio (33.88) (Table & Fig.05). The highest soluble solids contents were estimated from the fruits of cv. Ganesh (14.71 ^{o}brix), while cv. Mridula had lowest level of soluble solids (8.81 ^{o}brix). A number of reports were available which indicated that chemical composition of pomegranate fruit was significantly varied from cultivar to cultivar. The total soluble solids content in pomegranate cultivars measured by Meena, 2003;

Wani *et. al.*,2014; Akbarpoul *et. al*., 2009 were also in the range of 11.11 to 16.38 ^{o}brix, 12.82 to 13.87 ^{o}brix and 15.17 to 22.03 ^{o}brix, respectively. The variation in TSS among pomegranate cultivars depends upon the stage of maturity and ripening of fruit, genetic constitution of cultivars, orchard management practices, environmental conditions etc. Even in the same cultivar wide variation in TSS is measured at different stages of maturity and ripening of fruit. Increase in TSS in fruit during ripening might be associated with the transformation of pectin substances, starch, ripening hemi-cellulose or other polysaccharides in soluble sugar and dehydration of fruit (Singh, 2009). The levels of total sugars in the present study ranged from 11.44 to 12.98 %. Significantly maximum level of total sugars was estimated in cv. Arakta, while cv. Muskat Red had lowest level of total sugars. Wide variation in total sugars in fruits of different pomegranate cultivars was also reported by Meena, 2003; Wani *et. al.,* 2014. The level of total sugars estimated by these researchers was in the same range (8.55 to13.76 %) as estimated in the present study. In general, the variation in these chemical compositions might be either due to genetic makeup of the different genotypes, photosynthetic efficiency of different cultivars or due to variation in agro-climatic condition as reported by Khodadi *et.al.*,1990; Bist *et.al*.,1994 and Singh 2009.

The level of titratable acidity in the present study was lowest in cv. Jalore Seedless (0.30 %), while highest level of titratable acidity was recorded in cvs. Bhagwa and Arakta (0.34 %). In other cultivars (G-137, Phulerakta, Ganesh, Muskat Red and Mridula), the level of titratable acidity ranged from 0.31 to 0.33 %. Meena, 2003; Akbarpour *et. al.* 2009 while studying the fruit quality attributes had also observed wide variation in acidity content in different pomegranate cultivars. However, acidity content did not vary significantly in cvs. Arakta and Bhagwa in the present study. Data showed significant variation in TSS/Acid ratio in different pomegranate cultivars studied in the present study. Meena 2003 also observed significant variation in TSS/Acid ratio (11.82 to 47.71 %) in pomegranate cultivars.

Based on the results obtained on biochemical attributes of different pomegranate cultivars in the present study, cvs. Ganesh and Arakta were found promising over other cultivars in respect of TSS, acidity and total sugar contents in fruits.

Table 1: Total soluble solids in pomegranate cultivars

S. No.	**Cultivars**	**Total soluble solids (^{0}brix)**	**Increase or decrease in total soluble solids over Ganesh**	**Percent increase (+) or decrease (-) in total soluble solids over Ganesh**
1.	Ganesh	14.71	-	-
2.	Arakta	13.84	(-) 0.87	(-) 05.91
3.	Phulerakta	11.75	(-) 2.96	(-) 20.12
4.	G -137	12.71	(-) 2.00	(-) 13.59
5.	Jalore Seedless	12.22	(-) 2.49	(-) 16.92
6.	Mridula	08.81	(-) 5.90	(-) 40.10
7.	Muskat Red	10.52	(-) 4.19	(-) 28.48
8.	Bhagwa	10.11	(-) 4.60	(-) 31.27
Mean		**11.83**	-	-
S.Em. ±		0.2857	-	-
C.D at 5 %		0.8276	-	-

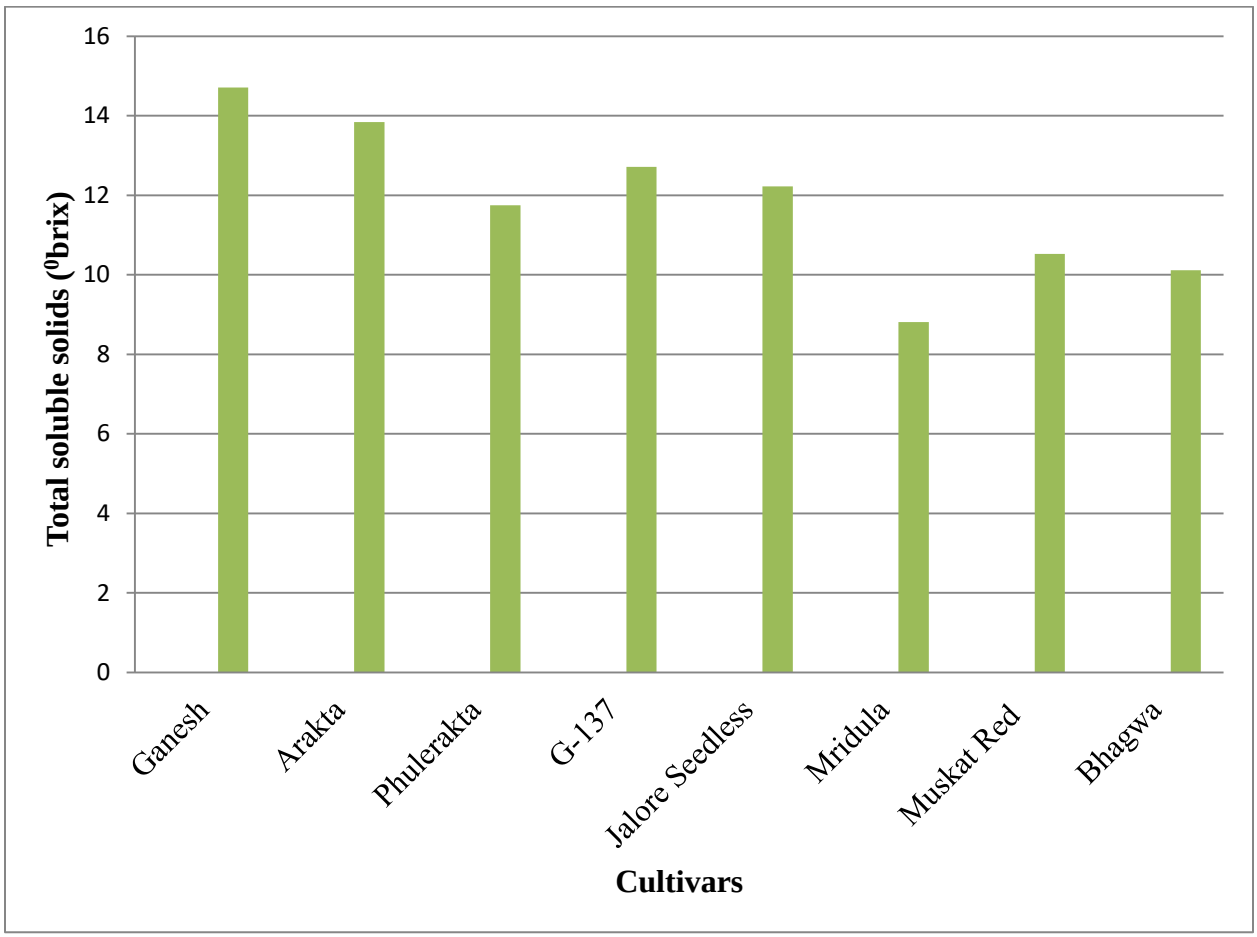

Fig.1.:Total soluble solids in pomegranate cultivars

Table 2: Total sugars content in pomegranate cultivars

S. No.	Cultivars	Total sugar (%)	Increase or decrease in total sugars over Ganesh	Percent increase (+) or decrease (-) in total sugars over Ganesh
1.	Ganesh	12.33	-	-
2.	Arakta	12.98	(+) 0.65	(+) 5.27
3.	Phulerakta	11.84	(-) 0.49	(-) 3.97
4.	G -137	11.62	(-) 0.71	(-) 5.75
5.	Jalore Seedless	11.82	(-) 0.51	(-) 4.13
6.	Mridula	12.36	(+) 0.03	(+) 0.24
7.	Muskat Red	11.44	(-) 0.89	(-) 7.21
8.	Bhagwa	11.52	(-) 0.81	(-) 6.56
Mean		**11.98**	-	-
S.Em. ±		0.19521	-	-
C.D at 5 %		0.56549	-	-

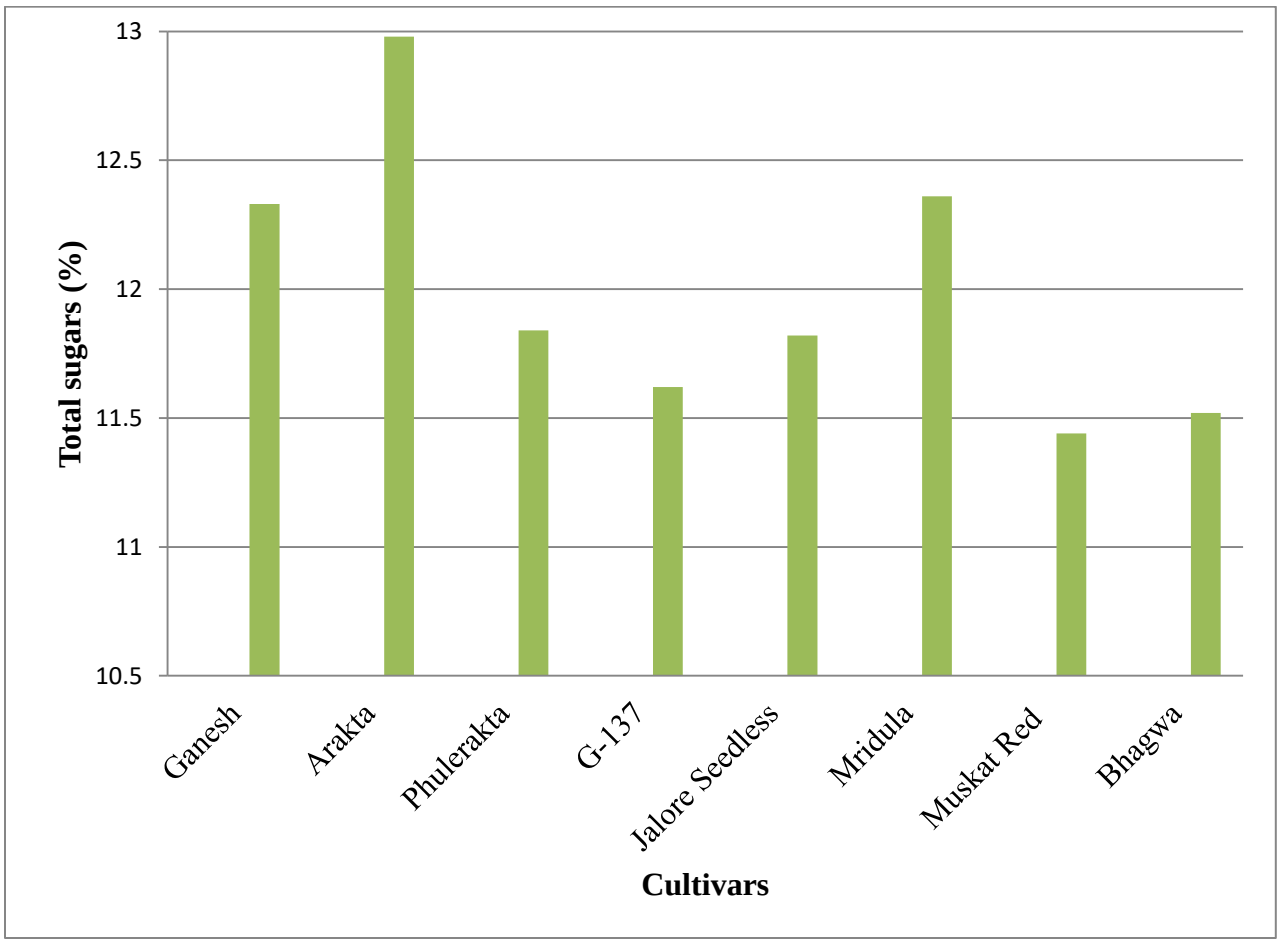

Fig. 2: Total sugars content in pomegranate cultivars

Table 3: Titratable acidity content in pomegranate cultivars

S. No.	Cultivars	Titratable acidity (%)	Increase or decrease in titratable acidity over Ganesh	Percent increase (+) or decrease (-) in titratable acidity over Ganesh
1.	Ganesh	0.32	-	-
2.	Arakta	0.34	(+) 0.02	(+) 6.25
3.	Phulerakta	0.33	(+) 0.01	(+) 3.12
4.	G -137	0.31	(-) 0.01	(-) 3.12
5.	Jalore Seedless	0.30	(-) 0.02	(-) 6.25
6.	Mridula	0.31	(-) 0.01	(-) 3.12
7.	Muskat Red	0.32	0	0
8.	Bhagwa	0.34	(-) 0.02	(-) 6.25
Mean		**0.32**	-	-
S.Em. ±		0.01	-	-
C.D at 5 %		0.02	-	-

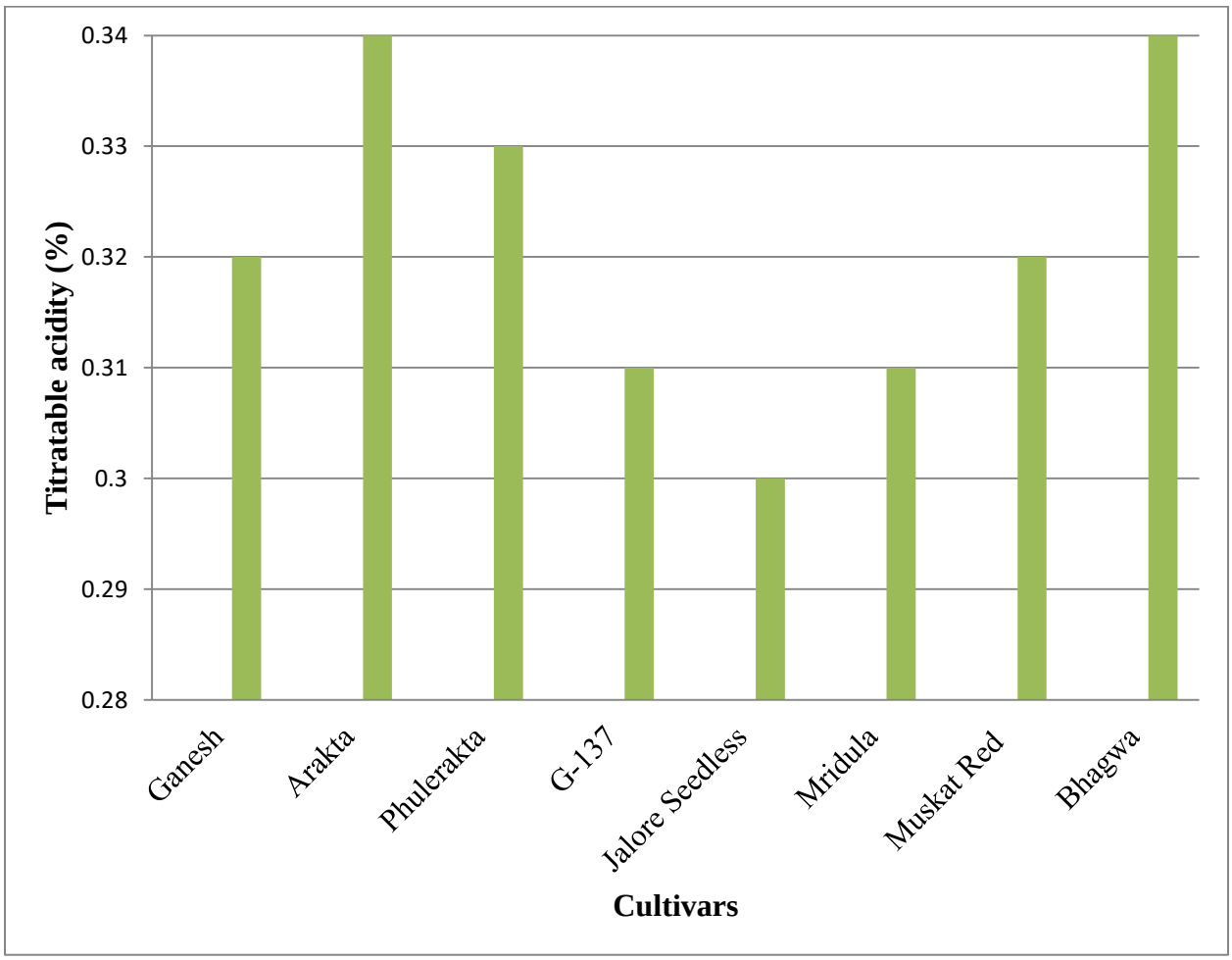

Fig 3: Titratable acidity content in pomegranate cultivars

Table 4: TSS: Acid ratio in pomegranate cultivars

S. No.	Cultivars	TSS:Acid ratio	Increase or decrease in TSS:Acid ratio over Ganesh	Percent increase (+) or decrease (-) in TSS:Acid ratio over Ganesh
1.	Ganesh	45.96	-	-
2.	Arakta	40.70	(-) 05.26	(-) 11.44
3.	Phulerakta	35.60	(-) 10.36	(-) 22.54
4.	G -137	41.00	(-) 04.96	(-) 10.79
5.	Jalore Seedless	40.73	(-) 05.23	(-) 11.37
6.	Mridula	28.41	(-) 17.55	(-) 38.18
7.	Muskat Red	32.87	(-) 13.09	(-) 28.48
8.	Bhagwa	29.73	(-) 16.23	(-) 35.31
Mean		**36.87**	-	-
S.Em. ±		0.495	-	-
C.D at 5 %		1.433	-	-

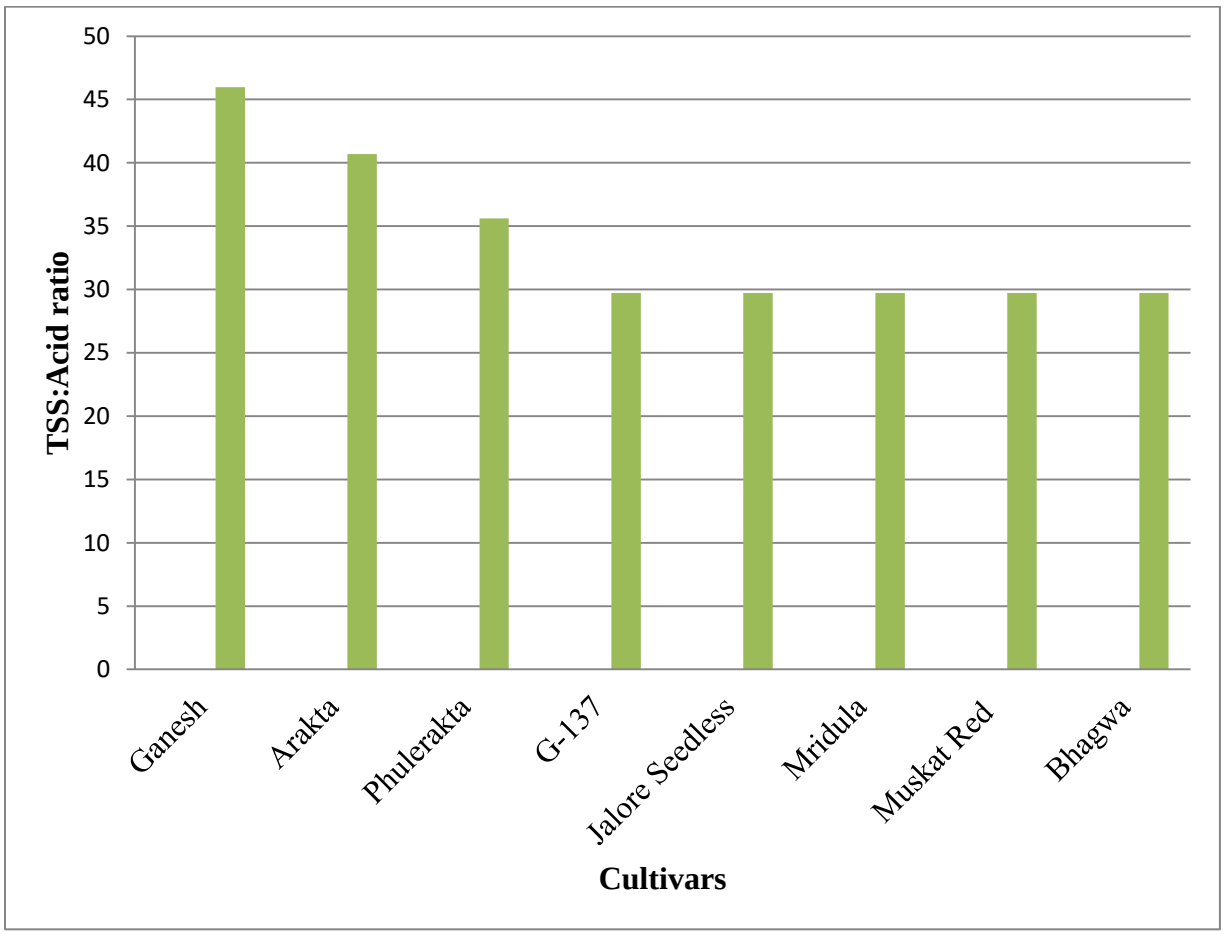

Fig 4: TSS:Acid ratio in pomegranate cultivars

Table 5: Sugar: Acid ratio in pomegranate cultivars

S. No.	Cultivars	Sugar:Acid ratio	Increase or decrease in Sugar:Acid ratio over Ganesh	Percent increase (+) or decrease (-) in Sugar:Acid ratio over Ganesh
1.	Ganesh	38.53	-	-
2.	Arakta	38.17	(-) 0.36	(-) 00.93
3.	Phulerakta	35.87	(-) 2.66	(-) 06.90
4.	G -137	37.48	(-) 1.05	(-) 02.72
5.	Jalore Seedless	39.4	(+) 0.87	(+) 02.25
6.	Mridula	39.87	(+) 1.34	(+) 03.47
7.	Muskat Red	35.75	(-) 2.78	(-) 07.21
8.	Bhagwa	33.88	(-) 4.65	(-) 12.06
Mean		**37.36**	-	-
S.Em. ±		0.00242	-	-
C.D at 5 %		0.00701	-	-

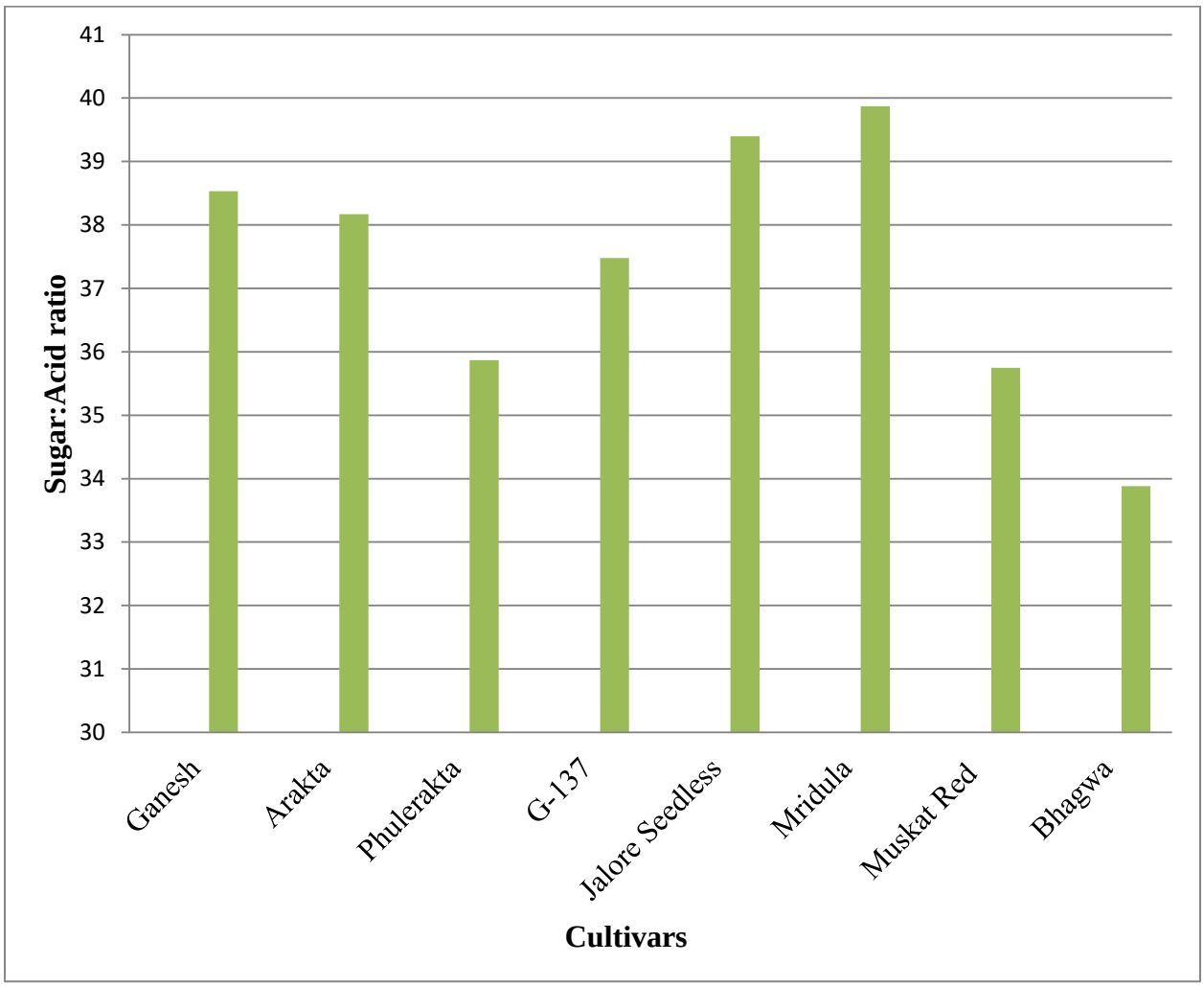

Fig 5: Sugar: Acid ratio in pomegranate cultivars

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RESEARCH ARTICLE

Growth and Yield of Chilli (*Capsicum annum* L.) as Affected by Different NADEP Compost and Chemical Fertilizers

Vipin Kumar*, Arvind Kumar, Manoj Kumar Singh and Mukesh Kumar

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Abstract:

The Utilization of organic fertilizer such as compost and manure fertilizer, they can ameliorate soil texture as well as supply macro and micronutrients needed by the chilli (*Capsicum annum* L.). The research aimed to study the effect of organic and inorganic fertilizers combination usage to plant growth and yield of chilli. This research arranged in randomized block design in triplicate with eleven treatments, which comprised NPK through chemical fertilizers and in combinations with different type of NADEP compost. Pant C-1 of chilli variety was used in this experiment. Observation on nine growth and yield characters like Plant height, No. of leaves per plant, Days to 50% flowering, days to first fruiting, length of fruit, diameter of fruit, number of fruits per plant, weight of fruit per plant and yield per hectare were taken. The results showed that combination of NADEP compost and chemical fertilizers gave the highest growth and yield. The best treatment in this study was combination of NADEP compost and chemical fertilizers (20:40:50 kg/ha of NPF +10 Ton of NADEP 1) per planting hole with the result obtained at 91.2 Q/ha yield and other yield attributing characters.

Keywords: Chilli, NADEP Compost, Chemical Fertilizers

Introduction

Chilli (*Capsicum annum* L.) belongs to the family solanaceae. It is one of the most valuable commercial annual spice crop grown in India. It is rich source of vitamin A and C. Chilli fruits having deep red colour, without pungency are used as paprika colour is the principal criterion for assessing its quality. It is grown to as green tender fruits and consumed as vegetables, salad and delicious hot spicy pickles. Beside these, the ripened fruits are also used as dry red chilli power. As spice, it is also used in different dishes. Chilli is famous for its pleasant aromatic flavour, pungency and high colouring substance. Among the spices, dry Chilli contributes the major share in India. Chilli is widely cultivated throughout the Western Uttar Pradesh. (Anonymous , 2018).

Fertilizer application has a pronounced influence on plant development, growth and marketable yield of many vegetable crops production like chillies. (Iqbal *et al.*, 2013). Inorganic fertilizer or fertilizer is any material of natural or synthetic origin (other than liming materials) that is applied to soils or to plant tissues (usually leaves) to supply one or more plant nutrients essential to the growth of plants. This also depends on its soil fertility as well as organic things such as humic acid, seaweed and worm castings. (Ghosh *et al.,* 2004).Yet today, farmers uses the various chemical fertilizers without any integrated use of manures which causes imbalance of nutrient uptake which resulting the yield of chilli is quite low and uncertainly due to imbalance of manure and fertilizers schedule. The present investigation was carried out to find out optimum dose of compost and chemical fertilizers to increase the sustainable yield potential.

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Material and Methods

The present investigation was carried out during 2015- 2016 at Horticultural Research Centre, of Sardar Vallabhbhai Patel University of Agricultural & Technology, Meerut to standardize the optimum dose and formulation of NADEP compost in combination with chemical fertilizers for obtaining best growth, flowering and production of yield. The experiment comprised of eleven treatments consisting of different type of NADEP compost made from different waste materials (Table 1) in combination with two levels of NPK, and the combination of was laid out in randomized block design with three replication. Pant C-1 variety of chilly was taken for the experiment. The treatments were as follows:

T1: 10:20:25 kg/ha of NPK + 10 t NADEP (N1)

T2: 10:20:25 kg/ha of NPK + 10 t NADEP (N2)

T3: 10:20:25 kg/ha of NPK + 10 t NADEP (N3)

T4: 10:20:25 kg/ha of NPK + 10 t NADEP (N4)

T5: 10:20:25 kg/ha of NPK + 10 t NADEP (N5)

T6: 20:40:50 kg/ha of NPK + 10 t NADEP (N1)

T7: 20:40:50 kg/ha of NPK + 10 t NADEP (N2)

T8: 20:40:50 kg/ha of NPK + 10 t NADEP (N3)

T9: 20:40:50 kg/ha of NPK + 10 t NADEP (N4)

T10: 20:40:50 kg/ha of NPK + 10 t NADEP (N5)

T11: 20:40:50 kg/ha of NPK Through chemical fertilizers (Control)

Table 1: Nutrients composition of compost obtained from different types NADEP composting.

Sl. No	Treatments	N (%)	P_2O_5 (%)	K_2O (%)	pH	EC (ms)
N_1	1500 kg. Dung + 420 kg. Dry cuttings of Parthenium + 1400 kg. Soil + 2000 lit. Water	2.18	0.41	1.42	8.56	0.355
N_2	1300 kg. Dung + 720 kg. green cuttings of parthenium+ 1200 kg. Soil + 1800 lit. Water	2.37	0.53	1.79	9.04	0.480
N_3	1500 kg. Dung + 420 kg. Dry cuttings of Bhang + 1400 kg. Soil + 2000 lit. Water	1.24	0.23	1.24	8.56	0.387
N_4	1300 kg. Dung + 720 kg. green cuttings of Bhang + 1200 kg. Soil + 1800 lit. Water	1.69	0.10	1.08	8.00	0.402
N_5	1500 kg. Dung + 420 kg. Waste material (Crop residue, leaves etc.) + 1400 kg. Soil + 2000 lit. Water	2.10	0.42	1.89	8.69	0.410

Basal application of ½ dose of N in the form of urea, full dose of P in the form of single super phosphate (S.S.P.) and K in the form of murate of potash, and NADEP compost as per the nutrient status was given with broad cast method. Rest half dose of N was applied 30 days after transplantation. During the experimentation, Five plants under each treatment combination were randomly selected and tagged for recording the observation on growth and yield characters (whenever required).

Results and Discussion

Growth of Chilli

Maximum plant height (52.6 cm) and number of leaves per plant (68.5) were also found with the T6 (20:40:50 kg/ha of NPK + 10 t NADEP (1)) whereas these were minimum 47.2, and 44.7 for number of leaves per plant and height of plant respectively in the controlled treatment T11 (20:40:50 kg/ha of NPK). Masud, (2009) ; Dorji *et al.,* (2011) found that the growth parameters were significantly affected by number of nodulation /plant, which is increased by inoculation treatments through organic compost.

Levels and type of NADEP compost had significant effect on growth and fruiting. Results shown in table 1 indicating that, minimum days taken to flowering (47.30 days) and fruiting (56.3 days) were recorded in T6 (20:40:50 kg/ha of NPK + 10 t NADEP (1) followed treatment 1(10:20:25 kg/ha of NPK + 10 t NADEP (1) and treatment 4 (10:20:25 kg/ha of NPK + 10 t NADEP (4) respectively for both flowering and fruiting characters. The deficiency of major nutrients stunted the plant growth, resulting the maximum days taken to flowering. Very optimum dose of NPK and combination with NADEP compost reduce the days taken to flowering up to a certain limit and vice versa. Similar results were coated by Naeem *et al*., (2002) in chilli.

Fruit size was also affected by the combination of NADEP compost and chemical fertilizers. Maximum length of fruit (7.2 cm) and diameter of fruit (4.2 cm) were also found with the T6 (20:40:50 kg/ha of NPK + 10 t NADEP (1) whereas these were minimum 3.9 and 3.0 for length of fruit and diameter of fruit respectively in the controlled treatment T11 (20:40:50 kg/ha of NPK). This might be due to enhanced production of growth promoting substances like gibberellic acid, Indole acetic acid and plant growth substances. N, P, K + organic manure increasing the soil biomass and helping the growth and development and enzymatic and hormonal activities of plants. The same results has been shown by Chandraprabha *et al.*,(2018) ; Abbasi *et al.,* (2015).

Yield of Chilli

Data recorded on yield characters indicated that, NADEP-compost levels also influenced the number of fruits per plant, fruit weight and ultimately yield of chilli. Number of fruits per plant (62.2) weight of fruit per plant (126.4 gm) and yield of fruits (91.2 Q/ha) were obtained with T6 (20:40:50 kg/ha of NPK + 10 t NADEP (1) followed by T1 (10:20:25 kg/ha of NPK + 10 t NADEP (N1)) and T7 (20:40:50 kg/ha of NPK + 10 t NADEP (N2) treatments under the present study and these were was minimum for number of fruits per plant (45.1), weight of fruits per plant (90.20 gm) and yield of fruits (68.5 q/ha) in controlled treatment T11 (20:40:50 kg/ha of NPK). Proper nutrients promote vigorous growth of the plant which ultimately increase the size of weight and size of fruit, which confirms the observation of Deore *et. al.,* (2008). The organic source applied to the soil through biofertilizers have influenced the soil nutrient availability through better microbial activities and releasing the nutrients from the soil and help in the process of absorption of ample nutrients and its utilization by the plants due to influence on yield. Similar results were reported by Masud, (2009) and Dorji *et al.,* (2005).

Conclusion

The above results showed that the pure chemical fertilizers could not result in highest yield and growth of chilli. The integration of biofertilizers along with chemical fertilizers in an appropriate dose not only has a positive effect on the yield attributes of chilli but also increasing soil fertility status and ecofriendly as well. A comparative study of the present findings led to the conclusion that sowing of chilli with the application of NADEP compost and chemical fertilizers @ 20:40:50 kg/ha of NPK + 10 t NADEP (1) kg/ha was found

most effective to best growth and yield of chilli crop under North west plain zones of Uttar Pardesh. As far as NADEP preparation is concerned, it may be concluded that parthenium may be a good source to enrich the NADEP composting.

Table2: Effect of NADEP-compost on yield characters of Chilli (*Capsicum annum* L.)

Sl No	Treatment	Plant Height (cm)	No. of leaves per plant	Days to 50% flowering	Days to first fruiting	Length of fruit (cm)	Diameter of fruit (cm)	Number of fruits per plant	Weight of fruit per plant (gm)	Yield of fruit q/ha
1	10:20:25 kg/ha of NPK + 10 t NADEP (1)	51.3	66.1	48.2	57.5	5.8	4.0	59.1	118.0	86.5
2	10:20:25 kg/ha of NPK + 10 t NADEP (2)	46.0	56.2	54.2	62.1	5.5	3.5	53.6	102.1	73.0
3	10:20:25 kg/ha of NPK + 10 t NADEP (3)	47.1	63.4	52.9	63.4	5.2	3.5	55.7	112.2	78.9
4	10:20:25 kg/ha of NPK + 10 t NADEP (4)	50.0	64.8	50.3	59.1	5.9	3.7	56.8	114.2	82.2
5	10:20:25 kg/ha of NPK + 10 t NADEP (5)	48.9	64.1	52.1	60.2	5.5	3.6	56.4	113.9	81.4
6	20:40:50 kg/ha of NPK + 10 t NADEP (1)	52.6	68.5	47.3	56.3	7.2	4.2	62.2	126.4	91.2
7	20:40:50 kg/ha of NPK + 10 t NADEP (2)	50.2	65.0	49.0	58.1	6.2	3.8	57.6	115.7	84.3
8	20:40:50 kg/ha of NPK + 10 t NADEP (3)	46.5	59.3	55.1	61.2	4.7	3.4	54.2	106.5	75.6
9	20:40:50 kg/ha of NPK + 10 t NADEP (4)	47.0	62.0	53.1	60.2	4.5	3.4	55.0	108.5	76.5
10	20:40:50 kg/ha of NPK + 10 t NADEP (5)	45.2	51.9	56.7	65.2	4.1	3.5	52.1	100.0	72.8
11	20:40:50 kg/ha of NPK	44.7	47.2	57.2	68.2	3.9	3.0	45.1	90.2	68.5
	CD (5%)	**2.07**	**3.08**	**2.28**	**3.00**	**0.20**	**0.26**	**2.29**	**4.06**	**2.11**

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RESEARCH ARTICLE

Studies on Release of Fluconazole from different Water Soluble PEG Ointment formulations

Ranu Agrawal, Swati Singh and R. K. Soni

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Abstract

Antifungal ointment is a topical medication designed to treat fungal infections. Antifungal ointments work by exploiting differences between mammalian and fungal cells to kill the fungal microorganism without dangerous effects on the host. In the present study PEG was used for the preparation of different formulation and their viscosity was determined using Brook field Viscometer and diffusion rate was studied by *in vitro* release through dialysis. Antifungal activity was also determined using agar plate diffusion assay. In water soluble bases Polyethylene Glycols ointment is the only pharmaceutical preparation. In this experiment F2 formulation showed maximum fluconazole release within two hours. *In vivo* testing method also showed that the F2 formulation has inhibited the growth of fungus. Since in *in vivo* condition plates were incubation for the 24-48 hours so there is not very much differences in measured inhibition zone diameter.

Key words: Ointment, Polyethylene glycol, Fluconazole, Antifungal activity, Formulation.

Introduction

There are varieties of vehicles ranging from solids to semisolids and liquid penetration as creams, gels, ointments, pests, aerosols and solutions used in topical products for the treatment of dermatological diseases (Mekkawy *et.al.*, 2013). Antifungal ointment is a current medication which is designed to treat fungal infections for external purposes applying directly on the skin. Topical formulations like Cream and ointments are recommended for better patient observance and hence become more satisfactory to patients(David and Anthen, 1990). Cream is an emulsified semisolid dosage formulation which contains more than 20% water and volatiles substances or more than 50% of hydrocarbon, waxes or polyethylene glycols as the vehicle for external application to the skin(Shrivastava, 2006). Two types of emulsion may be made i.e, oil in water cream, in this water is as continuous phase and water in oil, in that oil is as continuous phase. Creams are non transparent, viscous and mild-greasy to non greasy, tend to generally evaporate or may be absorbed when rubbed onto the surface.

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Ointments are semisolid substances prepared for application to the skin. They may be oleaginous e.g., white ointment; they can be formed without oleaginous substances for example polyethylene glycol ointment, or they may be prepared as emulsions of fatty or wax which contain relatively high proportion of water e.g., hydrophilic ointment.Generally, cream is chosen by some investigators in azole group with different formulations. (Muynck and Remon, 1987 ; Ismail *et al.*, 1990; Shiva and *et al.*, 2009). The azole antifungals such as ketoconazole or itraconazoledepletes ergosterol synthesis in fungal disruption(Shecher *et al.*,1990) and can be both substrates and inhibitors of the P-glycoprotein, which (among other functions) excretes toxins and drugs into the intestines.[9] Azole antifungals also are both substrates and inhibitors of the cytochrome P450 family CYP3A4 (Russell *et al.*, 2010) causing increased concentration when administering, for example, calcium channel blockers, immunosuppressants, chemotherapeutic drugs, benzodiazepines, tricyclic antidepressants, macrolides and SSRIs.Antifungalointment are available, with relatively small concentrations of an active ingredient which is antifungal in nature as econazole, tioconazone, clotrimazone, and miconazole. Antifungal ointment works by attacking the fungus itself, while trying to minimize the damage done to the person using the ointment. Because there are many different types of fungal infections occur so many different types of antifungal creams are available. Generally a small amount will be applied, and it may be left on for a brief time and then washed off, or it may be left on for an extended period of time until it washes off on its own. Some antifungal creams may also be intended for internal application, as is the case with creams used to treat yeast infections.

One set of major concerns for environmental hazard is, like that for human hazard, associated with mycotoxin production(Samson *et. al.*, 2004; Skvens, 2007). Toxins from *A. niger*may affect other vertebrates and plants as well. There is one early report of crown rot of peanuts by *A. niger* under specific growth conditions. However, it is not a significant pathogen in the environment. In addition, *A. niger* is one of many common place spoilage-associated fungi, which can cause severe economic effects.

In the present study PEG was used for the preparation of different formulation (Ugrine *et al.*, 1989; farouk *et al.*, 1989). In water soluble bases Polyethylene Glycols ointment is the only pharmacopeial preparation. Polyethylene Glycols (PEGs) which are known as macrogels are widely used in topical pharmaceutical formulations since this chemicals are stable,

hydrophilic substances that are essentially nonirritant to the skin and easily removed from the skin by washing. The viscosity was determined of different formulations. Antifungal activity of fluconazole was determined using these PEG formulations.

Materials And Methods

Materials

Chemical: Fluconazole (Cipla, Manufactured by Cipla Ltd.), Dialysis membrane 110 (HiMedia) membrane cut off 12000 to 14000 (HiMedia Laboratories Pvt Ltd.). Propylene glycol, Polyethene Glycol (PEG) 400, Polyethene Glycol 4000 (PEG) (Sisco Research Laboratories Pvt. Ltd., Mumbai)

Microorganism: Fungus species- *Aspergillus niger*.This species were procured from the department of Botany, CCS University Meerut and Department of Microbiology CCS University, Meerut. These were grown in our laboratory on SDA media.

Preparation of fluconazole gel formulation

The composition of the prepared ointment and cream formulation bases containing 1% W/W fluconazole is shown in Table 1 and Figure 1.

Table 1: Composition of the prepared ointment formulation containing 1% Fluconazole

Composition %	PEG Ointment Bases				
	F1	F2	F3	F4	F5
Propylene glycol	20	0	15	10	5
PEG 4000	20	40	25	30	35
PEG 400	60	60	60	60	60

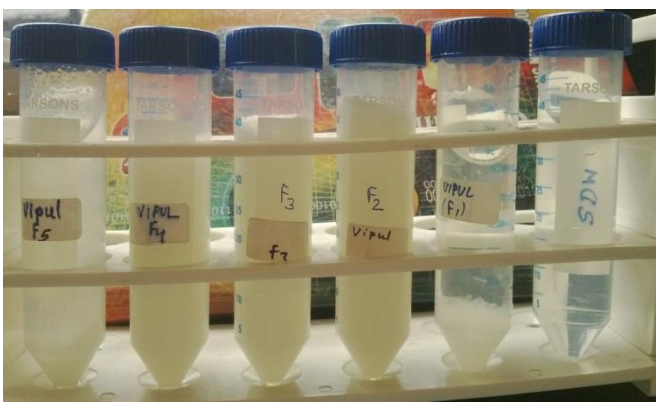

Figure 1. Prepared ointment formulations

Preparation of water soluble ointment

The specified concentration of polyethylene glycol (PEG) 4000 was melted in a porcelain dish over a boiling water bath. PEG 400 was heated to approximately temperature and added to the melted PEG 4000. The mixture was then removed from heat and stirred. Then, fluconazole (1% w/w) dissolved in 20% propylene glycol (which is slightly heated) was added to the PEG's mixture and stirred.

Evaluation of the prepared fluconazole gel formulation

Viscosity

The viscosity of the prepared gel formulations was determined using BrookField viscometer (Chrisopher and Brain, 2010). The viscosity was measured in centipoises (cps) at 50 rpm for 1 minute and temperature 32°C using spindle no. 5.

In vitro release studies

The *In vitro* release of fluconazole from the prepared gel formulations was studies using dialysis method (Luo *et al.*, 2011). A one gram sample of each formulation was accurately weighed and placed on a semi permeable cellophane memebrane. Cellophane membrane was charged in boiled distilled water until to occupy a circle of 2.5 cm diameter. The one end of membrane was tightly closed with the thread and the material was poured into the membrane. After filling the dialysis tubing the other end was also tied with the thread. Both ends were tied on the outside of the beaker and dialysis tubing was immersed into the 25 ml phosphate buffer pH 7.4 which is the released medium (donor compartment). Phosphate buffer in the beaker was stirred on the magnetic stirrer. The system was maintained for 2 hours at 37°C±2°C. Samples of 5 ml were withdrawn at intervals of 0.25, 0.5, 0.75, 1.0, 1.5 and 2 hours. The volume of each sample was replaced by the same volume of fresh buffer to maintain constant volume. Samples were analysed for fluconazole content spectrophotometrically at λ_{max} 261 nm against phosphate buffer blank.

In vitro antifungal activity

2.5.1 SDA medium and SD broth was prepared for the growth of fungi as standard protocol. Saline solution (0.85% NaCl) was prepared.

Subculturing: After incubation, appearance of the discrete, wells separated colonies has been examined; the next step is to subculture some of the cells from one of the colonies to separate agar plates with sterilized loop for further examination and use. Each of these new cultures represents the growth of single species and it is called a pure or stock culture.

Determination of zone of inhibition

Zone of inhibition was determined by using Agar cup plate method (Boyan *et al.*, 2008). The *in vitro* antifungal activity of the PEG ointment bases against *Aspergillus niger* was studied. Agar cup plate method is a simple method to evaluate the effectiveness of compound against the selected test organism. 1ml of spore suspension was mixed in 10 ml SDA broth and its 1 ml was spread over solidified SDA agar petriplate. Than after wells were created with the help of sterile cork borer a porer of size 1 cm and filled with 0.5 gm of each formula. The plate was incubated at 30°C±2°C for 2 days. After incubation agar plates were examined for the zone of inhibition diameter which is an indicator for the antifungal activity. Plain formulation (normal saline) was also tested as a positive

growth control result. The mean values of zone of inhibition diameter of two plates were calculated.

Statistical analysis

All studies were performed in duplicate and the values were expressed as mean ± Standard Deviation. The data were analysed by one way ANOVA at a significance level of 0.05.

RESULT AND DISCUSSION

Evaluation of the prepared Fluconazole Gel Formulations

Viscosity of the different PEG based formulation was measured. The viscosity is illustrated in Figure 2. The data has been given in Table 2.0. According to data F2 formulation is most viscous. This formulation does not contain propylene glycol and contains 40% PEG 4000. F1 formulation which has 20% propylene glycol and 20% PEG 4000 is least viscous. Viscosity of formulations decreased through decreasing percentage of PEG 4000 from 40% to 20%.

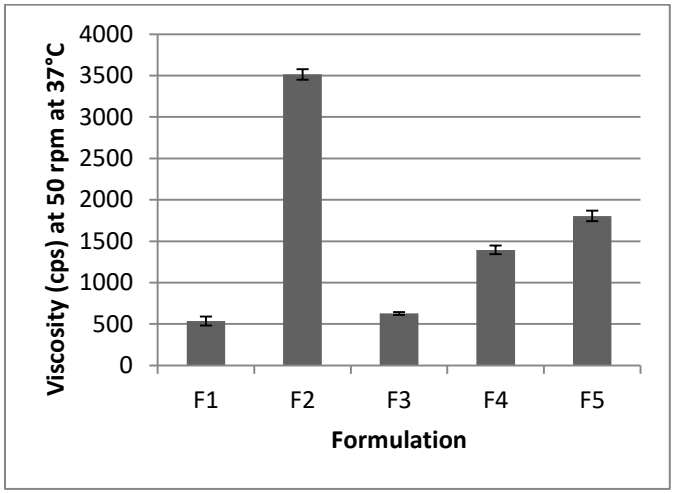

Figure 2: Viscosity of different formulation of PEG based ointment

Table 2: Viscosity of different ointment formulations by Brook filed Viscometer at 32°C and 50 rpm

S. No.	Formulation name	N1 Value	N2 Value	Average ± SD
1	F1	500	576	538 ±53.74
2	F2	3470	3560	3515±63.64
3	F3	616	640	628±16.97
4	F4	1360	1432	1396±50.911
5	F5	1760	1850	1805±63.63

***In vitro* release studies**

The percentage of fluconazole from water soluble PEG based ointment was released over a period of two hours from the prepared ointment containing 1% w/w fluconazole is shown in figure 3. Absorbance data recorded at different time interval have been shown in Table 3. All data suggested that the diffusion is present is all formulations. But diffusion rate is different. These data revealed, F2 formulation that has highest value of PEG 4000 showing maximum absorbance of outer compartment and the absorbance F5, F4 and F3 is respectively lower. F2 formulation diffusion is constant at 1.5 hrs and 2 hrs. In all formulation maximum diffusion has been achieved at 1.5 hrs.

Table 3 :*In vitro* release studies

Formulations Names	Absorbance at λ_{max} 261nm wavelength at different time intervals					
	0.25 hrs	0.5 hrs	0.75 hrs	1 hrs	1.5 hrs	2 hrs
F1	0.044	0.077	0.090	0.093	0.128	0.130
F2	0.057	0.079	0.117	0.156	0.189	0.188
F3	0.017	0.034	0.059	0.079	0.085	0.072
F4	0.057	0.086	0.108	0.114	0.123	0.118
F5	0.016	0.039	0.066	0.097	0.126	0.136

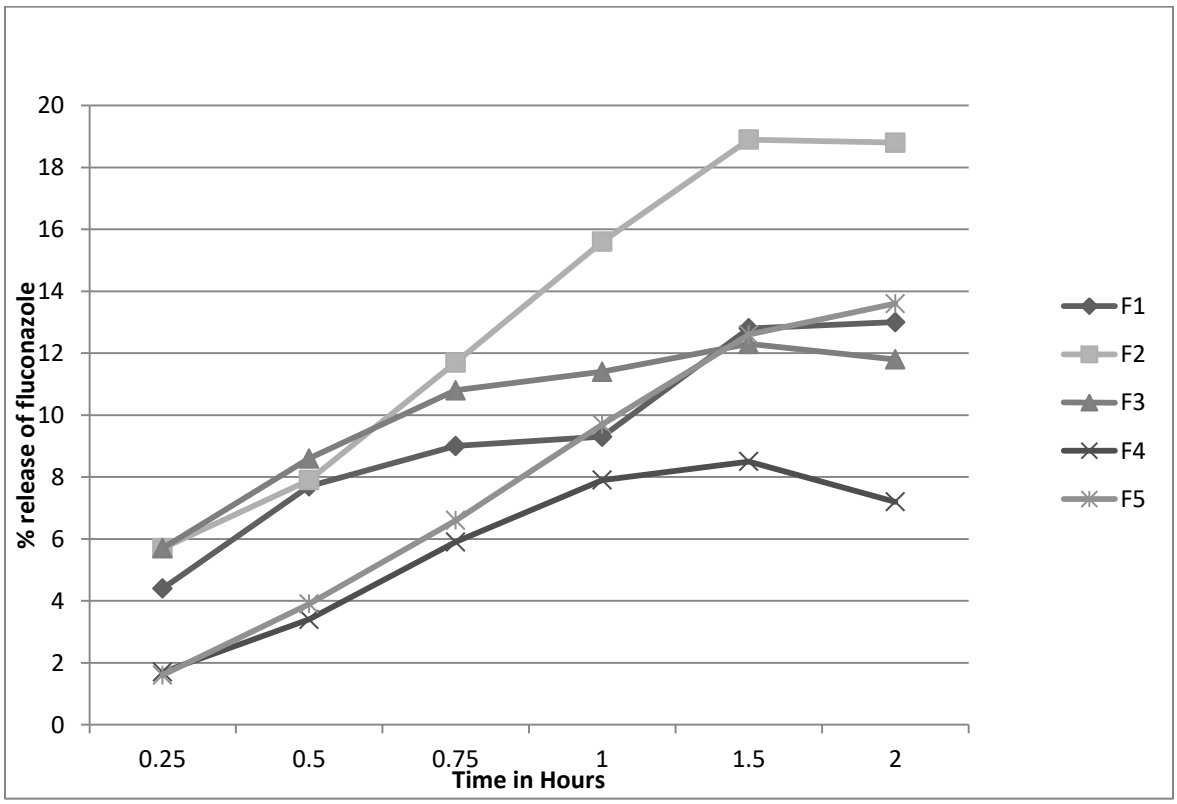

Figure 3: % release of fluconazole from different PEG formulations

***In vitro* antifungal activity**

The antifungal activity of all formulation F1 to F5 having 1% fluconazole w/w was determined against fungus *Aspergillus niger*. Antifungal activity was determined by Agar cup plate method. Figure 5 showed the petri plates showing zone of inhibition in presence of all formulations. The antifungal activity is described in Table 4.

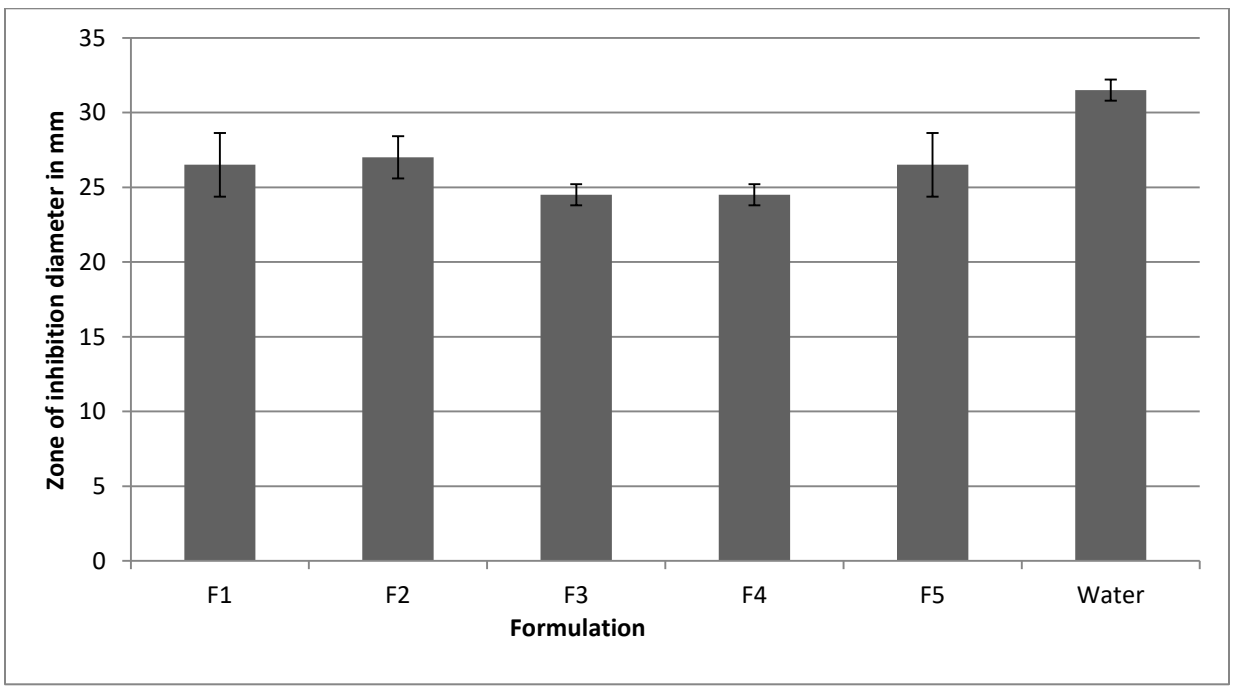

Figure 4: *In vitro* antifungal activity against A*spergillus niger* of different formulations using agar cup-plate method.

Table 4: *In vitro* antifungal activity against A*spergillus niger* of different formulations using agar cup-plate method.

Formulation name	Measurement of zone of inhibition in mm		
	N1	N2	Average ± SD
F1	28	25	26.5±2.121
F2	26	28	27.0±1.41
F3	25	24	24.5±0.707
F4	28	24	24.5±0.707
F5	28	25	26.5±2.121
Water only	32	31	31.5±0.707

All illustrated in **Table 3** the tested formulation exhibited a good growth inhibition zone for test fungus. It was found the formulation F1 have showed also a good fungal growth inhibition. There is no longer difference among all formulation to show normal growth inhibition. All data have been described also in the graphical format (Figure 4).

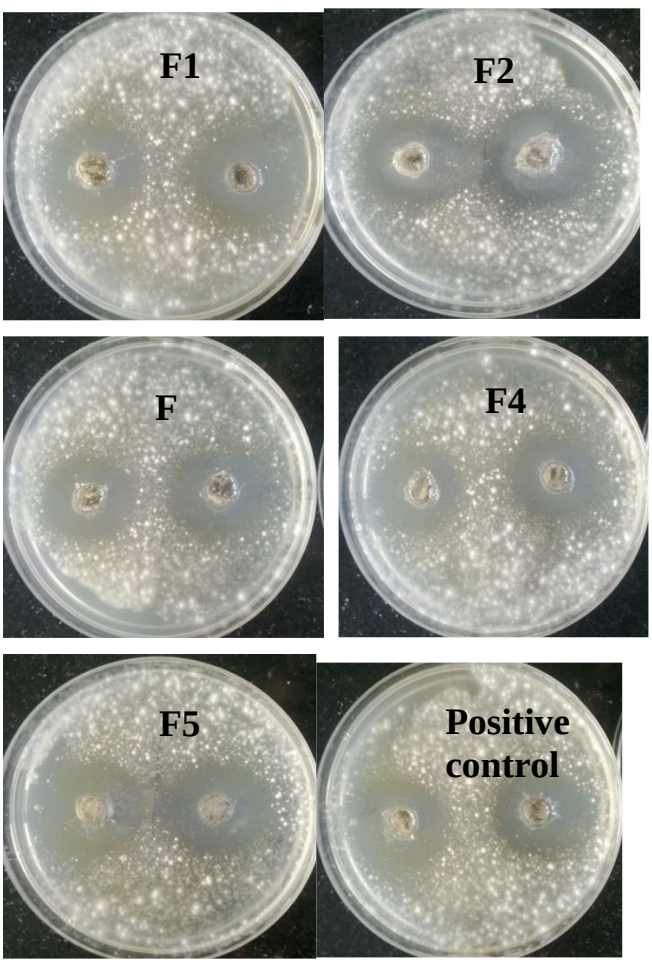

Figure 5: In vitro antifungal activity of different formulated ointment against *Aspergillus niger*

Antifungal ointment is a topical medication designed to treat fungal infections. Antifungal ointments work by exploiting differences between mammalian and fungal cells to kill the fungal organism without dangerous effects on the host. In the present study PEG was used for the preparation of different formulation. In water soluble bases Polyethylene Glycols ointment is the only pharmaceutical preparation. Polyethylene Glycols (PEGs) which are known as macrogels are widely used in topical pharmaceutical formulations since this chemicals are stable, hydrophilic substances that are essentially nonirritant to the skin and easily removed from the skin by washing.

In this study different formulation of PEG ointment bases (F1, F2, F3, F4 and F5) were prepared, and their viscosity was determined using Brook field Viscometer and diffusion rate was studied by *in vitro* release through dialysis. Antifungal activity was also determined using agar plate diffusion assay.

Formulation F2 was found to most viscous. In the dialysis assay the percentage of fluconazole from water soluble PEG based ointment was released over a period of two hours from the prepared ointment containing 1% w/w fluconazole. In this experiment F2 formulation showed maximum fluconazole release within two hours. *In vivo* testing method also showed that the F2 formulation has inhibited the growth of fungus. Since in *in vivo* condition plates were incubation for the 24-48 hours so there is not very much differences in measured inhibition zone diameter.

Conclusion

In conclusion, the diffusion of any drug through the different bases depends on the nature and the composition of the bases. So, the release rate can be altered by changing the nature and the composition of the bases. Present work has been done to explore a PEG ointment base having better diffusion rate. The obtained results showed that in *in vitro* condition formulation F2 was showing best diffusion capacity within 2 hours. *In vivo* testing method also showed that the F2 formulation has inhibited the growth of fungus. Since in *in vivo* condition plates were incubation for the 24-48 hours so there is not very much differences in inhibition zone diameter. This study further can be extended also by exploring a better O/W base ointment.

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RESEARCH ARTICLE

Submerged Fermentation of *Lactobacillus delbrueckii* NCIM - 2025 exposed to Alloxan monohydrate for production of Lactic acid from molasses

Pranabesh Maji[1]* and Md, Selim[2]

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Abstract

The potency of Alloxan monohydrate on Submerged Fermentation (SmF) of *Lactobacillus delbrueckii NCIM - 2025* for production of lactic acid from molasses was assessed. Alloxan monohydrate was found to be enhanced the yield when 22% (w/v) molasses solution was allowed to ferment at pH 5.9, temperature 35^0C and optimum incubation period of 6 days. It was observed that there is a gradual increase in the product of lactic acid with increasing concentration of Alloxan monohydrate upto 6.0×10^{-5}M. The compound Alloxan monohydrate gave the maximum yield of lactic acid, i. e., 10.286 g/100 ml at the concentration mentioned which is 11.248% higher with respect to control.

Keywords: Alloxan monohydrate, Submerged Fermentation, *Lactobacillus delbrueckii NCIM – 2025*, Lactic acid, Molasses

Introduction

There are some organic molecules which when introduced to the fermentation medium can effect the enzymes responsible for the biosynthesis of micro and macro molecules in the microbial cells as well bioconversion of raw substrate into desired products and such organic compounds may be referred to as physiologically active organic compounds (Raveendran, 2018; Nkhata, *et al.,* 2018). It has been found that a few physiologically and pharmacologically active organic molecules are very active and play biological properties of vital importance in the biosynthesis of some useful micro and macro organic molecules (Warne, *et. al.,* 2014 ; Sadh, *et. al.,* 2018). In pharmacology, biological activity or pharmacological activity describes the beneficial or adverse effects of a drug on living matter. When a drug is a complex chemical mixture, this activity is exerted by the substance's active ingredient or pharmacophore but can be modified by the other constituents.

Some organic molecules which when introduced to the submerged fermentation medium can effect the enzyme responsible for the biosynthesis of lactic acid (Mazzoli, *et. al*.2014; Dumbrepatil, *et. al*., 2008). Glusac, *et al.,* 2015 observed that a slight variation in fat composition of *L. delbruecki* is stimulated by the addition of fatty acids. *L. acidophillus* also needs malvalic acid (Tamura, 1957) having highly strained cyclopropene ring as a growth factor. Some authors reported that p-hydroxybenzoic acid useful for *E. coli* and Oleic acid requirements by lactic acid bacteria (Broberg, *et al.,* 2007 ; Partanena, *et al.,* 2001). A group of workershave studied some organic compounds having barbiturate nucleus in their structure and found them most significant and effective for different industrial fermentation processes. It was also reported barbitone as a growth promoter and enzyme stimulant for many biological fermentative enzymes induction (Ishibashi, *et al* ., 1981; Maji, 2020).

As a result of such interesting and conflicting observations it is obvious that much work has been done on different active organic molecule of importance and their requirements by different bacteria, fungi and yeasts but no substantiate work has been done on SmF production of lactic acid by *Lactobacillus delbrueckii* NCIM - 2025 exposed to Alloxan monohydrate molecule. Presntly in this paper an attempts has been made to study the influence of alloxan monohydrate on SmF production of lactic acid by *Lactobacillus delbrueckii* NCIM - 2025.

Experimental

The influence of Alloxan monohydrate on SmF production of lactic acid by *Lactobacillus delbrueckii* NCIM - 2025.

In this present experiment the composition of the fermentation medium was prepared as follows :

Molasses	:	22% (w/v)
Malt Extract	:	1.75%
Yeast Extract	:	1.75%
Peptone	:	1.75%
$(NH_4)_2HPO_4$	:	1.75%
$CaCO_3$	:	10 %
pH	:	5.9
Distilled water	:	To make up 100 ml.

The pH of the medium was adjusted to 5.9 by adding requisite amount of phosphate-buffer solution, and the pH was also ascertained by a pH meter. The above compostion medium represents volume of a fermentor flask, i. e., "100ml" production medium for lactic acid fermentation.

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Now, the same produciton medium for SmF production of lactic acid by *Lactobacillus delbrueckii* NCIM - 2025 was prepared (Maji, *et.al.,* 2016) for 99 fermentor flasks, i. e., each fermentor flask containg '100 ml' of production medium. The fermentor flasks were then arranged in ten sets, each comprising 9 fermentor flask. Each set was again rearranged in three subsets, each comprising of 3 fermentor flasks. The remaining nine fermentor flasks out of 99 fermentor flasks were kept as control and these were also rearranged in three subsets each consisting of three fermentor flasks.

Now $1.0x10^{-3}$M solution of Alloxan monohydrate was prepared and 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0 and 10.0 ml of this solution was added to the fermentor flasks of 1st to 10th sets respectively. The control fermentor flasks containg no alloxan monohydrate. Now the total volume in each fermentor flask were made up to 100ml by adding requisite amount of distil water. Thus, the concentration of alloxazine in 1st, 2nd, 3rd, 4th, 5th, 6th, 7th, 8th, 9th and 10th subsets were taken from 1.0×10^{-5}M to 10.0×10^{-5} M increased by 1.0×10^{-5}M in each case. The fermentor flasks were then sterilized, cooled, inoculated, incubated and analysed after 6 days for lactic acid formed and molasses sugars left unfermented.

Results

The results obtained in the study of the influence Alloxan monohydrate on SmF production of lactic acid by *Lactobacillus delbrueckii* NCIM - 2025 are tabulated in the Tables 1 and 2.

Table - 1 : SmF production of lactic acid by *Lactobacillus delbrueckii* NCIM - 2025 exposed to alloxan monohydrate

Concentration of AOM 1 X 10^{-5} M to 10 x 10^{-5}M	**Yield of lactic acid *in g/100 ml**	**Molasses left * unfermented in g/100ml**	**% of lactic acid increased(+) in 6 days of incubation period**
Control	9.246	1.759	------
1.0×10^{-5}	9.280	1.725	(+) 0.367
2.0×10^{-5}	9.362	1.683	(+) 1.254
3.0×10^{-5}	9.580	1.425	(+) 3.612
4.0×10^{-5}	9.823	1.177	(+) 6.240
5.0×10^{-5}	10.128	0.872	(+) 9.539
6.0×10^{-5}**	10.286***	0.714	(+) 11.248
7.0×10^{-5}	10.008	0.998	(+) 8.241
8.0×10^{-5}	9.743	1.257	(+) 5.375
9.0×10^{-5}	9.445	1.559	(+) 2.152
10.0×10^{-5}	9.319	1.689	(+) 0.8003

* Each value represents mean of three trials.

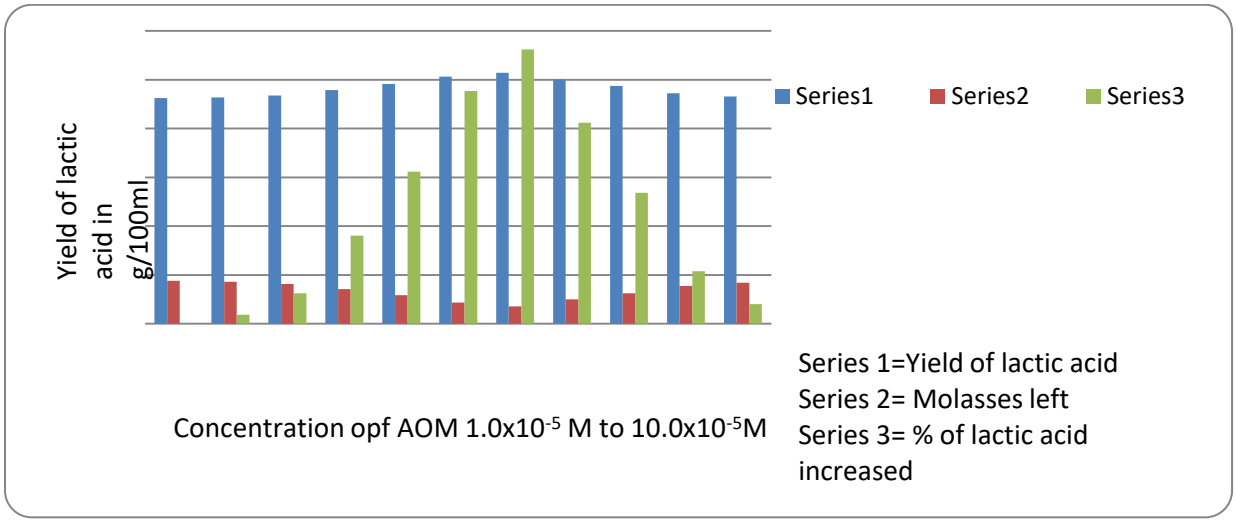
** Optimum concentration of Alloxan monohydrate (AOM) .

*** Optimum yield of lactic acid

(–) Values indicate % decrease in the yield of lactic acid

Experimental deviation $\pm$ 2.5 – 3.5%

Table - 2 : SmF production of lactic acid by *Lactobacillus delbrueckii* NCIM - 2025 exposed to alloxan monohydrate by graphical presentation.



Discussion

Barbiturates has been found to be most effective and useful for various industrial fermentations process. It has also many significant roles in different biological processes and a lot of questions are still unsettled and open concerning the mode of action of these barbiturate molecules on the enzymes catalysed systems involved in the pathways leading to the mode of enzyme functions. However, whatever their biological functions may be, these organic molecules should be incorporated in to the fermentation medium for the better functioning of the process and improved yield of the desired products. It is a secondary factor that influences the fermentation technique associated with enzymes of *Lactobacillus delbrueckii* NCIM - 2025

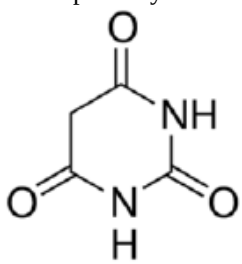


Figure 1: Chemical structure of Barbituric acid

Further, a group of researchers have reported stimulatory effect of barbituric acid and its derivatives possessing barbiturate nucleus. Since the organic molecule, i. e., 5,5-diphenylhydantoin also possess part structure combination of barbiturate nucleus, it may influence critically the outcome of lactic acid by the bacterial strain of *Lactobacillus delbrueckii* NCIM – 2025.(Kumari , 2018; Yadav, 2018) .

The influence of alloxan monohydrate in lactic acid fermentation :

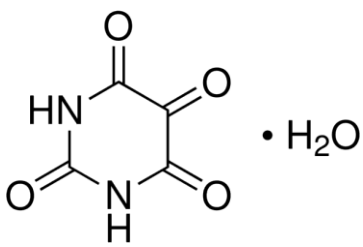


Figure 2: Chemical structure of Alloxan Monohydrate

The data recorded in the Table-1 shows that the addition of alloxan monohydrate into the lactic acid fermentation medium enhances the production of lactic acid significantly. It has been observed that there is also a gradual increase in the yield of lactic acid with gradual stepping up of alloxan monohydrate till the maximum yield of lactic acid is reached which is 10.286 g/100ml (11.248%) higher in comparison to control fermentor flasks, i. e., 9.246 g/100 ml at 6.0 x 10^{-5}M molar concentration of the compound alloxan monohydrate in 6 days of optimum incubation period.

It has been observed that the compound alloxan monohydrate is a very important active organic molecule and its biological activities may be attributed to the active>C = O groups associated with six membered hetero organic molecule and -CO-NH-CO-linkage. Since no clear evidence could be put forward regarding its activity and stimulating properties of lactic acid fermentation process the compound alloxan monohydrate is considered to influence critically some metabolic enzymatic pathways intimately concerned with the fermentative biosynthesis of lactic acid by using the bacterial strain of *Lactobacillus delbrueckii* NCIM-2025 .

The compound alloxan monohydrate bears more than two >C=O groups and -NH-CO-NH-linkage which serves as a most effective energy source and influences significantly the growth and activity of the lactic acid bacteria *Lactobacillus delbrueckii* NCIM-2025 and thereby enhances the fermentative biosynthesis of lactic acid. It was also interesting to note that almost at all the concentrations of compounds % of lactic acid produced was higher than control. The favourable and significant response of the compound may also be attributed to the fact that in compound ,at least at the bonds where oxygen is attached with carbon of compound there is a chance and probability of accepting protons given by the different enzyme surface area itself thereby getting increased electronegativity. The bacterial activity under the above circumstances is expected to go more exogeneously because the increase of electronegativity more and more of related enzymes are expected to participate and to take the

position for reaction with active sites of the compound and because of this stream of mobility population of the cell enzymes, most of them are expected to occur at the surroundings area of bacterial cells or enzymes elaborated by the strain *Lactobacillus delbrueckii* NCIM-2025 in the fementation medium.

Although the exact mechanism of the role of compound alloxan monohydrate is still some what uncertain and at present it is very difficult to predict the real reason of the significant response of the compound on SmF production of lactic acid by *Lactobacillus delbrueckii* NCIM - 2025 because there may be wide spread probable possibilities in this regard as follows :

Incorporation of compound alloxan monohydrate in fermentation medium may cause an alternation in the structure and behaviour of enzymes that geometrically fits with the molasses substratum which is vital force as well source of lactic acid production by *Lactobacillus delbrueckii* NCIM-2025. It may also cause an enhancement of the quantum of the enzymes which may thus increase the efficiency per unit of the elaborated enzyme and hence fermentative biosynthesis of lactic acid by utilizing a major percent of molasses substrate, i. e., molasses in the fermentation medium.

Conclusions

It may, therefore, be concluded that addition of alloxan monohydrate to the production medium has stimulatory effect at all its concentrations used, i.e., from 1.0 x 10^{-5} M to 10.0 x 10^{-5}M and the yield of lactic acid has been found greater in each case in comparison to control fermentor flasks. However, incorporation of alloxan monohydrate at higher concentration level is not encouraging for SmF production of lactic acid by *Lactobacillus delbrueckii* NCIM - 2025.

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RESEARCH ARTICLE

Combining Ability Analysis for Yield and Yield Attributing traits in Maize (*Zea Mays* L.)

Viqar-un-nisa, Wajhat-un-nisa, Z.Adar, M.A. Wani and M.A. Rather

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Abstract

Maize (Zea mays L., 2n=20) belong to family poacea and is recognised as "queen of cereals".Total production of maize is standard greater than wheat and rice in addition being the staple crop to many countries. Maize is without delay fed on in the form of cereal treats, alcohol, feed to animals, and other stuff such as starch and syrup. Conveniently, there are six specific sorts of maize like flint corn, dent corn, pop corn, popcorn, flour corn and sweet corn. Sweet corn is most preferred by way of people while field corn types are fed to animals. It has constantly been higher in involvement to industrial uses. It is viewed very versatile crop with wide adaptability and extensive genetic variability.

Key words : Maize, corn Genetic variability

Introduction

Maize is regarded in India as an solely cereal crop in the world with an annual manufacturing of about 854.6 million tonnes over an region of 168.4 million hectares and common productivity of 5.07 tonnes per hectare (Anonymous, 2015). In 2014 whole world production of maize was once 1.04 billion tonnes. Maize is is primarily cultivated in America with 361 million metric tons grown in the states. In India complete maize production in 2018 was 27.8 million tonnes and in the world, it used to be 1.15 billion tonnes led through USA with 34.2 percent, accompanied by way of china with 22.4 percent (FAO STAT, 2018)

Nutrition is prerequisite for the properly being of human beings, but still malnutrition is hassle so overcome dietary deficiency maize has performed an necessary role in day to day lives of mankind. Maize serves as meal for mankind as nicely as animal feed .Total of day by day energy in maize is about 56 percent, Proteins stored in pericarp in grain of maize is about 6 percent, about 82 percentage of proteins are current in endosperm. Maize acquire is specially supply of concentrated power it pertains to about 71 percent.But then again on an common basis endosperm contains albumis (3 percent), globulins (3percent), Zeins (60 percent), and glutelins (34 percent), zeins are believed to have pretty balanced quantity of lysine and tryptophan. Maize kernel incorporates about four percent of oil .

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Heterozygosis is accountable for diversity which makes the crosses to range from each other. Where as homozygosis is diminished mechanically due to inbreeding (Dar *et al.,* 2017).

Generally F1 hybrids of "outcrossed" inbred strains show a incredible enlarge in vigour and viability. Even if the inbred lines have, are degenerated due to inbreeding, and there will be returning of vigour. This phenomenon is termed heterosis or hybrid vigour. So for assessment in deciding the parents, crosses and any future breeding programme.

The capacity of a line to pass on enviable overall performance to its progenies is known as combining ability. Sprague and tatum had been first who used combining potential for the calculation of gene motion and variances due to gene action. General combining ability measures the breeding value it can be measured thru the overall performance of inbred in series of crosses with testers and therefore calculating the average out of all the crosses, whilst individual performance of a inbred line in a particular pass gives how a great deal combining ability is existing in a specific genotype and offers the notion of dominant component of variance whilst conventional combining potential offers the thought of additive thing of variance. Main significance of mating design is (1) Genetic manage of a trait is manifested. (2) A excellent breeding population is developed (3) Genetic attain is calculated. (4) Helps in comparison of parents, and Performance of their progeny is primary interest of breeder. Based on combining capacity outcomes and with better mean performance suitable inbreds are chosen.The information of the estimates of combining ability and genes actions is impotant for a successful breeding programme.Line x tester is nothing but extension of topcross while in Line x Tester design more than one tester is involved. Kempthorne in 1957 first gave the thought of Line x Tester mating design. This plan includes move between traces usually detailed as females (f) and extensive based totally testers particular as males (m) in f x m manner involving one line and one tester (Sharma, 2006). L x T provides information of each full-sibs and half-sibs simultaneously as evaluate to pinnacle cross which gives statistics of solely half-sibs. Line x Tester provides information of SCA for every cross, furnish facts of GCA of now not only lines but also for the testers . More over in addition, Line x Tester is used in inference of a number of types of gene actions that are good sized in the expression of a range of meteric traits (Rashid *et al*., 2007). The current investigation entiled "Genetic studies of early maturing maize (Zea mays L.) inbred lines" made to recognize the genetic nature of morphological, matuarity, yield and yield associated

components through studies involving ten diverse strains and three extensively adapted testers in Line x Tester mating design with following objective.

1. To assess Combing ability of newly derived inbred lines.

Materials And Methods

Ten diverse maize inbred lines viz., KDM-340,KDM-440,KDM-916,KDM-927A,KDM-895,CML-470,CML-474,CML-425,KDM-347,KDM-930 and three widely adapted testers V-351,V-335,KDM-914A were crossed line x tester mating design in order to obtain set of thirty crosses during Rabi-2018 at Winter Nursery ,Agricultural centre of Research Institute, Rajendranagar, Hyderabad then F1 was evaluated at dry land agriculture research station Srinagar during kharief - 2019 in randomized block design with three replications with experimental plot of 75 x 20 cm. The data was recorded on morphological, maturity, yield and yield attributing traits viz., Days to 50 per cent tasseling , Days to 50 per cent silking, Days to maturity (Seed to seed), Plantheight (cm), Earheight (cm): 6 Earlength (cm), Eardiameter (cm), No. of kernel rows ear^{-1}: No. of kernels row^{-1}, Prolificacy index: Grain yield plant^{-1} (g) Combinig ability procedure was done according to kempthrone, (1957)

Table 1 Analysis of variance for yield, and yield attributeing traits.

Source of variation	df	Ear length	Ear diameter	Number of kernels ear^{-1}	Number of kernelsrow^{-1}	100 grain weight	Grain yield plant
Lines	9	17.40**	9.548**	13.561**	106.40**	80.198**	4210.10**
Testers	2	6.00	20.542	0.610	12.38	15.999	79.330
Line xTester	18	1.96**	3.091	4.327**	30.107**	19.568**	1847.474**
Error	58	0.79	0.629	0.080	0.045	0.258	5.102
∂^2lines		0.462	2.697*	0.463*	7.747**	4.765**	387.080
∂^2Tester		0.225	1.321	0.002	0.699	0.933	8.5206
∂^2gca		0.687	0.06**	0.127**	2.621**	1.978*	111.764**
∂^2sca		1.371	1.400**	2.978**	22.079**	9.848**	1069.989
∂^2A		1.374	0.12	2.253	5.243	3.956	223.582
∂^2D		1.371	1.400	2.978**	22.079**	9.848	1069.989
Degree of Dominance	1.002	0.0	1.14	2.052	1.577	2.187	

*,** significant at 5 % and 1 % levels respectively.

The analysis of variance was carried out using data of 12 agro morphological traits for thirty crosses sown in RBD during *Kharif* - 2019. The mean squares for replications were non significantly different for all traits. Larger amount of significantly important differences were found for all traits among lines and line x testers which indicated that the parental material exhibited good amount of diversity**.**

Genetic variability is key thing which determines development from selection Estimates of factors of genetic variance and their corresponding widespread errors have been estimated for all traits.Estimates of components of genetic variance cautioned that dominant factor played predominant role in Days to fifty percent tasseling , plant height, quantity of kernels per row, wide variety of kernels per cob, ear diameter, grain yield and days to maturity ,while additive element performed considerable role in Days to fifty percent silking ,ear height, ear length. Degree of dominance was unity for majority of traits except days to fifty percentage silking ,ear peak ,ear diameter, and prolificacy this was in agreement with results of Joshi *et al*., (1998) and Vasal *et al.,* (1992a) file that non additive gene consequences performed a critical role in the inheritance of kernels row-1, 100-grain weight, grain yield hectare-1 which had been in line with above findings. Abadi *et al.,* (2011) and their studies on maize mentioned incidence of non-additive gene action gene action for grain yield plant-1, kernels ear-1, 1000- grain weight. Alike penalties have been recommended by using General combining ability is estimated to know the genetic really worth of parents. The estimates of GCA outcomes are listed in Table - 3 and close promixation of consequences published that neither of the parents showed familiar combining ability in preferred path for all the features below consideration. However, parents like KDM-930, KDM-914, CML-425and V-351 showed general combining ability for characters like flowering and consequently confirmed mostly massive and negative normal combining potential effects. Most suitable parent for early maturity traits was once recognized KDM-914 and facet by using aspect additionally confirmed preferred estimates of universal combining results for days to 50 per cent tasseling, days to 50 per cent silking and cob top have been considered as top prevalent combiners. For grain yield plant^{-1} CML-474 used to be documented as good general combiner observed through KDM-440.These can be used straight as parents for developing excessive yielding single cross hybrids, KDM-914 showed absolute best frequent

combining potential for grain yield plant^{-1} which was accompanied with good sized typical combining ability in favored direction for 100-grain weight, kernels row^{-1}, flowering features viz., days to 50 per cent tasseling, days to 50 per cent silking however confirmed negative combining potential for morphological traits viz., plant height and ear height; prolificacy and kernel rows cob^{-1}. Similarly nice and massive usual combining ability consequences were mentioned for grain yield plant^{-1}, 100-grain weight, number of kernel rows ear^{-1}, quantity of kernels ear^{-1} whereas sizeable poor everyday combining ability outcomes for days to 50 per cent tasseling, days to 50 per cent silking, plant height and ear height (Singh *et al*., 2012) and (Pavan *et al*., 2011). For kernel rows cob^{-1}, KDM-440was determined as most magnificent combiner which too most and suitable combining capacity for grain yield plant^{-1}, kernels row^{-1}, 100-grain weight

Table 2 Estimates of General combining ability effects of lines and testers for yield and yield attributing traits in maize (*Zea mays* L.)

Parents plant[1]	Ear length(cm)	Ear diameter	Number of kernel Rows ear^{-1}	Number of kernels row^{-1}	100 grain weight	grain yield
CML-470	2.438*	-0.027	0.321**	1.990**	--1.231**	16.131*
CML-474	-0.251	-0.024	-0.771	1.760**	-2.914**	27.384*
CML-425	-2.39**	-0.014	-0.108	-0.415	-2.984**	19.089*
KDM-927	0.095	-0.162**	0.760**	5.314*	2.536**	1.736*
KDM-916A	-2.341*	-0.003	0.521**	1.364**	1.036**	17.893*
KDM-347	3.755**	0.076*	-1.938	-1.264	1.584**	-13.934*
KDM-895A	-0.616	0.156*	-1.005	1.680**	-0.197	-10.489*
KDM-440	-1.198	0.059	0.987**	1.550**	2.169**	26.733*
KDM-930A	2.863**	0.061*	0.871**	-1.876	1.453**	-12.830*
KDM-340	2.977*	0.069*	-0.153	2.377**	1.876*	11.731*
V-351	0.270	-0.012	-0.039	-0.607	2.999*	-0.717*
KDM-914 A	4.087**	-0.044	-0.146	0.986	1.233*	6.398*
V-335	1.345	0.312**	0.085**	0.732*	-1.342	1.906*
S.E.g_i (lines)	1.080	0.030	0.223	0.326	0.211	0.518
S.E.gi(testers)	1.596	0.045	0.329	0.482	0.129	0.739
No. of parents showing desirable gca effects	6	5	5	7	8	11

** significant at 1 %,* significant at 5% respectively

The results of outcomes due to specific combining ability for the thirty crosses for various traits, given in Table-4, printed that none of the cross combination infatuated high and suitable outcomes due to unique combining for majority of the traits(Meseka and Ishaaq, 2012). However, crosses which showcase essential and desirable particular combining ability effects incorporated are CML-470 x V -351, CML-474 x V - 335 ,CML-425 x KDM- 914 A,KDM-927 A x KDM- 914 A ,KDM-916 A x KDM-914 A ,KDM-916 Ax V-335 ,KDM-347 x KDM -914 A ,KDM-895 x V-351 ,KDM-440 x KDM- 914 A , KDM-930 x V-351, KDM-930 x KDM- 914, KDM-9130 x V-335 for Ear height; for Kernel rows cob^{-1}; KDM-347xV-351, CML-470xKDM-914A,CML-474xV-351, KDM-927 AxV-351 ,KDM-930 xV-335 for Kernels row^{-1}; KDM-347xV-351,KDM-440xV-351 KDM-916 A x KDM-914 CML-470 xV-351,CML-474xV-335 for Grain yield plant^{-1}.KDM-347x V351, KDM-440 xV335 ,CML470 x KDM-914A, KDM-895 x KDM914A ,KDM927A x KDM-914 A whereas whilst familiar combining capability was used to realize the overall performance of parents , it used to be determined that the majority of the precise cross combinations had been the result of crosses between low × high or low × low or low × medium or high × excessive or excessive × medium usual combiners (Kanagarasu *et. al*.,2012).

Among these crosses KDM-347x V351 which confirmed the superb and best possible particular combining potential effect for yield and had high× high combiners; KDM-440 x KDM – 914 had high × medium excellent well-known combiners; KDM-895 x KDM-V- 351 had low × high right combiners , suggesting that participation of one proper common combiner appears to be imperative to get the better unique combination. The results are in universal argument with the result of numerous employees (Ali *et al*., 2007 ; Dass *et al*., 1997;

Aguiar *et al.,* 2003; Vasal *et al.,* 1998). The gain of crosses touching on low × medium or high × low combiners as parents could be explained on the center of interface between superb alleles from properly prevalent combiner and destructive alleles from the terrible combiner as parents (Deitos, 2006)

Table 3 Estimates of Crosses combining ability effects of lines and testers for yield and yield attributing traits in maize (*Zea mays* L.)

Crosses	**Ear length(cm)**	**Ear diameter**	**Number of Kernel scob^{-1}**	**Number of ^{1} Kernels row^{-1}**	**100grain weight**	**grain yield plant^{-1}**
CML470 x V-351	2.666*	0.500*	4.890	5.152**	2.000**	10.452*
CML-470 XKDM-914A	2.559	0.617**	4.610 **	0.081	1.959**	29.380*
CML-470 XV-335	3.070*	0.620**	3.176	7.633**	2.293*	18.954*
CML-474 XV-351	3.005	0.251*	-4.396 **	5.992**	3.174**	4.380*
CML-474 X KDM-914A	-0.080	1.209**	-2.319	2.848**	1.620*	4.489
CML-474 XV-335	2.530	0.659**	-2.824*	5.974**	-1.128	-4.978
CML-425 X V-351	9.362*	0.372**	2.681*	3.652**	1.904*	7.545*
CML-425 XKDM-914A	1.802	0.194	0.753	2.205*	1.589*	-7846*
CML-425 XV-335	-10.313*	0.548**	3.896	4.831**	2.558*	0.401
KDM-927A XV-351	0.145	0.571**	3.110**	5.599**	0.665	14.960*
KDM-927A XKDM-914A	2.502*	-0.270**	-1.319	3.170**	-0.046	19.549*
KDM-927A XV-335	-1.002	-0.017	-2.033	3.099**	1.611	6.788*
KDM-916A XV-351	6.009	1.123**	3.176	2.848**	0.695	9.826*
KDM-916A XKDM-914A	10.109*	1.128**	-0.747	6.117**	2.325**	23.434*
KDM-916A XV-335	-1.280	0.552**	0.681	0.492	-0.978	15.908*
KDM-347 XV-351	4.302	0.968**	4.967**	7.544**	3.324**	34.197*
KDM-347 XKDM-914	1.741	0.208*	5.110	2.099*	2131**	4.238
KDM-347 X V-33	-4.623	0.276**	0.253	3.724**	2.991**	14.109*
KDM-895 X V-351	4.084	0.463**	4.890	3.402**	0.674	8.291*
KDM-895 X KDM-914A	0.441	0.128	1.181	4.224**	2.337**	25.383*
KDM-895 X V-335	4.902	0.500**	-1.324	-0.848	1.770*	9.257*
KDM-440 X V-351	1.087	0.259**	2.538*	6.438**	0.009	29.275*
KDM-440 X KDM-914A	0.752	0.186	-0.890	2.937**	3.556**	18.928*
KDM-440 X V-335	0.112	0.121	1.538	4.527**	1.733*	28.933*
KDM-930A XV-351	4.945	0.528**	5.176	4.152**	3.165**	10.940*
KDM-930 X KDM-914A	1.884	0.173	-0.104	2.831**	2.280**	16.234*
KDM-930 X V-335	-0.230	0.235	-2.896	-0.098	0.159	-2.009
KDM-340 X V-351	2.977	0.280**	3.962**	4.331*	0.192*	4.398*
KDM-340 XKDM-914A	3.834	0.960**	-0.747	2.867**	0.044*	9.153*
KDM-340 X V-335	-0.902	-0.439**	-0.824	2.992**	0.030	15.391*
S.E±(Sij)	4.596	0.102	0.743	1.086	0.098	1.615
Number of crosses	9	22	16	28	19	25

Showing desirable sca effects

** significant at 1 %,* significant at 5% respectively

From the results of the existing study, the following conclusion are drawn:

- Analysis of variance qualities below learn about published distinctly massive mean sum of squares for maize inbred lines which indicated material had emmense amount of variability for anumberoftraits.
- Analysis of variance for typical and unique combining ability used to be tremendous for almost all the features studied which depicted that the parents and crosses in the existing investigation had been diverse.
- Parents with great and favourable GCA for silking,tasseling, plant height,ear height and days to maturity (KDM-930 ,CML-425, KDM-914 A and KDM-916 A) may additionally be regarded might also be used to enhance inbred an accelerated supply of population.
- Desirable crosses with maximum SCA (KDM-347 x V-351, KDM-440 x KDM-914 A ,) heterosis (CML - 470 x KDM-914 A ,CML-474 x V-351,KDM-440 x V-335) should be tested for multilocation to arrive valid conclusion related to their use in commercial maize cultivation

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REVIEW ARTICLE

Computational Modeling in Biotechnology

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Abstract

Computational modeling can be a constructive collaborator in biotechnology. Such modeling promotes development through nano scale views of biomolecules and devices which are not attainable through experimental imaging methods. Computational systems aims to develop and use efficient algorithms, data structures, visualization and communication tools with the goal of computer modeling of biological systems. It involves the use of computer simulations of biological systems, including cellular subsystems to both analyze and visualize the complex connections of these processes. We exemplify the role of computational modeling through some recently developed computational models such as Coarse grained modeling, multi scale modeling and mesoscale modeling.

Keywords : Coarse Grained Model, Multi Scale Model, Mesoscale Model

Biological systems engage an incredibly large diversity of molecules, reactions and interactions. Computer technology allows us to measure and obtain biological data on a large scale at many different scales and levels: from molecules to whole organisms, and from tiny bacteria to complex forms. However, these massive data sets are often diverse in nature. Our brain is not able to deal with such complexity on its own, and the challenge ahead is to integrate and make sense of the data in order to understand and predict biological processes and their applications. Computational modeling is enormously indispensable for this daunting task *(*Melnik and Roderick ,2015*)*. For comparison of a specific sequence with those of other sequences of the data base, the same function may have to be repeated a million times. A computer only can handle this function with accuracy within a quick time frame. The goal is to create accurate real-time models of a system's response to environmental and internal stimuli. Computers are critical for analysis and modeling of these data (Warshel *et al.*, 2015). Computational modeling can amply expedite the process of designing biodevices and biological machines, however modeling tools and methods are significantly less developed than those available in the mainstream life sciences. This paper provides an overview of the computational methods and tools recently developed with the aim of drastically improving the proficiency of computer modeling in several areas of biotechnology.

Coarse Grained Modeling

Coarse-grained modeling or coarse-grained models, aim at simulating the behavior of non simple systems using their simplified representation. This framework involves an integrated, iterative approach to collect information from different modules. Coarse-grained models are widely used for molecular modeling of biomolecules at various granularity levels (*Badaczewska et al., 2020*). A wide range of coarse-grained models have been proposed till date. These models are usually dedicated to computational modeling of specific molecules. The primary steps, which coincide with key areas of method development, include developing first-pass coarse-grained models guided by experimental results, performing numerous large-scale coarse-grained simulations, identifying important interactions that drive emergent behaviors, and finally reconnecting to the molecular scale by performing all-atom molecular dynamics simulations guided by the coarse-grained results. Coarse-grained models have found practical applications in molecular dynamics simulations.

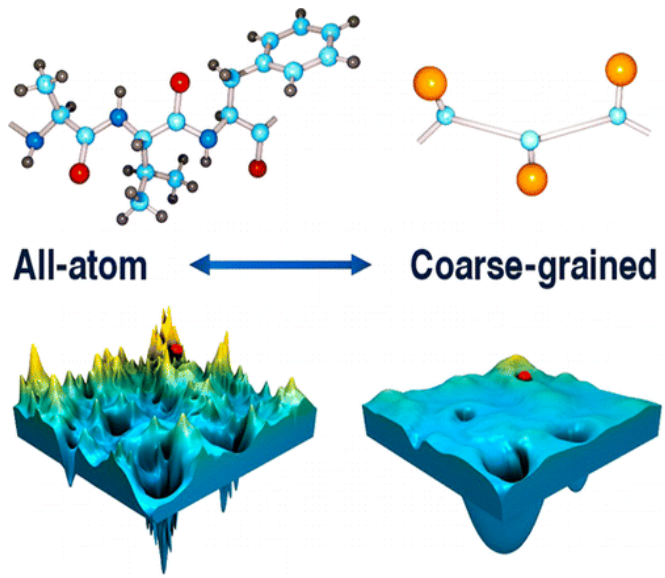

Figure 1: The Figure illustrates the effect of the smoothening of energy landscape into a coarse-grained model.

When designing coarse-grained modeling methods, the representation of atomistic structures on a coarse-grained level requires precise definition. The choice of representation determines to a large extent the possible options of force field and sampling, i.e., the compromise between accuracy and computational efficiency.

The smaller the number of explicitly treated united atoms (or pseudo atoms) representing fragments of protein chains, the faster simulation, and the lower accuracy. Very efficient models based on three/four united atoms per amino acid

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residue accelerate simulations by 3−4orders of magnitude in comparison with classical all-atom MD simulations. Nevertheless, it is useful to develop even more simplified models dedicated to large protein systems with realistic connection with all-atom resolution schemes (López *et al.*, 2009*)*.

Multi scale modeling

Multi scale modeling combines models of different scales of a system in order to obtain an overall model of desired quality or computational efficiency which is difficult to achieve by a single scale model. This modeling paradigm is widely regarded as a promising and powerful tool in various disciplines, including process engineering, material science, computational mechanics, system biology, and biomedical engineering. In principle, every simulation that allows transfer of information between at least two different levels of granularity can be considered multi scale. Multi scale methods are more efficient and enable analysis of larger systems in a longer time scale with a simultaneous ability to preserve a high level of details when necessary (Ingolfsson *et al.*, 2014*)*.. This idea was applied to biological objects perhaps for the first time by Levitt and Warshel in 1976 in their study of mechanisms of enzyme action. Since then multi scale modeling has found successful applications in the modeling of proteins, membranes, ribonucleic acids and large proteins. Depending on the given scientific problem, several combinations of methods have been proposed. In practice, the most common are QM/MM (quantum mechanics/molecular mechanics) and all-atom/coarse-grained.

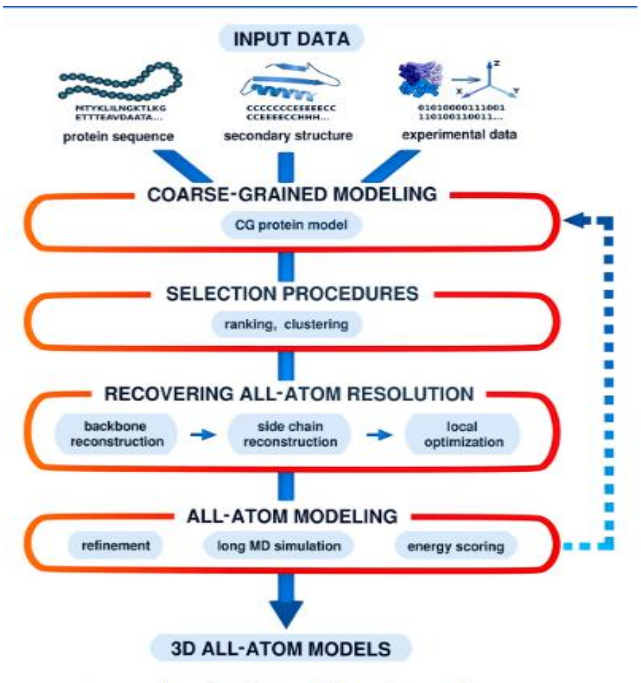

Figure 2: Typical multi scale modeling scheme that merges coarse-grained and all atom modeling.

QM/MM modeling was historically the first multi scale approach used for chemical computation. Successful multi scale modeling, regardless of the type, needs efficient and reliable algorithms for transferring information between calculations with different resolutions. Multi scale dynamic modeling is even more demanding since proper calculation ought to be fast enough so as not to hinder the benefit of coarse-graining. Till date many different approaches that define the concept of a boundary have emerged. However, since information exchange methods are the key limiting factors (Parson *et al*., 2005), new theories that would fill in these gaps are still of great need.

Mesoscale modeling

Mesoscale methods are being used to investigate techniques for micro fluidic sorting and enrichment of biological cells and other particles. Fluids are involved in practically all physiological activities of living organisms. However, biological and bio related flows are hard to analyze due to the inherent combination of interdependent effects and processes that occur on a multitude of spatial and temporal scales. Mesoscale modeling provides an engaging and accessible overview of this comparatively new field of mesoscale biosimulation, which is set to increase its importance because imaging tools in the biological sciences are increasingly able to visualize these length-scales. Recent advances in mesoscale simulations enable researchers to tackle problems that are central for the understanding of such flows (Lee *et al.*, 2016).

Examples of Mesoscale modeling

Computational modeling effectively facilitates the development of novel therapeutic approaches. Among other methods, Dissipative Particle Dynamics (DPD) and the Lattice Boltzmann Method (LBM) have become increasingly popular during recent years due to their ability to solve a large variety of problems.

Dissipative Particle Dynamics (DPD):

Dissipative Particle Dynamics (DPD is an off-lattice mesoscopic simulation technique which involves a set of particles moving in continuous space and discrete time (Levitt *et al.*,1975). It is a stochastic simulation technique for simulating the dynamic and rheological properties of simple and complex fluids. Particles represent whole molecules or fluid regions, rather than single atoms, and atomistic details are not considered relevant to the processes addressed. The particles' internal degrees of freedom are integrated out and replaced by simplified pair wise dissipative and random forces, so as to conserve momentum locally and ensure correct hydrodynamic behavior (Hadley *et al.*, 2012*)*. The main advantage of this method is that it gives access to longer time and length scales than are possible using conventional molecular dynamics simulations.

Lattice Boltzmann method (LBM):

The lattice Boltzmann method (LBM) is a computation and modeling method different from traditional numerical methods. It has unique features that other numerical methods do not have due to its micro-particle characterization (*Levitt, Michael, 2014*). The Lattice Boltzmann method has the capability to study the multi-scales models. Such models are more realistic and satisfy the current challenges of optimization, theory and research. For example, LBM is used in the field of oncology, it can swiftly demonstrate the complex fluid flows of biological problems, furthermore, this

technique has been successfully used to model the flow through the bone tissues at the pore scale.

Examples of methods	Advantages	Disadvantages
DPD	• Exactly conserves mass and momentum • Designed to model hydrodynamics • Captures basic features of molecular architecture • Effective for modeling of multicomponent systems	• Require mapping to specific physical system • Soft interaction potentials allow particles to overlap • Computationally expensive • Reduced resolution compared to molecular dynamics • Limited to low Schmidt number
LBM	• Efficient for flows in complex geometries and with particles • Computationally efficient including massive parallel computing • Models multi-phase systems • Eliminates statistical noise • Easy to implement	• Range of viscosities is limited by method stability • Typically used for incompressible laminar flows • Difficult to implement certain boundary conditions

Figure 3: Table illustrating the advantages and disadvantages of DPD and LBM methods.

Conclusion

Modeling such advanced computational devices requires force fields that are sufficiently accurate for both the inorganic and organic components, a development effort that will need to be pursued for years to come. Other challenges are posed by the large sizes and long time scales of devices and processes, involving millions of atoms and millisecond to second duration. Advanced computational technology and new concepts in simplifying models using “coarse-graining” allow these challenges to be addressed. Many hurdles must yet be overcome to hone the computational approach, but the examples given above show how computational modeling can already be a useful partner in biotechnology.

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RESEARCH ARTICLE

Effect of Protected Regime and Organic Amendments on Physiology and Yield of Three Green Verdant Vegetables

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Abstract

The point of this investigation was to assess the effect of protected systems and organic treatments on leaf reenness, physiology and development parameters of three green verdant vegetables (Indian spinach, Fenugreek and Amaranthus). Split-split plot designed was utilized to review the results of protected system (S), organic treatment (OT), crop types (C) and their interaction on the expansion and yield of vegetables for three growth stages. Data analysis of SPAD (Soil Plant Analysis Development) meter, chlorophyll quantification, IRGA (Infrared Gas Analyzer) and growth measurements were performed in triplicates (n=3) using agricolae package in R. Two-way variance assessment with mean separation by Tukey's Least Significant Difference (LSD) at $\alpha = 0.05$ was used to compare variations between structures (S1-2), organic treatments (OT1-5) and relationships between them. Results showed values for SPAD, chl. content and photosynthesis rate in polyhouse (S1) were significantly higher ($p<0.05$) than in shadenet (S2) in all 3 crops. For all three crops, the net assimilation rate and dry weight in the polyhouse were considerably greater ($p<0.05$) at all 3 growth phases. Higher SPAD values and values variation not only stated the vegetables ' better output, but also helped to predict the correct time for their harvest. The findings showed that the polyhouse is appropriate compared to the shadenet for increasing short-lived leafy vegetables. Pre-soil sampling, detailed assessment of soil nutrient and biochemical content of organic leafy vegetables, however, would be helpful in making any recommendations on the selection of protected system type and organic applications.

Keywords: Dry Weight, Green Leafy Vegetables, Physiology, SPAD, Protected System

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Introduction

Green leaved vegetables are the mostsupply of minerals (including iron, calcium, potassium, and magnesium), amino acids and vitamins, as well as vitamins K, C, E, and many of the B vitamins (Gupta and Wagle, 1988; Gupta *et al*., 1989; Lintas, 1992).For example, lettuce, rocket, and spinach constitute nitrate (Hord *et al*., 2009) and proof suggests that dietary nitrate is also a cardio-protective (Bondonno *et al*., 2014). Indian spinach (*Beta vulgaris*var. *bengalensis* Hort.) with succulent leaves is one amongstthe foremostwidespread vegetables of tropical and subtropical region and is bigwide in India (Padmanabha *et al*., 2008). *Trigonella foenum-graecum* L. (Fenugreek) is native to Asia and southern Europe illustriouscookery and medicative uses within the history of previous civilizations (Khan *et al*., 2014). *Amaranthus* sp. (Amaranth) is an annual or short-lived perennial plant used in many nations, including India, as leafy vegetables, grain, decorative, leafy vegetables, medicinal plants and forage crops (George *et al.*, 2014; Hauptli *et al.*, 1977). There is a growing demand on the market for such vegetables. Protected vegetable cultivation optimizes the agricultural productivity both in terms of quality (Gruda, 2005) and quantity (Negi *et al*., 2013), helps to prolong the harvest period, shortens the increasing growing period and makes the item accessible in the off-season market (Singh and Sirohi, 2004). Leafy vegetables are herbaceous plants that are short-lived and can readily be cultivated in a short time. If cultivated under protected structures, it is possible to harvest new leafy vegetables throughout the season. Such vegetables, however, are very often susceptible to microbial contamination (Franz *et al*., 2008 ; Gil *et al*., 2015 ; Liu *et al*., 2013), illness (Correll *et al*., 1994) and pest attack (Morelock and Correll, 2008 ; Mureithi *et al*., 2017), including fungicide and pesticide remains (Gonzlez-Rodrguez *et al*., 2008). Growing them under protected schemes will not only deter them from these issues, but will also render vegetables accessible sooner than most other plants for new harvest. In the physiology, efficiency and economy of green plants, chlorophyll pigment plays a distinctive function (Palta, 1990). Nutrient accessibility and multiple environmental variables such as light intensity (Fu *et al*., 2012); temperature and stress affect the amount of chlorophyll in leaf tissue. The content of chlorophylls can be determined photometrically after pigment extraction using an organic solvent such as acetone or dimethyl sulfoxide (DMSO) or by means of a handheld device SPAD (Soil Plant Analysis Development) meter and the connection between leaf chlorophyll concentration and SPAD-

502 chlorophyll meter measurements (Uddling *et al*., 2007; Markwell *et al*., 1995). There was a strong correlation between chlorophyll content and SPAD values for overall nitrogen content (Netto *et al*. 2005; Piekkielek and Fox, 1992) and crop photosynthesis (Torres Netto *et al*., 2002). Photosynthesis and transpiration rates are based on CO_2 and H_2O variations. The rate of absorption of CO_2 is used to evaluate the rate of assimilation of photosynthetic carbon, while the rate of loss of water is used to evaluate transpiration rate. Attempts to forecast crop quality and yield based on SPAD values have also been produced (Bail *et al*., 2005 ; Costa *et al*., 2001). Organic treatments in field plants such as wheat (Saikia *et al*., 2015), corn (Agegnehu *et al*., 2016) and vegetables such as cabbage have been observed to improve biomass (Vimala *et al*., 2006; Islam *et al*., 2017). Although increasing vegetables under protected systems are not new, very little technical assessment on organic leafy vegetables under protected cultivation is seen. The obectives of this research were therefore (i) to assess three organic leafy green vegetables for yield and quality, (ii) to analyze physiological reactions to microclimate circumstances and (iii) to compare their development parameters between two protected structures treated with five distinct organics.

Material And Methods

Study area

The experiment was conducted on the organic field site (Plot No: C 52-A ; Coordinates: 15 ° 29′50.695″ N ; 74 ° 58′40.96″ E, 768.0 masl elevation) at the Organic Farm Institute of Organic Farm, Agricultural Science University, Dharwad, Karnataka, India.

Crops and cultivation

Two protected system and five different organic treatments (Control (OT1), Vermicompost (OT2), Vermicompost + Neem Cake (OT3), Vermicompost + Neem Cake (OT3), Vermicompost + Neem Cake + Rock Phosphate (OT4) and Compost (OT5)) were selected to assess their interactions on three green leafy vegetables (Indian Spinach (C1), Fenugreek (C2) and (C3) Amaranth). Split-split plot layout (S1 and S2 as the two primary plots, crop type (C1, C2 and C3) as split plot and organic treatment (OT1, OT2, OT3, OT4 and OT5) as split-split plot design).

Each plot length of 18 x 1 m was split equally into 5 sub-sub plots in 3 replications. Organics were applied for each type of crop based on the recommended dose (Table 1). Using easy tools such as spade and hand held harrow, soil beds were prepared. Coarse sand and stones have been separated from the beds and removed. Seven days before seed was sown, organics were introduced. Seeds with horizontal row spacing of 11.5 cm and inter-row spacing of 22.5 cm were shown in four rows. In the spring of 2018, crops were cultivated in red soil and medium black loam soil in S1 and green colored S2 (40% shade factor). The average temperature recorded in S1 and S2 was 35.2 and 36.2 and relative humidity (percent) in S1 and S2 was 43 and 41 respectively during the experimental era using Vartech THM-B2 Digital Thermo Hygro Meter. Local organic seeds were collected from an organic market at Dharwad City, North Karnataka, India.

Table 1 Recommended dose of organic nutrients added with respect to crops.

Crop	N (kg/ha)	P(kg/ha)	K(kg/ha)
Spinach (C1)	150.00	100.00	100.00
Fenugreek(C2)	100.00	50.00	0.00
Amaranthus (C3)	100.00	50.00	50.00

Data gathering

SPAD readings were recorded for 3 growth stages (GSs) at 20 Days After Sowing (DAS), 30 DAS and 40 DAS (harvest) representing every sample plant treated with organic nutrients in the field. The leaf SPAD values acquired during this research were the average of 4 readings (2 on each side of the midrib leaf) wherever possible, taking into account the size of the leaves. The SPAD meter (SPAD-502, KONICA MINOLTA, INC.) is a widely used handheld device for immediate and non-destructive measurement in agricultural research of leaf chlorophyll content (Ling *et al*., 2011). The SPAD measures the transmission through the leaf of red (650 nm) and infrared (940 nm) radiation and calculates a comparative SPAD meter value corresponding to the quantity of chlorophyll in the sample leaf (Minolta, 1989). LI-6400 Portable Photosynthesis System (LI-COR, Lincoln, Nebraska, USA) is an open system design photosynthetic gas exchange system in which air moves through the leaf chamber continually to maintain CO_2 concentration and explains the operating principle (Long *et al*., 1996). Readings for all tagged plant samples in the field were taken from 9 am-12 midnight. IRGA measurements were also taken from 9 a.m. to 12:30 p.m., choosing every 4th medium sized leaf from the top of every 3 tagged plant samples wherever possible for 2 GSs. Photosynthetic rate was recorded once the CO_2 concentration was stabilised. The pigments of photosynthesis and their abundance differ between crops. Chl *a* in the species of vegetables helps in oxygen photosynthesis, while other pigments assist to absorb light and transfer light radioactive energy to reaction centers (Costa *et al*., 2010). The leaves sample used for SPAD measurements was weighed to 0.05gm and used for assessment of chlorophyll content. 0.05gm of new leaf specimens were put in a 5mL DMSO test tube (Sudhakar *et al*., 2016) and retained overnight. Absorbances have been recorded for chl *a* at 645 nm. and chl *b* 663 nm for further assessment of chlorophyll content, using Bio Spectrophotometer with Touch Screen and Built-in PC (Elico Limited).

The quantity of chlorophyll in the sample is mg chlorophyll per g (mg g^{-1} f.wt.) of fresh weight as per formula (Arnon, 1949). In the laboratory, measurements of chlorophyll quantification and growth parameters were performed. Stems, leaves and roots were separated from each plant sample and left for 5 days to dry for shade. Overnight oven drying was done at 45°C. Growth parameters were evaluated in accordance with (LAR (cm^2g^{-1}), Radford (1967), (LWR (gg^{-1}) and SLA (cm^2g^{-1}), Kvet and Marshall (1971), (SLW (gcm^{2-1}), Pearce *et al*. (1969)), (NAR ($gg^{-1}t^{-1}$), Williams (1946)) and total DW (gm) at 20, 30 (DAS) and harvest estimated after oven drying.

Table 2 Effect of protected systems and organic nutrients on SPAD and chl. Content (mgg^{-1}f.wt.) of green leafy vegetables

Values (Mean of 3 GSs)	SPAD values	Chl*a*	Chl*b*	T.chl.	Chl *a/b* ratio
S1	42.95^{a}	1.16^{a}	1.51^{a}	2.70^{a}	0.79^{a}
S2	36.77^{b}	1.10^{a}	1.32^{b}	2.45^{b}	0.83^{b}
LSD at 5%	1.88***	0.085^{ns}	0.15***	0.23***	0.03***
Crops (C)					
C1	31.61^{c}	0.80^{c}	0.91^{b}	1.71^{b}	0.85^{a}
C2	51.77^{a}	1.25^{b}	1.63^{a}	2.93^{a}	0.78^{b}
C3	36.21^{b}	1.36^{a}	1.70^{a}	3.09^{a}	0.80^{b}
LSD at 5%	1.90**	0.08**	0.12**	0.20**	0.01**
Nutrient management (OT)					
OT1	38.69^{a}	1.14^{a}	1.42^{ab}	2.57^{a}	0.80^{bc}
OT2	40.16^{a}	1.18^{a}	1.50^{a}	2.70^{a}	0.79^{c}
OT3	40.02^{a}	1.15^{a}	1.46^{ab}	2.63^{a}	0.81^{ab}
OT4	40.43^{a}	1.11^{a}	1.39^{ab}	2.53^{a}	0.80^{bc}
OT5	40.01^{a}	1.10^{a}	1.31^{b}	2.44^{a}	0.83^{a}
LSD at 5%	1.76^{ns}	0.12^{ns}	0.18^{ns}	0.30^{ns}	0.01^{ns}
Interactions (S:OT)					
OT1:S1	42.20^{a}	1.15^{a}	1.49^{ab}	2.64^{abc}	0.79^{de}
OT2:S1	42.89^{a}	1.20^{a}	1.63^{a}	2.83^{a}	0.76^{f}
OT3:S1	42.52^{a}	1.17^{a}	1.60^{a}	2.77^{ab}	0.79^{de}
OT4:S1	42.80^{a}	1.16^{a}	1.51^{ab}	2.67^{abc}	0.79^{e}
OT5:S1	44.38^{a}	1.13^{a}	1.48^{abc}	2.60^{abc}	0.81^{cd}
OT1:S2	35.18^{c}	1.12^{a}	1.39^{abc}	2.51^{abc}	0.82^{bc}
OT2:S2	37.43^{bc}	1.15^{a}	1.41^{abc}	2.57^{abc}	0.83^{abc}
OT3:S2	37.52^{bc}	1.13^{a}	1.38^{abc}	2.50^{abc}	0.84^{ab}
OT4:S2	38.07^{b}	1.07^{a}	1.32^{bc}	2.39^{bc}	0.82^{bc}
OT5:S2	35.65^{bc}	1.07^{a}	1.21^{c}	2.29^{c}	0.85^{a}
LSD at 5%	2.48**	0.17^{ns}	0.26**	0.43**	0.02**
Growth Stages (GSs)					
20 DAS	37.99^{c}	1.029^{b}	1.23^{c}	2.26^{b}	0.83^{a}
30 DAS	39.45^{b}	1.184^{a}	1.60^{a}	2.76^{a}	0.76^{b}
Harvest	42.14^{a}	1.19^{a}	1.41^{b}	2.68^{a}	0.84^{a}
LSD at 5%	1.36***	0.095**	0.13***	0.24***	0.01***

[a]Means with the same letter are not significantly different according to the LSD test at p<0.05.

^{ns,*,**,***}= non-significant, p<0.05, p<0.01, p<0.001 respectively

Results And Discussion

Statistical analysis

All assessment was carried out in triplicates (n=3) corresponding to the two protected systems (S1 and S2). Two-way variance (ANOVA) assessment with Tukey's Least Significant Difference (LSD) mean separation at $\alpha = 0.05$ was used to determine important differences between systems (S1-2), treatments (OT1−5) and their interactions (S1-2: OT1−5). Mean values among (S1-2), (OT1−5) and their (S1-2: OT1−5) were compared using LSD at $P \leq 0.05$. Data analyzes were carried out using the agricolae package R (R Core Team, 2018, de Mendiburu, 2017).

SPAD values and chlorophyll content

There was a significant difference ($p<0.01$) between two systems and between the three crops in SPAD values (Table 2). The mean comparison with the interaction (S1-2: C1−3) and (S1-2: OT1−5) was significant (***). For 3 growth phases (GSs), adjusted R^2 for SPAD values was 0.866. Over 3 growth stages, SPAD values improved significantly (***) except for C3. Chl *a*, *b* and total chl. significantly varied (S1-2, OT1−5 and C1−3)-wise. (S1-2: OT1−5) interaction was also significant (*) and adjusted R^2 was 0.874. Total chl. content was significantly higher (*) in S1 compared to S2. Chl *a* /*b* ratios have been studied to compare how their content varies under different treatments (OT1−5) and systems (S1-2). Chl *a* /*b* ratio was higher in S2 compared to S1 (Table 2). Interactions between the (S1-2: OT1−5 and C1−3: S1-2) for SPAD values and total chl. content were significant (**) over all 3 growth stages. However, effects of organic treatments on SPAD values and chl content were insignificant ($p>0.01$)

Physiology

LSD tests for Photosynthetic rate (*A*) (μmol CO_2 m^{-2} s^{-1}), Stomatal conductivity (g_s) (mol H_2O m^{-2} s^{-1}), Intercellular CO_2 (*Ci*) (μmol CO_2 mol^{-1}), Transpiration (*E*) (mmol H_2O m^{-2} s^{-1}) and Leaf temperature (°C) were significant over different stages (Table 3). The photosynthetic rate (A) was significantly higher (***) in S1 compared to S2. Effects of protected systems and organic nutrients on *A*, g_s, C_i , *E* and leaf temp of green leafy vegetables for 2 GSs was significant ($p<0.01$). (S1-2 : C1−3) interaction was also significant ($p<0.01$). Adjusted R^2 for *A* for 2 GSs was 0.753. A sharply decreased after 30 DAS. Almost all 3 crops were ready for harvest in S1. Crops maturity in S2 was delayed by a week. Interactions between (S1-2: OT1−5) was insignificant ($p>0.01$). *A* was recorded higher in C3 consistently followed by C1 and C3. *A* was unaffected by treatments however the (S1-2: OT1−5) and (S1-2 : C1−3) interactions was significant (**). $g_s$was not affected by S1-2 and interactions. However, $g_s$was higher in S1 compared to S2. Adjusted R^2 for g_s was 0.687 for 3 GSs. C_i decreased with the increase in *A* and g_s. Adjusted R^2 was 0.739 for C_i for 3 GSs. *E* was higher in S2 compared to S1 whereas relative humidity was vice-versa. *A* decreased with decrease in *E*. Adjusted R^2 is 0.474 for *E* for 2 GSs. Leaf temperature was significantly higher in S2 compared to S1. Adjusted R^2 for *E* for 2 GSs was 0.623. Its (S1-2: OT1−5) interaction was not significant ($p>0.01$).

Table 3 Effects of protected systems and organic nutrients on photosynthetic rate *A* (*μ* mol CO_2 m^{-2} s^{-1}), stomatal conductance g_s(mol H_2O m^{-2} s^{-1}), intercellular CO_2*Ci* (*μ*mol CO_2 mol^{-1}), transpiration *E* (mmol H_2O m^{-2} s^{-1}) and leaf temperature °C of green leafy vegetables for 2 GSs.

Mean of 3 GSs	*A*	g_s	C_i	*E*	Leaf temp.
S1	16.81[a]	0.33[a]	291.40[a]	6.25[a]	32.53[b]
S2	12.20[b]	0.31[a]	298.95[a]	7.91[a]	34.03[a]
LSD at 5%	1.61***	0.10[ns]	18.56[ns]	2.13[ns]	1.46***
Crops (C)					
C1	13.77[b]	0.50[a]	348.41[a]	9.08[a]	31.75[c]
C2	10.36[b]	0.31[b]	334.92[a]	6.79[b]	33.07[b]
C3	19.37[a]	0.16[c]	202.21[b]	5.37[c]	35.03[a]
LSD at 5%	3.47***	0.06***	29.83***	0.91***	0.56***
Nutrient management (OT)					
OT1	13.98[b]	0.34[a]	303.93[a]	7.08[a]	33.17[b]
OT2	15.15[a]	0.34[a]	302.05[a]	6.98[a]	33.10[b]
OT3	15.10[a]	0.33[a]	304.06[a]	6.89[a]	32.90[b]
OT4	15.54[a]	0.36[a]	288.90[a]	7.45[a]	32.72[b]
OT5	12.75[c]	0.26[b]	276.94[a]	7.00[a]	34.61[a]

LSD at 5%	0.71^{ns}	0.08^{ns}	33.49^{ns}	1.39^{ns}	0.59^{ns}
Interactions (S:OT)					
OT1:S1	17.30^{b}	0.34^{a}	289.04^{a}	5.91^{cd}	32.30^{cd}
OT2:S1	16.35^{b}	0.32^{a}	289.40^{a}	5.52^{d}	32.38^{c}
OT3:S1	17.27^{b}	0.34^{a}	302.83^{a}	5.93^{cd}	32.12^{cd}
OT4:S1	18.75^{a}	0.40^{a}	297.94^{a}	6.25^{cd}	31.51^{d}
OT5:S1	14.37^{c}	0.28^{ab}	277.80^{a}	7.63^{abc}	34.33^{ab}
OT1:S2	10.67^{e}	0.34^{a}	318.81^{a}	8.26^{ab}	34.04^{b}
OT2:S2	13.95^{c}	0.35^{a}	314.71^{a}	8.44^{a}	33.62^{b}
OT3:S2	12.93^{d}	0.33^{a}	305.30^{a}	7.85^{abc}	33.68^{b}
OT4:S2	12.34^{d}	0.33^{a}	279.87^{a}	8.65^{a}	33.93^{b}
OT5:S2	11.13^{e}	0.19^{b}	276.09^{a}	6.36^{bcd}	34.89^{a}
LSD at 5%	1.00^{***}	0.12^{ns}	47.36^{ns}	1.97^{***}	0.83^{***}
Growth Stages					
30 DAS	20.35^{a}	0.34^{a}	269.26^{b}	8.13^{a}	32.91^{b}
Harvest	8.66^{b}	0.30^{a}	321.10^{a}	6.03^{b}	33.65^{a}
LSD at 5%	0.45^{***}	0.05^{ns}	21.18^{***}	0.88^{***}	0.37^{***}

[a]Means with the same letter are not significantly different according to the LSD test at $p<0.05$.

^{ns,*,**,***}= non-significant, $p<0.05$, $p<0.01$, $p<0.001$ respectively

Table 4 Effects of protected systems and organic nutrients on LAR (cm^2g^{-1}), LWR (gg^{-1}), SLW (gcm^{2-1}), SLA (cm^2g^{-1}), NAR ($gg^{-1}t^{-1}$) and DW (gm) of green leafy vegetables for 3 GSs.

Mean 3 GSs	**LAR**	**LWR**	**SLW**	**SLA**	**NAR**	**T.DW**
S1	26.77^{b}	0.27^{b}	0.002^{a}	54.78^{b}	1.052^{a}	2.70^{a}
S2	36.51^{a}	0.29^{a}	0.001^{b}	64.32^{a}	0.39^{a}	0.93^{b}
LSD at 5%	8.16^{***}	0.02^{***}	0.0003^{***}	9.23^{***}	0.77	0.99^{***}
C1	42.23^{a}	0.32^{a}	0.002^{b}	73.11^{a}	1.35^{a}	2.43^{a}
C2	29.05^{b}	0.29^{b}	0.002^{a}	53.67^{b}	0.17^{b}	0.42^{b}
C3	23.63^{b}	0.23^{c}	0.002^{a}	51.87^{b}	0.65^{b}	2.59^{a}
LSD at 5%	5.65^{**}	0.02^{***}	0.0002^{**}	8.75^{**}	0.63^{**}	0.9^{**}
Nutrient management (OT)						
OT1	31.20^{a}	0.28^{ab}	0.002^{ab}	58.15^{a}	0.69^{a}	1.49^{b}
OT2	29.70^{a}	0.26^{b}	0.002^{a}	59.27^{a}	1.10^{a}	2.78^{a}
OT3	32.08^{a}	0.28^{ab}	0.002^{a}	58.13^{a}	0.74^{a}	1.92^{ab}
OT4	31.62^{a}	0.28^{ab}	0.002^{a}	59.34^{a}	0.46^{a}	1.23^{b}
OT5	33.60^{a}	0.30^{a}	0.001^{b}	62.86^{a}	0.61^{a}	1.65^{b}
LSD at 5%	6.50^{ns}	0.04^{ns}	0.0001^{ns}	9.01^{ns}	0.76^{ns}	1.14^{ns}

Interactions (S:OT)						
OT1:S1	28.37^{bcd}	0.26^{ab}	0.0022^{bcd}	57.34^{abc}	1.10^{ab}	2.53^{ab}
OT2:S1	24.15^{d}	0.25^{b}	0.0024^{ab}	53.80^{c}	1.74^{a}	4.07^{a}
OT3:S1	27.82^{bcd}	0.28^{ab}	0.0023^{abc}	54.21^{bc}	0.88^{ab}	2.60^{ab}
OT4:S1	27.23^{bcd}	0.26^{ab}	0.0025^{a}	52.09^{c}	0.59^{b}	1.77^{bc}
OT5:S1	26.27^{cd}	0.29^{ab}	0.0023^{abc}	56.43^{bc}	0.95^{ab}	2.53^{ab}
OT1:S2	35.26^{abc}	0.30^{a}	0.0020^{de}	58.96^{abc}	0.28^{b}	0.45^{c}
OT2:S2	34.02^{abc}	0.27^{ab}	0.0020^{e}	64.73^{abc}	0.46^{b}	1.50^{bc}
OT3:S2	36.34^{ab}	0.29^{ab}	0.0021^{cde}	62.05^{abc}	0.61^{b}	1.25^{bc}
OT4:S2	36.00^{ab}	0.29^{ab}	0.0019^{e}	66.59^{ab}	0.33^{b}	0.69^{c}
OT5:S2	40.93^{a}	0.30^{a}	0.0017^{f}	69.30^{a}	0.28^{b}	0.76^{c}
LSD at 5%	9.19^{ns}	0.05^{ns}	0.0002^{ns}	12.75^{ns}	1.08^{ns}	1.6*
Growth Stages (GSs)						
20 DAS	16.26^{c}	0.50^{a}	0.001^{c}	74.46^{a}	-	0.61^{b}
30 DAS	29.12^{b}	0.18^{b}	0.002^{b}	54.66^{b}	0.37^{b}	1.05^{b}
Harvest	49.53^{a}	0.16^{b}	0.003^{a}	49.53^{b}	1.07^{a}	3.78^{a}
LSD at 5%	5.03***	0.03***	0.0001***	6.98***	0.48***	0.88***

[a]Means with the same letter are not significantly different according to the LSD test at $p<0.05$.

^{ns,*,**,***}= non-significant, $p<0.05$, $p<0.01$, $p<0.001$ respectively

-It was not measured

Growth parameters

Tukey's Honest Significant Difference (Tukey's HSD) method to make all the pair-wise comparisons was used to compare all the means of LAR, LWR, SLW, SLA, NAR and DW (gm) (Table 4). In general, LSD test for growth parameters were significant over growth period and between interactions. Total dry matter production was comparatively higher in OT2 followed by OT3 and OT5. Total DW was significantly low for OT4 followed by OT1. Total dry weight (DW) of all 3 crops (C1, C2 and C3) was significantly higher ($p<0.01$) in S1 compared to S2. Treatments- and system-wise total DW increased significantly over the growth stages, however interaction between system: treatment within same system (S1-2: OT1−5) remained insignificant ($p>0.01$). LAR was higher in S2 compared to S1. Adjusted R^2 was 0.614 for LAR mean of 3 GSs. LWR was another growth parameters measured for the analysis which was significant (***) for treatments, system and crops for all 3 GSs. LWR was higher in S2 compared to S1. However interaction between (S1-2: OT1−5) was insignificant. Adjusted R2 was 0.717 for LWR mean of 3 GSs. LWR was significant for 3 GSs. SLW was higher in S1 compared to S2. Interactions were insignificant. Adjusted R^2 was 0.399 for SLW of mean of 3 GSs. SLW was also significant (***) for 3 GSs. SLW increased over 3 GSs. SLA was significant (***) for 3 GSs. Adjusted R2 was 0.378 for SLA mean of 3 GSs. NAR significantly higher in S1 compared to S2. NAR was significant (***) for (S1-2: OT1−5) and (S1-2: C1−3) interactions. NAR steadily increased over 3 GSs for all 3 crops. NAR of C3 was significantly higher (***) compared to C1 and C2. Adjusted R^2 was 0.306 for NAR of mean of 3 GSs. Total DW of C3 was significantly higher than C1 and C2. System-wise and treatment-wise, total DW was significant (***) for all 3 GSs. (S1-2: OT1−5) interaction was insignificant ($p>0.01$). Adjusted R^2 was 0.525 for total DW of mean of 3 GSs.

Discussion and correlation

SPAD mean values were significantly higher for all 3 crops in S1 (polyhouse) than in S2 (shadenet). Factorial impacts (Abdelhamidg *et al*., 2003) including micro-climate within the protected scheme can be ascribed to a steady rise in SPAD values. Higher SPAD values also show higher amount of N content in the leaf (Uchino *et al*., 2013 ; Nyi *et al*., 2012 ; Wood *et al*., 1993 ; Xiong *et al*., 2015) with a strong correlation between DW in rice and spinach which is reported by Yang *et al*. (2014) and Liu *et al*. (2006) respectively. It could also imply that the values of SPAD and crop health are strongly correlated. There was a strong and negative correlation between shading intensity and leaf relative chlorophyll content (SPAD value) indicating a strong positive correlation between leaf chlorophyll content and yield (OMBDI *et al*., 2015). The SPAD values for C1 (Indian

spinach), C2 (Fenugreek) and C3 (Amaranthus) were significantly distinct. The C2 SPAD values were greater than those of C1 and C3. Similar variations in SPAD measurements were also recorded in coffee leaves (Netto *et al*., 2005) and Jatropha leaves (Nyi *et al*., 2012) SPAD values increased consistently over the 3 growth phases until harvest (40 DAS) with the exception of Amaranthus, which had matured in polyhouse 10 days previously compared to shadenet. This trend was noted during reproductive and senescence phases in quinoa and amaranth (Riccardi *et al*., 2014), during which SPAD values declined. With respect to texture of the leaves, crops in shadenet have thinner lamina with softer texture as compared to polyhouse crops. This could have been as a result of light intensities variation in polyhouse and shadenet (Ili *et al*., 2015). The content of leaf chlorophyll in S1 and S2 plants improved steadily from 20 DAS to harvest. The content of chl*a* in S1 was comparatively higher than in S2, as was the total production of DW in S1 compared to S2. Similar linear relationships between SPAD measurements and chl *a*, *b*, or total chl content have been recorded (Wang *et al*., 2005). This could be owing to the content of chl *a* and *b*, which are vital pigments for turning light energy into chemical energy (Steele *et al*., 2008) for main plant products. As anticipated from chl *b* in crops, their content in S2 and S1 was comparatively higher than chl *a* owing to low light intensities (Lei *et al*., 1996; Schiefthaler *et al*., 1999). But in S2 chl *b* content was much higher between two protected systems. Similarly, the chl *a* : chl *b* ratio in S2 across all three crop kinds was significantly higher. Boardman, 1977 ; Murchie and Horton, 1997 ; Kosma *et al*., 2017 ; Vandana and Bhatt, 1999 reported such differences in chl *b* content.

The influence on growth condition of protected systems was significant. LAR, LWR, SLA and total DW. NAR was significant over growth period with system interaction. Except for NAR and total DW, effect of organic treatments on other growth parameters was insignificant (P>0.05). However, there was a significant effect of organic treatments on total production of DW. Similar effect in sunflower nitrogen treatment was reported (Kumar *et al*., 2010). There was significant effect on total DW including on other growth parameters as a result of system: treatment interactions. Treatment with OT2, followed by OT3 and OT5 resulted to higher total DW production irrespective of system influence. SPAD values with LAR, LWR and SLA were negatively correlated. Marenco *et al*. (2009) reported this as well. The response of crops to organic treatments in combination with OT4 was poor. This may be due to the fact that the efficacy of rock phosphates as fertilizer depends on soil characteristics, particularly acidity, and the status of calcium and phosphate (Le Mare, 1991 ; Bolland and Gilkes, 1990).

In S1 NAR and total production of DW were significantly higher than S2, which increased proportionately over the growth stages. Results from the study of growth parameters also showed a decrease in LWR and SLA that could have been affected by spacing between the rows of plants (Papadopoulos and Pararajasingham, 1997). For organic growers, vermicompost, neem cake, rock phosphate and compost are important organic treatments. Vermicompost is a nutrient-rich organic manure readily available to plants with a low C: N ratio, high porosity and high water-holding capacity, capable of boosting soil fertility and significantly improving plant growth by the nutrients it contains (Lazcano and Dom'ınguez, 2011 ; Lim *et al*., 2015 ; Saadatian *et al*., 2017 ; Hernandez *et al*., 2010). Neem cake is another potential source of organic manure, the by-product obtained after the extraction of neem oil, with a sufficient amount of NPK including essential micronutrients. It is also used as an effective agent for bio-control. Compost is a soil conditioner although not rich in essential nutrients (Rashid, 2011) and an important source of organic nutrients based on its decomposition stage has the potential to improve the soil condition in the long term. In terms of yield and production, crops under protected cultivation with organic nutrients perform better.

Crops under protected cultivation with organic nutrients perform better in terms of their yield and production. Close correlation among *A*, g_s, *E* and $C_i$are known facts (Miyashita *et al*., 2005; Jarvis and Davies, 1998). *A* was significantly higher in S1 relative to S2 but declined from 30 DAS to harvest over the GSs, whereas intercellular CO_2 (C_i) was comparable in system variations. Stomatal conductance (g_s) was not affected by S1-2 and C1-3 interactions. Like *A*, $g_s$was higher in S1 compared to S2. Similar observation is made by (Broadley *et al*., 2001). Total chl and chl *b* and was observed to be higher in S1 than in S2 (Table 1). Higher chlorophyll contents have positive correlation with higher *A*, g_s, and *E* (Lee *et al*., 2007). This could have led in higher *A* in polyhouse, attributing higher total production of DW in polyhouse relative to shadenet. While optimizing the greenhouse environment is aimed at optimizing the plant photosynthetic process, it is not enough to conclude that single factors such as polyhouse interactions and shadenet interactions with organic nutrients would affect the *A*, g_s, *E* and $C_i$of the crops under experiment.

Conclusion

Higher SPAD values, chlorophyll content, physiological parameters and their differences during growth phases in protected green leafy vegetables not only stated an increase in DW but were also helpful in anticipating harvest time among vegetables. Crop matured earlier by 10 days in polyhouse compared to shadenet. Green leafy polyhouse vegetables have done much better than shadenet vegetables. The organic treatments less affected the total DW of all 3 leafy vegetables relative to the system: crop interaction. In the short time for marketable yield, crops under polyhouse performed significantly better than crops in shadenet. Except for vermicompost-treated green leafy vegetables, other organic treatments have insignificant effect. This could be because of their slow nutrient release pattern or could be the residual effect from previous use of organics in the same plot. If further research is to be carried out on the effects of organic treatments on leafy vegetables, understanding the soil nutrient status before new treatments, past crop history, pests and diseases would be helpful to start the study under protected cultivation.

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REVIEW ARTICLE

River Ganga- the miraculous Natural Resource is indispensable for Human being and the Environment

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Abstract

This research manuscript is prepared to explain the values, relevance and immense significance of water, the amazing and miraculous Natural Resource on the planet- Earth, for the better and quality life to human beings and the environment. Further, the river Ganga and her amazing water qualities are imperative and indispensable for the good health of the people and associated communities. The well being of environment is also directly influenced by the water of this life line river of India. Various issues related to water and their qualities, utilities including therapeutics have been elaborately discussed with experimental facts. The pollution scenario of entire river Ganga, both in upper stretches and lower stretches, have been statistically portrayed. The panic of water pollution and associated several fatal diseases caused in human beings find ample space for deliberation. I have scanned literature related to lethal and detrimental effects of metallic pollution, house hold/municipal and industrial effluents' pollution leading the causation of several dreaded diseases and health complications in human beings and the associated communities. The analysis made here provides sufficient back ground to conclude that the health of river Ganga is alarmingly bed ridden and calls for an emergent attention for rectification and improvement. The rejuvenation of Ganga is not only necessary for the river survival and health but also for the good of ours and the environment and even generations ahead.

Key words:

Water, Human Growth and Civilization, River Ganga, Pollution Scenario of Ganga, Human health and Diseases, Health remedial measures.

Introduction

Water is the most important resource needed for growth, development and sustenance of life on earth. The water resources and quality affect the economic, social and political development of the society. Ganga is the cultural heritage and symbol of faith for billions in India and the world. Ganga water is believed to be the purest water since time immemorial according to Indian mythology. Ganga water is thought of self purifying/rectifying nature and thus is of uniqueness.

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The river Ganga water is frequently used for drinking, cooking and bathing purposes because of sustenance of its purity features even after prolonged periods of storage. Most religious beliefs involve some ceremonial use of ''holy'' water and in India the water of Ganga is so revered with enormous faith. Under continuous Saraswati Indus civilization, prior to 7500 BC, Ganga is described in Rigveda (Hausler, 2006). Ganga plain is one of the most densely populated regions of the world, due to its availability of water and fertile soil and suitable landscape. Density of river is high in eastern UP. Due to the ample availability of water throughout the year, it has played a significant role in the growth of Indian civilization and economy (Paul and Sinha, 2013) accounting for 25% of Indian water resources. Ganga basin houses and supports the lives of more than 400 million people in India, Nepal and Bangladesh (Gopal, 2000) and has very rich heritage, cultural and religious values. This river drains about 1,060,000 km2 area and is the fifth largest in the world (Welcomme, 1985). The river system irrigates about one fourth of Indian subcontinents thereby making Indo-Gangetic plains as the most fertile zones in our country.

Peculiar features of water

Water is our cultural heritage and life without water is beyond imagination. Dynamism on earth is due to water. Life evolved in water. Approximately, 79 % on earth is water and same quantity is found in our body. Human body thus resembles with Limnology- the fresh water Ecology. Liquidity, fluidity, flexibility etc., in all the systems, are being maintained and managed by water. The bio-molecular organization of body is a reflection of significant water interactions. Human civilization is a kind and generous gift of water to human beings. Further, its time immensely realized to go back again to watering holes for more than satisfying the water needs because smiling friends and glittering dreams are there to revitalize and re-energize us.

Significance of Ganga

River Ganga directly and indirectly affects almost 1/3rd of Indian human population, besides nurturing and caring for the vast aquatic biotic community. Ganga is the life line of Indians including the religious, emotional, social and spiritual aspects. Note worthy to mention that the managerial and maintainable influences of Ganga are on major area of the environment. The following legendary admirations from foreign intellectual further make us feel immense pride on Ganga.

"There is not a river in the world which has influenced humanity or contributed to the growth of material, civilization of social ethics, to such an extent as the Ganges. The wealth of India has been concentrated on its valley and beneath the shade of trees whose roots have been nourished by its waters. The profoundest doctrines of moral Philosophy have been conceived, to be promulgated a far for the guidance of the world" (Stevenson, 1860-1922).

A **B** **C**

Pictures showing pollution free scenes from Gangotri Dham, the home land of Ganga. Note the silver shining water of Ganga. In A and C pictures, I am collecting water sample for research study. Amazing scenes of multicolored sun rays coming on earth as blessings.

Other issues

Several other issues related to water, like nutrition, drowning, droughts, zoonoses, changes in labor requirements (women and children), perceptions of changes in human health, conflicts over to water, increased income, tourism, environmental hazards, are of paramount relevance and concern for human and the world.

Water Quality

Several needs of quality water are irrigation**,** human consumption and other uses like laundry, animals etc. Ganga river is life line of Kanpur also since its water is used for domestic and agriculture purposes and therefore, effective maintenance of water quality is required through appropriate measurements. Physicochemical and microbiological characteristics are worth describing the quality of water (Sinha, 1986) and therefore, an analysis on these issues was carried out by many workers (Mehrotra, 1990, Sinha *et al*., 2000).

D **E** **F**

Pictures showing immense value of water in life. In Picture D- child is trying to quench the thirst by risky way. In Picture E- child is collecting water for drinking purpose and in F- animals are rushing to river for water drinking and bathing purpose for diminishing their increased body temperatures.

Water Scarcity

The anticipated next world war will be only for water. Absolute scarcityis being faced because of too less rainfall in comparison with evaporative demand. Erratic rainfallcauses dry spells and droughts. Likewise, soil disturbance occurs due to reduced soil permeability. Population growth is unarguably the sole reason for all types of scarcities. The combinatorial consequences of hydro climatic limitations and population growth lead to several complications.

Pollution Scenario of Ganga in India

The domestic house hold wastes, municipal sewage sludge wastes etc., containing nearly all types of pathogenic coliform bacteria, are considered as the biggest cause of about 85% pollution of river Ganga while, about 15% Ganga pollution is due to industrial effluents. The majority of the Ganga pollution is organic waste, sewage, trash, food and human and animal remains. River Ganga is badly polluted in lower stretches and hence, it is ironically called the journey of pollution from Haridwar onwards to the bay of Bengal. Approximately, 90 cities of various sizes and thousands of villages are located along the Ganga banks in its closest vicinity and nearly all of their sewage/ wastes, over 1.3 billion liters per day, directly get drained in the river along with thousands of animal carcasses, mainly cattle (Bharadwaj *et al*., 2011). Some of the prominent cities like Kanpur discharges nearly 259 MLD wastes, Varanasi nearly 250 MLD wastes, Patna about 150 MLD wastes and West Bengal nearly 700 MLD wastes. Surprisingly, nearly 45 MLD untreated waste water of 234 cities is discharged in Ganga and 142 industries of Uttar Pradesh add approximately 260 millions of liters of industrial effluents/wastes in Ganga/day. Coliforms in Ganga water at Varanasi were observed as 10,000 % higher i.e. 50,000 bacteria/100 ml of water (CFU). It was stated in 2012, during the meeting of Ganga Basin Authority, that every day 29,000 trillion litresof wastes, dirty and polluted water and industrial effluents are drained in the river Ganga in her journey of nearly 2525 kilometers from Gangotri to the bay of Bengal. Domestic and industrial wastewater constitute as a constant polluting source, whereas surface runoff is a seasonal incidence of pollution controlled mainly by the climate (Singh *et al.*, 2004).

Ganga Pollution in Upper Himalayan Regions

Nearly 860 million litres daily domestic and municipal sewer are being drained in Ganga in Himalayan regions thereby turning the situations panic amounting to serious pollution problems. Thus, less polluted Ganga in upper Himalayan regions, is becoming more polluted like the downstream Ganga (Uttarakhand State Report, 2005-2009). Approximately 26 MLDsewage in alaknanda, from shree Badrinath to Devprayag, comes in. Nearly, 10 million population resides nearby Ganga and 80% sewage is drained into Ganga from Uttarkashi, Srinagar, Devprayag, Rishikesh to Haridwar (about 250 Km. distance). The seven districts of Garhwal Himalaya, namely- Uttarkashi, Chamoli, Rudraprayag, Tehri, Dehradun, Pauri and Haridwar discharge daily 13.768, 10.926, 0.364, 5.449, 11.979, 2.660 and 41.804 million litres of sewer/domestic wastes directly and indirectly to Ganga respectively. Over the past century, city populations along the Ganga have grown at an accelerating rate, while waste control infrastructure has remained relatively unchanged.

G **H** **I**

Pictures, **G, H, I** showing damaging and detrimental anthropogenic activities challenging the survival of healthy Mountain Ecosystem and the river Aquatic Ecosystem in Garhwal Himalayan regions.

River Ganga Pollution in Rishikesh

Rishikesh, the International Capital of Yoga and most liked tourist destination, is scenically located at the Gateway to Garhwal Himalaya. River Ganga reaches here from the upper Himalayas. Rishikesh is an important tourist destination globally because of piligrimages, adventure sports, yoga etc. Reviewing the pollution situation at Rishikesh is significant due to many times more influx of transient human population (tourists etc.) almost round the year and it increases enormously in Monsoon Season during Kawar Yatra (nearly 35 millions kawarias). Rishikesh is filled almost entirely with budget hotels and most accommodations and restaurants are in and around Lakshman Jhula, Swarg Ashram and along the banks of river Ganga where tourists stay. Further, city wastes as hospital garbage, polythenes and plastic bags/ pouches, human sputum, fecal matter, open urinals and pollutants like Lead, Mercury, Cadmium, polychlorinated biphenyls etc., coming from electronic wastes, computers, mobiles, refregerators etc. find their ultimate home in Ganga.There is an alarming increase in the number of patients of diarrhoeal diseases in the city mainly in rainy season and elsewhere (Zwane and Kremer, 2007, World Health Organization, 2008, Kosek, *et al.*, 2003). Domestic house hold wastes and

municipal sewage sludge wastes are considered as the biggest cause of about 85% pollution of river Ganga. Rishikesh is situated on the bank of holy river Ganga but around 20% of population does not have sewer lines and hence dirty domestic waste water is drained in Ganga amounting to around 85% of pollution specially the bacterial pollution. This research manuscript is extremely significant because of dealing with the consequences of ever increasing human population, urbanization, construction activities and immense anthropogenic interventions' caused pollution in river Ganga in Himalayan regions. Further, devising new strategic measures, based on experiences of this study, for effective control of mitigating the pollution crises enhances far more the importance of this research work.

J **K** **L**

Pictures **J K L** are showing the brutal and unethical human actions by direct drainage of municipal/ domestic sewage sludge in Ganga at Laxman Jhula Rishikesh.

M **N** **O**

Pictures **M N O** from Chandreshwar Nagar, Rishikesh are again showing the heart breaking scenes of human devil acts with Ganga.

P **Q** **R**

Pictures **P Q R** showing merger of the biggest drain with city waste/ municipal and domestic sewage sludge in Ganga at Triveni Ghat Rishikesh in 2015.

Drinking Water Standards (EPA)

Chemical	Maximum Contaminant Level (mg/L)
1. Lead	0.015
2 .Mercury	0.002
3. Nitrate (as N)	10.0
4. Nitrite (as N)	1.0
5. Alcohol (lasso)	0.002
6. Aldicarb	0.007
7. Atrazine	0.003
8. Carbofuran (furadan)	0.04

Relevant human health issues

Water quality**,** Water availability: quantity & accessibility**,** Hygiene behaviorandVector borne diseases are issues of paramount concern to human beings for their good health and quality life.

Water and human health

Biological water quality is adversely affected by various sources of contamination, lack of sanitation, upstream activities and transport and in house contamination and consequently poses threatening and damaging effects up to 95% in the form of several prominent fatal water borne diseases like gastroenteritis, conjunctivitis, encephalitis, dermatitis, giardiasis, amoebiaosis, colitis, diarrhoea of several types like bacterial, viral and protozoan, malaria etc. Mainly the bacterial diarrhea including *Salmonella*; *E. coli*; *Pseudomonas*are being transmitted through contaminated water and prevalent in developing countries with poorer hygienic conditions and low socioeconomic status. Close proximity of drinking, bathing, sewage, washing etc. are envisaged as the main precipitating factors for such diseases. An estimated 80% of all health problems and 1/3rd of deaths in India are attributable to water borne diseases (Air pollution rising in Kanpur CSE BS Reporter/New Delhi Dec 8, 2009). Further, 1/3rd of deaths are caused due to water pollution. It is a fundamental truth that good water quality produces healthier humans than the poor water quality.

Water borne diseases become more panic and severe when water bodies get densely contaminated and polluted. Approximately 85% Ganga pollutionis due to domestic wastes**(**Uttarakhand State Report, 2005-2009**)**. Nearly 90%human diseases are water borne (Zwane and Kremer, 2007, World Health Organization, 2008; Kosek *et. al.*, 2003). Recent water samples collected from Ganga in Varanasi revealed the fecal coliform counts of about 50,000 bacteria per 100 milliliters of water (CFU), 10,000% higher than the government standard for safe river bathing. The result of this pollution is seen in the form of aetiological factors responsible for the causation of an array of water borne diseases including cholera, hepatitis, typhoid and amoebic dysentery. Malariaand other mosquito transmitted diseases like dengue, yellow fever, filariasis are water quality dependent and malaria only is responsible for more cases of morbidity and mortality than any other tropical disease**.** Water plays crucial roles in transmission of many infections and diseases. High humidity and stagnant waterare accountable for about 2 billion people being exposed to this disease and nearly 25% of children deaths.

Ganga pollution and human health

Industrial effluents and sewage/domestic wastes entering the water bodies are among the main sources of environmental toxicity damaging and destroying the aquatic biota and deteriorating the water quality (Tripathi, 1993, Sinha and Paul, 2012, Sinha *et.al.*, 2016). The heavy metals' caused river water pollution, in terms of toxicity, is one of the serious threats posed in most of the metropolitan cities of India and other developing countries. These heavy metals are of serious concerns due to not being readily degradable and accumulate in the animal as well as human bodies through the food chain causing serious environmental problems (Parveena *et.al*., 2010). Heavy metals in water systems are hazardous to human health including the developmental retardations, nephrological complications, cardiac troubles, various cancers and even deaths **(**Paul, 2017, Jaishankar, *et.al.*, 2014; Solenkova *et.al.*, 2014; Vaishaly *et.al.*, 2015). Major pollutants found in water include volatile, biodegradable and recalcitrant organic compounds, toxic metals, plant nutrients, suspended solids, microbial pathogens and parasites (Bitton, 1994; Sinha and Paul, 2016). The effects of heavy metals on various immune structures and their functions have been thoroughly investigated and damaging alterations in the lymphoid organs were found (Khangarot and Tripathi, 1990 and 1992; Khangarot *et. al.*, 1999). Further, the humoral and cell mediated immune responses were also found badly damaged (Khangarot and Tripathi, 1991; Khangarot *et.al.*, 1999). The findings of various researchers have shown that the practice of discharging waste from ever increasing number of industries and untreated domestic sewage into the aquatic ecosystem is continuously increasing the concentration of heavy metals in river water (Wang *et.al.*, 2011; Martin *et.al.*, 2015; Ali *et.al.*, 2016; Capangpangan *et.al.*, 2016). The faunal diversity of river Ganga was surveyed extensively (Sinha, 2014) from Haridwar to Farakka (1929 Km) and 87 species of zooplankton, 83 species of fishes were observed.

Clinical Application of Ganga Water

Water has been used, from time immemorial, for remedial and curative purposes. The river Ganga water has wide medicinal uses in local therapy. Further, outbreaks of acute diarrheal disease have been identified as causes of fatal disease dating back as far as the Sanskrit literature and during Hippocratic times (McMahan and Dupont, 2007). Though invisible, it was possible to show that this principle was particulate and D' Herelle called it bacteriophage (Herelle, 1922). Hippocrates, going back to 500 BC, described the healing of disease with Ganga water. Kloss (1929) observed the curative effect of Ganga water bath in the treatment of leprosy. Thus, the world credits Ganges water for discovery of bacteriophages. Overuse in human medicine and for agricultural purposes has become a recognized medical problem and scientists have become

increasingly concerned about the occurrence of antibacterial resistance in the environment.

The most unfortunate part of this curative and therapeutic roles played by Ganga water in present day scenario is refuted and wildly negated by the famine of water pollution.The scientific researches based observations, on curative and clinical effects of Ganga water, are all about hundred years old when Ganga was pure enough with all divine features and pollution was not even in the stage of inception. We have to leave no stones unturned for restoration of Ganga health by making it free from pollution menace if we desire to enjoy the clinical applications and curative relevance of Ganga for ourselves and associated communities.

Conclusion

This research manuscript comprehensively investigates various impacts of pollution, specially the water pollution, on the health of the most revered river of this planet- Earth, Ganga and the environment, beginning with the mythological features, cultural and civilizational relevances, philosophical implications on human life and moving towards the dependence of human life and biotic and abiotic associates and finally reaching to the destination of environmental contributions in mitigating the water pollution crises for the good of the health of Ganga directly and dependent communities and components indirectly. The present review portraits the prevailing national scenario of pollution including metallic pollution and associated effects on human health and Ganga. Further, the details on sources and discharge of sewer and other wastes, coming from several sites in Upper Himalayan regions, have been put forth with a particular focus on the most favored tourist destination- Rishikesh, also known as the World Capital of Yoga - the holy city of India. The present manuscript explains the observations of several experimentations performed by various workers on heavy metal pollution in Ganga. An elaborative research survey has been conducted on various experimental findings related to toxicants and their detrimental effects directly on Ganga, by threatening its health and survival and indirectly the various dependent and associated biotic and abiotic components of the environment including the human beings. Several water borne diseases have been found caused due to the pollution related complications in Ganga. Based on the foregoing deliberations and discourses, it becomes evident that the river Ganga is passing through critical stage of survival and an alarming call, henceforth, is made to preserve, conserve and finally reserve this heavenly and life line river of India unique in the world so that our and the environmental quality health missions may be achieved peacefully and successfully.

Future Recommendations

This research review paper is being aimed at identifying pollution crises looming large over the most magnificent, historical, revered, benevolent and highly valuable river Ganga- the cultural and rich heritage and marvelous natural resource of our country, being the life line for billions. Heeding on the foregoing discourse and the literature scanned along with my own research contributions in this direction, some remedial measures are very strongly recommended for effective execution. Further, minimizing the pollutant load, stopping the direct discharge of wastes of all types, construction and establishment of effective sewage treatment plants, maintenance and sustenance of the normal required water flow throughout and the natural biological and physicochemical properties and finally the cleaning targets and launching the effective result oriented awareness programmes can be effective endeavors to combat and mitigate the pollution crises in Ganga. In present prevailing scenario, execution of an active research program is need of the hour. It may be concluded that by protecting Ganga and the environment, we will be conserving our own future and generations ahead.

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REVIEW ARTICLE

A review on Environmental Implications of Disposal of Plastic Waste in Special Reference to Polyethylene terephthalate

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Abstract

In the present era the increased consumption of plastic materials in each and every aspect of human life and the environmental hazards created due to their disposal after ashort term use is the key concern. The present review represents a general overview the best way of disposal of most widely used plastic of them all, polyethylene terephthalate (PET). Further in this study the main problem of plastic pollution in this regard, the preparation, properties and various routes for the disposal like degradation, recycling, potential for biodegradation are also included.

Keywords: Disposal; Biodegradation; Polymers; Poly (ethyleneterephthalate); Environment

Introduction

Plastics have a wide range of applications in almost each and every field and became waste after use. Because of being nonbiodegradable in nature still having major environmental hazards. Due to this plastic pollution it has become an issue of global concern. This plastic pollution affects both terrestial as well as aquatic world. Knowingly or unknowingly this plastic waste have been generated from various sources such as domestic, industrial, hospital, packaging etc. and creating the pollution which is increasing day by day mainly through inappropriate dumping or throwing the plastic after use and by not subjecting it to the proper ways of disposal. Such pollution results in a number of deleterious repercussions.

Plastics: Their Impact And Disposal

Plastic pollution is the cause of several hazardous and ecologically harmful effects in the environment. No aspect of life remains untouched and uneffected. Plastics are being used in a wide variety of applications because they are exceptionally stable and durable because of being non bio-degradable in the environment .

This is perhaps unsurprising, as one of the primary reasons for the popularity and widespread application of many polymers is their exceptionally high stability and durability .

There are four mechanisms by which plastics degrade in the environment: photodegradation, thermooxidative degradation, hydrolytic degradation and biodegradation by microorganisms (Aianye *et al.*,2012) . Generally speaking, natural degradation of plastic begins with photodegradation, which leads to thermo oxidative degradation. Ultraviolet light from the sun provides the activation energy required to initiate the incorporation of oxygen atoms into the (Feng Jing *et al.*,2007) This causes the plastic to become brittle and to break into smaller and smaller pieces, until the polymer chains reach sufficiently low molecular weight to be metabolised by microorganisms (Grayce *et al.*,2010). These microbes either convert the carbon in the polymer chains to carbon di oxide or incorporate it into biomolecules. However, this entire process is very slow, and it can take 50 or more years for plastic to fully degrade (Koji *et al.*,2019). This is not aided by the fact that the photodegradative effect is significantly decreased in seawater due to the lower temperature and oxygen availability and that the rate of hydrolys is of most polymers is insignificant in the ocean (Maciej *et al.*, 2008) .

Poly (ethyleneterephthalate)

Preparation

Commercial synthesis of PET is done in two ways; first is the chemical reaction between Ethylene glycol (EG) with (1) terephthalic acid and second is the chemical reaction between Ethylene glycol (EG) with (2) dimethyl terephthalate (DMT). First reaction takes place at 240–260 °C and 300–500 kPa, while second reaction takes place at 140–220 °C and 100 kPa. End product of both of the above reactions is bis (hydroxyethyl) terephthalate (BHET) (Jiajing *et al.*, 2007). After this initial reaction few polymerisation steps are carried out to obtain the required molecular weight polymer as shown in Figure 1.

The first polymerisation step is transesterification between BHET molecules, displacing EG, at 250–280 °C and 2–3 kPa (Tomoaki *et al.*, 2009). The resulting oligomers are then polycondensed at 270–280 °C and 50–100 kPa . At this stage, the polymer is suitable for applications that do not require high molecular weight chains, however if higher molecular weight is required, the polymer is subjected to a third, solid state polymerisation, at 200–240 °C and 100 kPa. After synthesis of the raw polymer, it can then be processed into the required form, via extrusion, injection moulding or blow moulding (Tomoaki *et al.*, 2009)

Atmospheric CO_2 emissions for PET production, from cradle to gate, equate to approximately 2440 kg per 1000 kg of PET

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resin produced (Yoshikuni *et al.,* 2006). Advances in the catalytic synthesis of p-xylene, however, may work to reduce this environmental cost. P-xylene is an important constituent used in the production of terephthalicacid, with recent research showing that p-xylene has the potential to be produced from renewable sources, such as cellulose and hemicellulose biomass (Sasa Andjelic *et al.,* 2003).

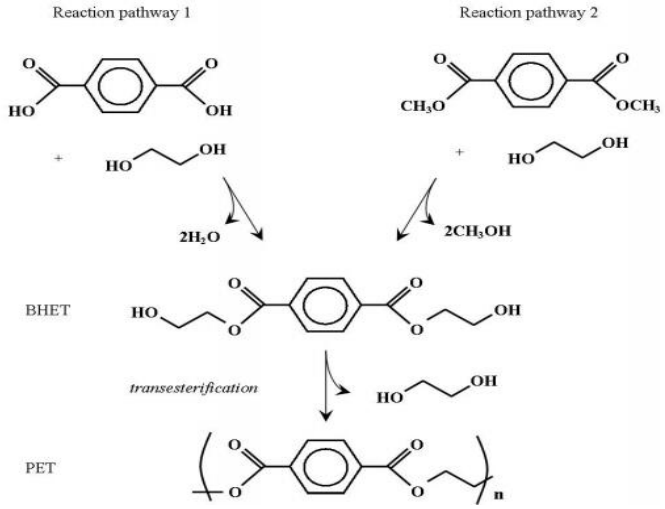

Figure 1: Preparation of PET

Properties

Poly (ethylene terephthalate) is a semicrystalline, thermoplastic polyester (Junji Watanabe *et al.,* 2003). PET is strong and durable, chemically and thermally stable, has low gas permeability and is easily processed and handled.This combination of properties makes PET a desirable material for a wide range of applications and a significant component of worldwide plastic consumption. PET is primarily used as fibres, sheets and films, and more specifically, it is used in food and beverage packaging (especially, soft-drink and water bottles), electronics, automotive parts, houseware, lighting products, power tools, sports goods, photographic applications, X-ray sheets and textiles. Depending on the intended application and desired properties, PET can be manufactured to specification by controlling the polymerisation conditions. Some of the specific properties of PET are summarised in Table 1.

Disposal

Plastic waste is managed mainly in three ways namely landfill, incineration and recycling (Hideto Tsuji *et al.,*2020). Biodegradation of PET waste is also finding its base in many ways. However each method has its own limitations.

Landfill

First and foremost way of disposal of plastic waste is in landfill in which waste occupies that space which may be used for agriculture etc Since plastics are non biodegradable so this space is unavailable for years and years, even more than 20 years.The reason behind this may lead to the fact that in landfills there is less oxygen and the environment all around is anaerobic. Plastics in landfillcontains various volatile chemicals particularly BPA (Bis Phenol A). which produces hydrogen sulphide by sulphate-reducing bacteria in soil populations (Hideto *et al., 2018*). High concentrations of hydrogen sulphide are potentially lethal.

Table - 1: Intrinsic properties of PET polymers

Property	**Value***
Average molecular weight	30,000–80,000 $gmol^{-1}$
Density	1.41g cm^{-3}
Melting temperature	255–265°C
Glass transition temperature	69–115°C
Young's modulus	1700 MPa
Water absorption(24h)	0.5%

*****Values taken from Awaja and Pavel . Values with ranges indicate properties, which vary depending on crystallinity and degree of polymerisation.

Incineration

Incineration is another frequently used technique for disposal of PET waste. When compared to landfill this technique has a potential as no space is rquired here as well as some heat energy is also produced in this process. But the drawback associated with this process is that when plastics are incineratedalong with heat energy many harmful chemicals like heavy metals, toxiccarbon-and oxygen-based freeradicals, not to mention significant quantities of green house gases, especially carbondioxide etc.are also produced and many of them are released to the atmosphere . Thus, due to numerous drawbacks associated with these two processes of disposal of plastic waste namely landfill and incineration, the third process was developed that is recycling process.

Recycling

Recycling of PET waste can be achieved following two methods and these are mechanical recycling and chemical recycling. In mechanical recycling of PET, it is recycled and again extruded into new products. The steps involved in mechanical recycling are removal of contaminants by first sorting the plastic waste and separate PET waste then PET is converted into flakes and washed either using 2% NaOH and detergent at 80°C, rinsed in cold water, or using tetrachloroethylene and dried under desiccation at ~170 °C for six hours.Now these flakes are ready to subject to extrusion. In chemical recycling PET is subjected to chemolysis with number of compounds, resulting in depolymerisation. Depolymerisation can be carried out by hydrolysis (using water), methanolysis (methanol), glycolysis (EG) or aminolysis (e.g., methylamine, ethylamine etc.,) A variety of monomers can be recovered by following these processes and these monomers can be used in many other applications.

Even after being a better solution of disposal of plastic waste than landfill and incineration these processes also have certain limits as these processes are relatively time taking and expensive The presence of additives and impurities also complicate the recycling and decrease the yield and quality of the end product.

Biodegradation

Biodegradation is an alternate way for waste disposal, as it is a cheaper process and does not produce secondary pollutants, such as those associated with incineration and landfill. Many studies have investigated the degradability of a wide range of polymers (Cong Yan *et al.*,2020; Adam *et al.*,2019). Many observed that in most cases, polymers with pure carbon backbones are particularly resistant to most methods of degradation, but polymers that include heteroatoms in the backbone (e.g., polyesters, polyamines) show higher susceptibility to degradation. Though aromatic polymers tend to be resistant to degradation, despite the presence of bonds that are normally readily hydrolysed (Hideto *et al.*,2018; Mezzanotte *et al.*,2003). In PET polymer; the ester bonds that form part of the polymer chain could normally be quite easily broken by a number of mechanisms, however, due to its aromatic groups, the polymer is essentially non-degradable under normal conditions.

Conclusions

Emergent use of plastics, its accumulation and appropriate disposal in order to make it usable in an environment friendly manner are the major issues all over the world. PET (polyethylene terephthalate) is the major component of this plastic waste stream because it has a wide range of applications. PET is very stable and durable, resisitant to biodegradation, transparent. All these properties make this polymer very popular for various applications like textiles, packaging etc. But due its nonbiodegradability it is being accumulated and adversely effecting our environment. The plastic waste disposal can be managed properly by following three methods namely, landfill, incineration and recycling. However each method has its own limitations and drawbacks like landfill occupies more and more space for long duration of time, Incineration is associated with other harmful volatile chemicals and recycling processes are time taking, expensive etc. Biodegradation seems to be an attractive and most effective route for disposal as it fullfills all above disadvantages and seems to be the most viable technique for plastic waste management and thereby making them environment friendly.

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REVIEW ARTICLE

Hairy Root Culture: A Novel Promising Approach for the Production of Highly Valuable Secondary Metabolites

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Abstract

Secondary metabolites production using hairy root culture (HRCs) has shown immense potential for industrial applications since the last several decades. Generally, the hairy root cultures are generated through the transformation of plant parts by a gram negative soil bacterium called *Agrobacterium rhizogenes.* Keeping the potential of HRCs for the production of pharmaceutically important compounds, several scientists are engaged in the development of methods that could be feasible for generating high frequencies of hairy roots. HRCs have been proved an attractive choice for secondary metabolites production due to the cost reduction, higher yields and genetic stability of *in vitro* cultures and hairy roots could be effectively grown in bioreactors. Therefore, HRCs are being utilized for producing valuable secondary metabolites of pharmaceutical importance at commercial scales. The present review paper would describe the current status of the hairy roots developed in several plant species, their advantages, challenges and prospects briefly.

Keywords: *Agrobacterium spp.,* Bioreactors, Culture environment, Elicitors, Secondary Metabolites.

Introduction

Most of the secondary metabolites are present in plant's body at lower to moderate levels and some others synthesized in specific tissues only at slower rates. So their production and extraction from plants at higher levels requires more time and demands higher downstream costs (Kim *et al.,* 2015). Plant tissue culture techniques are considered as potential tools of biotechnology because of their significant roles in agriculture as well as pharmaceutical industries. The utilization of plant tissue culture techniques such as hairy root cultures (HRCs) have pave the way to enhance the production of secondary metabolites at lower costs. Keeping the potential of (HRCs) in mind scientists have established efficient methodologies in several plant species. Hairy root cultures (HRCs) are generated through genetic transformation of plant tissues by the bacterium *Agrobacterium rhizogenes*. Nowadays, HRCs have become the choice for secondary metabolite production because of rapid growth rates in comparison of cell cultures and higher genetic stability (Eapen and Mitra, 2001; Bapat and Ganapathi, 2005). Hairy root cultures are considered as viable and cost effective options for producing secondary metabolites at larger scales, heterologous expression of plant proteins, identification of novel genes, studying gene functions and for phyto-remediation studies also (Georgiev *et al.,* 2012). HRCs offer advantages for secondary metabolite production as- (1) It reduces the dependency on natural resources for secondary metabolite production. (2) Faster and higher production in comparison of plant body. (3) Higher genetic and biosynthetic stability. (4) Advantages of accumulation of novel compounds. (5) Cost effective.The present review summarizes the overview of secondary metabolites production by using hairy root cultures.

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Agrobacterium rhizogenes: The Player of Hairy Roots

Agrobacterium rhizogenes is an important gram negative soil bacterium. It identified in 1930 (Riker *et al.,* 1930). It resides nearby plant roots and is responsible to cause hairy root disease in plants (Guillon *et al.,* 2006). Like as *A. tumefaciens*, A. *rhizogenes* also contains a plasmid that is called root inducing plasmid or Ri plasmid. The size of Ri plasmid is 250 Kb. It has left and right borders i.e. TL and TR separated by 15 Kb segment. TR has genes for auxin synthesis and for agropine synthesis whereas the TL has the genes for root locus i.e. rol A (279-423 bp), rol B (762-837 bp), rol C (~540 bp) and rol D (~1032 bp) (Meyer *et al.,* 2000). Only TL DNA is sufficient for hairy root induction in explants or in wounded plant parts. The plasmid also has the virulence region (vir region) which is responsible for the T DNA transfer (Chandra, 2012). The T DNA transfer mechanism of Ri plasmid is similar to the Ti plasmid because the T DNA of the both plasmids have high similarity (Moriguchi *et al.,* 2001).

A. *rhizogenes* genetic transformation process as per the description given by Georgiev *et al.* (2012) is accomplished in the following steps:

(1) Recognition of phenolic compound acetosyringone by bacteria and attachment to the plant body.
(2) Formation of T-DNA complex inside the bacteria.
(3) Transfer of T-DNA complex into plant cells.
(4) T-DNA integration and expression in plant genome.
(5) Formation of hairy roots at wounding sites.

Furthermore, for better understanding of induction of hairy root and its application, a pictorial representation is shown in Fig.-1.

Hairy Root Cultures for Secondary Metabolites

Hairy root cultures have been utilized for industrial production of secondary metabolites (Guillon *et al.,* 2006). Globally, several scientific groups are engaged in the development of efficient methods for establishing high frequency hairy root cultures through the modulation and manipulation of culture environmental conditions. Till date, advanced techniques have been utilized for the regeneration of HRCs for producing several important metabolites like as podophyllotoxin, zerumbone and resveratrol which possess anti-cancerous properties (Nandagopal *et al.,* 2017). HRCs for the production of several secondary metabolites have been developed in several plants which have been mentioned in Table-1. Some of the notable examples are being discussed in this review. In case of *Withania somnifera* hairy roots were induced by *A. rhizogenes* strain R1601 from cotyledons and leaf explants for the production of withanolide A, a steroidal lactone of medicinal and therapeutic value (Murthy *et al.*, 2008). Production of tropane alkaloids, which are important natural compounds used as pharmaceuticals ingredients, was also reported to have produced from HRCs of *Datura stramonium* (Ling *et al.,* 2011). In *Isatis tinctoria* 24-day-old HRCs yielded 438.10 µg/g dry weight of total flavonoid (rutin, neohesperidin, buddleoside, liquiritigenin, quercetin, isorhamnetin, kaemp- ferol, and isoliquiritigenin), which was significantly greater (341.73 µg/g) than that of 2-year-old field-grown roots (Gai *et al.*, 2015). *Rauwolfia serpentina* and *Solanum khasianum* are two important medicinal plants that contain alkaloids like ajmaline, ajmalicine, solasodine, and α-solanine (Srivastava *et al.,* 2016). The hairy roots of *Ophiorrhiza pumila* produce camptothecin (CPT), a monoter- penoid indole alkaloid used as a precursor in the synthesis of chemotherapeutic drugs (Udomsom *et al.,* 2016). Hairy root lines were also developed in grapevine (*Vitis vinifera* cv Pinot Noir 40024), wherein its ability to produce various stilbenes and elicitation was demon strated, which proved it as potentially valuable system for producing resveratrol derivatives (Tisserant *et al.,* 2016). Moharrami *et al.,* (2017) suggested that oxide nanoparticles (FeNPs) could be an effective elicitor in hairy root cultures of *Hyoscyamus reticulatus* for increasing the production of hyoscyamine and scopolamine (tropane alkaloids). The artemisinin accumulation in *Artemisia vulgaris* and *Artemisia dracunculus* "HRCs" were reported by Drobot *et al.,* (2017). The development of HRCs of *A. vulgaris* and *A. dracunculus* having higher yield of artemisinin and its derivatives and sugars than the mother plant is remarkable for *in vitro* production of valuable secondary metabolites. Pilaisangsuree *et al.,* (2018) established hairy root cultures for stilbene production in *Arachis hypogaea*. HRCs have also been developed in Macleaya cordata for the production of Benzyl isoquinoline Alkaloids Huang *et al.,* (2018).

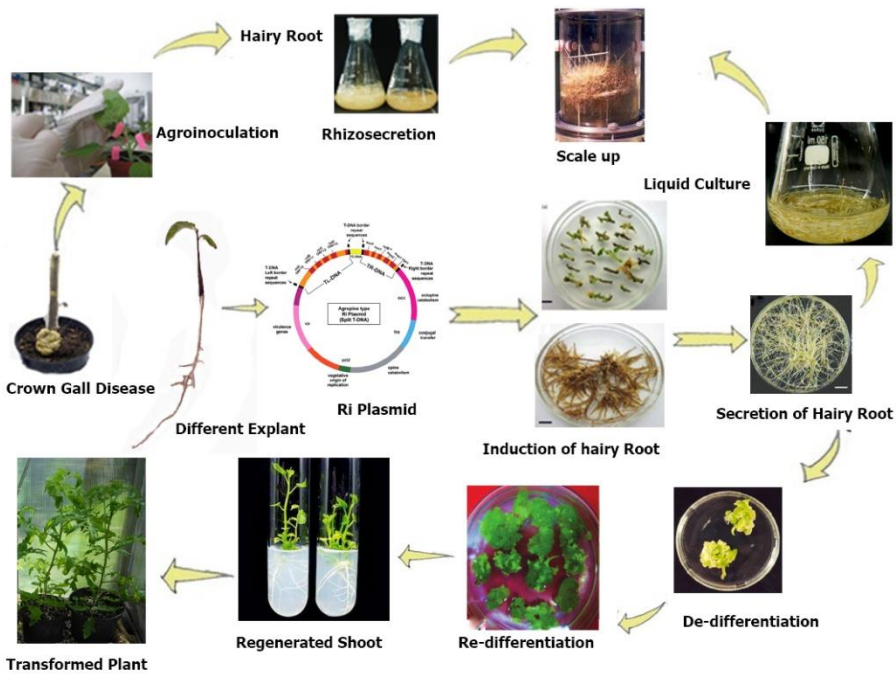

Fig.-1 Induction of hairy roots and their applications

Table-1: Production of secondary metabolites in various plant species by using hairy root cultures (HRCs)

S. No.	Accumulated Metabolites	Targeted Plant Species	Reference
1	Phytoecdysteroids	*Ophiorrhiza pumila*	Okuzumi *et al.* (2003)
2	Triterpenoid Saponins	*Panax ginseng* *Panax ginseng*	Jung *et al.* (2003) Choi *et al.* (2005)

		Panax ginseng	Kim *et al.* (2009)
3	Tropane Alkaloids	*Datura metel,* *Hyoscyamus muticus*	Moyano *et al.* (2003) Hakkinen *et al.* (2016)
4	Camptothecins	*Ophiorrhiza pumila*	Yamazaki *et al.* (2004)
5	Phenolic Acids	*Salvia miltiorrhiza* *Salvia miltiorrhiza*	Yan *et al.* (2006) Xiao *et al.* (2010)
6	Glucosinolates	*Tropaeolum majus*	Wielanek and Urbanek (2006)
7	Camptothecins	*Ophiorrhiza pumila*	Sirikantaramas *et al.* (2007)
8	Azadirachtin	*Azadirachta indica*	Satdive *et al.* (2007)
9	Dolichols	*Coluria geoides*	Skorupinska-Tudek *et al.* (2008)
10	Anthocyanins	*Medicago truncatula*	Pang *et al.* (2008)
11	Tropane Alkaloids	*Atropa baetica*	Jaber-Vazdekis *et al.* (2008)
12	Lignans	*Linum corymbosum*	Bayindir *et al.* (2008)
13	Glucosinolates	*Brassica rapa*	Kastell (2009)
14	Indole Glucosinolates	*Chinese cabbage*	Zang *et al.* (2009)
15	Rosmarinic Acid	*Coleus blumei* *Prunella vulgaris*	Bauer *et al.* (2009) Ru *et al.* (2016)
16	Gossypol	*Gossypium hirsutum*	Verma *et al.* (2009)
17	Psoralen	*Psoralea corylifolia*	Baskaran and Jayabalan (2009)
18	Diterpenoid	*Salvia sclarea*	Kuzma *et al.* (2009)
19	Paclitaxel	*Taxus x media var. Hicksii*	Syklowska-Baranek *et al.* (2009)
20	Hydroxybenzoates	*Daucus carota*	Sircar and Mitra (2009)
21	Proanthocyanidins	*Vitis vinifera*	Terrier *et al.* (2009)
22	Pyridine Alkaloids	*Nicotiana tabacum, Nicotiana glauca*	Kajikawa *et al.* (2009) De Boer *et al.* (2011)
23	Tanshinones	*Salvia miltiorrhiza* *Salvia miltiorrhiza*	Gao *et al.* (2009) Yang *et al.* (2012)
24	Shikonin	*Arnebia hispidissima*	Chaudhury and Pal (2010)
25	Flavonoids	*Glycine max* *Glycine max* *Glycine max* *Glycine max* *Glycyrrhiza uralensis,* *Astragalus membranaceus*	Jiang *et al.* (2010) Yi *et al.* (2010) Theboral *et al.* (2014) Han *et al.* (2017) Zhang *et al.* (2009) Gai *et al.* (2016)
26	Monoterpene Indole Alkaloids	*Catharanthus roseus* *Catharanthus roseus*	Zhou *et al.* (2010) Goklany *et al.* (2010)
27	Betalains	*Beta vulgaris*	Georgiev *et al.* (2010)
28	Drimartol A	*Artemisia annua*	Zhai and Zhong (2010)
29	Resveratrol	*Arachis hypogaea* *Arachis hypogaea*	Abbott *et al.* (2010) Yang *et al.* (2015)
30	Anisodamine	*Brugmansia candida*	Cardillo *et al.* (2010)
31	Triterpenoids	*Centella asiatica*	Kim *et al.* (2010a)
32	Catharanthine	*Catharanthus roseus*	Wang *et al.* (2010)
33	Alkaloid	*Catharanthus roseus*	Zhou *et al.* (2010)
34	Hyoscyamine	*Datura stramonium* *Hyoscyamus reticulatus*	Amdoun *et al.* (2010) Moharrami *et al.* (2017)
35	Rutin	*Fagopyrum esculentum*	Kim *et al.* (2010b)
36	Gentiopicroside	*Gentiana macrophylla*	Zhang *et al.* (2010)
37	Ginsenoside	*Panax quinquefolius*	Mathur *et al.* (2010)
38	Daidzein and Genistein	*Psoralea corylifolia*	Shinde *et al.* (2010)
39	Plumbagin	*Plumbago indica*	Gangopadhyay *et al.* (2011)
40	Tanshinone	*Salvia miltiorrhiza* *Salvia miltiorrhiza*	Yan *et al.* (2011) Kai *et al.* (2011)
41	Tropane Alkaloids	*Anisodus acutangulus*	Kai *et al.* (2012)
42	Gymnemic acid	*Gymnema sylvestre*	Nagela *et al.* (2013)
43	Withanolide A, Withanone, and Withaferin A	*Withania somnifera* *Withania somnifera*	Sivanandhan *et al.* (2013)

44	Phenylpropanoid	*Fagopyrum tataricum*	Thwe *et al.* (2013)
45	Bacoside A	*Bacopa monnieri*	Bansal *et al.* (2014)
46	Caffeic Acid	*Rhaponticum carthamoides*	SkaBa *et al.* (2015)
47	Rotenoid	*Mirabilis himalaica*	Lan *et al.* (2015)
48	Stilbene	*Nicotiana tabacum* *Arachis hypogaea*	Hidalgo *et al.* (2017) Pilaisangsuree *et al.* (2018)
49	Anthraquinone	*Rubia tinctorum*	Perassolo *et al.* (2017)
50	Benzylisoquinoline Alkaloid	*Macleaya cordata*	Huang *et al.* (2018)

Advantages of HRCs

For extracting high-value secondary metabolites, harvesting of plant roots can cause damage to whole plants. Therefore, hairy root culture has been explored as promising approach in producing secondary metabolites. High branching and elevated growth rate of hairy roots make it more appropriate for commercial upscaling in the bioreactor (Hu and Du, 2006). It has the ability to produce potentially faster without needing an external supply of growth hormones (auxins). In few cases, they do not required incubation under light. All HRCs are stable in metabolite production because of their great genetic stability.

HRCs of *Lithospermum erythrorhizon*, and *Scopolia parviflora* were explored in bubble column bioreactors for secondary metabolites such as shikonin, ginsenosides, and alkaloids (Min *et al.*, 2007; Ludwig-Muller *et al.*, 2008). Secondary metabolite production can be enhanced by addition utilization of several methods like biotranformation, precursor feeding, elicitation and cell immobilization of HRCs. Also, HRCs can generate secondary metabolites over consecutive generations without dropping genetic or biosynthetic strength (Giri and Narasu, 2000). Further more cell suspension culture, organ culture, adventitious roots, etc. were effectively induced in several plant species and explored for the production of high-value secondary metabolites but such methods have significant limitations including their genetic stability and slow growths.

In *Hyoscyamus muticus* hairy roots, a consecutive subculturing result in the production capacity of hairy roots was considered (Häkkinen *et al.,* 2016). Their study shows, hairy roots producing high tropane alkaloid levels upto sixteen year follow-up with regard to genetic and metabolic stability. Further, they also used cryopreservation method to preserve the hairy roots of *H. muticus* to replace hard subculturing.

Challenges in Developing and Maintaining HRCs

Hairy roots induction is depending upon several factors like, the bacterial strain, genotype of the plant and culture condition. Therfore, developing a appropriate protocol for hairy root induction is a prerequisite for HRCs. One of the major challenges for HRCs is scaling-up of culture for large volume production because of delicate, branched, and sensitive nature of hairy roots. Designing suitable bioreactors for appropriate culture of hairy root lines is required for the commercial practice. Bioreactors explored for large scale production of hairy roots can be separated as liquid-phase, gas-phase, or hybrid kind of reactors (Srivastava and Srivastava, 2007). Many bioreactors with variable designs utilized for the growth of hairy roots for *in vitro* production using HRCs like bubble column, stirred tank, airlift bioreactors etc.

Understanding the biosynthetic pathways of secondary metabolites is needed for appropriately modulation of biosynthetic pathways using metabolic engineering. HRC systems have a immense potential for large scale commercial production of several secondary metabolites as well as recombinant proteins. Moreover, the current development in high- throughput genomics approaches helps in a immense way to recognize the regulation of secondary metabolism. Mehrotra *et al.*, (2010) revealed that the global information from various "omic" platforms in relation to structural, functional aspects and pathway architecture, of important enzymes and genes that can sustain the design of sets of engineering, ensuing the generation of wide-ranging views of DNA sequence-to-metabolite passageway networking and their manage to obtain desired results. The recent molecular biology tools for e.g. the CRISPR/Cas9 technology, trigger a strong perspective for development of HRCs. According with PubMed Central database, the first transformation of HRs using CRISPR/Cas9 strategy has been successful in 2014. In this report transgenic tomato HRs producing eGFP were published (Ron *et al.*, 2014).The strong optimization of the process in terms of production capacity, bioreactor size, and ability to modify HRCs to produce tailored-made complex molecules overlay the way to a rigid place as a potential biotechnology tool of this expertise in plant molecular farming.

Prospect of HRCs

Hairy root exhibits a high degree of chromosomal stability over prolonged culture period. The steadiness of hairy roots posses a significant advantage for both research and large scale industrial purposes. Stability reveled in terms of growth DNA analysis, characteristics, gene expression and secondary metabolites production. Genetically transformed HRs offer several practical advantages in experimental studies, such as ease of initiation, culture, and maintenance, indefinite propagation of material resulting from the same parent plant, and genotypic and phenotypic stability. HRCs is a tool that provide significant technique for generation of synthetic seeds, under *in vitro* and *in vivo* conditions. This technology is significantly valuable for growth and conservation of finest agricultural and rare medicinal plant species, which are hard to regenerate. HRCs revealed its potential in the production of the insufficient or high-value plant bioactive molecules of pharmaceutical and commercial significance.

These systems with the support of genetic engineering methods open novel possibilities for significant enhancement

of productivity. Now a day modulation of pathway is possible, one can redirect metabolic flux, changed limiting steps of pathways and control inefficient pathways. Development of large-scale culture protocols using bioreactors has made generation of secondary metabolites at industrial level (Murthy *et al.*, 2008). Sophisticated bioreactor derived bioactive molecules grasp the prospect of generating remedies for humanizing human health (Sivakumar , 2006). HRCs have significant role for the production of recombinant proteins due to its properties such as rapid growth, genotypic and phenotypic stability, and hormone-free growth. HRC based biotransformation systems are being considered as extremely effective in terms of a variety of plants exogenous substrates as well as diverse chemical reactions. HRCs has come to an stage of functional research in increasing pharmaceutical lead compounds by making chemical amendment with the assist of its inherent enzyme resources (Banerjee *et al* .,2012).

Conclusion

HRC have exclusive application in plants for higher production of valuable products of commercial significant. The several reports by various authors in this article proved that laboratory production of hairy roots are cost effective, high yielding and generate several secondary metabolites in less time as compared to conventional way. By using genetic engineering fabrication hairy root produces pharmaceutically important products, better quality of vitamins, proteins used in bioaccumulation, biofortification of heavy metals, phytoremediation and production of artificial seeds. Now a days HRC becomes an economically viable proposition for *in vitro* production of high value secondary metabolites.

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Recent News

International Team of Researchers Identify Evolutionary Origins of SARS-CoV-2

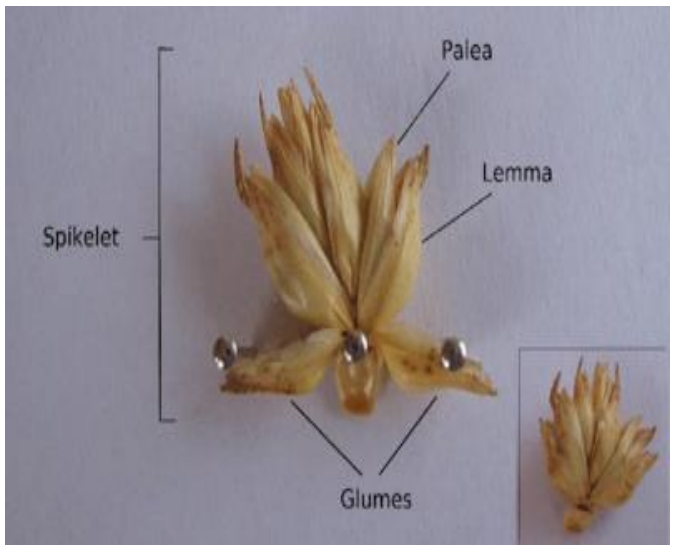
An international team of researchers from China, Europe, and the United States has discovered that the lineage that gave rise to SARS-CoV-2, the virus that is responsible for the COVID-19 pandemic, has been circulating in bats for decades and likely includes other viruses with the ability to infect humans. The team, with combined expertise in recombination, phylogenetic dating, virus sampling, and molecular and viral evolution, found that the lineage of viruses to which SARS-CoV-2 belongs diverged from other bat viruses about 40-70 years ago. Importantly, although SARS-CoV-2 is genetically similar (about 96%) to the RaTG13 coronavirus, which was sampled from a *Rhinolophus affinis horseshoe* bat in 2013 in Yunnan province, China, the team found that it diverged from RaTG13 sometime in 1969. The team also found that one of the older traits that SARS-CoV-2 shares with its relatives is the receptor-binding domain (RBD) located on the Spike protein, which enables the virus to recognize and bind to receptors on the surfaces of human cells.

David L. Robertson, professor of computational virology at the University of Glasgow said this means that other viruses that are capable of infecting humans are circulating in horseshoe bats in China. He added that SARS-CoV-2's RBD sequence has so far only been found in a few pangolin viruses and SARS-CoV-2's ability to infect humans has not yet been seen in another close bat relative of the SARS-CoV-2 virus.

Robertson further explained that while it is possible that pangolins may have acted as an intermediate host facilitating transmission of SARS-CoV-2 to humans, no evidence exists to suggest that pangolin infection is a requirement for bat viruses to cross into humans. Their research suggests that SARS-CoV-2 likely evolved the ability to replicate in the upper respiratory tract of both humans and pangolins.

CRISPR-Cas9 Sheds Light on Spikelet Development in Rice

Researchers from China National Rice Research Institute used CRISPR-Cas9 and revealed that MORE FLORET 1 plays an important role in the regulation of organ identity and spikelet determinacy in rice. The findings are reported in Plant Physiology.



Rice spikelets exhibit a unique inflorescence structure and the mechanisms behind their development remain to be unclear. Researchers believe that strategies in regulating spikelet formation could impact grain yield. Thus, Deyong Ren and the research team identified and characterized the recessive spikelet mutant more floret 1 (mof1). The mof1 mutant showed a delay in the transition from spikelet to floral meristem, which includes the development of extra lemma-like and palea-like organs. Furthermore, the main body of the palea was reduced, while the sterile lemma was enlarged and partially acquired hull identity.Through map-based cloning, the team identified the MOF1 locus and which was confirmed by complementation and by generating new mof1 alleles using CRISPR-Cas9 genome editing.

Scientists Describe Protocol for Multi-gene Genome Editing in Maize

University of Massachusetts Amherst scientists developed an efficient assembly of large multiplex CRISPR-Cas9 guide arrays for genome editing of maize. Details of the protocol are published in Bio-Protocol.CRISPR-Cas9 has been known as an effective tool for genome editing, which makes many researchers wishes to use the tool for targeting multiple genes. To address this, researchers developed vectors that target multiple genomic loci from a single transformation event. One of the proposed systems is called MoClo, which is an elaboration of Golden Gate cloning and is particularly fit for assembling larger multiplexed Cas9 guide arrays. Jarret Man and Madelaine Bartlett described the steps for designing and building a custom guide array targeting any number of maize loci using the MoClo standard components and syntax. Instructions for using variations of the maize and rice U6 to drive guide RNA expression were also provided.

CRISPR Nucleases Evaluated for Genome Editing in Maize

CRISPR-Cas9 and Cas12a (Cpf1) nucleases are two of the most powerful genome editing tools in plants. Iowa State University researchers compared the activities of these two nucleases by targeting the maize glossy2 gene coding region. The research team, led by Keunsub Lee, introduced the SpCas9-guide RNA (gRNA) and LbCas12a-CRISPR RNA (crRNA) into maize inbred embryos using Agrobacterium-mediated transformation.

Analysis showed that 90-100% of the Cas9-edited T0 plants carried mutations, 63-77% of which were homozygous or biallelic mutants. Meanwhile, 0-60% of Cas12a-edited T0 plants had the on-target mutations. Analysis of potential off-target sites for the two nucleases identified 18 and 67 potential off-targets for SpCas9 and LbCas12a, respectively. Further analysis of the off-target sites revealed no detectable mutations in the T1 plants.

These results suggest that the CRISPR-Cas9 system used in this study is highly efficient and specific for genome editing in maize. On the other hand, CRISPR-Cas12a needs to be improved for genome editing.

Gene Switches for Height Identified in Plants

Scientists were able to identify two key genes responsible for the plant height in rice plants, opening up numerous possibilities of breeding varieties with desirable height and yield traits of not just rice but other crops that also carry the same genes.The scientists divided their work into two investigations: one focused on deep-water rice variety and the other focused on shallow-water rice variety and both were studied under greenhouse conditions. They were able to identify two important genes. First, the accelerator of internode elongation (ACE1) that turns on when the deep-water variety is covered in water and stimulates cell division in stems causing the plant to grow. This was found to be mutated in the shallow-water variety. The second is decelerator of internode elongation (DEC1) which suppresses stem growth. This was found to be active in the shallow-water variety even when the plant was submerged in water, but was found to stop expressing in deep-water variety when exposed to flooding. Scientists described the two genes as switches for plant height.

Another interesting fact is that the two genes are not only found in rice, but they are also present in other plants such as sugarcane, barley, and the grass *Brachypodium distachyon*. ACE1 is also found in corn, and the crop also has the gene-equivalent of DEC1. The genes' discovery can lead to the development of plant varieties that are resistant to stress factors brought by climate change. The possibilities vary from developing improved low-yield varieties already adapted to seasonal flooding to improved high-yield shorter varieties that can withstand flooding.

Study: Heartburn Medicine Doesn't Work as COVID-19 Antiviral

Anecdotal reports from China indicated that COVID-19 patients who were taking famotidine for heartburn had reduced rates of intubation and mortality compared to those who took a different kind of antacid drugs. However, preliminary results of a study conducted by Boston University and partners showed that famotidine has no antiviral effect against SARS-CoV-2.

The researchers systematically analyzed the effect of famotidine on viral proteases and virus replication and found that famotidine neither binds with nor inhibits SARS-CoV-2 proteases. Furthermore, no direct antiviral activity of famotidine was found at concentrations of up to 200 μM, when tested against SARS-CoV-2 in two different cell lines, including a human cell line originating from lungs, a primary target of COVID-19. However, the researchers said they are not concluding that the drug might be helpful in other ways.

"We're not challenging that famotidine might help...We're saying that the mechanism of action is not antiviral," said Mohsan Saeed, a virologist from Boston University and one of the authors of the study.

Varieties developed through use of genomic resources:

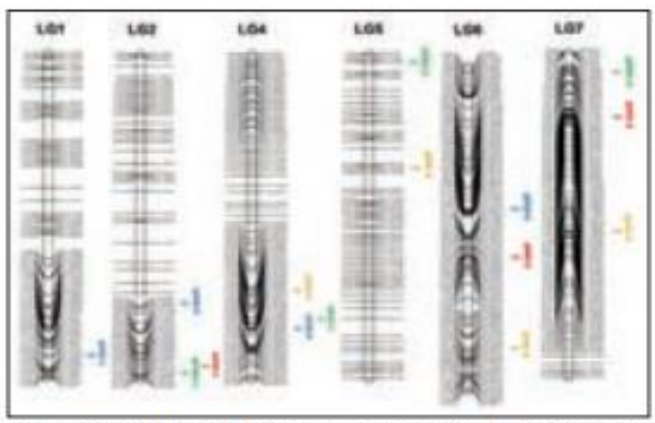
Six varieties of different cereal and pulse crops, viz. wheat (PBW 771), maize [Pusa HQPM-5 Improved (APQH 5), Pusa Vivek Hybrid-27 Improved (APH 27), Pusa HQPM-7 Improved (APQH 7)] and chickpea [Pusa Chickpea 10216 (BGM 10216), Super Annigeri-1 (MABC-WR-SAI)] have been developed through genomic assisted selection. These varieties have been improved by introgression of traits for quality, disease and drought tolerance.

Use of genomic approach for herbicide tolerance and quality improvement: Marker-assisted backcross breeding was employed to transfer a herbicide tolerance mutant allele of ALS gene from Robin, an Imazethapyr tolerant EMS-induced mutant of Nagina 22, into two elite Basmati rice varieties, viz. Pusa Basmati 1121 and Pusa Basmati 1509. Genome wide association study (GWAS) was employed in rice to map significant QTLs for Fe and Zn for enhancing the endosperm mineral micronutrient density as rice is widely consumed after polishing. In pearl millet, QTLs for grain Fe and Zn content were mapped using 210 RILs (PPMI 683 × PPMI 627).

Standardization of CRISPR-Cas9 Technology: The CRISPR-Cas9 genome editing technology was standardized for different crops to enhance stress tolerance and nutritional quality. In rice, CRISPR-Cas9 genome editing was employed to develop mutants of DST (DROUGHT AND SALT TOLERANCE) gene for improving salt and drought tolerance. To reduce the seed phytate content, CRISPR-Cas9 genome editing was used to mutate GmIPK1 gene. T0 lines showed 6–9-fold reduction in phytate content. Overexpressing abscisic acid receptor OsPYL10 and Isopentenyltransferase 9 (IPT9) genes were found to confer drought and salt stress tolerance to transgenic rice.

Insect resistance genes from Cajanus platycarpus against Helicoverpa armigera: Cajanus platycarpus is one of the non-crossable wild relatives of pigeonpea possessing resistance to polyphagous insect Helicoverpa armigera. Hence, C. platycarpus was used for understanding the mechanism of resistance to H. armigera and identification of candidate genes to mitigate the menace of the herbivore. RNA-seq and differential gene expression analysis were carried out between C. platycarpus and cultivated pigeonpea cultivar TTB7, at different time points after challenge with the insect larvae. Fifteen herbivory response-specific genes with >2-fold differential expressions have been selected. These genes with probable role in: (i) insect structural destruction, (ii) interference in digestion, (iii) reduction in availability of nutrients, and (iv) transcription factors have been shortlisted for validation. These putative insect resistance genes have been cloned from C. platycarpus into binary vectors and are being validated in Nicotiana tabacum.

QTL mapping of drought stress tolerance in chickpea: Genotyping-by-Sequencing approach was used for the large scale SNP discovery and simultaneous genotyping of recombinant inbred lines (RILs) of anintraspecific mapping population (Pusa 362 × SBD 377) of chickpea contrasting for drought related traits. The chickpea genome annotation project database was used to delineate the location of the GBS derived 3,267 SNPs in the genomic regions: intergenic, genic (exons), intragenic (introns) and UTRs. The occurrence of both types of transitions—C/T and A/G – was higher than any of the transversions. The SNP genotyping data was utilized to construct one of the most saturated intraspecific genetic linkage maps of chickpea having 3,267 SNPs on 8 linkage groups. The map was utilized to identify 15 quantitative trait loci (QTLs) associated with drought traits (membrane stability index, relative water content, seed weight and yield under stress condition) accounting for phenotypic variations ranging from 11.8% to 27.1%.



Location of QTLs on the genetic linkage map of chickpea developed from the cross Pusa 362 × SBD 377. QTLs are depicted as coloured vertical bars to the right of the linkage groups. MSI (Membrane stability index), RWC (Relative water content), SW (100 seed weight) and YLD (Yield under stress condition).

Molecular diversity in jackfruit: A set of primer sequences from SSR flanking regions were identified for the validation of SSRs in a jackfruit germplasm set. Primer sets for 200 genic-SSRs have been custom synthesized in jackfruit for their validation, and molecular characterization of the 224 jackfruit accessions collected primarly from Jharkhand was carried out using these SSR markers. A total of 81 alleles were detected in 224 jackfruit accessions by using 27 SSR markers. The number of alleles ranged from 2 to 4, with an average of 3

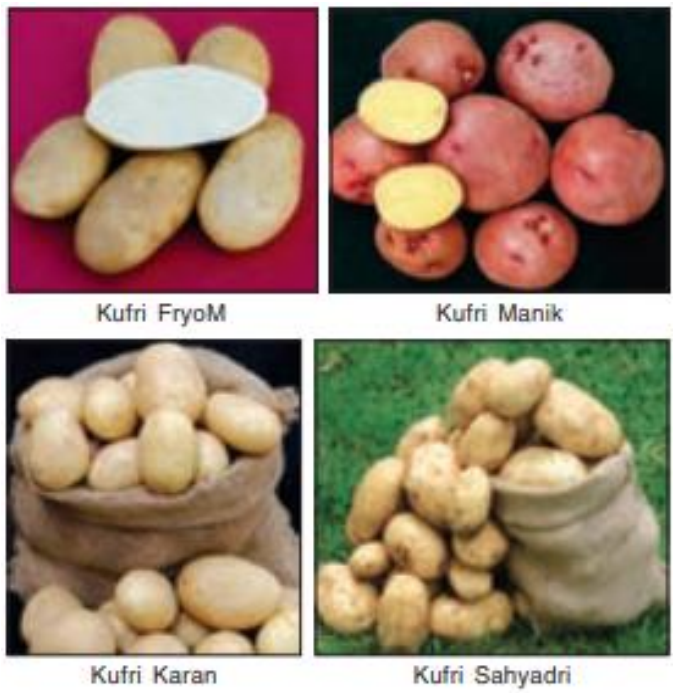
alleles per locus. In population structure studies significant, genetic admixing was observed in the jackfruit accessions.

Phenomics for abiotic stress breeding in field crops: Phenomics was used to identify germplasm and breeding lines with high water use efficiency (WUE) and drought tolerance in the major food crops. Genotypes which use significantly less water than that of Nagina 22 in rice and RILs of wheat superior to C 306 in WUE were identified. Elevated CO2 (EC) mediated decrease in nitrate uptake and assimilation was found to be a cause for reduction in grain quality under EC conditions. Genome-Wide Association Studies (GWAS) and linkage-mapping analyses were used to identify QTLs for stress tolerance and yield in different field and horticultural crops.

Event selection trial of transgenic pigeonpea and chickpea harbouring Bt gene(s): Event selection trials of five transgenic events each of pigeonpea and chickpea harboring Bacillus thuringiensis-crystal 1Ac/ cry1Aabc genes for gram pod borer resistance trait were conducted to identify the best event in each crop, based on trait efficacy (resistance to gram pod borer), expression of Bt protein at various stages and related agronomic characters including yield. Correlated with protein expression, 61.57–87.77% reduction was observed in the transgenic pigeonpea events over control variety ICPL 87119 (Asha) and 72.51–85.16% reduction in transgenic chickpea events over control variety DCP 92-3. Genome-wide identification of nodule-specific cysteine rich (NCR) peptides in chickpea: Symbiotic nitrogen fixation (SNF) ability of legumes can make them self-reliant for N-requirement, however, that is not the case due to lack of knowledge about critical process of bacteroid differentiation during nodule development, which is mediated by plant derived nodule specific cysteine rich (NCR) peptides. In the present investigation, 67 putative NCR peptides including 30 unique sequences of Cicer arietinum, were identified and characterized. Each sequence possesses at least one conserved latenodulin domain. Nine putative NCR peptides of Cicer arietinum contain single motif, 24 sequences had two motifs and 34 sequences had three motifs. The generated information will help in developing tools for optimizing the symbiotic efficiency under natural farming environment. QTL mapping for foliar fungal disease resistance: 84 polymorphic SSR markers were used in mapping foliar fungal disease resistance and 70 of them were mapped on 14 linkage groups (LGs). The genotypic and phenotypic data was used for QTL analysis. Two major QTLs (LLSQTL1 and LLSQTL2) were detected for Late Leaf Spot resistance and one major QTL (RustQTL) for rust resistance. Validation of newly developed markers for LLS and rust diseases: Twenty-four markers were used for validation in 12 groundnut varieties resistant to both late leaf spot and rust diseases and 9 varieties resistant to rust only to find out any alleles other than alleles from GPBD 4 which can be utilized for breeding. All markers differentiated resistant varieties justified by their phenotypes (disease score). After screening with all the markers of targeted genomic region, it was observed that all the 12 groundnut varieties resistant to both LLS and rust diseases, carry resistant alleles. These markers were able to clearly differentiate resistant and susceptible varieties specified by their allelic pattern.

Tropical tuber crops

New varieties of different tropical tuber crops (three cassava, one sweet potato, three greater yam, three aerial yam and four taro) were identified by the ICAR-AICRP on Tuber Crops.



Cassava: TCa13-1(CAU C-1 Nungha) matures in 8– 9 months with 24–31 t/ha yield; recommended for release and cultivation in Manipur. TCa13-7(9S 125), a hybrid (CR43-11 × Mankozhunthan), with high (35 t/ha) yield, tolerant to cassava mosaic disease; recommended for cultivation in Kerala, Manipur and Chhattisgarh. TCa13- 4 (S4) has 27.33 to 31.2 t/ha fresh tuber yield. It is tolerant to cassava mosaic virus; recommended for cultivation during kharif in Chhattisgarh.

Sweet potato: TSp12-6 (BCSP–10) has 20.1 t/ha tuber yield; less affected by sweet potato weevil (0.8 t/ha) damage and it is recommended for cultivation in Bihar.

Onion

Arka Bheem has bulb yield potential up to 47 t/ha in 130 days. It has been recommended for cultivation in Karnataka. Bhima Shakti (DOGR-1156) has 32–36 t/ha yield in rabi; very good bulb storability (up to five months); notified for cultivation in Andhra Pradesh, Chhattisgarh, Karnataka, Madhya Pradesh, Maharashtra and Odisha. DOGR-HT-1, an advanced breeding line of short day onion with 30 t/ha bulb yield during rabi, matures in about 120–125 days after transplanting. It is suitable for dehydration and produces seed under short day conditions. NHRDF Fursungi (L-819) is tolerant to Stemphylium blight; 380–400 q/ha yield during rabi; notified for cultivation in Delhi, Rajasthan, Haryana, Jammu & Kashmir, Punjab, Gujarat and Maharashtra. JWO-11-5-7 has 230 q/ha marketable yield and can be stored up to 4 months. It has been notified for cultivation during rabi in Gujarat, Jammu, Punjab, Delhi, Haryana, Rajasthan, Madhya Pradesh, Chhattisgarh, West Bengal and Maharashtra.

Garlic: Bulbs of Yamuna Purple (G-404) are light purple; 25–30 cloves/bulb; marketable yield ranges from 165 to 175 q/ha. It has been notified for cultivation during rabi in Jammu, Punjab, Delhi, Haryana, Rajasthan, Madhya Pradesh, Chhattisgarh, West Bengal and Maharashtra.

Mushrooms

Button mushroom: Two improved strains, NBS-5- 1084 with 13.34% biological efficiency and NBS-5-1077 with 12.92% biological efficiency as against 12.69% in control were identified. Oyster mushroom: PSCH-35 is a crossbred strain of Pleurotus sajorcaju. The average biological efficiency (BE) of the strain is 58.65%with 2.71% superiority than check and suitable for culture at 24–28°C.

Shiitake mushroom: DMRO-356 is a selection having 46.7% biological efficiency.

Palak: In Thar Hariparna, fresh leaf yield is 154.72 q/ha.

Banana: The genetically modified and bio-fortified banana cv. Rasthali and Grande Naine with enhanced pro-vitamin-A (range 20 µg/100 g dry weight) were developed. The protocol for large scale micropropagation of banana Elakki Bale using embryogenic cell suspension (ECS) was developed (1 lakh plants/ embryogenic calli). Field evaluation of embryo derived plantlets showed normal phenotype as compared to sucker or shoot tip plants.

Drought Tolerant HB4® Wheat approved for cultivation in Argentina

Argentina's Ministry of Agriculture has granted approval of Bioceres Crop Solutions' H_B4 wheat event for growth and consumption. The HB4 trait increases wheat yields by up to 20% and is currently the only drought tolerance technology for wheat and soybean crops in the world. Argentina is Latin America's largest wheat producer and the world's first country to adopt HB4 drought tolerance technology for wheat.

Argentina's regulatory clearance follows the approval of HB4 soybean which has been approved in the United States and Brazil. Commercialization of HB4 wheat in Argentina is contingent upon import approval in Brazil, which purchases just over 85% of its wheat from Argentina. Currently, regulatory processes for HB4 wheat are advancing in the US, Uruguay, Paraguay, and Bolivia. Bioceres also intends to initiate regulatory processes in Australia and Russia, as well as certain countries in Asia and Africa.

Drought-tolerant HB4 Wheat is a patented seed technology developed by Trigall Genetics, Bioceres' joint venture with Florimond Desprez, a global leader in wheat genetics. In field trials conducted during the last 10 years, HB4 seed varieties increased wheat yields by 20%, on average, during growing seasons impacted by droughts. HB4 is integrated with top-selling wheat germplasms and branded as EcoWheat®. In preparation for the commercial launch of EcoWheat, around 17,300 acres (7,000 hectares) of different varieties have been planted by participating growers. Mr. Federico Trucco, Chief Executive Officer of Bioceres, said, "Our EcoWheat® and EcoSoy® products will enable food production companies and retailers the opportunity to offer consumers foods that are carbon-neutral, in addition to other environmental benefits they increasingly desire. Importantly, our HB4 technology

does not translate into higher costs for consumers, making sustainable foods widely accessible."

Emmanuelle Charpentier and Jennifer A. Doudna Awarded 2020 Nobel Prize in Chemistry

On October 7, 2020, The Royal Swedish Academy of Sciences awarded the Nobel Prize in Chemistry jointly to Emmanuelle Charpentier of the Max Planck Unit for the Science of Pathogens and Jennifer A. Doudna of the University of California, Berkeley for the development of a method for genome editing. This method, the CRISPR-Cas9 genetic scissors, is one of gene technology's sharpest tools.

Modifying genes in cells used to be time-consuming, difficult, and sometimes impossible work. CRISPR-Cas9 has made it possible to change the code of life over the course of a few weeks. Claes Gustafsson, chair of the Nobel Committee for Chemistry said, "There is enormous power in this genetic tool, which affects us all. It has not only revolutionized basic science, but also resulted in innovative crops and will lead to ground-breaking new medical treatments."

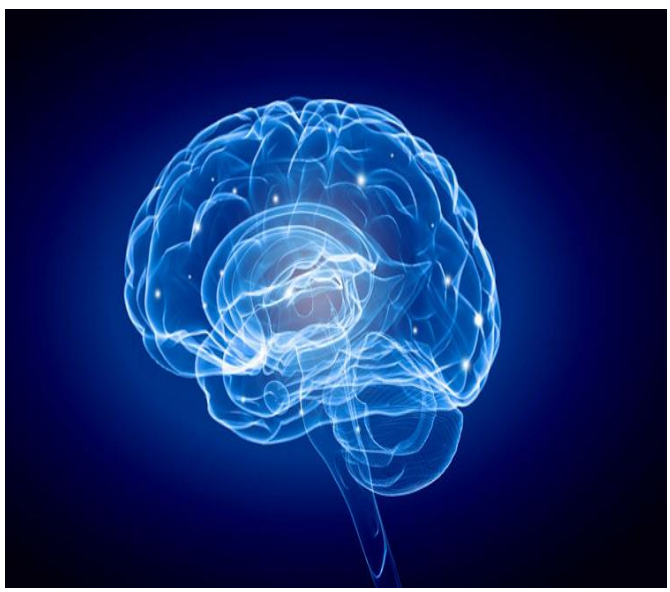
CRISPR-Cas9's discovery was unexpected. Emmanuelle Charpentier's studies of *Streptococcus pyogenes*, one of the bacteria that cause the most harm to humanity, led her discovery of a previously unknown molecule, *tracrRNA*. Her work showed that *tracrRNA* is part of bacteria's ancient immune system, CRISPR-Cas, that disarms viruses by cleaving their DNA. Charpentier published her discovery in 2011 and initiated a collaboration with Jennifer Doudna, an experienced biochemist with vast knowledge of RNA. Together, they succeeded in recreating the bacteria's genetic scissors in a test tube and simplifying the scissors' molecular components so they were easier to use.

Photo Souce: Nobel Media. Ill. Niklas Elmehed.

Since Charpentier and Doudna discovered the CRISPR-Cas9 genetic scissors in 2012 their use has exploded. This tool has contributed to many important discoveries in basic research, and plant researchers have been able to develop crops that withstand mold, pests, and drought. In medicine, clinical trials of new cancer therapies are underway, and the dream of being able to cure inherited diseases is about to come true. These genetic scissors have taken the life sciences into a new epoch and, in many ways, are bringing the greatest benefit to humankind

CRISPR-Cas9 a promising way for Drug Addiction Treatment

BChE is a natural enzyme that degrades cocaine into harmless and inactive components in humans. However, its usage for therapy is hampered by difficulty in delivery and making sure the enzyme maintains its function. Thus, using skin to produce modified BChE to continuously supply the body with the enzyme is a promising tool. The researchers find that skin-grafted mice injected with lethal doses of cocaine were able to live compared with control samples. Preference for cocaine is also low, and relapse is not observed after 25 days of withdrawal.



The researchers also test the expression of the enzyme in human skin cells. They find high expression of *BChE* in these cells, proving the promising effectiveness of the method. Further studies on possible side effects of the treatment is being considered by the group. If proven effective, this technology may be used in other types of drug and alcohol addiction in humans.

Seed Weight are Maternally Controlled in Canola: A study

The basis of seed weight variation in rapeseed or canola is explored through genetic, morphological, and cytological analyses. This variation is deemed important for domestication and crop improvement, but its regulatory mechanism is poorly understood. Although seed weight is theoretically influenced by the mother and the zygote itself, the exact contribution of each is unclear.

Researcher Hanzhong Wang from Oil Crops Research Institute of the Chinese Academy of Agricultural Sciences and colleagues primarily use RNA expression analysis to determine the mechanism responsible for seed weight. Results show correlation among pod length, pod wall photosynthetic area, carbohydrate content, and final seed weight. RNA expression is increased in genes related to seed development, cell division, nutrient reservoir, and ribosomal proteins in large seeds. The researchers concluded with seed weight being controlled primarily by the maternal source, specifically the mother's pod size.

Plant-based Milk Bottles Launched in New Zealand

The milk bottles are made from sugarcane, which is natural, renewable, and sustainably sourced from Brazil. Brazil is one of the biggest producers of sugarcane. One good characteristic of sugarcane is that it captures carbon dioxide from the atmosphere as it grows. Thus, the bottles have a lower carbon footprint. The plant-based milk bottles are initially sold in North Island, for further expansion in distribution depending on the consumer response. Milk in recyclable plant-based bottles are now available in New Zealand which opens new way for environment protection.

Microbial diversity below seafloor is as rich as on Earth's surface

For the first time, researchers have mapped the biological diversity of marine sediment, one of Earth's largest global biomes. The research team discovered that microbial diversity in the dark, energy-limited world beneath the seafloor is as diverse as in Earth's surface biomes in the University of Rhode Island

CRISPR meets Pac-Man: New DNA cut-and-paste tool enables bigger gene edits

Gene editing for the development of new treatments, and for studying disease as well as normal function in humans and other organisms, may advance more quickly with a new tool for cutting larger pieces of DNA out of a cell's genome, according to a new study conducted by University of California - San Francisco

Pinpointing the 'silent' mutations that gave the coronavirus an evolutionary edge

RNA folding may help explain how the coronavirus became so hard to stop after it spilled over from wildlife to humans

Researchers have identified a number of 'silent' mutations in the roughly 30,000 letters of the COVID-19 virus's genetic code that helped it thrive once it made the leap from bats and other wildlife to humans -- and possibly helped set the stage for the global pandemic discovered by Duke University

Advancing wildlife genomics through the development of molecular methods

A team of scientists report a new method for identifying any genome sequence located next to a known sequence. Sonication Inverse PCR (SIP) can be used to characterize any DNA sequence (near a known sequence) and can be applied across genomics applications within a clinical setting as well as molecular evolutionary analyses discovered by Forschungsverbund Berlin

The road to uncovering a novel mechanism for disposing of misfolded proteins

The discovery of the cause of a rare liver disease in babies led to uncovering a novel cellular mechanism for disposing of misfolded proteins that has implications for neurodegenerative conditions of older age by the Baylor College of Medicine

Machine learning uncovers potential new TB drugs

Using a machine-learning approach that incorporates uncertainty, researchers identified several promising compounds that target a protein required for the survival of the bacteria that cause tuberculosis discovered by the Massachusetts Institute of Technology

Novel antiviral strategy for treatment of COVID-19

A research team led by Professor Hongzhe SUN, Norman & Cecilia Yip Professor in Bioinorganic Chemistry, Department of Chemistry, Faculty of Science, and Professor Kwok Yung YUEN, Henry Fok Professor in Infectious Diseases, Department of Microbiology, Li Ka Shing Faculty of Medicine of the University of Hong Kong (HKU), has discovered a novel antiviral strategy for treatment of COVID-19.

They discovered that a class of metallodrugs currently used in the treatment of other infectious diseases is showing efficacy

to potently suppress SARS-CoV-2 replication and relieve viral-associated symptoms in an animal model.

The findings provide a new and readily available therapeutic option with high clinical potential for infection with SARS-CoV-2. This ground-breaking work has been published online in a top-class scientific journal *Nature Microbiology*. A related patent has been filed in the US.

Boost to develop microalgae into health foods

A new discovery may provide the crucial link that helps accelerate development of microalgae into beneficial human health supplements.

Dietary supplementation of fatty acids produced from microalgae have wide-reaching health benefits for humans, including the ability to reduce obesity, diabetes and fatty liver disease, preventing hair loss, and assisting wounds to heal discovered by Flinders University

Scientists engineer bacteria-killing molecules from wasp venom

Potential new antibiotics work by disrupting bacterial membrane and summoning immune cells in animal models

Scientists have engineered powerful new antimicrobial molecules from toxic proteins found in wasp venom. The team hopes to develop the molecules into new bacteria-killing drugs, an important advancement considering increasing numbers of antibiotic-resistant bacteria discovered by the University of Pennsylvania School of Medicine

A team led by scientists in the Perelman School of Medicine at the University of Pennsylvania has engineered powerful new antimicrobial molecules from toxic proteins found in wasp venom. The team hopes to develop the molecules into new bacteria-killing drugs, an important advancement considering increasing numbers of antibiotic-resistant bacteria which can cause illness such as sepsis and tuberculosis.

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Sirova, D., Adamec, L. and Verba,J. (2013). Enzymatic activities in trops of four aquatic pieces 3 of the Carnivorous genus Vlricularia, *New Phytology* 159(3) : 669-675.

Sengar, R.S., Sharma, A.K., Chaudhary, R. and Kureel, R.S. (2009). Biodiesel plant Jatrophaneed for future. Procedings of "5th World Congress of Cellular & Molecular Biology (WCCMB, 2012)", November 02-06, School of Biotechnology, Devi Ahilya University Indore, India & World Society of Cellular & Molecular Biology, France. 142-143.

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