1. Name of the Department/Section: Plant Biotechnology Centre

Salient features: 1. Spacious and well planned building.

2. Well equipped laboratories
3. Trained and devoted teaching staff
4. Opportunity for independent work

2. About Department:

University has established an independent Plant Tissue Culture Laboratory in 1995. Thetrained and devoted scientist having background of Biochemistry, Molecular Biology, Genetics and plant breeding, Genetic Engineering, Tissue Culture and Plant Transformation techniques were pooled from the various places in the jurisdiction of the university and placed under one umbrella at central campus Dapoli in 2000. Consequently from the university receipts and the *Swaminathan Foundation Project* and Mega seed project some of the major equipments required for Biotechnology research were purchased and research work initiated. After realizing the necessity and importance of biotechnology in Agriculture and research work done by the team of the scientists with limited resources, Government of Maharashtra has kindly extended the financial support for the construction of an additional building for Biotechnology along with all well equipments required for the research and education in frontier areas of biotechnology fromthe year 2007-08.

3. Academic Programmes:

Masters Programme

Name of the programme: M. Sc. (Molecular Biology and Biotechnology)

Semester	Course	Course No.	Title of the course offered by the department	Credits
I.	Major : 9 Credits	MBB 501	Principles of Biotechnology	3+0=3
		MBB 502	Fundamentals of Molecular Biology	3+0=3
		MBB 504	Techniques in Molecular Biology-1	0+3=3
	Minor : 6 Credits	GPB 502 PP 504	Principles of plant breeding Physiological and molecular responses of	2+1=3
			plants to abiotic stress	2+1=3
	Supporting	-	-	-
	NCCC : 2 Credits	PGS-501	Library and Information Services	0+1=1
		PGS-504	Basic Concepts in Laboratory Techniques	0+1=1
			Total Course Credits	15+2=17
II.	Major : 9 Credits	MBB 503	Molecular Cell Biology	3+0=3
		MBB 505	Omics and system biology	2+1=3
		MBB 507	Techniques in molecular biology - II	0+3=3
	Minor : 3 Credits	PL PATH 505	Principles of plant pathology	2+1=3
	Supporting 7 Credits	STAT-511 BIOCHEM 501	Experimental Designs Basic Biochemistry	2+1=3 3+1=4

	NCCC: 1 Credit	PGS-502	Technical writing and communication skills	1+0=1
			Total Course Credits	9+3+7+1=20
III.	Major : 3 Credits	MBB508	Introduction to bioinformatics	2+1=3
	Research	MBB599	Master's Research	0+15=15
	15 Credits			

	NCCC 2 Credits	PGS-503 PGS-505	Intellectual property and it's management in agriculture Agricultural Research Ethics and Rural Development Programmes	1+0=1 1+0=1
	Total Course Credits			3+15+2=20
IV.	Seminar 1 Credit	MBB 591	Master's Seminar	0+1=1
	Research 15 Credits	MBB-599	Master's Research	0+15=15
			Total Course Credits	1+15=16
			Grand Total I +II+ III+ IV	17+20+20+16=73

Course Curricula syllabi:

MBB 501 PRINCIPLES OF BIOTECHNOLOGY 3+0

Theory

Unit I

History, scope and importance of Biotechnology; Specializations in Agricultural Biotechnology: Genomics, Genetic engineering, Tissue Culture, Bio-fuel, Microbial Biotechnology, Food Biotechnology etc. Basics of Biotechnology, Primary metabolic pathways, Enzymes and its activities.

Unit II

Structure of DNA, RNA and protein, their physical and chemical properties. DNA function: Expression, exchange of genetic material, mutation. DNA modifying enzymes and vectors; Methods of recombinant DNA technology; Nucleic acid hybridization; DNA/RNA libraries; Applications of gene cloning in basic and applied research, Plant transformation: Gene transfer methods and applications of GM crops.

Unit III

Molecular analysis of nucleic acids -PCR and its application in agriculture and industry, Introduction to Molecular markers: RFLP, RAPD, SSR, SNP etc, and their applications; DNA sequencing, different methods; Plant cell and tissue culture techniques and their applications. Introduction to genomics, transcriptomics, ionomics, metabolomics and proteomics.

Unit IV

Introduction to Emerging topics: Genome editing, gene silencing, Plant microbial interactions, Success stories in Biotechnology, Careers, and employment in biotechnology. Public perception of biotechnology; Bio-safety and bioethics issues; Intellectual property rights in biotechnology.

MBB 502 FUNDAMENTALS OF MOLECULAR BIOLOGY 3+0

Theory

Unit I

Historical developments of molecular biology, Nucleic acids as genetic material, Chemistry and Nomenclature of nucleic acids; Structure of DNA: primary structure; secondary structure, Forms of DNA: A, B, Z and their function; Structure and Types of RNA Genome organization in prokaryotes and eukaryotes; DNA Topology; DNA re-association kinetics, Types of repeat sequences.

Unit II

Central dogma of Molecular Biology; DNA replication- Classical experiments, Models of DNA replication; DNA replication, Origin and Steps in DNA replication - initiation, elongation and termination; Enzymes and accessory proteins and its mechanisms; Eukaryotic DNA replication in brief. Types of DNA damages and mutations; DNA repair mechanisms, Recombination: Homologous and non-homologous, Genetic consequences.

Unit III

Prokaryotic transcription, initiation, elongation and termination, promoters, Structure and function of eukaryotic RNAs and ribosomal proteins. Eukaryotic transcription – RNA polymerase I, II and III, Elongation and Termination, Eukaryotic promoters and enhancers, Transcription factors, Post transcriptional processing, Splicing: Catalytic RNAs, RNA stability and transport, RNA editing.

Unit IV

Genetic code and its characteristics, Universal and modified genetic code and its characteristics, Wobble hypothesis; Translational machinery; Ribosomes in prokaryotes and Eukaryotes. Initiation complex formation, Cap dependent and Cap independent initiation in eukaryotes, Elongation: translocation, trans-peptidation and termination of translation; Co- and Post-translational modifications of proteins; Translational control; Protein stability -Protein turnover and degradation.

Unit V

Gene regulation in prokaryotes, Constitutive and Inducible expression, small molecule regulators; Operon concept: lac and trp operons, attenuation, anti-termination, stringent control. Gene regulation in eukaryotes– regulatory RNA and RNA interference mechanisms, Silencers, insulators, enhancers, mechanism of silencing and activation; Families of DNA binding transcription factors: Helix turn-helix, helix-loop-helix etc. Epigenetic regulations

MBB 504 TECHNIQUES IN MOLECULAR BIOLOGY-1 0+3

Objective

To get a basic overview of molecular biology techniques, good lab practices and recombinant DNA technology

To get a hands on training in chromatography, protein analysis, nucleic acid analysis, bacterial and phage genetics

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Practical's

1. Good lab practices, preparation of buffers and reagents.
2. Principle of centrifugation and spectrophotometry.
3. Growth of bacterial culture and preparation of growth curve, Isolation of Genomic DNA from bacteria.
4. Isolation of plasmid DNA from bacteria.
5. Growth of lambda phage and isolation of phage DNA.
6. Isolation and restriction of plant DNA (e.g. Rice / Moong / Mango / Marigold).
7. Quantification of DNA by (a) Agarose Gel electrophoresis and (b) Spectrophotometry
8. PCR using isolated DNA.
9. PAGE Gel electrophoresis.
10. Restriction digestion of plasmid and phage DNA, ligation, Recombinant DNA construction.
11. Transformation of E. coli and selection of transformants
12. Chromatographic techniques
    i. TLC
    ii. Gel Filtration Chromatography,
    iii. Ion exchange Chromatography,
    iv. Affinity Chromatography
13. Dot blot analysis, Southern hybridization, Northern hybridization.
14. Western blotting and ELISA.
15. Radiation safety and non-radio isotopic procedure.

MBB 503 MOLECULAR CELL BIOLOGY 3+0

Theory

Unit I

Origin of life, History of cell biology, Evolution of the cell: endo-symbiotic theory, tree of life, General structure and differences between prokaryotic and eukaryotic cell; Similarities and distinction between plant and animal cells; different kinds of cells in plant and animal tissues.

Unit II

Cell wall, cell membrane, structure and composition of bio-membranes, Structure and function of major organelles: Endoplasmic reticulum Ribosomes, Golgi apparatus, Mitochondria, Chloroplasts, Lysosomes, Peroxisomes, Micro-bodies, Vacuoles, Nucleus, Cyto-skeletal elements.

Unit III

Membrane transport; Diffusion, osmosis, ion channels, active transport, mechanism of protein sorting and regulation of intracellular transport, transmembrane and vesicular transport - endocytosis and exocytosis; General principles of cell communication: hormones and their receptors, signaling through G-protein coupled receptors, enzyme linked receptors; signal transduction mechanisms and regulation, Cell junctions, Cell adhesion, Cell movement; Extracellular matrix.

Unit IV

Chromatin structure, Cell division and regulation of cell cycle; Mechanisms of cell division, Molecular event sat M phase, mitosis and cytokinesis, Ribosomes in relation to cell growth and division, Extracellular and intracellular Control of Cell Division; abnormal cell division: cancer- hall marks of cancer and role of onco genes and tumor suppressor genes in cancer development - Programmed cell death (Apoptosis).

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Unit V

Morphogenetic movements and the shaping of the body plan, Cell diversification, cell memory, cell determination, and the concept of positional values; Differentiated cells and the maintenance of tissues and organ development; Stem cells: types and applications; Basics of Animal development in model organisms (C. elegans; Drosophila); Plant development.

MBB 505 OMICS AND SYSTEMS BIOLOGY 2+1

Theory

Unit I

Forward and Reverse Genetics, structural and functional genomics, principles of various sequencing methods; Different methods of genome sequencing, principles of various sequencing chemistries, physical and genetic maps, Comparative and evolutionary genomics, Organelle genomics, applications in phylogenetics, case studies of completed genomes, preliminary genome data analysis, map based cloning, basics of ionomics analysis, different method

Unit II

Protein-basics: primary-, secondary- and tertiary structure, Basics of X-ray crystallography and NMR, Principal and Applications of mass spectrometry, Proteomics: Edman degradation peptide sequencing, Peptide fingerprinting, Gel based (2D PAGE) and gel free (HPLC/MS), Basics of software used in proteomics, MASCOT,PD-Quest, etc., Study of protein interactions, Prokaryotic and yeast-based eXpression system and purification

Unit III

Metabolomics and its applications, Metabolite extraction-High throughput Analysis and interpretation, chromatography (HPLC/GC/LC),UV-Visible Spectroscopy; Use of 1D/2D NMR and MS in metabolome analysis, Multivariate analysis and identification of metabolite as biomarkers, Study of ionome using inductively coupled plasma – mass spectroscopy (ICP-MS), X-Ray Fluorescence (XRF), Neutron activation analysis (NAA), Data integration using genome, transcriptome, proteome, metabolome and ionome with phenome. High Throughput phenotyping with Sensors.

Unit IV

Introductory systems Biology - The biochemical models, genetic models and systems model, Molecules to Pathway, Biological oscillators, Genetic oscillators, Quorum Sensing, Cell-cell communication, Drosophila Development, Pathways to Network, Gene regulation at a single cell level, transcription network, REGULATORY CIRCUITS, Negative and positive auto-regulation, Alternative Stable States, Bimodal Switches, Network building and analysis

Practical

1. Isolation of HMW DNA and brief overview of sequencing, Primary information on genome data analysis.
2. BSA Standard curve preparation, Extraction of protein and estimation methods.
3. Quantification of proteins from different plant tissues using spectrophotometry.
4. 2-D Gel Electrophoresis, 2-D Image analysis.
5. Experiments on protein-protein interaction (Yeast 2-hybrid, Split Ubiquitin system).
6. Demonstration on MALDI-TOF.
7. Demonstration on ICP-MS, AAS, Nitrogen estimation using various methods.

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MBB 507 TECHNIQUES IN MOLECULAR BIOLOGY-II 0+3

Objective

To get a basic overview of molecular biology techniques, good lab practices and molecular markers.
To get a hands on training in RNAi, microarrays, yeast 2 hybrid and immunological techniques.

Practical's

1. Construction of gene libraries (c-DNA and Genomics).
2. Synthesis and cloning of c-DNA.
3. Real time PCR and interpretation of data.
4. Molecular markers
   i. RAPD.
   ii. SSR.
   iii. AFLP/ISSR and their analysis.
5. Case study of SSR markers-construction of linkage map.
6. QTL analysis using genotypic data based on SSR.
7. SNP identification and analysis.
8. Micro array studies and use of relevant software.
9. Proteomics
   i. 2Dgels,
   ii. Mass spectrometry
10. RNAi- designing of construct, phenotyping of the plant.
11. Yeast1and2-hybridinteraction.
12. Generation and screening of mutants.
13. Transposon mediated mutagenesis.
14. Immunology and molecular diagnostics: Ouchterlony double diffusion, Immunoprecipitation, Radiation Immunodiffusion, Immunoelectrophoretic, Rocket Immunoelectrophoretic, Counter Current Immunoelectrophoretic, ELISA, LateX Agglutination, Immunohistochemistry.

MBB 508 INTRODUCTION TO BIOINFORMATICS 2+1

Objective

To get a basic overview of computational techniques related to DNA, RNA and protein analysis.

To get a hands-on training in software's and programs used to analyse, assemble or annotate genomes, phylogenetics, proteomics etc

Theory

Unit I

Bioinformatics basics, scope and importance of bioinformatics; Biological databases for DNA and Protein sequences -PIR, SWISSPROT, Gene Bank, DDBJ, secondary database, structural databases–PDB, SCOP and CATH, Specialized genomic resources, Microarray database.

Unit II

Bioinformatics Tools Facilitate the Genome-Wide Identification of Protein-Coding Genes, Sequence analysis, Sequence submission and retrieval system- SEQUIN, BANKit, SAKURA, Webin, Sequence alignment, pair wise alignment techniques, multiple sequence alignment; Tools for Sequence alignment-BLAST and its variants; Phylogenetic analysis-CLUSTALX, CLUSTALW, Phylip, Tcoffee

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Unit III

Sequencing of protein; Protein secondary structure prediction- Chousfasman, GOR Method, Protein 3D Structure Prediction: Evaluation of models-Structure validation and refinement-Ramachandran plot, Force field calculations, SAVES. Protein function prediction- sequence and domain based, Primer designing- principles and methods. Drug discovery, Structure Based Drug Design-Rationale for computer aided drug designing, basic principles, docking, QSAR.

Practical

1. Usage of NCBI resources
2. Retrieval of sequence / structure from data bases and submission
3. Different Databases, BLAST eXercises.
4. Assembly of DNA and RNA Seq data
5. Annotation of assembled sequences, Phylogenetics and alignment
6. Visualization of structures, Docking of ligand receptors
7. Protein structure analysis and modeling

4. Infrastructure:

a. Laboratories: Art-of-state laboratory facilities are created by the department. Independent laboratories for Molecular biology, plant tissue culture, genetic engineering, media preparation and sterilization are available. Each lab is spacious and 20 students can work at a time.

b. Name of the important instruments/facilities:

Sr. No.	Name of the equipment	Sr. No.	Name of the equipment
1	Horizontal gel electrophoresis	14	Refrigerators
2	Vertical gel electrophoresis	15	Particle gun
3	Thermal cyclers	16	Rotary incubator shaker
4	Laminar Flow cabinet	17	Oven
5	Gel documentation unit	18	Incubators
6	Hybridization chamber	19	Rotary shaker
7	High speed refrigerator centrifuge (Table top model)	20	Culture racks
8	Weighing balance	21	Autoclaves
9	Refrigerated Micro centrifuge	22	Quartz double distillation unit
10	ELISA reader and washer	23	Ice flaking machine
11	UV visible spectrophotometer	24	Milli Q water purification system
12	Tube Rotator	25	Thermostatic water bath
13	-20^{0}C, -40^{0}C and -86^{0}C deep freeze	26	Green house type III

Teachings Aids

1. LCD	3. Over head projector
2. Computers	

c. Activities:

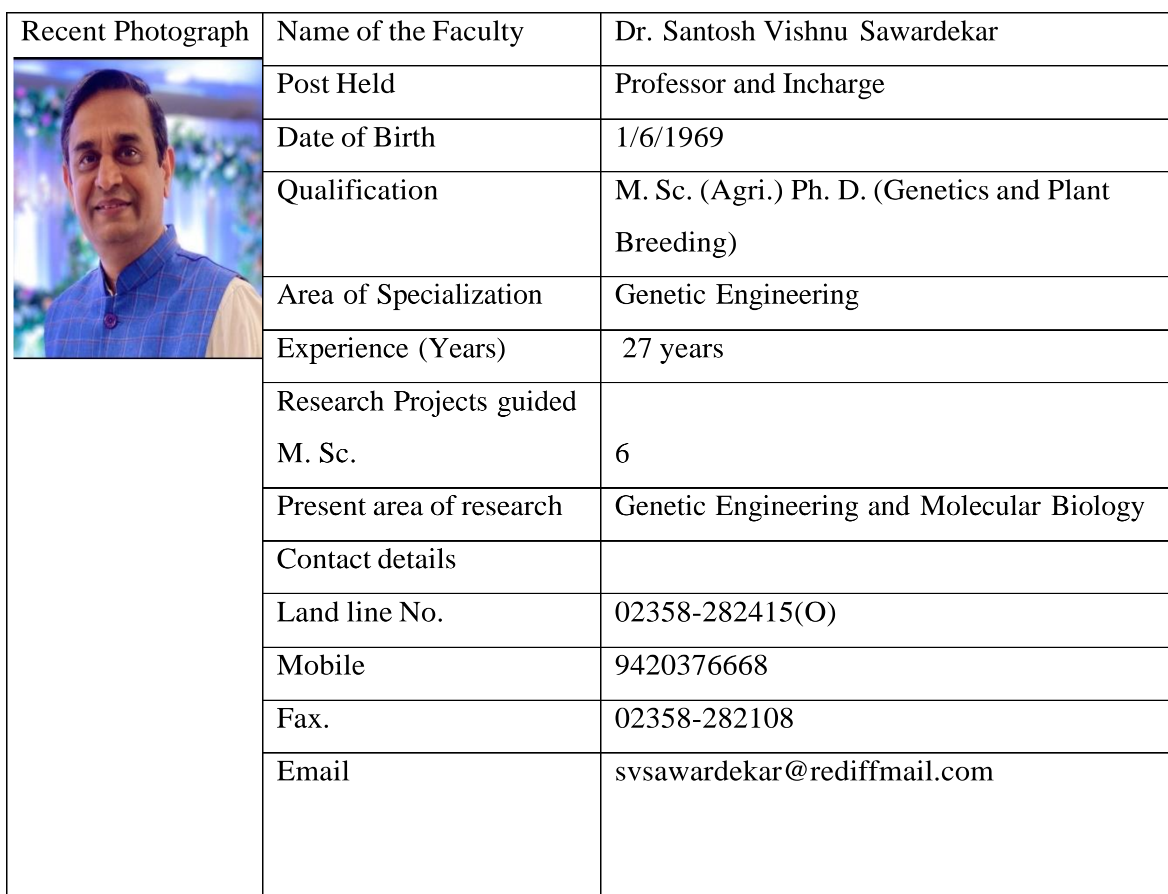
1. Micropropogation technique of commercially important horticulture crop like banana Cv.Grand naine and Safed velchi.
2. Micropropogation technique of endangered forest species.
3. Creation of variability in small millets through callus culture.
4. Marker Assisted Selection (MAS) in rice for developing resistance against biotic and abioticstress.
5. Development of transgenic in pulses for pod borer resistance.
6. Finger printing of released varieties university through molecular markers.

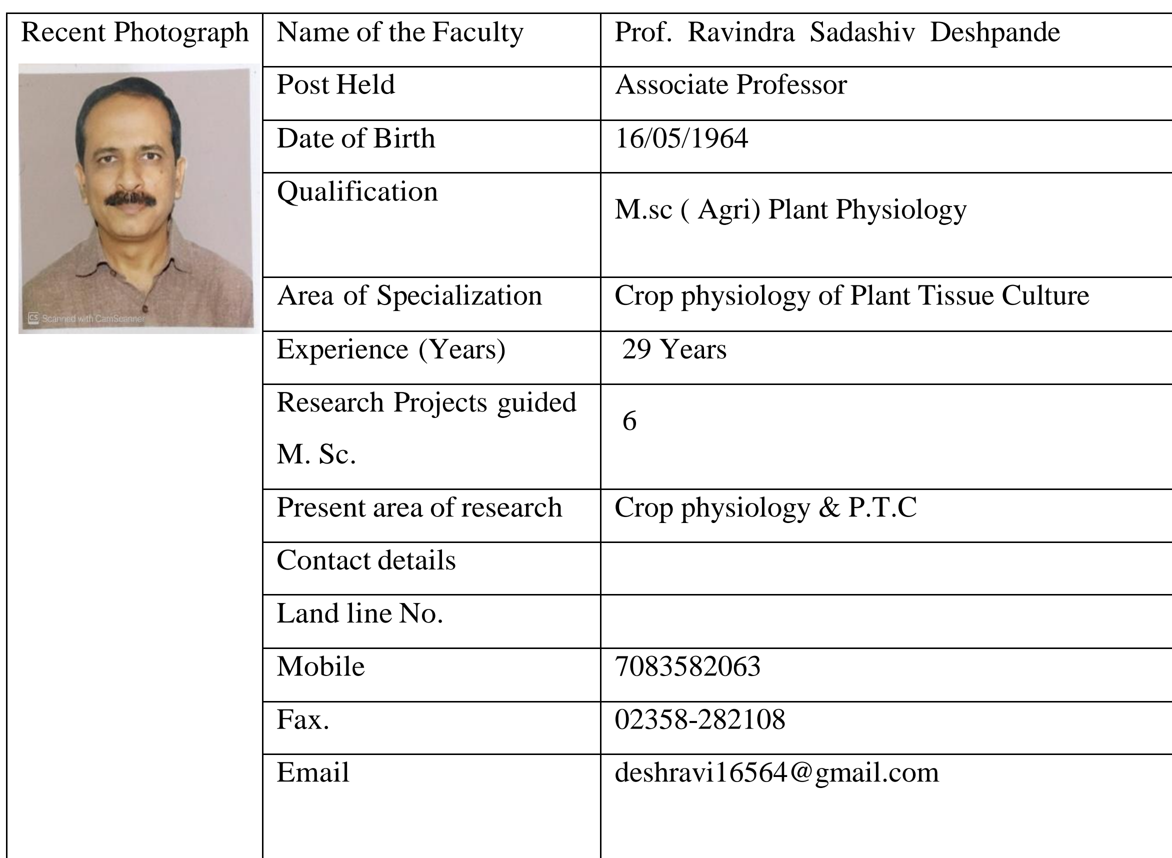
d. photographs:

S

5. Faculty

A. Academic staff: Assistant Professor and above with the details of the staff as given below

Recent Photograph	Name of the Faculty	Dr. Santosh Vishnu Sawardekar
	Post Held	Professor and Incharge
	Date of Birth	1/6/1969
	Qualification	M. Sc. (Agri.) Ph. D. (Genetics and Plant Breeding)
	Area of Specialization	Genetic Engineering
	Experience (Years)	27 years
	Research Projects guided M. Sc.	 6
	Present area of research	Genetic Engineering and Molecular Biology
	Contact details	
	Land line No.	02358-282415(O)
	Mobile	9420376668
	Fax.	02358-282108
	Email	svsawardekar@rediffmail.com



Recent Photograph	Name of the Faculty	Prof. Ravindra Sadashiv Deshpande
	Post Held	Associate Professor
	Date of Birth	16/05/1964
	Qualification	M.sc (Agri) Plant Physiology
	Area of Specialization	Crop physiology of Plant Tissue Culture
	Experience (Years)	29 Years
	Research Projects guided M. Sc.	6
	Present area of research	Crop physiology & P.T.C
	Contact details	
	Land line No.	
	Mobile	7083582063
	Fax.	02358-282108
	Email	deshravi16564@gmail.com

a. Research staff: The name of the research staff member like SRA and JRA. **NIL**

6. Instructional Farm

Availing the facilities available with Dr. B. S. Konkan Krishi Vidyapeeth, Dapoli.

7. Research Activities and Achievements (including projects)

a. Variety/Implements released: -

Title : Use of irradiation technique for creation of variability in nagli and assessment of mutants through molecular markers.

Background information : Finger millet is the staple food of 77 million tribal and millions of other people in India. It is an important food security crop and is important nutritionally. Inspite of the importance of ragi to the livelihoods of millions it has been neglected by national and international agriculture research and development system due to lack of realization of potential productivity and commercial value. Though even number of superior genes presenting different genotypes could not bring in one genotype because of small and tiny flowers is the barrier of hybridization. Similarly, lack of seed of improved varieties, non-availability of resistance source for blast and minimum genetic variability creates major problems for improvement of ragi through hybridization. Mutation breeding appears effective and potential in

highly self-fertilized small millets to rectify a character, to improve a character, to generate polygenic variation and to induce partial to complete sterility for increasing recombination frequency. Following gamma radiation, early mutants of finger millet, Hamsa, with increased finger number and grain-bearing area were obtained.

DNA based markers are helpful for assessment of genetic variability, selection of desirable mutants and screening for blast disease resistance. If high yielding genotypes with increased nutritional value are made available for cultivation it will meet the problem of malnutrition in tribals. It is gaining lot of importance in manufacture of weaning foods as it is a rich source of calcium and iron which can be exploited commercially for helping the tribals to gain additional income.

Objectives : 1) Irradiation of promising genotypes of finger millet.

2) To develop blast resistant mutant in finger millet.

3) Assessment of genetic variability of mutants through molecular markers.

Project Scientist : :. N. B. Gokhale, Incharge, Plant Biotechnology Centre, College of Agriculture, Dapoli.

Co-Scientists associated : 1. Dr. S. V. Sawardekar, Associate Professor,

2. Shri. D. M. Patil, Jr. Research Fellow

3. Sou. S. S. Sawant, Jr. Research Assistant.

Experimental Details

Materials used : Selection of Genotype: Dapoli Nagli 1

- Wider adaptability
- High yielding
- Dark red kernel colour
- Susceptible to Blast at seedling stage and flowering stage

- Various chemicals for DNA isolation.
- PCR components
- RAPD and ISSR primers (Table 1 and Table2)
- Thermocycler
- Refrigerated centrifuge machine
- -20 0C refrigerator
- Water bath
- Gel electrophoresis system
- Gel documentation system

Methodology:

i) Optimization of LD50:

Twenty five gram seeds of Cv. Dapoli Nagli 1 were irradiated at varying dose of gamma rays (400, 500, 600 and 700 -Gy) at NA & BTD, BARC, Mumbai. The irradiated seeds were subjected to paper fold germination test and field emergence test for optimization LD50 in replicated trial. After eight days of growth LD50 dose was determined based on seedling height and germination percentage.

ii) Raising of M1:

400, 500, 600, and 700 Gy dose treated seeds along with control were sown in kharif, 2012 and individual panicle/ plant from M1 population were harvested. Ten seedlings were selected randomly for taking observations. Chlorophyll mutants were scored and classified. Desirable mutants from M1 generation were selected on the basis of their phenotypical characters and harvested separately.

iii) Raising of M2 generation:

The seeds harvested in M1 generation was grown on raised bed and 20 plants of each line were transplanted to observe mutant plants in M2 generation.

iv) M3 generation:

Eighty three sister lines were raised in M3 generation during kharif 2013. All recommended package of practices were followed during growth period of the crop. Newly evolved characters were recorded in M3 generation. Observations on days to 50% flowering and maturity duration was recorded on line basis and for other morphological characters were recorded on 5 randomly selected plants from each line

v) Raising of M4 generation:

58 desirable mutant lines were selected from M3 generation for raising M4 generation in Rabi 2014. All the 58 mutants lines with control DPL-1 were grown in two replications. Observations on quantitative characters were recorded by selecting 5 plants from each line in each replication. Stability of blast free mutant line and other desirable characters were also observed in this generation. Mean values of recorded observations are presented in Table 12.

vi) Raising of M5 generation:

High yielding and morphologically superior 23 lines were selected from M4 generation and grown in two replications with control parent DPL-1 during kharif 2014. Ten Mutant lines (1, 2, 3, 4, 11, 12, 14, 17, 18 and 21) with good morphological features are selected for stability analysis of mutants in IVT (Initial Varietal Trial) during next season.

vii) Genetic Diversity Analysis:

a) DNA isolation: The genomic DNA used for analysis was isolated from green leaves of 23 field grown desirable mutants and one control parent (Dapoli-1) by following the protocol of Doyle and Doyle (1990) i.e. Rapid Method.

b) DNA amplification and gel electrophoresis:

A set of 15 primers (Table 4) composed wholly of defined, short tandem repeat sequences with anchor and representing different microsatellites (di and tri-repeats) have been used as generic primers in PCR amplification of inter simple sequence repeat (ISSR) regions as according to the method of Adawyet al., (2002). Amplification was achieved in eppendorf thermal cycler using 20

μl reaction mixture containing 3U of Taq DNA polymerase (BangloreGenei Ltd.), 2.5 μl of 10x Taq assay buffer with 2.5 mM of MgCl2, 1 μl of 10 mM of dNTPs, 0.5 μl of 25 mM MgCl2, 1 μl

of 5 pmoles concentration of oligonucleotide primer and approximately 50 ng of 1μl of template DNA. The PCR thermal cycler programmed for initially 5-minute denaturation step at 94°C, followed by 30 cycles of denaturation at 94°C for 20-sec, annealing 58°C for 60-sec and extension at 72°C for 60 sec and finally 72°C for 7 min. The PCR product were mixed with 2 μl of gel loading dye and electrophoresed alongside a molecular weight marker (1.3 kb) on 2 per cent agarose gel in 1 X TAE buffer at 100 volts. The gels were photographed under UV light using Pentax K 312 nm camera. The images of gels were also taken by the documentation systems (Uvi-Tech. Fire reader, Cambridge, England) and saved in computer for further analysis.

c) Data analysis:

ISSR markers across the 23 mutant lines and one control parent (Dapoli-1) were scored for their presence (1) or absence (0) of bands for each primer. The binary data so generated was used to estimate the levels of polymorphism by dividing the number of polymorphic bands by the total number of scored bands. Jaccard's similarity co-efficients for each pairwise comparison between mutants were calculated and similarity co-efficient matrix was generated. This matrix was subjected to Unweighted Pair Group Method for Arithmetic Average analysis (UPGMA) to construct a dendrogram. The similarity co-efficient analysis and dendrogram construction were carried out by using MVSP-A Multivariate Statistical Package_5785 (Version 3.1)

viii) Estimation of Protein, Ca and Fe content of promising mutant lines selected from M5 generation:

10 promising mutant lines obtained from M5 generation along with control (Dapoli-1) were used for quantitative estimation of Protein, Calcium and Iron. The nitrogen content of the sample was assessed by Kjeldahl method using Pelican Kelplus equipment and crude protein was calculated by multiplying with a factor 6.25 (AOAC, 1990). The calcium content of 100 mesh millet powder was estimated by Versenate method while iron content in the digested sample was estimated by using atomic absorption spectrophotometer (Tandon, 1993). Details of quantitative estimation are presented in Table 8.

Results:

i) Optimization of LD50 dose:

It was observed that the non-irradiated seeds of Cv. Dapoli Nagli 1 (Control) showed 90% germination with maximum root and shoot length (5.01 and 4.67 cm, respectively). Based on this germination percentage, root and shoot length the germination percentage and shoot and root length of treated seed was calculated. It was observed that the germination percentage was reduced to 55% with the 500 Gy irradiation treatment. The root and shoot length also reduced substantially to 2.47 and 2.48, respectively.

Table 1: Paper germination test for optimization of LD50.

Sr. No.	Dose	Root length (cm)	Shoot length (cm)	Germination percentage
1	400 Gy	3.84	3.12	70
2	500 Gy	2.47	2.48	55
3	600 Gy	1.15	1.58	40
4	700 Gy	1.34	1.41	30
5	Control	5.01	4.67	90

ii) Raising of M1:

Desirable mutants from M1 generation were selected on the basis of their phenotypical characters and harvested separately. The details of lines harvested dose wise are given in Table No. 2.

Table 2: No. of lines harvested in M1 generation.

Sr. No.	Dose	No. of lines grown for M2 Generation
1	400 Gy	184
2	500 Gy	156
3	600 Gy	108
4	700 Gy	124
5	Control	112
Total M2 population (20 plants per line)		**13680**

iii) Study of M2 generation.

The dosewise ancillary data was recorded and presented in Table 3.The maximum days to first flowering (115.57days) was recorded in 700 Gy dose. Desirable changes was observed in all yield attributing characters in seeds treated with 500 Gy dose of irradiation. Individual mutant plants were tagged at appropriate stage and their seeds were harvested separately after maturity.

Table 3: Mean values of quantitative characters in M2 generation.

Sr. No	Character	Control	400 Gy	500 Gy	600 Gy	700 Gy	SEm ±	CD at 5%
1)	**Days to first flowering**	93.66	100.50	105.21	106.40	115.57	0.19	0.75
2)	**Days to first panicle maturity**	127	135.72	138.04	145.01	150.13	0.85	3.36
3)	**Plant height (cm)**	36.28	30.07	30.67	29.66	33.13	0.95	3.74
4)	**Tillers/plant**	1.85	4.48	4.53	3.58	3.05	0.21	0.82

5)	**Panicles/plant**	1.15	4.08	5.76	4.05	3.26	0.61	2.38
6)	**Fingers/panicle**	6.35	8.40	8.93	7.53	8.66	0.39	1.54
7)	**Finger length (cm)**	5.77	8.16	8.23	7.91	7.70	0.41	1.61
8)	**Panicle Weight/plant (gm)**	1.84	1.93	1.91	1.81	1.85	0.47	1.86
9)	**Grain density/cm**	46.66	44.95	48.9	47.95	45.15	3.58	14.06
10)	**Yield per plant (gm)**	3.121	4.2526	4.7494	4.1817	4.2972	0.24	0.94

iv) M3 generation:

Morphological data were collected and based on phenotypic observations 58 lines were selected for raising in M4 generation (Data not shown).

v) M4 generation:

Stability of blast free mutant line and other desirable characters were also observed in this generation (Data not shown).

vi) M5 generation:

Ten Mutant lines (1, 2, 3, 4, 11, 12, 14, 17, 18 and 21) with good morphological features areselected. All 10 mutant lines showed variable morphological characters and were superior to parent variety Dapoli Nagli 1 in terms of no. of fingers per panicle, length of panicle and yield per plant. Four lines also showed blast tolerance where most of population infested by blast (Table 5).

vii) Diversity analysis:

The ISSR pattern of genomic DNA of 23 mutants and one control parent (Dapoli-1) were analysed with respect to the fragments, in formativeness of the markers and polymorphism for the assessment of genetic diversity present among the genotypes. For the present study 15 ISSR primers were used for molecular characterization and to assess genetic diversity and all of them were polymorphic. The ISSR profile generated by each primer (Plate 2) was analysed using standard DNA marker (1.3 kbp and 100 bp) and compared with their respective banding patterns. A total of 1321 scorable DNA fragments were produced among them 1201 were found to be polymorphic across the finger millet mutants and its control parent. The size of amplified fragments ranged from 450-1100 bp (Table. 5). The minimum number of polymorphic fragments produced by the primer was 53 (UBC-816) while the maximum number of polymorphic fragments were found to be 153 (UBC- 891) and an average number of 80.06 polymorphic bands were observed per primer. The average percentage polymorphism across the 15 primers among the mutants and control was found to be 91.87 per cent. It indicates that ISSR markers having great potential to show the polymorphism among the finger millet mutants. The percentage of polymorphism across the finger millet genotypes ranged from 52.94-100 per cent. The average number of alleles per primer was 11.86 with primer UBC-811, UBC-816, UBC-825, UBC-834, UBC-848 and 891 showed unique fragments.

e) Genetic relationship among the mutants line:

The similarity co-efficient ranged from 0.131 (between mutant lines 2 & 23) to 0.683 (between mutant line 16 and 17), indicated the distinctness of these mutants.

f) Cluster analysis:

In the present study, 23 mutants and one control parent (Dapoli-1) were subjected to cluster analysis for assessing the molecular diversity based on UPGMA analysis. The cluster analysis band on the similarity co-efficient clearly distinguished all the 23 mutants and one control in to two groups (Fig 1). Both the clusters were subdivided into two sub clusters. The genetic diversity in this study might be useful in future strategies for evolution of desired genotypes.

viii) Estimation of Protein, Ca and Fe content of promising mutant lines selected from M5 generation:

10 promising mutant lines obtained from M5 generation along with control (Dapoli-1) were used for quantitative estimation of Protein, Calcium and Iron (Table 4). Mutant line 2,3,4 and 11 showed increased protein percentage (5.25, 5.77,6.12,6.47 respectively) to parent variety Dapoli Nagli1(5.07%). Similarly, mutant line 2,3,14 and 21 showed increased calcium content (252, 248, 242 and 234 mg/100g) over parent variety (231 mg/100 g). The Iron content was observed maximum in mutant line 1, 2 and 14 (12, 11 and 10 mg/100g).

Table 4. ISSR primers and their sequences.

Sr. No.	**Primer**	**Primer sequence**	**Annealing temperature**	**GC Content**
		(5' – 3')	**(0C)**	**(%)**
1	UBC-807	AGAGAGAGAGAGAGAGT	50.4	47.1
2	UBC-810	GAGAGAGAGAGAGAGAT	49.5	50.0
3	UBC-811	GAGAGAGAGAGAGAGAC	52.8	52.9
4	UBC-816	CACACACACACACACAT	51.1	47.1
5	UBC-820	GTGTGTGTGTGTGTGTC	50.3	52.9
6	UBC-825	ACACACACACACACACT	50.4	47.1
7	UBC-827	ACACACACACACACACG	54.9	52.9
8	UBC-828	TGTGTGTGTGTGTGTGA	53.2	47.1
9	UBC-834	AGAGAGAGAGAGAGAGT	45.4	47.1
10	UBC-836	AGAGAGAGAGAGAGAGC	52.6	52.9
11	UBC-844	CTCTCTCTCTCTCTCTC	46.5	52.9
12	UBC-848	CACACACACACACACAT	54.8	47.1
13	UBC-857	ACACACACACACACACCG	52.0	55.5
14	UBC-878	GGATGGATGGATGGAT	48.5	50.0
15	UBC-891	AGATGTGTGTGTGTGTG	50.0	47.05

Table 5 : Morphological features of promising mutant lines and their nutritional status.

Sr. No.	Mutant Line	Days to Maturity	Plant Height (cm)	Length of Finger (cm)	Tillers/ Plant	Finger/ Panicle	Grain Density	Yield/Plant(gm)	Special Features	Nutritional Status		
										Protein (%)	Calcium (mg/100gm)	Iron(mg/100gm)
1	1	131	114.1	10	2.1	7.2	44.3	9.30	Fully opened panicle, less affected by blast	4.90	225	12
2	2	116	99.7	12.8	2	8.2	52.8	11.80	Early, Fully opened panicle, less affected by blast	5.25	252	11
3	3	127	94.1	11.2	1.9	11.3	53.7	15.19	Dwarf, Fully opened panicle, less affected by blast, high yield	5.77	248	8
4	4	133	106.5	8.5	1.5	8.5	60.6	11.51	Good growth with better grain density	6.12	216	7
5	11	105	103.5	8.95	1.8	6.6	74.9	6.24	Very early, good plant stature	6.47	172	7.7
6	12	127	111.4	12.6	2	10.6	60.6	9.48	Fully opened panicle, more fingers, blast tolerant	4.90	226	6.5
7	14	126	107.9	9.9	1	5.2	77.9	8.10	Blast tolerant	4.02	242	10
8	17	123.5	113.5	9.9	1.2	5.9	58.5	12.27	Blast tolerant, high yield	4.20	168	9
9	18	124.5	108.4	9.9	1.1	6.7	83.9	7.92	Blast tolerant	4.72	198	4.5
10	21	129	113.5	10.2	1.2	5.6	104	9.67	Blast tolerant, more grain density	4.37	234	9
11	DPL-1	132	110.7	8.7	3.1	6.7	64.5	7.58	Late, small panicle, highly affected by blast	5.07	231	9.6

Table 6. Molecular analysis of 23 mutant lines of finger millet using 16 primers.

Sr. No.	Primer	Total No. of Bands	No. of Polymorphic Bands	Per cent Polymorphism	Range of Amplification (bp)	Unique Fragments	No. of Alleles
1.	UBC 807	72	72	100	800-1300	-	10
2.	UBC 810	108	108	100	350-1250	-	16
3.	UBC 811	117	93	79.48	300-1350	Mutant line-1 (1175 bp)	17
4.	UBC 816	53	53	100	500-850	Mutant line -1 (575 bp)	8
5.	UBC 820	57	57	100	600-1050	-	10
6.	UBC 825	105	105	100	150-850	Mutant line-8 (375 bp)	15
7.	UBC 827	58	58	100	350-900	-	10
8.	UBC 828	123	75	60.97	250-1050	-	11
9.	UBC 834	62	62	100	800-500	Mutant line-9 (625 bp)	7
10.	UBC 836	157	133	84.71	200-1100	-	18
11.	UBC 844	70	70	100	350-750	-	8
12.	UBC 848	79	79	100	300-900	Mutant line-5 (875 bp)	11
13.	UBC 857	51	27	52.94	950-1400	-	7
14.	UBC 878	56	56	100	700-1400	-	8
15.	UBC 891	153	153	100	400-1600	Mutant line - 14 (575 bp)	22
	Total	1321	1201				178
	Average	88.06	80.06	91.87	450-1100	-	11.86

Table 7. Clustering Pattern

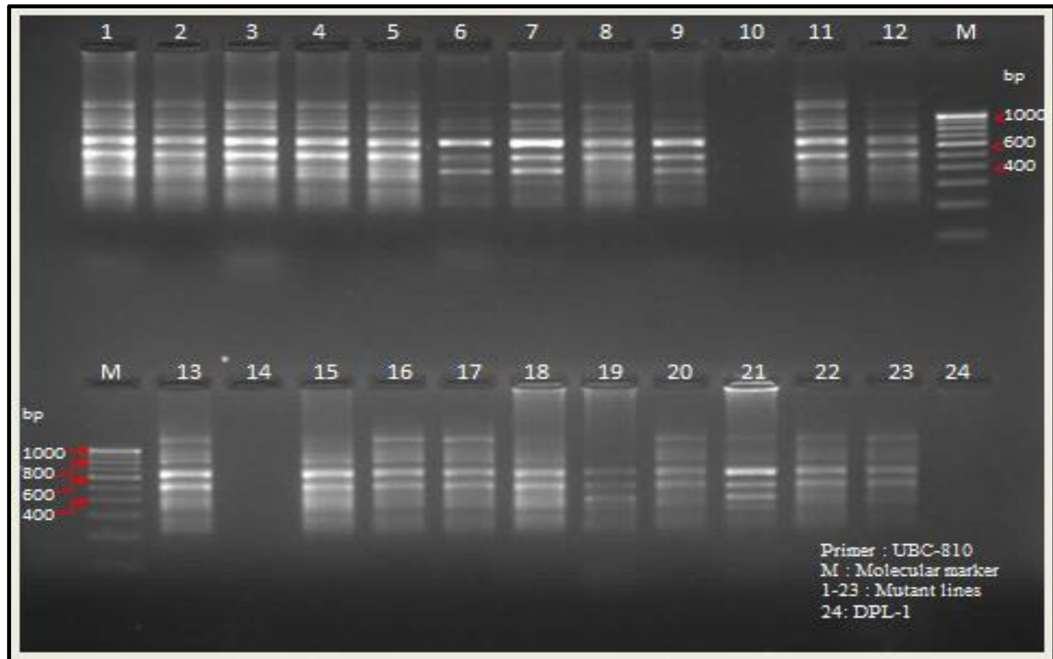
Cluster		No. of Mutant lines	Name of Mutant line
I	IA	6	Mutant line-19, Mutant line-20, Mutant line-21, Mutant line-22, Mutant line-23, Control (DPL-1).
	IB	6	Mutant line-13, Mutant line-14, Mutant line-15, Mutant line-16, Mutant line-17, Mutant line-18.
II	IIA	8	Mutant line-5, Mutant line-6, Mutant line-7, Mutant line-8, Mutant line-9, Mutant line-10, Mutant line-11, Mutant line-12.
	IIB	4	Mutant line-1, Mutant line-2, Mutant line-3, Mutant line-4.

Plate: 1 Comparative features of desirable mutant line as compared to the control(Dapoli-1).

Mutant, free from blast incidence

Blast affected mutant

Plate 2 : ISSR Assay of Mutant selected in M_5 generation


1 2 3 4 5 6 7 8 9 10 11 12 M
bp
1000
600
400
M 13 14 15 16 17 18 19 20 21 22 23 24
bp
1000
800
600
400
Primer : UBC-810
M : Molecular marker
1-23 : Mutant lines
24: DPL-1

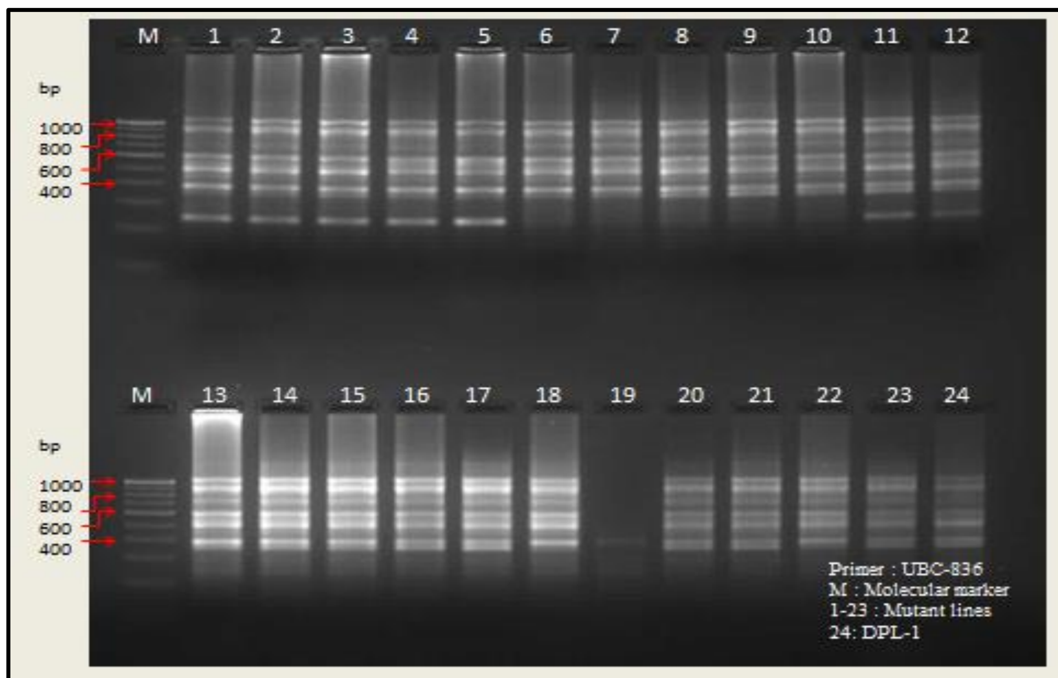
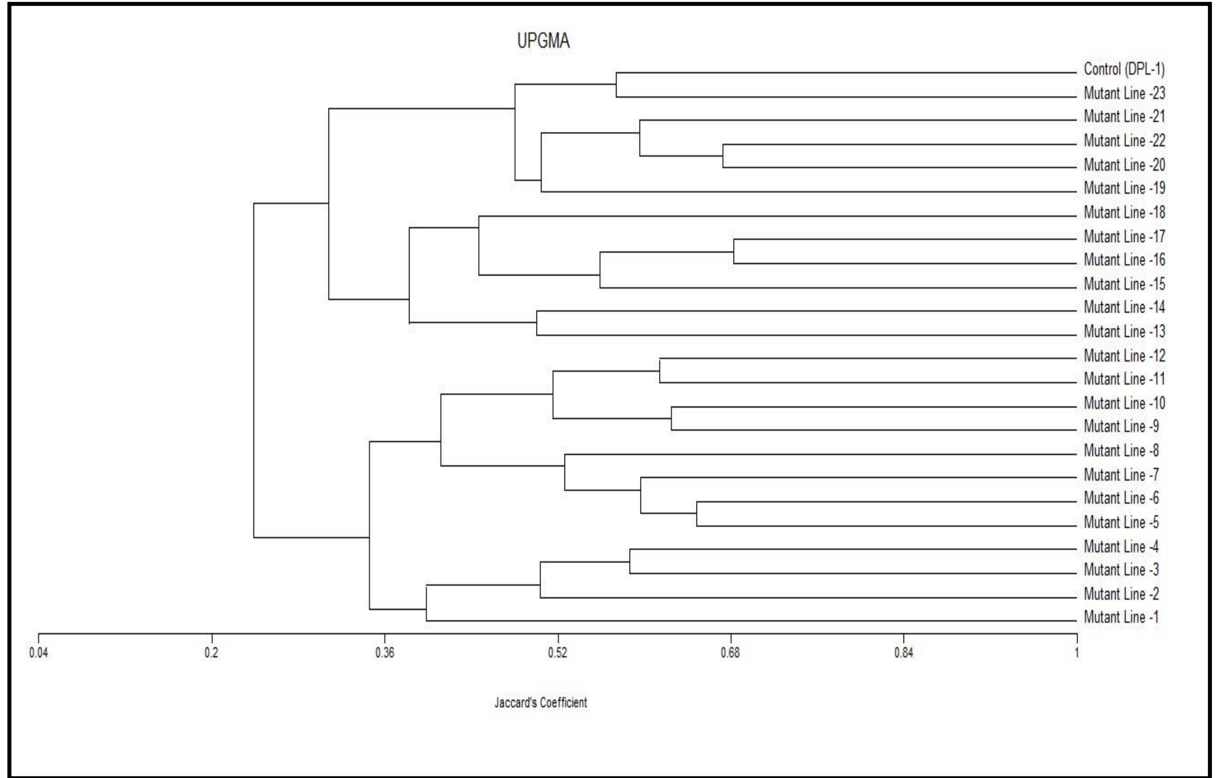

M 1 2 3 4 5 6 7 8 9 10 11 12
bp
1000
800
600
400
M 13 14 15 16 17 18 19 20 21 22 23 24
bp
1000
800
600
400
Primer : UBC-836
M : Molecular marker
1-23 : Mutant lines
24: DPL-1

Fig 1. UPGMA dendrogram showing clustering of 24 mutant lines based on 15 ISSR primers.



Conclusion:

It was observed that at 500 Gy there was 50% reduction in shoot and root length ratio and seed germination as compared to the control. The mutants selected from 500Gy dose showed desirable variability in terms earliness in duration, more no. of fingers per panicle, increased panicle length and blast tolerance.

Application:

After studying stability performance of promising mutant lines the high yielding, blast tolerant and nutritionally superior line can be released as a variety for commercial cultivation.

Recommendation

For finger millet 500 Gy dose of γ radiation is optimum for creation desirable variability.

शिफारस

नाचणीपिकासाठी ५०० जीवाय गॅमा किरणोत्सर्गाच्या भागाचा वापर केल्यास चांगल्या प्रकारची विविधता निर्माण करता येते.

B) Year of commencement of Experiment	:	2011
C) Development of variable mutants	:	The generation study of mutant lines were followed upto M5 generation and 10 desirable and variable mutant lines are developed.
D) Identification of LD 50	:	It was observed that at 500 Gy there was 50% reduction in shoot and root length ratio and seed germination as compared to the control.
Whether recommended by	:	
I. Pre-Research Review Committee	:	Yes
II. Research Review Committee	:	Yes
III. Variety Release committee	:	Yes

b. Research Recommendations: -

Title	:	Optimization of in vitro sterilization technique in variety Konkan Safed Velchi of banana (Musa paradisica L.).
Background information	:	Banana (Musa paradisica L.) is an important fruit crop of the Musaceae family. It evolved in the humid tropical region of South East Asia with India as one of its centre of origin. Modern edible varieties like Musa paradisica have evolved from the two species Musa acuminata and Musa balbisiana and their natural hybrids. It ranks 4th in the category of staple food products and is rich source of energy (128Kcal/100g), carbohydrate (27%), crude fiber (0.5%), protein (1.2%) and moisture (70%) and is also rich in vitamins A, B and C particularly, vitamin B. It is also a good source of sodium, potassium, phosphorus, calcium and magnesium. Banana is a triploid and it is vegetatively propagated through suckers which are the rhizome cut off from the mother plant. It is done so, because they are parthenocarpic and practically seedless or seed sterile and therefore propagation by seeds is not possible. The traditional method of mass multiplication of banana is slow and cumbersome process and varieties are found contaminated in the field with several

		diseases when exposed to adverse environmental conditions. Therefore, in order to accelerate multiplication of stocks in a controlled environment and to obtain disease free healthy suckers, in vitro propagation is preferred. Konkan Safed Velchi variety of banana is recently developed by Dr. B. S. Konkan Krishi Vidyapeeth, Dapoli. This variety has tremendous demand because of its peculiar taste and small fruit size. But the availability of plantlets is limiting factor for its large scale cultivation. In vitro propagation is one of the methods of choice since it provides a rapid reliable system for production of large number of genetically uniform disease free plantlets. In general, micropropagated banana plants establish faster, grow more vigorously, are taller, have a shorter and more uniform production period and produce higher yields than conventional propagules. It can be continued throughout the year irrespective of the season. Bacterial contamination is a major problem hindering tissue culture application. Darkening of the culture medium due to release of phenolic compound from the tissue of the explants is another obstacle that affects the growth of the explants by getting accumulated in the surrounding medium. These problems reduce the number of plants in further tissue culture techniques. Therefore, the objective of the present investigation was standardization of sterilization protocol for aseptic inoculation of explants, and in vitro propagation of Konkan Safed Velchi cultivar of banana.
Objectives	:	1. To standardize sterilization technique for aseptic inoculation of explants. 2. To develop in vitro propagation technique for mass multiplication of Konkan Safed Velchi. 3. To compare rate of multiplication of Konkan Safed Velchi with Grand naine.
Budget	:	6,66,660/-

Project Scientist	:	Dr. S. V. Sawardekar, Associate Professor, Plant Biotechnology Centre, Dapoli.
Co-Scientists associated	:	Dr. N. B. Gokhale, Incharge, Plant Biotechnology Centre, College of Agriculture, Dapoli.
Experimental Details		
A. Materials used	:	• 50 Sword suckers of Konkan Safed Velchi and 50 sword suckers of Grand Naine • Various chemicals for sterilization and in vitro culture. • Desired glasswares and plastic wares • Autoclave • pH meter • Refrigerated centrifuge machine • Laminar air flow • Culture room with required physical conditions

For the present study two varieties of banana (Musa paradisica) Cv. Grand naine and Konkan Safed Velchi were used. Two to three months old sword suckers were excised from healthy disease free mother plant. These suckers were thoroughly washed under running tap water. Shoot tips were prepared by trimming roots and outer leaf sheaths from the suckers. The explants were kept immersed in tap water for 5 min, then they were pre-treated with 5 ml Tween 20 for 10 min and a combination of 5 ml Dettol and 45 ml Savlon for 30 min with constant swirling and subsequently draining off the disinfectants by washing them with tap water 2-3 times to remove the traces of the disinfectants. The explants were then washed under running tap water for 60 min.

Standardization of surface sterilization methods was carried out by treating the explants with various combinations and concentrations of different chemical sterilizing agent for different durations of exposure time as mentioned in Table No.1. The explants were cultured on the initiation and proliferation media up to 9 subcultures after which they were transferred on rooting media. Media combinations used for establishment, proliferation, multiplication and rooting of the explants are as mentioned in Table 2 and Table 3.

As all studies were done in laboratory under well-defined conditions of the medium, growth, temperature and light and all the data was analyzed under Factorial Completely Randomized Design (FCRD) by using OP STATS software.

Results:-

Effect of sterilization treatment combination on per cent contamination, per cent survival and days to shoot bud initiation:

The observation of per cent contamination has been presented in Table 1. The treatment combination T3 was found to be best to achieve lowest per cent contamination (3.33) for both Konkan Safed Velchi and Grand naine. While treatment combination T1 showed highest per cent

contamination of 50 and 30 for Konkan Safed Velchi and Grand naine, respectively. The treatment combination T3 was found to be best combination to achieve highest percentage of contamination free healthy cultures, showing highest per cent survival (96.67) for both the varieties. While treatment combination T1 showed lowest per cent survival of 50 and 70 for Konkan Safed Velchi and Grand naine, respectively.

Effectiveness of surface sterilization depends on many factors like explant type, concentration of sterilizing agents used, duration of treatments with various sterilizing agents and sequence of treatments. It was observed that on an average treatment combination T3 resulted in minimum number of days (21.84) for shoot bud initiation. The treatment T1 recorded maximum number of days (31.17) for shoot bud initiation in respect to the both varieties (Table No.1).

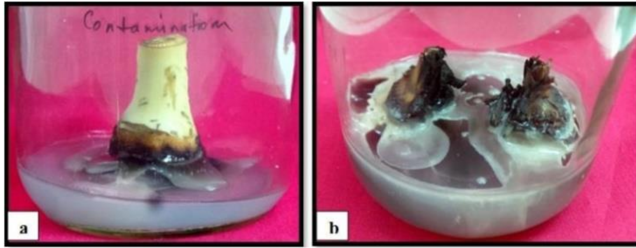
Effect of establishment and proliferation media combination on shooting and rooting

The highest average number of shoots were recorded on media combination E4 (17.67) followed by E5, E6 and E3. The lowest average number of shoots were recorded on media combination E2. No shoots were observed on E1 treatment (control). Media combination E4 (MS + 6 mgL-1 BAP + 1 mgL-1 NAA) was observed to be best for Grand naine and Konkan Safed Velchi as it gave on an average 19 shoots and 16.33 shoots per explants, respectively. This media combination was found to be suitable as it was observed that increase or decrease in the concentration of BAP beyond 6 mgL-1 leads to decrease in the average number of shoots per explant.

Table 3 indicates that the optimum medium for regeneration of roots was found to be R5 (1/2 MS + 1 mgL-1 BAP + 2 mgL-1 NAA + 2 mgL-1 IBA) with an average of 17 roots per shooted plant generated for Konkan Safed Velchi and 18 roots per shooted plant generated for Grand naine. It was also observed that, with an increase or decrease in the concentration of auxins (NAA and IBA) the average number of roots decline. For Konkan Safed Velchi, highest per cent root response was recorded as 85, whereas for Grand naine it was recorded as 86.67 on media combination R5. The lowest per cent root response for Konkan Safed Velchi was recorded as 30 and for Grand naine it was recorded as 40 on media combination R2.

In Konkan Safed Velchi it was observed that one cultured shoot tip can produce 299.4 shoots on an average after six subcultures and the number of multiple shoots increases during the successive subcultures up to 7th subculture only after which the number of multiple shoots decreases (Table 4).

While in case of Grand naine one cultured shoot tip can produce 865.8 shoots on an average after nine subcultures and the number of multiple shoots increases till the 9th subculturing.



a) & b) Bacterial contamination

A)

B)

C)

D)

E)

F)

G)

H)

I)

Fig. 1 :- Sterilization technique for Konkan Safed Velchi.

A) Treatment with Carbendazim
B) Treatment with Ethanol
C) 1st cut given to explants
D) Treatment with Sodium hypochlorite
E) 2nd cut given to explants
F) Treatment with Cefotaxime
G) Explant inoculated in media
H) Shooting and rooting
I) Establishment of plantlets

Table 1 : Effect of sterilization treatment combination on per cent contamination, per cent survival and days to shoot bud initiation

Treatment	Sterilants	Concentration	Exposure time (min)	Per cent contamination			Per cent survival			Days to shoot bud initiation		
				Variety		Mean	Variety		Mean	Variety		Mean
				Konkan Safed Velchi	Grand Naine		Konkan Safed Velchi	Grand Naine		Konkan Safed Velchi	Grand Naine	
T1	Carbendazim	0.50%	45	50.00 (45)	30.00 (33.00)	40.00 (39.00)	50.00 (45)	70.00 (57.00)	60.00 (51.00)	35.33	27	31.17
	Ethanol	70%	1									
	Sodium Hypochlorite	5%	5									
	Cefotaxime	200 mg/L	15									
T2	Carbendazim	0.50%	60	23.33 (28.78)	23.33 (28.78)	23.33 (28.78)	76.67 (61.22)	76.67 (61.22)	76.67 (61.22)	31	23	27
	Ethanol	70%	1									
	Sodium Hypochlorite	5%	15									
	Cefotaxime	200 mg/L	30									
T3	Carbendazim	1%	30	3.33 (6.14)	3.33 (6.14)	3.33 (6.14)	96.67 (83.86)	96.67 (83.86)	96.67 (83.86)	23	20.67	21. 84
	Ethanol	70%	1									
	Sodium Hypochlorite	5%	10									
	Cefotaxime	250 mg/L	30									
	SEm					**4.0575**			**4.05755**			**0.50917**
	CD at 1%					**17.5277**			**17.5277**			**2.19952**

Table 2 : Effect of establishment and proliferation media combinations on shoot regeneration and per cent shoot response

Tr. No.	Treatment Details	Shoot regeneration			Per cent shoot response		
		Variety		Mean	Variety		Mean
		Konkan Safed Velchi	Grand Naine		Konkan Safed Velchi	Grand Naine	
E1	MS	0	0	0	0 (0)	0 (0)	0 (0)
E2	MS + 2 BAP + 1 NAA	1.33	1.67	1.5	6.67 (14.76)	8.33 (16.60)	7.50 (15.68)
E3	MS + 4 BAP + 1NAA	7.67	6.67	7.17	38.33 (38.24)	33.33 (35.25)	35.83 (36.75)
E4	MS + 6 BAP + 1NAA	16.33	19	17.67	81.67 (64.69)	95.00 (77.08)	88.33 (70.89)
E5	MS + 8 BAP + 1 NAA	11.33	13.67	12.5	56.67 (48.85)	68.33 (56.03)	62.50 (52.44)
E6	MS + 10 BAP + 1 NAA	10	7.67	8.83	50.00 (45.00)	38.33 (38.24)	44.17 (41.62)
	SEm			**0.518188**			**1.610427**
	CD at 1%			**2.049676**			**6.369996**

Table 3 : Effect of rooting media combinations on root regeneration and per cent root response

Tr. No.	Treatment Details	Root regeneration			Per cent root response		
		Variety		Mean	Variety		Mean
		Konkan Safed Velchi	Grand Naine		Konkan Safed Velchi	Grand Naine	
R1	½ MS + 1 BAP	0	0	0	0 (0)	0 (0)	0 (0)
R2	½ MS + 1 BAP + 1 NAA + IBA	6	8	7	30.00 (33.00)	40.00 (39.15)	35.00 (36.07)
R3	½ MS + 1 BAP + 1 NAA + 2 IBA	12.33	13.33	12.83	61.67 (51.81)	66.67 (54.89)	64.17 (53.35)
R4	½ MS + 1 BAP + 2 NAA + 1 IBA	15	17	16	75.00 (60.07)	85.00 (67.40)	80.00 (63.74)
R5	½ MS + 1 BAP + 2 NAA + 2 IBA	17	18	17.5	85.00 (67.40)	86.67 (69.55)	85.83 (68.48)
R6	½ MS + 1 BAP + 2 NAA + 3 IBA	9	10	9.5	45.00 (42.12)	50.00 (45.00)	47.50 (43.56)
	SEm			**0.757677**			**2.564103**
	CD at 1%			**2.996968**			**10.142233**

Table 4. Number of multiple shoots regenerated in various subcultures for Konkan Safed Velchi

CLONES	NUMBER OF EXPLANTS INOCULATED	SUB-CULTURE								
		1st	2nd	3rd	4th	5th	6th	7th	8th	9th
1	1	1	3	9	22	68	136	165	122	87
2	1	0	1	4	15	73	149	193	168	128
3	1	2	4	10	26	87	181	293	258	214
4	1	1	2	8	23	75	168	242	197	155
5	1	2	6	12	29	109	218	368	318	273
6	1	0	0	4	16	56	115	154	97	64
7	1	1	2	8	24	101	213	317	267	232
8	1	1	4	12	28	121	245	404	359	314
9	1	2	6	15	31	137	277	**452**	408	353
10	1	1	3	11	29	123	255	406	356	311
Mean	**1**	**1.1**	**3.1**	**9.3**	**24.3**	**95**	**195.7**	**299.4**	**255**	**213.1**

Table 5. Number of multiple shoots regenerated in various subcultures for Grand naine

CLONES	NUMBER OF EXPLANTS INOCULATED	SUB-CULTURE								
		1st	2nd	3rd	4th	5th	6th	7th	8th	9th
1	1	2	5	14	42	126	252	405	656	811
2	1	1	3	9	28	90	180	256	421	686
3	1	1	4	12	39	119	257	409	653	899
4	1	2	6	22	68	205	409	621	842	978
5	1	2	5	18	53	160	278	561	784	937
6	1	0	2	7	22	82	153	390	525	679
7	1	1	3	10	35	107	319	542	704	958
8	1	2	6	20	61	183	347	511	645	770
9	1	3	7	25	73	223	450	667	825	959
10	1	2	4	15	48	153	343	593	738	**981**
Mean	**1**	**1.6**	**4.5**	**15.2**	**46.9**	**144.8**	**298.8**	**495.5**	**679.3**	**865.8**

CONCLUSION:

For *in vitro* regeneration of banana Cv. Konkan Safed Velchi the treatment in the sequential order of Carbendazim 1% followed by Ethanol 70%, Sodium Hypochloride 5% and Cefotaxime 250 mg/L for the period of 30, 1, 10 and 30 min. produces 96.67% aseptic culture.

Media combination of MS + 6 mgL^{-1} BAP + 1 mgL^{-1} NAA was found to be best for establishment and proliferation of cultures, whereas media combination of ½ MS + 1 mgL^{-1} BAP + 2 mgL^{-1} NAA + 2 mgL^{-1} IBA was found to be best for root regeneration for both the varieties. Among the two varieties, Konkan Safed Velchi was efficient in producing multiple shoots only up to six subcultures as compared to Grand naine which produced multiple shoots up to nine subcultures.

Application:

This sterilization technique could be utilized for large scale *in vitro* multiplication of Konkan Safed Velchi a cultivar of banana.

Recommendation

For *in vitro* regeneration of banana *Cv*. Konkan Safed Velchi the treatment in the sequential order of Carbendazim 1% followed by Ethanol 70%, Sodium Hypochloride 5% and Cefotaxime 250 mg/L for the period of 30, 1, 10 and 30 minutes, respectively produces aseptic culture.

शिफारस

केळीच्या कोकण सफेद वेलची या जातीची उती संवर्धनाने अभिवृध्दी करण्यासाठी क्रमाने कार्बेन्डॅझिम 1 %, इथेनॉल 7 0 %, सोडीयम हायपोक्लोराईड 5 %, आणि सिफोटॅक्सीम 2 5 0 मि.ग्रॅ. प्रति लिटर यांची मात्रा अनुक्रमे 3 0 , 1 , 1 0 आणि 3 0 मिनीटे कालावधीसाठी दिल्यास निर्जतूक संवर्धक तयार होते.

Details of grants received and utilized so far (Rupees):

Sr. No.		**Sanctioned**	**Opening**	**Received**	**Total**	**Spent**	**Unspent**
1st	**(2015-16)**		Nil				
1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
1.	Staff Salaries	6,66,660/-	Nil	4,99,995/-	4,99,995/-	2,51,073/-	2,48,922/-
2.	Labour						
3.	Consumables						
4.	Miscellaneous						
5.	**Total:**	**6,66,660/-**	**Nil**	**4,99,995/-**	**4,99,995/-**	**2,51,073/-**	**2,48,922/-**
2nd	(2016-17)		2,48,922/-	2,48,922/-	2,48,922/-	75,909/-	1,73,013/-
3rd	(2017-18)		1,73,013/-	1,73,013/-	1,73,013/-	10,000/-	1,63,013/-

Title : Optimization of *in vitro* sterilization technique in variety Konkan Safed Velchi of banana (*Musa paradisica* L.).

Background information : Banana (*Musa paradisica* L.) is an important fruit crop of the Musaceae family. It evolved in the humid tropical region of South East Asia with India as one of its centre of origin. Modern edible varieties like *Musa paradisica* have evolved from the two species *Musa acuminata* and *Musa balbisiana* and their natural hybrids. It ranks 4th in the category of staple food products and is rich source of energy (128Kcal/100g), carbohydrate (27%), crude fiber (0.5%), protein (1.2%) and moisture (70%) and is also rich in vitamins A, B and C particularly, vitamin B. It is also a good source of sodium, potassium, phosphorus, calcium and magnesium.

Banana is a triploid and it is vegetatively propagated through suckers which are the rhizome cut off from the mother plant. It is done so, because they are parthenocarpic and practically seedless or seed sterile and therefore propagation by seeds is not possible. The traditional method of mass multiplication of banana is slow and cumbersome process and varieties are found contaminated in the field with several diseases when exposed to adverse environmental conditions. Therefore, in order to accelerate multiplication of stocks in a controlled environment and to obtain disease free healthy suckers, *in vitro* propagation is preferred.

Konkan Safed Velchi variety of banana is recently developed by Dr. B. S. Konkan Krishi Vidyapeeth, Dapoli. This variety has tremendous demand because of its peculiar taste and small fruit size. But the availability of plantlets is limiting factor for its large scale cultivation. *In vitro* propagation is one of the methods of choice since it provides a rapid reliable system for production of large number of genetically uniform disease free plantlets. In general, micropropagated banana plants establish faster, grow more vigorously, are taller, have a shorter and more uniform production period and produce higher yields than conventional propagules. It can be continued throughout the year irrespective of the season.

Bacterial contamination is a major problem hindering tissue culture application. Darkening of the culture medium due to

release of phenolic compound from the tissue of the explants is another obstacle that affects the growth of the explants by getting accumulated in the surrounding medium. These problems reduce the number of plants in further tissue culture techniques. Therefore, the objective of the present investigation was standardization of sterilization protocol for aseptic inoculation of explants, and *in vitro* propagation of Konkan Safed Velchi cultivar of banana.

Objectives :

4. To standardize sterilization technique for aseptic inoculation of explants.
5. To develop *in vitro* propagation technique for mass multiplication of Konkan Safed Velchi.
6. To compare rate of multiplication of Konkan Safed Velchi with Grand naine.

Project Scientist : Dr. S. V. Sawardekar, Associate Professor, Plant Biotechnology Centre, Dapoli.

Co-Scientists associated : Dr. N. B. Gokhale, Incharge, Plant Biotechnology Centre, College of Agriculture, Dapoli.

Experimental Details

B. Materials used :

- 50 Sword suckers of Konkan Safed Velchi and 50 sword suckers of Grand Naine
- Various chemicals for sterilization and *in vitro* culture.
- Desired glasswares and plastic wares
- Autoclave
- pH meter
- Refrigerated centrifuge machine
- Laminar air flow
- Culture room with required physical conditions

For the present study two varieties of banana (*Musa paradisica*) *Cv*. Grand naine and Konkan Safed Velchi were used. Two to three months old sword suckers were excised from healthy disease free mother plant. These suckers were thoroughly washed under running tap water. Shoot tips were prepared by trimming roots and outer leaf sheaths from the suckers. The explants were kept immersed in tap water for 5 min, then they were pre-treated with 5 ml Tween 20 for 10 min and a combination of 5 ml Dettol and 45 ml Savlon for 30 min with constant swirling and subsequently draining off the disinfectants by washing them with tap water 2-3 times to remove the traces of the disinfectants. The explants were then washed under running tap water for 60 min.

Standardization of surface sterilization methods was carried out by treating the explants with

various combinations and concentrations of different chemical sterilizing agent for different durations of exposure time as mentioned in Table No.1. The explants were cultured on the initiation and proliferation media up to 9 subcultures after which they were transferred on rooting media. Media combinations used for establishment, proliferation, multiplication and rooting of the explants are as mentioned in Table 2 and Table 3.

As all studies were done in laboratory under well-defined conditions of the medium, growth, temperature and light and all the data was analyzed under Factorial Completely Randomized Design (FCRD) by using OP STATS software.

Results:-

Effect of sterilization treatment combination on per cent contamination, per cent survival and days to shoot bud initiation:

The observation of per cent contamination has been presented in Table 1. The treatment combination T3 was found to be best to achieve lowest per cent contamination (3.33) for both Konkan Safed Velchi and Grand naine. While treatment combination T1 showed highest per cent contamination of 50 and 30 for Konkan Safed Velchi and Grand naine, respectively. The treatment combination T3 was found to be best combination to achieve highest percentage of contamination free healthy cultures, showing highest per cent survival (96.67) for both the varieties. While treatment combination T1 showed lowest per cent survival of 50 and 70 for Konkan Safed Velchi and Grand naine, respectively.

Effectiveness of surface sterilization depends on many factors like explant type, concentration of sterilizing agents used, duration of treatments with various sterilizing agents and sequence of treatments. It was observed that on an average treatment combination T3 resulted in minimum number of days (21.84) for shoot bud initiation. The treatment T1 recorded maximum number of days (31.17) for shoot bud initiation in respect to the both varieties (Table No.1).

Effect of establishment and proliferation media combination on shooting and rooting

The highest average number of shoots were recorded on media combination E4 (17.67) followed by E5, E6 and E3. The lowest average number of shoots were recorded on media combination E2. No shoots were observed on E1 treatment (control). Media combination E4 (MS + 6 mgL^{-1} BAP + 1 mgL^{-1} NAA) was observed to be best for Grand naine and Konkan Safed Velchi as it gave on an average 19 shoots and 16.33 shoots per explants, respectively. This media combination was found to be suitable as it was observed that increase or decrease in the concentration of BAP beyond 6 mgL^{-1} leads to decrease in the average number of shoots per explant.

Table 3 indicates that the optimum medium for regeneration of roots was found to be R5 (1/2 MS + 1 mgL^{-1} BAP + 2 mgL^{-1} NAA + 2 mgL^{-1} IBA) with an average of 17 roots per shooted plant generated for Konkan Safed Velchi and 18 roots per shooted plant generated for Grand naine. It was also observed that, with an increase or decrease in the concentration of auxins (NAA and IBA) the average number of roots decline. For Konkan Safed Velchi, highest per cent root response was recorded as 85, whereas for Grand naine it was recorded as 86.67 on media combination R5. The

lowest per cent root response for Konkan Safed Velchi was recorded as 30 and for Grand naine it was recorded as 40 on media combination R2.

In Konkan Safed Velchi it was observed that one cultured shoot tip can produce 299.4 shoots on an average after six subcultures and the number of multiple shoots increases during the successive subcultures up to 7th subculture only after which the number of multiple shoots decreases (Table 4).

While in case of Grand naine one cultured shoot tip can produce 865.8 shoots on an average after nine subcultures and the number of multiple shoots increases till the 9th subculturing.

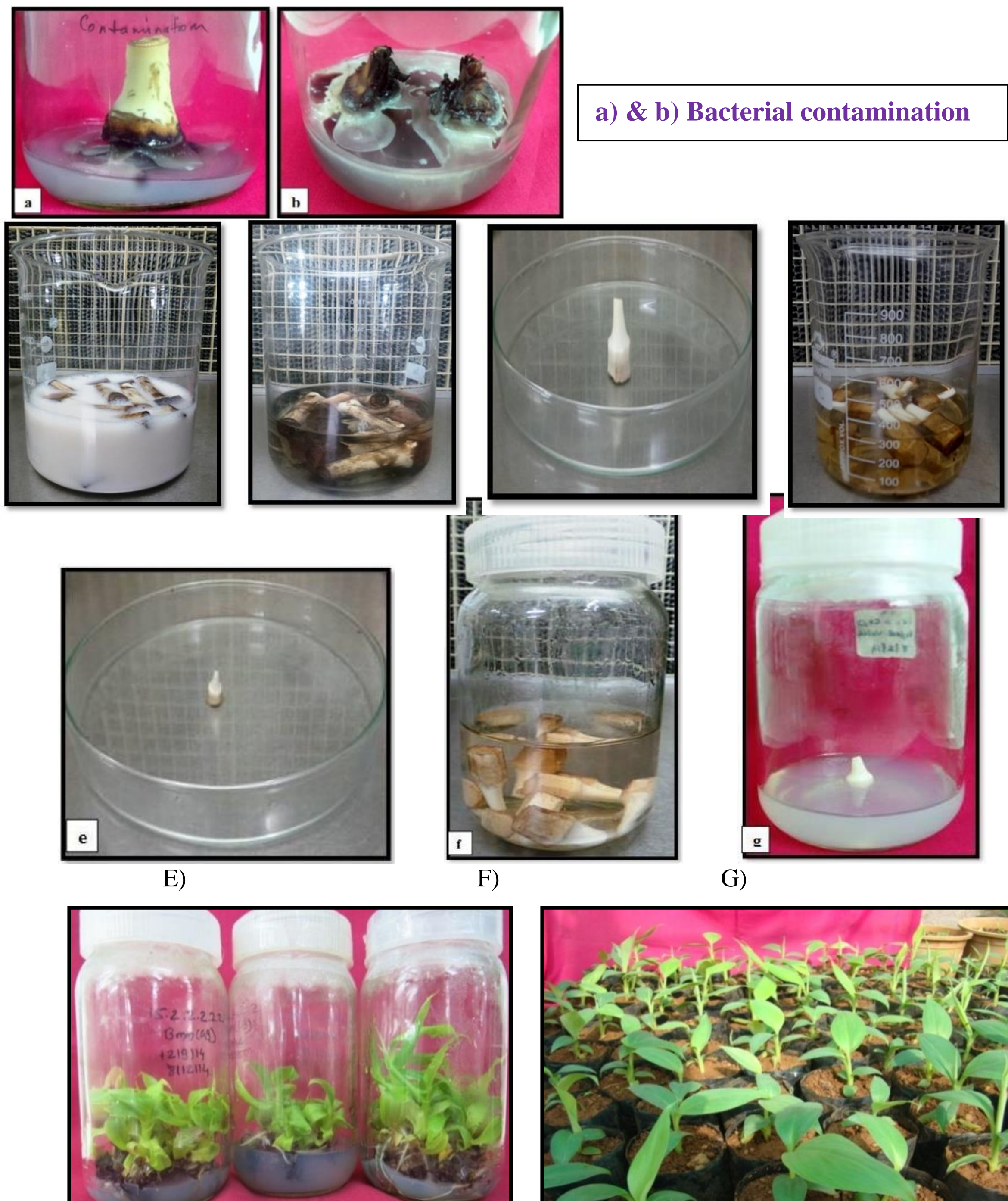


Fig. 1 :- Sterilization technique for Konkan Safed Velchi.

A) Treatment with Carbendazim
B) Treatment with Ethanol
C) 1st cut given to explants
D) Treatment with Sodium hypochlorite
E) 2nd cut given to explants
F) Treatment with Cefotaxime
G) Explant inoculated in media
H) Shooting and rooting
I) Establishment of plantlets

Table 1 : Effect of sterilization treatment combination on per cent contamination, per cent survival and days to shoot bud initiation

Treatment	Sterilants	Concentration	Exposure time (min)	Per cent contamination			Per cent survival			Days to shoot bud initiation		
				Variety		Mean	Variety		Mean	Variety		Mean
				Konkan Safed Velchi	Grand Naine		Konkan Safed Velchi	Grand Naine		Konkan Safed Velchi	Grand Naine	
T1	Carbendazim	0.50%	45	50.00 (45)	30.00 (33.00)	40.00 (39.00)	50.00 (45)	70.00 (57.00)	60.00 (51.00)	35.33	27	31.17
	Ethanol	70%	1									
	Sodium Hypochlorite	5%	5									
	Cefotaxime	200 mg/L	15									
T2	Carbendazim	0.50%	60	23.33 (28.78)	23.33 (28.78)	23.33 (28.78)	76.67 (61.22)	76.67 (61.22)	76.67 (61.22)	31	23	27
	Ethanol	70%	1									
	Sodium Hypochlorite	5%	15									
	Cefotaxime	200 mg/L	30									
T3	Carbendazim	1%	30	3.33 (6.14)	3.33 (6.14)	3.33 (6.14)	96.67 (83.86)	96.67 (83.86)	96.67 (83.86)	23	20.67	21. 84
	Ethanol	70%	1									
	Sodium Hypochlorite	5%	10									
	Cefotaxime	250 mg/L	30									
	SEm					**4.0575**			**4.05755**			**0.50917**
	CD at 1%					**17.5277**			**17.5277**			**2.19952**

Table 2 : Effect of establishment and proliferation media combinations on shoot regeneration and per cent shoot response

Tr. No.	Treatment Details	Shoot regeneration			Per cent shoot response		
		Variety		Mean	Variety		Mean
		Konkan Safed Velchi	Grand Naine		Konkan Safed Velchi	Grand Naine	
E1	MS	0	0	0	0 (0)	0 (0)	0 (0)
E2	MS + 2 BAP + 1 NAA	1.33	1.67	1.5	6.67 (14.76)	8.33 (16.60)	7.50 (15.68)
E3	MS + 4 BAP + 1NAA	7.67	6.67	7.17	38.33 (38.24)	33.33 (35.25)	35.83 (36.75)
E4	MS + 6 BAP + 1NAA	16.33	19	17.67	81.67 (64.69)	95.00 (77.08)	88.33 (70.89)
E5	MS + 8 BAP + 1 NAA	11.33	13.67	12.5	56.67 (48.85)	68.33 (56.03)	62.50 (52.44)
E6	MS + 10 BAP + 1 NAA	10	7.67	8.83	50.00 (45.00)	38.33 (38.24)	44.17 (41.62)
	SEm			**0.518188**			**1.610427**
	CD at 1%			**2.049676**			**6.369996**

Table 3 : Effect of rooting media combinations on root regeneration and per cent root response

Tr. No.	Treatment Details	Root regeneration			Per cent root response		
		Variety		Mean	Variety		Mean
		Konkan Safed Velchi	Grand Naine		Konkan Safed Velchi	Grand Naine	
R1	½ MS + 1 BAP	0	0	0	0 (0)	0 (0)	0 (0)
R2	½ MS + 1 BAP + 1 NAA + IBA	6	8	7	30.00 (33.00)	40.00 (39.15)	35.00 (36.07)
R3	½ MS + 1 BAP + 1 NAA + 2 IBA	12.33	13.33	12.83	61.67 (51.81)	66.67 (54.89)	64.17 (53.35)
R4	½ MS + 1 BAP + 2 NAA + 1 IBA	15	17	16	75.00 (60.07)	85.00 (67.40)	80.00 (63.74)
R5	½ MS + 1 BAP + 2 NAA + 2 IBA	17	18	17.5	85.00 (67.40)	86.67 (69.55)	85.83 (68.48)
R6	½ MS + 1 BAP + 2 NAA + 3 IBA	9	10	9.5	45.00 (42.12)	50.00 (45.00)	47.50 (43.56)
	SEm			**0.757677**			**2.564103**
	CD at 1%			**2.996968**			**10.142233**

Table 4. Number of multiple shoots regenerated in various subcultures for Konkan Safed Velchi

CLONES	NUMBER OF EXPLANTS INOCULATED	SUB-CULTURE								
		1st	2nd	3rd	4th	5th	6th	7th	8th	9th
1	1	1	3	9	22	68	136	165	122	87
2	1	0	1	4	15	73	149	193	168	128
3	1	2	4	10	26	87	181	293	258	214
4	1	1	2	8	23	75	168	242	197	155
5	1	2	6	12	29	109	218	368	318	273
6	1	0	0	4	16	56	115	154	97	64
7	1	1	2	8	24	101	213	317	267	232
8	1	1	4	12	28	121	245	404	359	314
9	1	2	6	15	31	137	277	**452**	408	353
10	1	1	3	11	29	123	255	406	356	311
Mean	**1**	**1.1**	**3.1**	**9.3**	**24.3**	**95**	**195.7**	**299.4**	**255**	**213.1**

Table 5. Number of multiple shoots regenerated in various subcultures for Grand naine

CLONES	NUMBER OF EXPLANTS INOCULATED	SUB-CULTURE								
		1st	2nd	3rd	4th	5th	6th	7th	8th	9th
1	1	2	5	14	42	126	252	405	656	811
2	1	1	3	9	28	90	180	256	421	686
3	1	1	4	12	39	119	257	409	653	899
4	1	2	6	22	68	205	409	621	842	978
5	1	2	5	18	53	160	278	561	784	937
6	1	0	2	7	22	82	153	390	525	679
7	1	1	3	10	35	107	319	542	704	958
8	1	2	6	20	61	183	347	511	645	770
9	1	3	7	25	73	223	450	667	825	959
10	1	2	4	15	48	153	343	593	738	**981**
Mean	**1**	**1.6**	**4.5**	**15.2**	**46.9**	**144.8**	**298.8**	**495.5**	**679.3**	**865.8**

CONCLUSION:

For *in vitro* regeneration of banana Cv. Konkan Safed Velchi the treatment in the sequential order of Carbendazim 1% followed by Ethanol 70%, Sodium Hypochloride 5% and Cefotaxime 250 mg/L for the period of 30, 1, 10 and 30 min. produces 96.67% aseptic culture.

Media combination of MS + 6 mgL^{-1} BAP + 1 mgL^{-1} NAA was found to be best for establishment and proliferation of cultures, whereas media combination of ½ MS + 1 mgL^{-1} BAP + 2 mgL^{-1} NAA + 2 mgL^{-1} IBA was found to be best for root regeneration for both the varieties. Among the two varieties, Konkan Safed Velchi was efficient in producing multiple shoots only up to six subcultures as compared to Grand naine which produced multiple shoots up to nine subcultures.

Application:

This sterilization technique could be utilized for large scale *in vitro* multiplication of Konkan Safed Velchi a cultivar of banana.

Recommendation

For *in vitro* regeneration of banana *Cv*. Konkan Safed Velchi the treatment in the sequential order of Carbendazim 1% followed by Ethanol 70%, Sodium Hypochloride 5% and Cefotaxime 250 mg/L for the period of 30, 1, 10 and 30 minutes, respectively produces aseptic culture.

शिफारस

केळीच्या कोकण सफेद वेलची या जातीची उती संवर्धनाने अभिवृध्दी करण्यासाठी क्रमाने कार्बेन्डॅझिम 1 %, **इथेनॉल** 7 0 %, **सोडीयम हायपोक्लोराईड** 5 %, **आणि सिफोटॅक्सीम** 2 5 0 **मि.ग्रॅ. प्रति लिटर यांची मात्रा अनुक्रमे** 3 0 , 1 , 1 0 **आणि** 3 0 **मिनीटे कालावधीसाठी दिल्यास निर्जतूक संवर्धक तयार होते.**

B) Year of commencement of Experiment	:	2013
C) Protocol optimized	:	*In vitro* sterilization protocol is optimized.
D) Identification of chemical sterilants	:	Sequential order of sterilizing agents is standardized.
Whether recommended by	:	
IV. Pre-Research Review Committee	:	Yes
V. Research Review Committee	:	Yes
VI. Variety Release committee	:	Yes

Title	:	**Standardization of *In-vitro* Regeneration Protocol on Local Cultivar Red banana (*Musa acuminata*).**
Objectives	:	1. Standardization of surface sterilization technique in Red banana 2. Standardization of protocol for Red banana. a) Direct organogenesis and embryogenesis studies in Red banana. b) Studies on hardening treatment on Red banana for survival. 3. Commercial scaling up of micropropagation technique for mass multiplication of Red banana.
Scientist involved	:	1. Dr. S. V. Sawardekar, Principal Investigator, Incharge and Professor (CAS), Plant Biotechnology Centre, College of Agriculture, Dapoli. 2. Dr. N. B. Gokhale, Co-principal Investigator, Deputy Director of Research, Dr. B. S. Konkan Krishi Vidyapeeth, Dapoli 3. Shri. V. G. Kelkar, JTA,Plant Biotechnology Centre, Dapoli
Associated Scientist	:	1. S. S. Sawant, Junior Research Assistant, Plant Biotechnology Centre, College of Agriculture, Dapoli. 2. Dr. B. R. Salvi, Head Department of Horticulture, College of Agriculture, Dapoli. 3 Dr. U. V. Mahadkar, Associate Dean, College of Agriculture, Dapoli. 4. Dr. S. A. Chavan, Ex-Associate Dean and Head Department of Agronomy, College of Agriculture, Dapoli.
Location	:	Plant Biotechnology Centre, College of Agriculture, Dapoli.
Year of commencement	:	2015
Year of completion	:	2017
Duration of experiment	:	Two years
Budget	:	5.6 lakh
Variety	:	**Local Cultivar Red banana**
Background information	:	**Red banana** (*Musa acuminata*) is a triploid cultivar (AAA Group). **Family-**Musaceae Red banana is having reddish-purple skin and yellowish pulp. In Bihar and other regions, it is popular as Lal Velchi while in Karnataka as Chandra Bale. The colour of the pseudostem, petiole, midrib and fruit rind is purplish red. Fruits are sweet, orange yellow coloured and with a pleasant aroma. It is highly susceptible to bunchy top, fusarium wilt and nematodes. Red bananas are also rich in fiber and helps in fulfilling about 16% of the dietary fiber

requirement of an individual. This fruit also contains large amount of Vitamin B6 a single red banana provides even 20% of the daily recommended amount of vitamin D and supports the metabolism of proteins and red blood cells (Food House, 2013). It also contains vitamin C, potassium and β-carotene.

In Konkan region, there is less area under red banana plantation due to the less availability of the plantlets.The area under expansion of red banana in Konkan region is a large constraint. The plantlets produced through tissue culture will help in meeting the increasing demand for farmers. This variety has tremendous demand because of its peculiar taste and nutrition. But the availability of plantlets are limiting factor for its large scale cultivation. This cultivar is also entitled as endangered species and it need to be protected. In *vitro* propagation is one of the methods of choice since it provides a rapid reliable system for production of large number of genetically uniform disease free plantlets. In general, micropropagated banana plants establish faster, grow more vigorously, are taller, have a shorter and more uniform production period and produce higher yields than conventional propagules. It can be continued throughout the year irrespective of the season.

Bacterial contamination is a major problem hindering tissue culture application. Darkening of the culture medium due to release of phenolic compound from the tissue of the explants is another obstacle that affects the growth of the explants by getting accumulated in the surrounding medium. These problems reduce the number of plants in further tissue culture techniques. Therefore, the objective of the present investigation was standardization of sterilization protocol for aseptic inoculation of explants, and *in vitro* propagation of Red banana cultivar .Through banana micropropagation, it is possible to get plantlets free from bacteria and other microorganisms. It is the one of suitable way to protect the endangered cultivar and produce disease free planting material. Micropropagation also be able to supply of quality planting material on regular basis for higher yield and shortening the harvesting period.

Experimental Details

Material and Methodology : For the present study the suckers of Red banana (*Musa acuminate*) local cultivar collected from the farmers field of Shrivardhan, Alibaug and Dive-Agar of Raigad District and used for experimental purpose.

Two to three months old sword suckers were excised from healthy disease free mother plant. These suckers were thoroughly washed under

running tap water. Shoot tips were prepared by trimming roots and outer leaf sheaths from the suckers.

The explants were kept immersed in water for 5 min, then they were pre-treated with 5 ml/L Tween 20 for 10 min and a combination of 5 ml/L Dettol and 45 ml/L Savlon for 30 min with constant swirling and subsequently draining off the disinfectants by washing them with DD water 2-3 times to remove the traces of the disinfectants. The explants were then washed under running water for 60 min.

Standardization of surface sterilization methods was carried out by treating the explants with various combinations and concentrations of different chemical sterilizing agent for different durations of exposure time as mentioned in Table 1. The explants were inoculated on the establishment media combination and established explants were subcultured on proliferation media after 21 days. Proliferated plantlets subculture on rooting media. Media combinations were used for establishment, proliferation and rooting of the explants as mentioned in Table 2, 3 and Table 4 respectively.

As all studies were done in laboratory under well-defined conditions of the medium, growth, temperature and light and all the data was analyzed under Completely Randomized Design (CRD) by using OP STAT software**.**

Results:-

Table 1: Effect of sterilization treatment combination on per cent contamination, per cent survival and days to shoot bud initiation

Treatment	Sterilizing Agents	Concentration	Exposure time (min)	Per cent contamination	Per cent survival	Days to shoot bud initiation
T1	Carbendazim	0.50%	45	55%	45%	29
	Ethanol	70%	1			
	Sodium Hypochlorite	5%	5			
	Cefotaxime	150 mg/L	15			
T2	Carbendazim	0.50%	60	44%	56%	25
	Ethanol	70%	1			
	Sodium Hypochlorite	5%	15			
	Cefotaxime	200 mg/L	30			
T3	**Tween20**	**5ml/L**	**10**	**32.2%**	**67.8%**	**22**
	Dettol + Savlon	**5 ml +45 ml/L**	**30**			
	Carbendazim	**1%**	**30**			
	Ethanol	**70%**	**1**			
	Sodium	**5%**	**10**			

	Hypochlorite					
	Cefotaxime (I)	**250 mg/L**	**20**			
	Cefotaxime (II)	**250 mg/L**	**40**			
T4	Tween20	5ml/L	10	34.2%	40%	-
	Dettol + Savlon	5 ml +45 ml/L	30			
	Carbendazim	1%	30			
	Ethanol	70%	1			
	Sodium Hypochlorite	5%	10			
	Cefotaxime (I)	250 mg/L	20			
	Cefotaxime (II)	250 mg/L	40			
	Cefotaxime (III)	250 mg/L	60			

Effect of sterilization treatment combination on per cent contamination, survival and days to shoot bud initiation:

The per cent contamination observed is presented in Table 1. The treatment combination T3 was found to be best to achieve lowest per cent contamination (32.20) for Red banana while, same treatment combination showed highest per cent survival 67.8 %. The treatment combination T3 was found to be best combination to achieve highest percentage of contamination free healthy cultures. While, treatment combination T1 showed lowest per cent survival of 45%. Effectiveness of surface sterilization depends on many factors like explant type, concentration of sterilizing agents and duration of treatments with various sterilizing agents and sequence of treatments. It was observed that on an average treatment combination T3 resulted in minimum number of days (22) for shoot bud initiation. The treatment T1 recorded maximum number of days (29) for shoot bud initiation.

Table 2: Effect of media combination on establishment of Red banana cultivar.

Tr. No.	Treatment Details	% Establishment
E1	MS	0.00
E2	MS + 2 mgL^{-1} BAP + 0.1 mgL^{-1}NAA+ 20 gmL^{-1} Ascorbic acid + 1 gL^{-1}Activated charcoal	0.00
E3	MS + 2.5 mgL^{-1} BAP +0. 1 mgL^{-1}NAA+ 20 mgL^{-1} Ascorbic acid +1 gL^{-1}Activated charcoal	0.00
E4	MS + 3.0 mgL^{-1} BAP + 0.175mgL^{-1}NAA+ 20 mgL^{-1} Ascorbic acid+1 gL^{-1}Activated charcoal	0.00
E5	MS + 3.5 mgL^{-1} BAP + 0.175mgL^{-1}NAA + 20 mgL^{-1} Ascorbic acid+1 gL^{-1}Activated charcoal	32.00
E6	MS + 4 mgL^{-1} BAP +0.1 mgL^{-1}NAA+ 20 mgL^{-1} Ascorbic acid+ 1 gL^{-1}Activated charcoal	37.33
E7	MS + 4 mgL^{-1} BAP + 0.1 mgL^{-1} IAA+ 20 mgL^{-1} Ascorbic acid+ 1 gL^{-1}Activated charcoal	44.67
E8	**MS + 4.5 mgL^{-1} BAP + 0.175 mgL^{-1}IAA + 20 mgL^{-1} Ascorbic acid +1 gL^{-1}Activated charcoal**	**78.00**
E9	MS + 5mgL^{-1} BAP + 0.15mgL^{-1}IAA + 20 mgL^{-1} Ascorbic acid+ 1 gL^{-1}Activated charcoal	55.33
E10	MS + 5.5 mgL^{-1} BAP + + 0.175 mgL^{-1} IAA + 20mgL^{-1} Ascorbic acid+1 gL^{-1}Activated charcoal	40.33

E11	MS + 6.0 mgL-1 BAP + 0.1 mgL-1 IAA + 20 mgL-1 Ascorbic acid+1 gL^{-1}Activated charcoal	35.67
	CD at 1%	**4.27**
	SE	**1.44**

Effect of media combination on establishment

In all eleven media combinations were used for establishment of explants are in Table 2. The surface sterilized ten explants were inoculated in aseptic condition on these media combinations and incubated in controlled environment for about 21 days. After 21 days established explants became round shaped. The observations were recorded and it was found that the highest establishment was observed on media combination E8 (MS + 4.5 mgL^{-1} BAP + 0.175 mgL^{-1}IAA + 20 mgL^{-1} Ascorbic acid +1 gL^{-1}Activated charcoal) 78% followed by E9 (MS + 5mgL^{-1} BAP + 0.15mgL^{-1}IAA + 20 mgL^{-1} Ascorbic acid+1 gL^{-1}Activated charcoal) 55.33%. The lowest per cent establishment were recorded on media combination E5 (32%). No explant establishment were observed on E1 E2, E3, and E4 media combination. The media combination (E8)MS + 4.5 mgL^{-1} BAP + 0.175 mgL^{-1}IAA + 20 mgL^{-1} Ascorbic acid +1 gL^{-1}Activated charcoal was observed to be best for establishment of explants.

Table 3: Effect of media combination on proliferation of Red banana cultivar after 8th subculture

Tr. No.	Treatment Details	No. of Shoots regeneration
S1	MS	0.00
S2	MS + 2 mgL^{-1} BAP + 20 gmL^{-1} Ascorbic acid	0.00
S3	MS + 3 mgL^{-1} BAP 20 gmL^{-1} Ascorbic acid	36.75
S4	MS + 3 mgL^{-1} BAP + 0.1 mgL^{-1} IAA + 20 gmL^{-1} Ascorbic acid	49.91
S5	MS +3.5 mgL^{-1} BAP + 0.15 mgL^{-1} IAA + 20 mgL^{-1} Ascorbic acid	58.65
S6	MS + 4.0 mgL^{-1} BAP + 0.175 mgL^{-1} IAA + 20 mgL^{-1} Ascorbic acid	111.37
S7	**MS + 4.5 mgL^{-1} BAP + 0.175 mgL^{-1} IAA + 20 mgL^{-1} Ascorbic acid**	**560.29**
S8	MS + 5 mgL^{-1} BAP + 0.175 mgL^{-1} IAA + 20 mgL^{-1} Ascorbic acid	250.27
S9	MS + 5.5 mgL^{-1} BAP + 0.175 mgL^{-1} IAA + 20 mgL^{-1} Ascorbic acid	226.52
S10	MS + 6mgL^{-1} BAP + 0.175 mgL^{-1} IAA + 20 mgL^{-1} Ascorbic acid+	147.24
S11	MS + 6.5 mgL^{-1} BAP + 0.175 mgL^{-1} IAA + 20 mgL^{-1} Ascorbic acid	96.73
	SE	**3.44**
	CD at 1%	**12.9**

Effect of media combination on proliferation

The established explants were cut into two pieces vertically and placed on eleven different media combinations are given in Table 3. After 21 days of inoculation the explants were proliferated. The proliferated explants were subcultured up to 8th subculture and observations were recorded. The treatment (S7) containing MS + 4.5 mgL^{-1} BAP + 0.175 mgL^{-1} IAA + 20 mgL^{-1} Ascorbic acid showed the maximum rate of multiple shooting (560.29) than other media combinations followed by MS + 5 mgL^{-1} BAP + 0.175 mgL^{-1} IAA + 20 mgL^{-1} Ascorbic acid (250.27) The lowest average number of shoots were recorded on media combination S3. None of the multiple shoots were observed on S1 (control), S2 treatment. The media combination S7 was observed to be best for Red banana proliferation.

Effect of media combination on root regeneration

The proliferated shoots subculture on different rooting media combinations. The observations was recorded .The results presented in Table 4 indicates that the best medium for regeneration of roots was found to be R8 (MS + 4.5 mgL^{-1} BAP + 0.175 mgL^{-1}IAA + 20 mgL^{-1} Ascorbic acid) with an average of 15.34 roots per shooted plant. The lowest per cent root response was recorded as 7.2 on media combination R2 (MS +1 mgL^{-1} BAP + 0.1 mgL^{-1}NAA+ 20 gmL^{-1} Ascorbic acid). None of the roots form on the media R1 (MS-control).

Table 4: Effect of media combination on root regeneration of Red banana cultivar

Tr. No.	Treatment Details	No. of roots regenerated/ explant
R1	MS	0.000
R2	MS +1 mgL^{-1} BAP + 0.1 mgL^{-1} NAA+ 20 gmL^{-1} Ascorbic acid	7.20
R3	MS + 1 mgL^{-1} BAP +0.5 mgL^{-1} NAA+ 20 mgL^{-1} Ascorbic acid +1 gL^{-1} Activated charcoal	7.26
R4	MS + 1 mgL^{-1} BAP + 0.6mgL^{-1} NAA+ 20 mgL^{-1} Ascorbic acid+1 gL^{-1} Activated charcoal	6.35
R5	MS + 1 mgL^{-1} BAP + 0.7mgL^{-1} NAA + 20 mgL^{-1} Ascorbic acid+1 gL^{-1} Activated charcoal	9.00
R6	MS + 1mgL^{-1} BAP +0.1 mgL^{-1} NAA+ 20 mgL^{-1} Ascorbic acid+1 gL^{-1} Activated charcoal	8.70
R7	MS + 1mgL^{-1} BAP + 0.15 mgL^{-1} IAA+ 20 mgL^{-1} Ascorbic acid+1 gL^{-1} Activated charcoal	10.01
R8	**MS + 4.5 mgL^{-1} BAP + 0.175 mgL^{-1}IAA + 20 mgL^{-1} Ascorbic acid**	**15.34**
R9	MS + 4 mgL^{-1} BAP + 0.2mgL^{-1} IAA + 20 mgL^{-1} Ascorbic acid +1 gL^{-1} Activated charcoal	10.430
	S.E.	**2.366**
	CD at 1%	**0.790**

Table 5. Effect of different potting mixtures on hardening of Red banana plantlets

Treatments			Number of plants shifted	Number of plants survived	Survivability (%)
Soil	**FYM**	**Sand**			
1.00	0.00	1.00	20	5	25.00
1.00	1.00	0.00	20	3	15.00
1.00	**1.00**	**1.00**	**20**	**18**	**90.00**
1.00	0.00	0.00	20	4	20.00

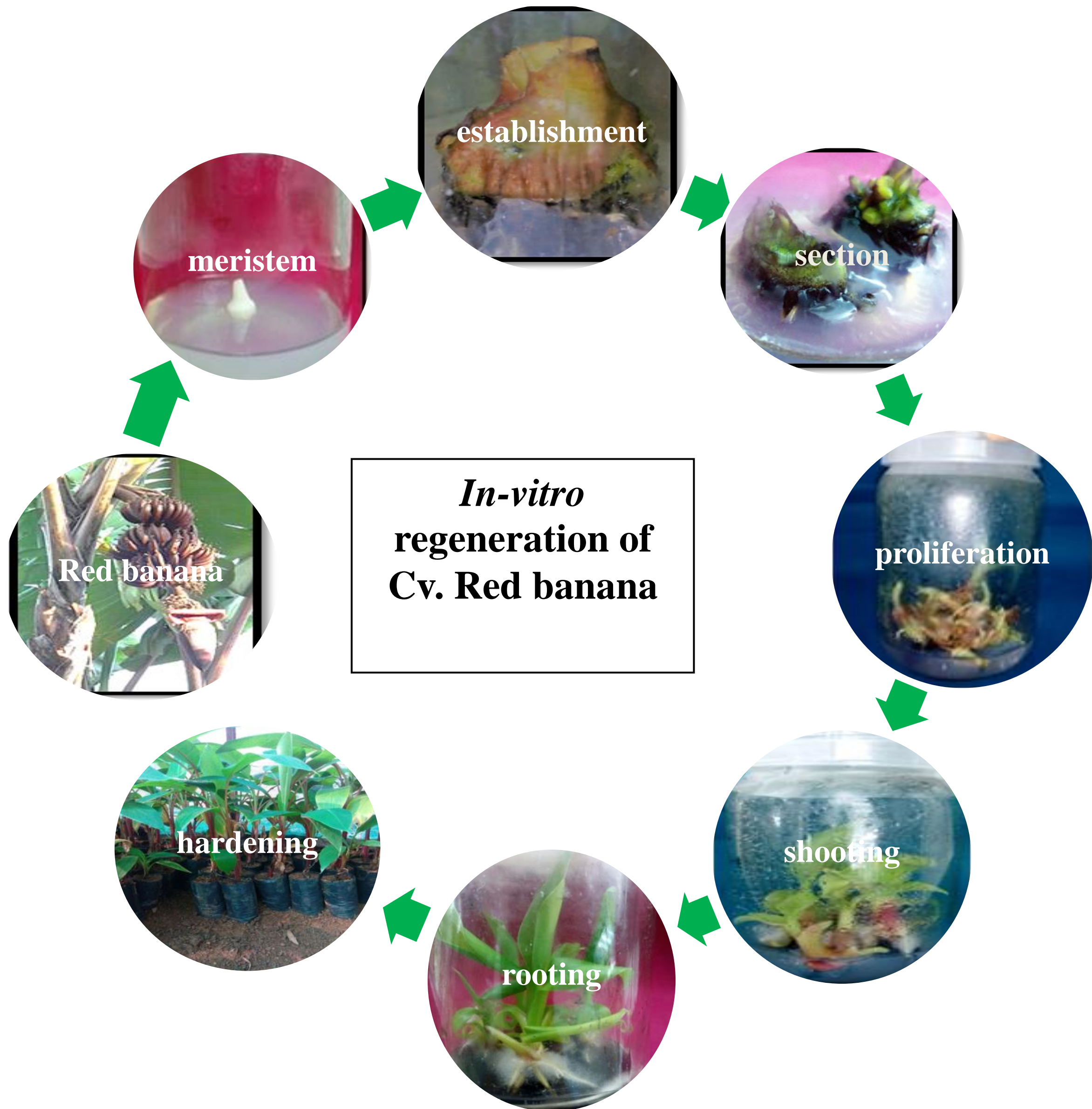
The rooted plants were transferred in (Table 5) potting mixture of soil, FYM and sand (Soil -Lateritic soil, FYM -Farm Yard Manuare) present in equal proportion (1:1:1) recorded 90 per cent survivability.

CONCLUSION:

For *in vitro* regeneration of *Cv*. Red banana the treatment in the sequential order of Tween 20 (5ml/lit) 10 min. Dettol + Savlon (5 ml +45 ml/lit respectively) 30 min.followed by Carbendazim 1%, Ethanol 70%, Sodium Hypochlorite 5% and Cefotaxime I- 250 mg/L and Cefotaxime II- 250 mg/L for the period of 30, 1, 10 and 20 min and 40 min. produces 67.8 % aseptic culture. The Media combination MS + 4.5 mgL^{-1} BAP + 0.175 mgL^{-1}IAA + 20 mgL^{-1} Ascorbic acid +1 gL^{-1}Activated charcoal was found to be best for establishment and same media excluding charcoal for proliferation and root regeneration . The plantlets hardened in the

potting mixture (1:1:1) of soil, FYM and sand (Soil -Lateritic soil, FYM -Farm Yard Manuare).

Application: This micropropagation technique may be utilized for large scale multiplication of *Cv*. Red banana.



Acknowledgment	**:**	The Plant Biotechnology Centre, Dr. B. S. Konkan Krishi Vidyapeeth, Dapoli acknowledge the financial assistance tendered by the Zilla Parishad, Raigad for the project with due to reverence and regards.
Recommendation	:	Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth developed tissue culture technology recommended for micropropagation of Red banana.
शिफारस	**:**	**डॉ. बाळासाहेब सावंत कोकण कृषि विद्यापीठ विकसित उतीसंवर्धन तंत्र लाल केळीच्या सुक्ष्म अभिवृध्दीसाठी शिफारस करण्यात येत आहे.**

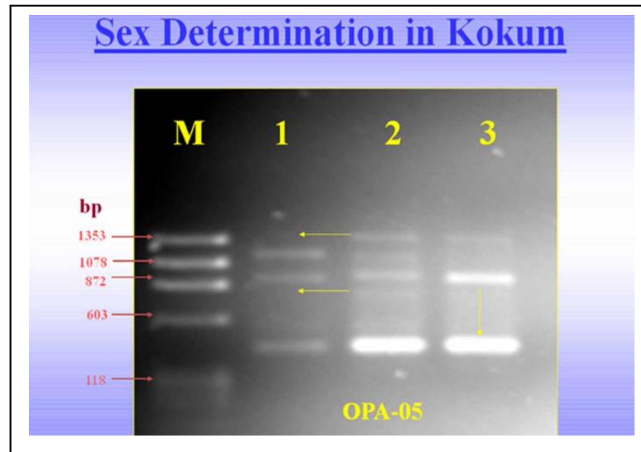
Details of grants received and utilized so far (Rupees):

Sr. No.		**Sanctioned**	**Opening**	**Received**	**Total**	**Spent**	**Unspent**
1st	**(2016-17)**						
1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
1.	Staff Salaries	1,96,000/-	Nil	1,96,000/-	1,96,000/-	1,06,178/-	89822/-
2.	Labour	64,000/-	Nil	64,000/-	64,000/-	33120/-	30880/-
3.	Consumables	2,50,000/-	Nil	2,50,000/-	2,50,000/-	133682/-	1,16,318/-
4.	Miscellaneous	50,000/-	Nil	50,000/-	50,000/-	29265/-	20,735/-
5.	**Total:**	**5,60,000/-**	**Nil**	**5,60,000/-**	**5,60,000/-**	**3,02,245/-**	**2,57,755/-**
2nd	(2017-18)	2,57,755/-	2,57,755/-	2,57,755/-	2,57,755/-	2,45,614/-	12,141/-

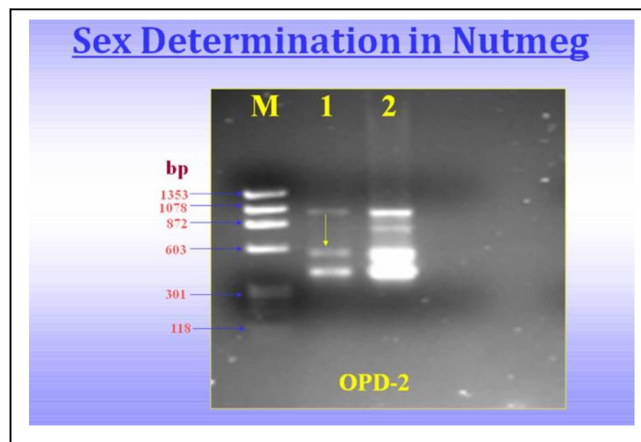
c. Research Outcome/Findings:

1. Standardized the micro propagation technique in banana and medicinal plants.

2. Sex determination in Kokum (*Garcinia indica*) through molecular markers.



3. Sex determination in Nutmeg. (*Myristica fragranns* Houff) through molecular markers.

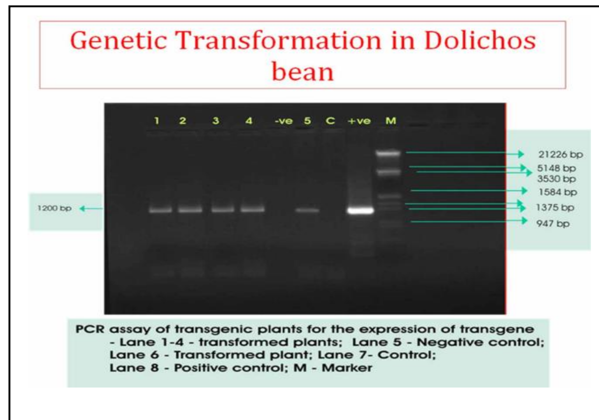


4. Developed *in vitro* somaclones in Nagli (*Eleusina coracana* L.) and analysed variation through RAPD.
5. Standardized *in vitro* Regeneration protocol in Pigeon pea.

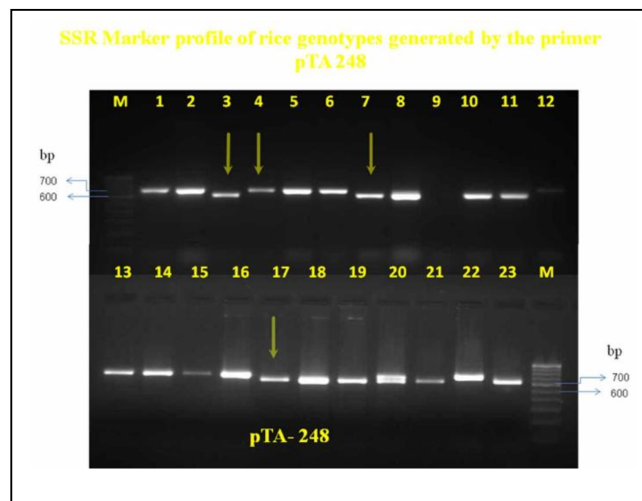
Fig 1.a Variable clones of Nagli.

6. Developed genetic transformation protocol in pigeon pea and dolichos bean.

7. Marker Assisted Selection in rice for biotic and abiotic stress.



8. Finger printing of released rice varieties of DBSKKV, Dapoli.



d. Completed Research Projects/ Programmes/ Schemes

Sr. No.	Name of project	Sponsoring Agency	Period	Amount (Lakh)
1)	Providing basic facilities for imparting education and conduct research in genetic engineering.	Government of Maharashtra	**2005-2008**	**102.00**
2)	Use of irradiation technique for creation of variability in fingermillet and assessment of mutants through molecular markers.	Board of Research on Nuclear Sciences, BARC, Mumbai	2011-14	17.66
3)	Confined field trial for event selection on salt tolerant (*Oryza sativa* L.) events namely OsN1 to OsN 25 containing Os NHX1 gene at KLRS, Panvel	Mahyco, Jalna	2014-16	**19.60**
4)	In vitro multiplication of banana Cv. Safed Velchi	Zilla Parishad, Alibaug	2015-16, 2016-17, 2017-18	5.60
5)	Standardization of Invitro regeneration protocol of Red banana	Zilla Parishad, Alibaug	2015-16, 2016-17, 2017-18	5.60
6)	Standardization of clonal propagation techniques in Mango (Mangifera indica L.) cv. Alphonso	District Planning Commission	2019-2021	10.0
7)	"Improvement of red banana (Musa acuminate) through mutation breeding and biotechnological intervention".	Board of Research and Nuclear Sciences, Department of Atomic Energy (DAE) Government of India.	25/08/2020 to 25/8/2023	34.38
8)	Development of explant regeneration pretissue culture technique in alphonso mango	DPDC, Ratnagiri. GOVT. Of Maharashtra	1/4/2021 to 31/3/2023	20.00
9)	Seedling development of alphonso mango through tissue culture	DPDC, Ratnagiri. GOVT. Of Maharashtra	1/4/2021 to 31/3/2023	20.00
10)	Advance Biotechnology	GOVT. Of Maharashtra	27/09/2023 to	100.00 till today

e. Ongoing Research Projects/Programmes/Schemes:

Title: Use of irradiation technique for creation of variability in finger millet and assessment of mutants through molecular marker

UR No.: No. 2011/35/11/BRNS/1452/20 Sep. 2011

Objectives: 1. Irradiation of promising genotypes of finger millet.

2. Assessment of genetic variability of mutants through molecular markers.

3. To develop blast resistant mutant in finger millet.

Name of PI: Dr. N. B. Gokhale

Co-PI: Dr. S. V. Sawardekar

Sponsoring Agency: Government of India, Department of Atomic Energy (DAE), Board of Research in Nuclear Sciences (BRNS)

Duration: 3 years

Total Outlay: Rs. 1766350/-

8. Repository of abstracts of the theses:

Name of the candidate: S. N. SABALE

Degree for which the thesis/project report submitted: M. Sc. (Ag. Biotechnology)

Year of submission: 2012

Name of the Guide/ Co guide: Dr. N. B. GOKHALE

Abstract:

Elimination of bacterial contamination by using antibiotics in micropropagtion of banana (*musa spp.*) Cv. Grand naine.

Abstract

The investigation entitled, “Elimination of bacterial contamination by use of antibiotics in micropropagtion of banana (*Musa spp.*) Cv. Grand naine” was undertaken in Completely Randomized Design with 3 replications. This experiment was carried out to eliminate the bacterial growth by using the antibiotics in culture media during the micropropagation of banana Cv. Grand naine. The four antibiotics namely, Augmentin, Amoxicillin, Rifampicin, Cefotaxime were tested for its efficiency to control the bacterial contamination occurring during micropropagation.

The media combination MS + 6.5 mgl^{-1} BAP + 1000 ppm Amoxicillin shown maximum elimination of bacterial contamination at 15 DAI. While, the media combination MS + 5 mgl^{-1} BAP + 2 mgl^{-1} IAA + 1000 ppm Amoxicillin shown maximum elimination of bacterial contamination at 30 DAI. Highest percent of explants establishment was on MS + 6.5 mgl^{-1} BAP + 1000 ppm Amoxicillin and highest percent of shoot initiation was on MS + 5 mgl^{-1} BAP + 2 mgl^{-1} IAA + 1000 ppm Amoxicillin. The minimum number of days to shooting was recorded

24.75 days on the media combination MS + 5 mgl^{-1} BAP + 2 mgl^{-1} IAA + 1000 ppm Cefotaxime and the maximum number of shoots was recorded 6.24 shoots per explant in the media combination MS + 5 mgl^{-1} BAP + 2 mgl^{-1} IAA + 750 ppm Amoxicillin at 30 DAI.

The media combination supplemented with 1000 ppm Amoxicillin was effective in elimination of maximum bacterial contamination without any phytotoxic effect on explants at 30 DAI, saving maximum cultures during establishment and proliferation ultimately increasing the plantlets during micropropagation.

Name of the candidate: K.A. Lipne

Degree for which the thesis/project report submitted: M. Sc. (Ag. Biotechnology)

Year of submission: 2012

Name of the Guide/ Co guide: Dr. S.G. Bhave

Abstract:

Genetic transformation of dolichos bean (*lablab purpureus* (l.) Sweet) for pod borer resistance

ABSTRACT

The present investigation was aimed to standardize regeneration and *Agrobacterium*-mediated genetic transformation protocols in popular dolichos bean variety Konkan bhushan. Different explants viz, mature embryo axes with single cotyledon, mature embryo axes and shoot tip were cultured on MS basal medium supplemented with different levels of BAP (2 to 6 mg/l), NAA (0.5 mg/l) and Kinetin (2 to 6 mg/l) for multiple shoot induction. Frequency of multiple shoot induction was significantly more in MEASC explants than in MEA and shoot tip. BAP at 2 mg/l and NAA at 0.5 mg/l induced shooting in most explants and recorded highest number of multiple shoots. Addition of 0.5 mg/l kinetin and 0.5 mg/l BAP in MS medium resulted in higher frequency of shoot elongation. The elongated shoots were rooted on MS medium supplemented with 0.1 mg/l NAA.

MEASC explants were co-cultivated with *Agrobacterium* strain EHA 105 carrying pBinBt3 construct having *cryIIAa* gene. The result of the present study indicate that highest transformation efficiency (0.952) could be achieved by 15 min colonization and 48 hour cocultivation followed by washing of explants in 400 mg/l cefotaxime. The genetic engineering approaches have potential of greatly enhancing the productivity of dolichos bean by increasing the resistance against pod borer. To be of value the transgenic plants must efficiently express transgene. However, the present study showed that *Agrobaterium*-mediated transformation is a possible approach to develop transgenic plant in dolichos bean.

Name of the candidate: V. D. Padwale

Degree for which the thesis/project report submitted: M. Sc. (Ag. Biotechnology)

Year of submission: 2012

Name of the Guide/ Co guide: Dr. N. B. Gokhale

Abstract:

Assessment of genetic variation in Mango (*Mangifera indica* L.) Cv. Alphonso by using RAPD markers

ABSTRACT

Assessment of genetic variation in Mango (*Mangifera indica* L.) Cv. Alphonso is fundamental for the conservation of genetic resources and utilization in breeding programme. The objective of this study was to assess the genetic variation in mango (Cv. Alphonso) at various locations in Konkan region.

RAPD profile for all Mango plants of various location (Cv. Alphonso) were generated with 38 random decamer primers. Out of 38 primers screened 10 primers gave scorable DNA fragments and each of the 10 random primers revealed polymorphism. These primers generated 201 DNA fragments in the average range of 348.3 bp to 812.2 bp, of which 140 were polymorphic. The average level of polymorphism generated by the primers was high (67.37%). The primers OPF-20, OPM-12, and OPU-08 produced distinct RAPD patterns (100% polymorphism) for all the Mango plants. The average discrimination power among the 10 primers was 52.5 per cent.

The overall range of the similarity among all Mango samples was found to be very wide, ranging from 0.086 to 0.571 which indicates there was high variability among the Alphonso cultivars under study. The cluster analysis based on RAPD data showed that Mango samples formed two main groups. The 900 Alphonso cultivars occupied a unique position and was most diverse from rest of the Alphonso cultivars. The study indicated that RAPD markers are suitable for the assessment of genetic diversity among Mango samples and identification of diverse sources in crop germplasm collection.

Name of the candidate: D. M. Patil

Degree for which the thesis/project report submitted: M. Sc. (Ag. Biotechnology)

Year of submission: 2012

Name of the Guide/ Co guide: Dr. S. V. Sawardekar

Abstract:

Genetic diversity analysis in cowpea (*vigna unguiculata* (l.) Walp.) By using RAPD markers

ABSTRACT

Assessment of genetic variability within *Vigna unguiculata* (L.) Walp. is fundamental for the conservation of genetic resources and its utilization in hybridization programme. The objective of this study was to estimate the genetic diversity among 30 genotypes of cowpea through molecular characterization by using RAPD markers.

RAPD profiles for all 30 genotypes were generated with 20 random decamer primers. Out of 20 primers screened 17 primers gave scorable DNA fragments and each of the 17 random primers revealed polymorphism. The primers generated 1238 DNA fragments in the average range of 381.94 bp to 1131.71 bp, of which 908 were polymorphic. The level of polymorphism generated by the primers was high (71.20%). The primers OPA-04, OPA-05, OPC-02, OPC-05 and OPC-08 produced distinct RAPD patterns (100% polymorphism) for all the 30 genotypes. These primers can be used for identification of the genotypes studied. The average discrimination power among the 17 primers was 62 per cent.

The overall range of the similarity among 30 genotypes was found to be very wide, ranging from 0.321 to 0.800 which indicates there was high variability among the cowpea cultivars under study. The cluster analysis based on RAPD data showed that genotypes formed two main groups. The genotype PCP-97223 occupied a unique position and was most diverse from rest of the genotypes. The study indicated that RAPD markers are suitable for the assessment of genetic diversity among group of genotypes and identification of diverse sources in crop germplasm collection. This study could identify the diverse genotype like DCP-11 and Pusa Phalguni for their use in hybridization programme of cowpea.

Name of the candidate: P. C. Ingale

Degree for which the thesis/project report submitted: M. Sc. (Ag. Biotechnology)

Year of submission: 2013

Name of the Guide/ Co guide: Dr. N. B. Gokhale

Abstract:

Sex determination in nutmeg (*myristica fragarns* houtt.) By using RAPD markers

ABSTRACT

Determination of sex in nutmeg is of utmost importance from the commercial agricultural point of view, since the sexuality cannot be distinguished prior to flower initiation. The use of bio-molecular techniques in sex determination of nutmeg presents a potential theoretical significance and economic value. For this analysis quality DNA is a prerequisite. Since this crop is having lot of phenolic compounds, optimization of DNA isolation protocol and use of RAPD markers to determine sex of nutmeg has been used in the present investigation.

The DNA was extracted from young leaves of nutmeg from 5 male and 5 female plants. The standardization of buffer constituents for DNA isolation was carried out. In Rapid method five components of the extraction buffer were standardized and in CTAB method three concentrations of CTAB powder were used. The results obtained using 0.900g glucose, 0.100g PVP,0.040g Sodium bisulphate, 0.050g Sodium Lauryl Sulphate, 500µl Sarcosine in Rapid method and 2% CTAB powder in CTAB method of DNA isolation are encouraging.

The most suitable combination of 100 mM Tris–HCl pH 8.0, 50 mM EDTA, 1 M NaCl as standardized buffer constituents showed clear and specific banding pattern. Modifications in extraction procedure indicated that the decrease in sample size, use of PVP, 0.3% mercaptoethanol, twice the volume isopropanol and washing with 70% ethanol produced better and clear bands when subjected to PCR.

Modifications carried out in PCR analysis showed that the most favorable conditions of dNTPs 1µM (1µl) concentration showed clear bands, Taq polymerase 0.5 units (1µl) proved better than others in proper band formation and annealing temperature 37^0C for 45 sec yielded good results.

The nutmeg DNA showed a poor amplification with RAPD primers studied. Of the total 60 decamer primers used in the investigation, only 14 primers showed amplification. Primers OPA-14, OPA-15, OPQ-16 and OPQ-04 showed polymorphism which can be used to differentiate male and female plants. A large number of RAPD primers failed to amplify. However, the information generated by few polymorphic primers was able to differentiate in between male and female plants. Such information will help the farmers to identify the sex of the saplings at an early stage and avoid their economic loss. Thus widening the probability ratio of male to female plants 1:9 as compared to present ration of 1:1 increasing more females, thereby increasing the yield of nutmeg almost two folds is possible.

Name of the candidate: S. S. Jadhav

Degree for which the thesis/project report submitted: M. Sc. (Ag. Biotechnology)

Year of submission: 2013

Name of the Guide/ Co guide: Dr. S. G. Bhave

Abstract:

Standardization of micropropagation technique in kartoli (*momordica dioica* Roxb.).

ABSTRACT

The present investigation was aimed to standardize micropropagation technique in kartoli. The three type of explants namely; apical bud, axillary bud and seeds were used for this experiment.

Treatment involving bavistin (0.1%) and $HgCl_2$ (0.1%) for 7 and 10 minutes respectively proved to be the best treatments in apical and axillary buds as these treatments recorded maximum proliferating cultures

along with better per cent of culture establishment. In seed explants, treatment of bavistin (0.5%) for 5 minutes and $HgCl_2$ (0.5%) for 5 minutes recorded maximum establishment of cultures. MS medium along with 10.0 mg/l BAP+ 80 mg/l Ads observed to be the best combination for establishment. On this combination apical buds showed 93.33% establishment whereas axillary buds showed 81.66% established cultures. The seed explants showed zero per cent germination on various establishment media.

The best multiple shoot initiation was observed on the media combination of MS+ 10.0 mg/l BAP+ 80 mg/l Ads. On this medium 88.33% cultures of the apical bud and 66.66% cultures of the axillary buds showed multiple shoot initiation. On the same media combination single kartoli culture of apical bud and axillary bud produced maximum of 15 and 11 multiple shoots per explant, respectively. MS with different levels of IAA, IBA and NAA were tried for root induction in which media combination MS+ 1mg/l IBA gave maximum rooting percentage (96.66%) and produced 15 roots per explant. The different potting mixtures like soil, sand, FYM and vermiculite were used for hardening among which vermiculite proved to be the best potting mixture for hardening of kartoli cultures.

Name of the candidate: T. A. Bagkar

Degree for which the thesis/project report submitted: M. Sc. (Ag. Biotechnology)

Year of submission: 2013

Name of the Guide/ Co guide: Dr. S. V. Sawardekar

Abstract:

Sex determination of Kokum (*Garcinia indica* Choisy.) by RAPD markers.

ABSTRACT

Determination of sex in Kokum (*Cv.* Konkan Amruta) is of utmost importance from the commercial agricultural point of view, since the sexuality cannot be distinguished prior to flower initiation. The use of bio-molecular techniques in sex determination of Kokum presents a potential theoretical significance and economic value. For this analysis, quality DNA is a prerequisite. Since this crop is having lot of phenolic compounds, optimization of DNA isolation protocol and use of RAPD markers to determine sex of Kokum has been used in the present investigation.

The DNA were extracted from young leaves of Kokum from 5 male and 5 female plants. The standardization of buffer constituents for DNA isolation was carried out. In rapid method five components of the extraction buffer were standardized and in CTAB method, three concentrations of CTAB powder were used. The results obtained using 0.900g glucose, 0.100g PVP, 0.040g sodium bisulphite, 0.050g sodium lauryl sulphate, 500µl sarcosine in Rapid method and 2% CTAB in CTAB method of DNA isolation a were satisfactory.

The most suitable combination of 100 mM Tris–HCl, pH 8.0, 50 mM EDTA and 1 M NaCl as standardized buffer constituents showed clear and specific banding pattern. Modifications in extraction

procedure resulted into better and clear banding pattern when subjected to PCR analysis.

Modification in PCR reaction mixture (1µl dNTPs, 0.5µl Taq polymerase) and thermoprofile (Annealing temperature for RAPD and ISSR markers 39 °C and 50 °C, respectively) showed clear and specific banding pattern.

Out of the total 60 RAPD and 11 ISSR decamer primers used in the investigation, 20 primers showed amplification. Out of which OPA-03, OPA-5, OPD-01, OPD-02, OPD-05, OPD-11, OPQ-09 and UBC-807 showed polymorphism between male and female plants of Kokum. Among these 8 primers, OPA-5 showed male specific banding pattern which was 80% reproducible.

Name of the candidate: H. G. Gilande

Degree for which the thesis/project report submitted: M. Sc. (Ag. Biotechnology)

Year of submission: 2014

Name of the Guide/ Co guide: Dr. N. B. Gokhale

Abstract:

Analysis of genetic variability of γ-irradiated mutant lines of finger millet using molecular markers

ABSTRACT

The molecular marker technology has a great potential for assessing genetic variability and relationship among the selected mutant lines of finger millet. In the present study, gamma radiation induced mutants of finger millet cv. Dapoli-1 showing distinct morphological differences were screened using 16 ISSR markers.

The DNA was extracted from the green leaf samples collected from 15 days old seedlings of finger millet from 31 mutant lines and one control parent line Dapoli-1 by rapid DNA extraction method. The results obtained using 900mg Glucose, 50mg SDS, 300mg PVP, 40mg Sodium bisulphite and 500 µl Sarcosine in rapid method. The most suitable combination of extraction buffer was found to be 200 mM Tris-HCL having pH 8.0, 25 mM EDTA, 250 mM NaCl which showed clear and specific banding pattern when subjected to PCR.

Initially the PCR master mix was standardized by changing the quantity of each component and the optimum concentration of each component in master mix was used for further ISSR analysis. In which 10 mM (1µl) dNTPs concentration and Taq polymerase 3 U (0.5 µl) gave better amplification. The annealing temperature ranging from 43.5^{0}C to 54.8^{0}C for 1 minute yielded good results.

The finger millet DNA showed better amplification with 16 ISSR primers studied. A total of 1808 bands amplified and out of which 1776 were polymorphic that means all of the 16 ISSR markers showed 97.43 % polymorphism. The primer UBC-827 showed 58.97 minimum per cent polymorphism while the average bands per primer were 113.

The ISSR profile generated by each of the primer was analyzed using standard DNA ladder (1353-310bp and 5000-250bp) and compared with their respective banding pattern. The average size of amplified

fragment ranged from 337bp to 1390bp. The minimum PIC value gave by the primer UBC-820 (0.33) and the maximum PIC value gave by the primer UBC-847 (0.89) and average polymorphic information content is 0.76 among the all 31 mutants. It indicates that ISSR markers having great potential to show the polymorphism among the finger millet mutants.

The data of 31 mutants and one control parent of finger millet were used to generate pair-wise matrix based on the Jaccard's co-efficient. The genetic distance was calculated on the basis of pooled data and the dendrogram was constructed. The similarity coefficient ranged from 0.096 (in between the mutant lines DML-18 and DML- 31) to 0.629 (in between mutant lines DML-1, DML-2 and mutant lines DML-17, DML-18) indicated the distinctness of these mutants.

Cluster analysis was carried out based on UPGMA analysis and it was divided 31 mutants and 1 control parent line into two main clusters and each having two subclasses. The first subclass of first cluster containing 9 mutants and the second subclass consist of 3 mutants. The first subclass of second cluster contains only one mutant line DML-31 while the second subclass of second cluster consist 19 mutant lines. In the present study it was observed that the mutant line DML-31 occupied a unique position and was most diverse from rest of 30 mutants and one control parent Dapoli-1.

Name of the candidate: S. R. Kamble

Degree for which the thesis/project report submitted: M. Sc. (Ag. Biotechnology)

Year of submission: 2014

Name of the Guide/ Co guide: Dr. S. V. Sawardekar

Abstract:

Callus mediated regeneration in rice (*Oryza sativa L.*) and its *in vitro* screening against salinity

ABSTRACT

In vitro experiment was conducted to assess the effect of salt stress on callus induction, survival and regeneration in salt sensitive genotype Karjat-5 and salt tolerant genotypes namely, Panvel-3 and FL-478. Among the different surface sterilizing treatments deployed, the use of alcohol (70%) for 1 minute and $HgCl_2$ (1%) for 10 minutes duration recorded maximum aseptic culture establishment in all three varieties.

Different media combinations created variable response for callus establishment. Early callus induction observed in Panvel-3 (12.41 days) as compared to FL-478 (16.41 days) and Karjat-5 (14.00 days). Callus induction frequency showed in all 3 genotypes was better on media combination, MS supplemented with 2,4-D and BAP. Among the all three varieties FL-478 showed better callus proliferation at higher NaCl concentration (10 $dSm^{-1)}$ fallowed by Panvel-3 (8 dSm^{-1}) and Karjat-5 (6 dSm^{-1}). In FL-478 weight of callus decreased up to 46 mg at 10 dSm^{-1} conc. of NaCl which is better performance as compared to other verities such as karjat-5 and panvel-3. Panvel-3 showed 89 mg weight reduction of callus during increased 121 mg on media without salt concentration. Drying of callus observed at salt conc.10 dSm^{-1}. The results revealed that

the salt tolerant genotypes panvel-3 recorded increase in weight of callus at increasing levels of NaCl concentrations than the salt susceptible genotype karjat-5. Similarly, somaclones regenerated from Panvel-3 showed variation in grain size as well as grain type. It has shown fine grain type as compare to the parent variety.The screening of these somaclones are also done at field condition which has shown tolerance upto 8 dSm^{-1}.

Name of the candidate: D. P. Nikam

Degree for which the thesis/project report submitted: M. Sc. (Ag. Biotechnology)

Year of submission: 2014

Name of the Guide/ Co guide: Dr. S. G. Bhave

Abstract:

Dna fingerprinting of released rice varieties of dr. B. S. Konkan krishi vidyapeeth, dapoli through ssr markers

ABSTRACT

The present investigation was carried out with an objective of characterization of rice varieties released by Dr. B. S. Konkan Krishi Vidyapeeth, Dapoli through SSR primers. A total of 35 SSR primer pairs distributed through out the genome were used for molecular analysis of 23 rice varieties. All 35 microsatellite markers were found to be polymorphic. The average size of amplified products ranged from 121.86 bp to 217.14 bp. A total of 184 alleles were obtained using 35 SSR primer pairs with an average of 5.26 alleles per primer. The number of alleles amplified for each primer pair ranged from 2 to 8. The markers RM-343, RM-112 and RM-224 generated a maximum number of alleles (8). While the primers RM-315 and RM-223 produced minimum number of alleles (2). The PIC values of primers ranged from 0.23 in SSR primer RM 315 to 0.78 in SSR primers RM 318, RM 276 and RM 343 with an average PIC value 0.58 of all the primers. Resolution factor (Rf) values of primers ranged from 0.574 in primer RM 318 to 0.763 in SSR primer RM 85 with an average Resolution factor (Rf) value 0.675 of all the primers. The minimum Resolution factor (Rf) value is 0.574 of the primer RM-318 while, maximum Resolution factor (Rf) value is 0.763 of the primer RM-85.

The Jaccard’s similarity coefficient values among these rice varieties are observed. The pair wise similarity values ranged from 0.016 to 0.578. Maximum similarity value of 0.578 was noticed between Ratnagiri-5 and Ratnagiri-4. Minimum similarity value of 0.016 was observed between Ratnagiri-4 and Karjat-184. From these studies, it is revealed that, rice varieties are more divergent indicating that large part of the genome may be dissimilar among themselves.

UPGMA cluster analysis was performed using Jaccard’s similarity coefficient matrices calculated from SSR markers to generate a dendrogram for 23 rice varieties. A pairwise similarity index (SI) was calculated and the UPGMA based dendrogram of 23 rice varieties generated with Multivariate Statistical Package (MVSP). UPGMA grouped 23 rice varieties into two main clusters which were further divided into two sub-clusters.

Name of the candidate: A. S. Chungada

Degree for which the thesis/project report submitted: M. Sc. (Ag. Biotechnology)

Year of submission: 2015

Name of the Guide/ Co guide: Dr. N.B. Gokhale

Abstract:

Molecular screening of rice germplasm for biotic and abiotic stresses

ABSTRACT

The present study was carried out to screen the rice germplasm for biotic and abiotic stresses tolerance in 55 genotypes using 18 microsatellite markers pairs distributed throughout the genome.

Blast resistance alleles were observed in the genotypes viz., Karjat-184, Karjat-1, IR 65598-112-2, Phule Maval, Bhogavati, Ratnagiri-5, Phule Samruddhe, Ratnagiri-24, Karjat-7, RNT-55-3-2, RNT-1-1-2-1, RNT-66-43-8, Karjat-3, Paras Sona, Panvel-3, Jaya, BR-827 and Karjat-5.

Salt tolerant genotypes include; Prabhavati, Ratnagiri-73, Karjat-1, Karjat-4, Paras Sona, RTN Purple, BR 827, IR 65598-112-2, Karjat-5, Ratna, Abhya, RNT-49-2-3-1-2, IR 68952-5-2-11-8-1, RNT 66-43-8, Jaya, Bhogavati, Phule Samruddhe, IR-46, Indrayani, Saysree, NPT-2, IR-54, RP-4-14, Panvel-1 and IR-44. Bacterial Blight resistant genotypes were; RNT 55-3-2, Ratnagiri-5, Karjat-184, Karjat-1 and KJT-6.

Two genotypes namely; Ratnagiri-60 and Ratnagiri-3 were observed tolerant to drought condition since it showed amplification at specific base pair. Gall midge resistant genotypes are Pawana, IR-68, Karjat-4, Ratnagiri-711, Abhya, Panvel-1, IR-44 and RP-4-14 observed in the present studies.

All the eighteen SSR primers used in this study amplified and showed the polymorphism in rice germplasm. A total of 231 loci were generated by 18 primers. Each primer thus produced on an average 12.83 loci in the size ranging from 169.5 bp to 317.22 bp in the 55 rice genotypes in relation to diversity assessment. UPGMA grouped 55 rice genotypes into two main clusters which were further divided into two sub-clusters.

This study will be helpful for selection of parental lines and development of new breeding population tolerant to specific traits through Marker Assisted Selection (MAS).

Name of the candidate: D V Rasam

Degree for which the thesis/project report submitted: M. Sc. (Ag. Biotechnology)

Year of submission: 2015

Name of the Guide/ Co guide: Dr. N. B. Gokhale

Abstract:

Molecular characterization of coconut (*cocos nucifera* l.) varieties by using molecular markers

ABSTRACT

The present study was carried out with an objectives to standardize the DNA isolation protocol of coconut and to characterize coconut varieties through molecular markers.

The DNA was extracted from tender leaf samples collected from the frond of five different trees of five coconut varieties. The standardization of buffer constituents for DNA isolation was carried out. Five components of the extraction buffer were standardized by using rapid method. The results obtained using 0.950 g glucose, 0.250 g PVP, 0.045 g sodium bisulphite, 0.055 g sodium lauryl sulphate, 500 µl were most suitable. Modifications in extraction procedure resulted into better and clear banding pattern when subjected to PCR analysis. Modification in PCR parameters like PCR master mixture and thermo profile showed clear and specific banding pattern.

The average per cent polymorphism showed by ISSR and SSR primers in between five varieties was 31.91 per cent and 92.86 per cent, respectively. In ISSR marker, the overall range of similarity between five varieties ranged from 0.657 to 0.775 whereas it was 0.037 to 0.304 in SSR primers.

Substantial average polymorphism was detected by ISSR primers among five samples of Banawali (23.16 %), Gangabondum Green Dwarf (24.18 %), Pratap (25.58 %), Konkan Bhatye Coconut Hybrid-I (27.08 %) and East Coast Tall (21.22 %). Average polymorphism was also detected by SSR primers among five samples of Banawali (85.71 %), Gangabondum Green Dwarf (86.90 %), Pratap (85.71 %), Konkan Bhatye Coconut Hybrid-I (100 %) and East Coast Tall (92.86 %).

Study indicates that genetic variation was found between five varieties and also within each variety of coconut. Analysis of both markers showed the superiority of SSR marker over the ISSR marker. Genetic diversity obtained within variety is not expected so there is need to maintain seed purity through artificial pollination.

Name of the candidate: Okello Moses

Degree for which the thesis/project report submitted: M. Sc. (Ag. Biotechnology)

Year of submission: 2015

Name of the Guide/ Co guide: Dr. S. V. Sawardekar

Abstract:

Molecular Screening of Rice Germplasm for Biotic and Abiotic Stresses

ABSTRACT

The present study was carried out to screen the rice germplasm for biotic and abiotic stress tolerance in 48 genotypes using 18 microsatellite markers pairs distributed throughout the rice genome.

Blast resistance alleles were observed in varieties viz., SR3-9, Norgual, Kamod-253, Waksal-2017, Jyoti, Sorty (Red Kernel), Surak, RTN Purpal, MO-9, Kolhapur Sunil, Chinoor, Panvel-61, CO-47-45-120, RS-113(Original), and Dular. RM-8225 primer was most prominent in the gene-specific amplicon.

Brown planthopper resistance alleles were observed in varieties viz., Kharamunga, Sonphala, Kolhapur sunil, Munga and Kothambir using the primer RM-6775.

Salt tolerant varieties includes; Kharamunga, SR3-9, Velchi, ManoharSali, Agni, Chinoor, Munga, Panvel-61, CO-47-45-120, RS-113(Original), Dular, MO-8, Kairli-PTB-49, Munga, TurgaBhat, Foxtail, Waksal-2017, Jyoti, Sorty (Red kernel), Kala rata, Ralak, Surak, RTN Purpal, Kolhapur sunil, MO-13, Rajkamal, Pak basmati, Patni-6, Bhada-79, Bamil, Pandy, Kharamunga, Kothambir, RP Bio-170.

Gall midge resistant varieties observed in the present studies are; Kamod-253, RS-113(Original), Kala rata, Surak, RTN purpal, Kolhapur sunil, Munga, Bhura rata, Pak basmati and Kharamunga.

Bacterial Blight resistant varieties ascertained were; Bela, MO-8, MO-9, Bhura rata, MO-5, MO-13, Pak basmati, Patni-6, Bhadas, Kairli-PTB-49 and RP Bio 197. RM-122 showed most prominence in identifying *Xa13* genes.

Drought tolerance alleles were observed in germplasm viz., Waksal-2017, Jyoti, Sorty (Red Kernel), Kala rata, and Pak basmati. RM-212 was most effective in screening drought tolerant genotypes.

All the eighteen SSR primers used in this study amplified and showed the polymorphism in rice germplasm. A total of 275 loci were generated by 18 primers. Each primer thus produced on an average 15.27 loci in the size ranging from 172.22bp to 329.44bp in the 48 rice varieties in relation to diversity assessment.

This study will be helpful for selection of parental lines and development of new breeding populations tolerant to specific traits through marker assisted selection (MAS).

Name of the candidate: P. P. Patil

Degree for which the thesis/project report submitted: M. Sc. (Ag. Biotechnology) Year of submission: 2015

Name of the Guide/ Co guide: Dr. S. G. Bhave

Abstract:

ABSTRACT

The molecular marker technology has a great potential for assessing genetic variability and relationship among the selected mutant lines. In the present study, gamma radiation induced mutants of finger millet cv. Dapoli-1 showing distinct morphological differences were screened using 15 ISSR markers.

The DNA was extracted from the green leaf samples collected from 15 days old seedlings of finger millet from 23 mutant lines and one control parent line Dapoli-1 by rapid DNA extraction method. The most suitable combination of extraction buffer was found to be 200 mM Tris-HCl having pH 8.0, 25 mM EDTA, 250 mM NaCl which showed clear and specific banding pattern when subjected to PCR.

Initially the PCR master mix was standardized by changing the quantity of each component and the optimum concentration of each component in master mix was used for further ISSR analysis. In which 10 mM (1µl) dNTPs concentration and Taq polymerase 3 U/µl (0.5 µl) gave better amplification. The annealing temperature ranging from 45.4^{0}C to 54.8^{0}C for 1 minute yielded good results.

The finger millet DNA showed better amplification with 15 ISSR primers studied. A total of 1321 bands were amplified and out of which 1201 were polymorphic which showed 91.87 % polymorphism. The primer UBC-857 showed 52.94 minimum per cent polymorphism while the average bands per primer were 88.06.

The ISSR profile generated by each of the primer was analyzed using standard DNA ladder (1353-310bp and 1000-100bp) and compared with their respective banding pattern. The average size of amplified fragment ranged from 450bp to 1100bp. The primer UBC-857 recorded minimum PIC value 0.11, whereas primer UBC-891 gave maximum PIC value 0.91 and average polymorphic information content is 0.73 among the all 23 mutants. It indicates that ISSR markers have a great potential to show the polymorphism among the finger millet mutants.

The data of 23 mutants and one control parent of finger millet were used to generate pair-wise matrix based on the Jaccard's co-efficient. The genetic distance was calculated on the basis of pooled data and the dendrogram was constructed. The similarity coefficient ranged from 0.131 (between the mutant lines DML-23 and DML- 02) to 0.683 (between mutant lines DML-17 and DML-16) indicating the similarities and distinctness of these mutants, respectively.

Cluster analysis was carried out based on UPGMA analysis and it divided 23 mutants and 1 control parent line into two main clusters and each having two sub-clusters. The first sub-clusters of first major cluster comprised of 6 mutants and the second sub-clusters also comprised of 6 mutants. The first sub-clusters of second major cluster had 8 mutants. While the second sub-clusters of second major cluster consisted of 4 mutant lines.

The mutant DML-6 with a low similarity coefficient of 0.140 with the parental line appears to be promising with tall, low tillering.

Name of the candidate: S. G. Mhatre

Degree for which the thesis/project report submitted: M. Sc. (Ag. Biotechnology)

Year of submission: 2015

Name of the Guide/ Co guide: Dr. S. V. Sawardekar

Abstract:

Analysis of callus generated somoclonal variation in proso millet (*Pannicum milliaceum* L) through molecular markers

ABSTRACT

The present investigation was carried out with an objective of ‘‘Analysis of callus generated somoclonal variation in proso millet (*Pannicum milliaceum* L) through molecular markers". A total of 16 ISSR primer were used for molecular analysis of 22 somaclones of sakhroli genotype and 16 somaclone of Asond with its control parent. Except UBC- 878, UBC-807 in Sakhroli and UBC- 816 in Asond genotype all ISSR markers were found to be polymorphic. The average size of amplified products ranged from 380 bp 1173 bp for Sakhroli and 300-1300 for Asond. A total of 158 alleles in Sakhroli genotype and 170 in Asond genotype were obtained with an average of 12 and 13 alleles per primer. The primer UBC-857 generated a maximum number of alleles (20) while the primers UBC-878 produced minimum number of alleles (2). The PIC values of primers ranged from 0.14 in ISSR primer UBC-844 to 0.88 in ISSR primers UBC-827 with an average PIC value 0.50 of all the primers sakhroli genotype while the PIC value of primers ranged from 0.39 in ISSR primer UBC-878 to 0.89 in ISSR primer UBC-891, with an average PIC value 0.69 in Asond genotype.

The Jaccard’s similarity coefficient values among these rice proso millet genotypes are observed. The pair wise similarity values ranged from 0.178 to 0.667in Sakhroli genotype while 0.120 to 0.536 in Asond . In Sakhroli genotype maximum similarity value of 0.667 was noticed between SSCN-5 and SSCN-6 while 0.536 was noticed between ASCN-16 and control parent in Asond. In Sakhroli genotype minimum similarity value of 0.178 was observed between SSCN-8 and control parent while 0.120 was noticed between ASCN-1 and ASCN-9. From these studies, it is revealed that, the ISSR marker is better tool for analysis of somaclonal variation in *in vitro* condition.

Name of the candidate: A. S. Badhe

Degree for which the thesis/project report submitted: M. Sc. (Ag. Biotechnology)

Year of submission: 2016

Name of the Guide/ Co guide: Dr. S. V. Sawardekar

Abstract:

Callus Mediated *In Vitro* Regeneration Studies in Banana (*Musa* sp.) cv. Safed velchi.

ABSTRACT

Present investigation aimed, to standardize sterilization technique for in vitro culture of banana (cv. Safed velchi) and to optimize media combinations for callus induction and in vitro regeneration. The study was conducted in Completely Randomized Design at Plant Biotechnology Centre, Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth, Dapoli, Dist. Ratnagiri (M.S.) during the academic year 2014-2016.

In surface sterilization, of Meristem explant, treatment involving Carbandazime (1%) for 30 min, Sodium Hypocloride (5%) for 10 min, Alcohol (70%) for 1 min and Cefotaxime (200mg/l) for 30 min duration

recorded maximum aseptic culture establishment (91.66%). In male flower explant, treatment involving Alcohol (70%) for 10 min duration recorded maximum aseptic culture establishment (88.33%). Among leaf, Male flower and Meristem explants, Meristem explant showed significantly better callus proliferation compared to leaf and Male flower explants. In leaf explant MS medium along with 2.0 mg/l 2,4-D + 0.5mg/ l BAP+ 0.5mg/l NAA, observed to be the best combination for callus establishment. On this combination, leaf explant showed 63.33% establishment. For male flower explant MS medium along with 2.5 mg/l 2, 4-D + 0.5mg/ l BAP + 0.5mg/l NAA showed 81.66% callus establishment. Whereas in meristem explant MS medium along with 4 mg/l 2, 4-D + 0.5mg/ l BAP + 0.5 mg/l NAA showed 88.33% callus establishment.

The proliferated callus were cut in to pieces and average 0.5 gm of callus put in to regeneration media. It has been observed that in all regeneration combinations tried, the media combination (MS + 5 mg/l BAP + 1.5mg/l NAA) showed development and shoots. The callus in all other media were greenish but did not showed regeneration.

Name of the candidate: S. S. Kadam

Degree for which the thesis/project report submitted: M. Sc. (Ag. Biotechnology)

Year of submission: 2016

Name of the Guide/ Co guide: Dr. S. V. Sawardekar

Abstract:

Fidelity testing in tissue culture developed banana plantlets of *Safed velchi* and detection of *BBTV* through ELISA

ABSTRACT

The present study was carried out with an objectives to test fidelity of tissue cultured banana plantlets through molecular markers, to standardize protocol for viral detection and to test tissue cultured banana plantlets for *Banana Bunchy Top Virus (BBTV)* through ELISA technique.

The DNA was extracted from tissue cultured tender leaf samples of banana plantlets. A total of 200 plant samples were selected for extraction of genomic DNA from 3 mother plants (mother plant no. 15, 16 and 21). The standardization of buffer constituents for DNA isolation was carried out. Modifications in extraction procedure and in PCR parameters like PCR master mixture and thermo profile showed clear and specific banding pattern.

ISSR primer UBC-891 showed 0.12 per cent average polymorphism in third lot of mother plant no. 15. All other primers produced monomorphic banding pattern in remaining lots of mother plant no. 15, 16 and 21. The results revealed that, the monomorphic banding patterns between the selected plantlets and respective mother plant indicate the uniformity of tissue culture banana plantlets. It also indicates that, primer UBC-891 will be helpful in future to identify variation in tissue cultured plantlets of *Safed velchi*.

For detection of *BBTV* infection, 200 plant samples were used from mother plant no. 15, 16 and 21. It was observed that, only positive control and *BBTV* infected sample of banana plants showed positive results. While, all the remaining sample test wells showed the negative results.

It is also observed that, ISSR-PCR analysis and ELISA Assay is an effective technique for testing fidelity/uniformity and for the detection of *BBTV* infection of tissue cultured plants, respectively. These two tests have been made mandatory to all the tissue culture laboratories so that genetically pure clones and virus free plantlets will be detected.

Name of the candidate: V. G. Kelkar

Degree for which the thesis/project report submitted: M. Sc. (Ag. Biotechnology)

Year of submission: 2014

Name of the Guide/ Co guide: Dr. S. V. Sawardekar

Abstract:

Analysis of genetic variability among germplasm of finger millet by using ISSR marker.

ABSTRACT

The molecular marker technology has a great potential for assessing genetic variability and relationship among the selected germplasms. In the present study forty germplasm of finger millet showing distinct morphological differences were screened using 15 ISSR markers.

The DNA was extracted from the green leaf samples collected from 15 days old seedlings of finger millet from 40 gremplasms by rapid DNA extraction method. The combination of extraction buffer used was 200 mM Tris-HCl having pH 8.0, 25 mM EDTA, 250 mM NaCl which showed clear and specific banding pattern when subjected to PCR.

Optimum concentration of each component in master mix was used for further ISSR analysis. In which 10 mM (1µl) dNTPs concentration and Taq polymerase 3 U/µl (0.5 µl) gave better amplification. The annealing temperature ranging from 40.4^{0}C to 56.7^{0}C for 1 minute yielded good results.

The finger millet DNA showed better amplification with 15 ISSR primers studied. A total of 1876 bands were amplified and out of which 1552 were polymorphic which showed 83.32 % polymorphism. The primer UBC-834 showed 29.82 minimum per cent polymorphism while the average bands per primer were 124.8.

The ISSR profile generated by each of the primer was analyzed using standard DNA ladder (1353-310bp) and compared with their respective banding pattern. The average size of amplified fragment ranged from 200bp to 1650bp. The primer UBC-872 recorded minimum PIC value 0.20, whereas primer UBC-841 gave maximum PIC value 0.88 and average polymorphic information content is 0.70 among the all 40 germplasms. It indicates that ISSR markers have a great potential to show the polymorphism among the finger millet germplasms.

The data of 40 germplasms of finger millet were used to generate pair-wise matrix based on the Jaccard's Similarity co-efficient. The genetic distance was calculated on the basis of pooled data and the dendrogram was constructed. The similarity co-efficient ranged from 0.197 (between germplasm Nagali-55 and KMR-204) to 0.679 (between germplasm VR-762 and PR-202) indicating the distinctness and similarities of these germplasm.

Cluster analysis was carried out based on UPGMA analysis and it divided 40 germplasms into two main clusters and each having two sub-clusters. The first sub-clusters of first major cluster comprised of 13 genotypes and the second sub-clusters also comprised of 20 genotypes. The first sub-clusters of second major cluster had 1 genotype. While the second sub-clusters of second major cluster consisted of 6 genotypes.

Name of the candidate: B. P.. Kamble

Degree for which the thesis/project report submitted: M. Sc. (Ag. Biotechnology)

Year of submission: 2017

Name of the Guide/ Co guide: Dr. S. V. Sawardekar

Abstract:

Evaluation of Rice(*Oryza sativa* L.) Germplasm for Biotic and Abiotic Stresses through SSR markers.

ABSTRACT

The present study was carried out to evaluatethe rice germplasm for biotic and abiotic stress tolerance in 50 rice germplasm using 13 microsatellite markers pairs distributed throughout the rice genome.

Bacterial Leaf Blight resistant germplasm ascertained were; RM-122 (*Xa13*) showed the most prominence in identifying *Xa13* genes with the presence of resistance linked allele in nine varieties; IRBL-3-CP4/RL, Pavani, Mahadi, RPHR-46-33-38, IRBL KH-K (CO), Jeeviga samba, ADT-27, Basmati 386, RPB-10-170, IR-1552.

Blast resistance alleles were observed in germplasm viz.Dular, BSL-307, IRBL-60, TRL-165, Sarleshwari, Bentaphool, IRBLa-C, Indira maheshwari, Jaldubi, RM-8225 primer was most prominent in the gene-specific amplicon.

There was two SSR primers RM-6775 and RM-5479 used to observed Brown planthopper resistance alleles linked in fifteen rice germplasm viz; IRBL-3-CP4/RL, SYE-18-1210, Junakolam, BSL-307, Pavani, RP-4331-6-1-1-1-3, Phuleradha, IRBL-ZT, IRBL1-LA(CO), Mahamaya, HS-479521BR, SPV-2, TRL-165, Jeevigasamba, CR-57MR-15-2 conferred resistance. RM-5479 at 152bp showed resistance in varieties viz; Junakolam, BSL-307, RPHR-46-33-38, IRBLKH-K-(CO), RP-4311-6-1-1-1-3, IRBL-ZT, RPB-10-170, WGL-75, IR-64, Siddhagiri, RP-4331-6-1-1-1-3, IR-1552, ADT-27, CR-57MR-15-2, Shamlausing the primer RM-6775.

Gall midge resistant varieties observed in the present studies are; IRBL9-W/RL, TRL-165, ADT-27, CR-57-MR-15-2, IRBL-60, IRBL5-M(CO), Bentaphool, IRBL-ZT, IRBL1-LA(CO), IRBL 1-F5, CN-1405-82-2-9, SPV-2, RTN-16-3-1-12, BSL-307, RPB-10-170, IRBL7-M(CO), IRBLa-C, Sarleshwari, Barleshwari, Ghansal, Phuleradha, Jaldubi, Mahadi, Shamla, Mahamaya, Siddhigiri, Indiramaheshwari, Jeevigasamba. Junakolam.

Salt tolerant varieties includes;IPN-24, Dular, Phuleradha, IRBLa-C, BSL-307, Pawani, Sarleshwari, Bentaphool, RP-4331-6-1-1-1-3, Jaldubi, Mhadi, Belgambasmati, RTN-16-3-1-12, Jeevigasamba, ADT-27 and CR-57-MR-12-5.

Drought tolerance alleles were observed in germplasm viz., Sagbhat, Najarbhat, SYE-18-1210, IRBL-60, Ghansal, RPHR-46-33-38, IRBL KH-K(CO), Indira Maheshwari, Belgambasmati, SPV-2, Sorty, ADT-27. RM-212 was most effective in screening drought tolerant genotypes.

All the thirteen SSR primers used in this study amplified and showed the polymorphism in rice germplasm. A total of 280 loci were generated by 13 primers. Each primer thus produced on an average 21.53 loci in the size ranging from 155.76 to 286.53bp in the 50 rice varieties in relation to diversity assessment.
This study will be helpful for selection of parental lines and development of new breeding populations tolerant to specific traits through marker assisted selection (MAS)

Name of the candidate: S. R. Kamble

Degree for which the thesis/project report submitted: M. Sc. (Ag. Biotechnology)

Year of submission: 2017

Name of the Guide/ Co guide: Dr. N. B. Gokhale

Abstract:

Genetic diversity analysis in cashew (*Anacardium occidentale* L.) varieties by using ISSR marker.

ABSTRACT

The molecular marker technology has a great potential for assessing genetic diversity and relationship among the selected varieties. In the present study nine varieties of cashew showing distinct morphological differences were screened using 27 ISSR markers.

The DNA was extracted from the green leaf samples collected from 9 cashew varieties. The standardization of buffer constituents for DNA isolation was carried out. Five components of the extraction buffer were standardized by using rapid method. The results obtained using 0.950 g glucose, 0.250 g PVP, 0.045 g sodium bisulphite, 0.055 g sodium lauryl sulphate, 500 µl were most suitable. Modifications in extraction procedure resulted into better and clear banding pattern when subjected to PCR analysis. Modification in PCR parameters like PCR master mixture and thermo profile showed clear and specific banding pattern.

The cashew DNA showed better amplification with 27 ISSR primers studied. A total of 1152 bands were amplified and out of which 882 were polymorphic which showed 73.52 % polymorphism. The primer UBC-843 showed 30.76 minimum per cent polymorphism while the average bands per primer were 42.66.

The ISSR profile generated by each of the primer was analyzed using standard DNA ladder (10kb) and compared with their respective banding pattern. The size of amplified fragment ranged from 300bp to 2000bp. The primer UBC-843 recorded minimum PIC value 0.25, whereas primer UBC-876 gave maximum PIC value 0.91 and average polymorphic information content is 0.70 among the all 9 varieties. It indicates that ISSR markers have a great potential to show the polymorphism among the cashew varieties.

The data of 9 varieties of cashew were used to generate pair-wise matrix based on the Jaccard's Similarity co-efficient. The genetic distance was calculated on the basis of pooled data and the dendrogram was constructed. The similarity co-efficient ranged from 0.381 (between varieties Vengurla-2 and Vengurla-8) to 0.649 (between varieties Vengurla-4 and Vengurla-5) indicating the distinctness and similarities of these varieties.

Cluster analysis was carried out based on UPGMA analysis and it divided 9 varieties into two main clusters and each having two sub-clusters. The first sub-clusters of first major cluster comprised of 1 variety and the second sub-clusters comprised of 2 varieties. The first sub-clusters of second major cluster had 3 varieties. While the second sub-clusters of second major cluster consisted of 3 varieties.

Name of the candidate: Miss. Mahadik Supriya Mahadev

Degree for which the thesis/project report submitted: M. Sc. (Ag. Biotechnology)

Year of submission: 2017

Name of the Guide/ Co guide: Dr. S. V. Sawardekar

Abstract:

Standardization of callus development technique in sarpagandha (*Rauwolfia serpentina*) for isolation of secondary metabolites

ABSTRACT

Present investigation aimed to standardize sterilization technique in various explants of *Rauwolfia serpentina*, to study callus induction technique of *Rauwolfia serpentina* and qualitative analysis and quantitative estimation of secondary metabolites in callus of *Rauwolfia serpentina*. The study was conducted in Factorial Completely Randomized Design at Plant Biotechnology Centre, Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth, Dapoli, Dist. Ratnagiri (M.S.) during the academic year 2015-2017.

The surface sterilization treatment T_9 (Carbendazim (0.1%) (10 min) + Ethanol (70%) (30 sec) + $HgCl_2$ (0.1%) (5 min)) showed the maximum frequency of aseptic callus developed in meristem explants (95.33%). Leaf disc, leaf node and meristem explants were transferred to MS medium containing different combinations of PGRs. Among the various combinations of 2, 4-D (0.5-5.0 mg/l) and BAP (1.0 mg/l) the

frequency of callus induction was highest (94.67%) on MS + 2.0 mg/l 2.4-D+1.0 mg/l BAP when leaf disc used as explant. During organogenic callus formation, different types of calli with variation in color and texture were noticed and among them, the light green, fragile calli responded well for the induction of shoots. Among the various combinations of BAP and NAA used the frequency of shoot regeneration was highest 98.33% in BAP (4.5 mg/l) + NAA (0.5) mg/l. Maximum shoots (15.33) per culture were obtained from the leaf callus inoculated in shooting medium BAP (4.5 mg/l) + NAA (0.5 mg/l). The rooting was induced in *in vitro* regenerated shoots in MS medium containing NAA (1.0 mg/l) + BAP (0.1 mg/l) + Activated Charcol (1 g/l) and 95.33 % rooting was obtained. The *in vitro* regenerated plantlets were transferred to the pots with 75% success. The reserpine content was measured in callus and root of *Rauwolfia serpentina*. Thin layer chromatography reveals that callus culture accumulated reserpine as in the roots of parent plant. Spectrophotometric estimation showed that concentration of reserpine observed in callus extract was higher (6.8 µg/ml) than the concentration of reserpine observed in root extract (6.4 µg/ml).

In the present investigation, the analytical TLC and UV spectrophotometric data revealed that reserpine present in callus as well as root of cultivated plant. Reliable protocol can be used for regeneration of sarpagandha from leaf, leaf node and meristem explants with much higher rate of multiplication.

Name of the candidate: Keskar Kiran Pandurang

Degree for which the thesis/project report submitted: M. Sc. (Ag. Biotechnology)

Year of submission: 2018

Name of the Guide/ Co guide: Dr. S. V. Sawardekar

Abstract:

“Standardization of in vitro regeneration technique in red banana (Musa acuminata)”

ABSTRACT

The present investigation entitled, “Standardization of in vitro regeneration technique in red banana (Musa acuminata)” was aimed to develop surface sterilization techniques and optimization of growth hormones for in vitro regeneration of red banana and fidelity testing of tissue cultured red banana plantlets through molecular markers. The study was undertaken in completely randomized design with 3 replications.

The surface sterilization treatment of 5ml/L Tween20 for 10min, (5ml Dettol + 45ml savlon)/L for 30min ,1% Carbendazim for 30 min, 70% Ethanol for 1 min, 5% NaOCl for 10 min, 250 mgL-1 cefotaxime (I) for 20 min, 250 mgL-1 cefotaxime (II) for 40 min was found to be best combination to achieve highest percentage of contamination free healthy cultures (67.80%) and gave best response for minimum days to shoot initiation i.e. 22 days.

Media combination MS + 4.5 mgL-1 BAP + 0.175 mgL-1 IAA + 20 mgL-1 ascorbic acid showed highest establishment (78%) of explants. The media combination MS + 4.5 mgL-1 BAP + 0.175 mgL-1

IAA + 20 mgL-1 ascorbic acid was found effective for maximum number of shoots initiation (96.67%) and maximum rate of multiple shooting (560.29). Same media combination with addition of 1gL-1 charcoal was also found effective for highest percentage of root regeneration (93.33) and maximum number of roots per shoot with an average of 15.34 roots per shooted plant.

The days required for shoot initiation ranged between 21-30 days and for rooting ranged between 14 to 20 days. The rooted plants transferred in potting mixture of sand, soil and FYM present in equal proportion (1:1:1) recorded 90 per cent survivability. The best month for inoculation of explants for culture was July for highest proliferation.

In fidelity testing of red banana, out of 20 ISSR primers, 11 primers resulted in clear and scorable amplification products. Average per cent polymorphism was 0. Range of size of product within bulked DNA was 200-2000bp. Average number of alleles produced per marker was 4.81 The monomorphic banding patterns between the selected plantlets from single mother plant indicates the uniformity of tissue culture banana plantlets.

Key word : red banana, surface sterilization, growth hormones, fidelity

Name of the candidate: Randive Pragati Mangesh

Degree for which the thesis/project report submitted: M. Sc. (Ag. Biotechnology)

Year of submission: 2018

Name of the Guide/ Co guide: Dr. N.B. Gokhale

Abstract:

Characterization and diversity analysis of Rice (*Oryza sativa* L.) genotypes for biotic and abiotic stresses through molecular markers

ABSTRACT

The present study was carried out to screen the rice germplasm for biotic (Blast, Bacterial Blight, Brown Plant Hopper, Gall Midge) and abiotic (Drought, Salt) stress tolerance in 50 genotypes using 19 SSR markers.

Blast resistance linked alleles, for gene Pi-z detected by primer RM8225 were observed in the genotypes viz., IRBB 53, IR 11 N 223, IRBB 57, IRBB 58 and for gene Pi-1 detected by primer RM5926 were observed in the genotypes viz., B 40, IRBB 13, IRBB 65, IRBB 66, 12 DS-GMEI-22, IR 64.

Bacterial Blight resistance linked alleles, for gene Xa5 detected by primer RM122 were observed in almost all the genotypes.

Brown Plant Hopper resistance linked alleles, for gene Bph25 detected by primer RM6775 were observed in the genotypes; IRBB 13, IRBB 14, IRBB 64, 12 DS-GMEI-22, HHZ5-DT20-DT3-Y2, IR 05 A 272, IR 64.

Gall midge resistance linked alleles, for gene Gm4 detected by primer RM547 were observed in the genotypes; IR 11 N 169, IR 11 N 239, B 40, IRBB 2, IRBB 4, IRBB 5, IRBB 10, IRBB 50 and for

gene Gm8 detected by primer 22709 were observed in the genotypes; IR 11 A 546, IR 11 A 581, IRBB 8, IRBB 10, IRBB 14, IR 11 N 223.

Saltol QTL detected by primer RM140 was observed in the genotypes; IR 11 A 546, IR 11 A 581, IR 11 N 121, IR 11 N 137, IR 11 N 169, B 40, IRBB 7, IRBB 50, IRBB 61, IRBB 62. Saltol QTL detected by primer RM1287 was observed in the genotypes; IR 552, IRBB 1, IRBB 2, IRBB 3. Saltol QTL detected by the marker RM3412 was observed in the genotypes ; IR 11 N 137, IR 11 N 169, B 40, IRBB 4, IRBB 5, IRBB 50, IRBB 51, IRBB 53, IRBB 54, IRBB 55, IRBB 56, IRBB 57, IRBB 60, IRBB 61, IRBB 62.

Drought tolerance linked alleles detected by the marker RM-212 were observed in the genotypes; IRBB 55, IR 11 N 400, IRBB 57, IRBB 62, IRBB 63.

Among all the screened genotypes; B 40 showed presence of multiple resistance traits for the Blast, Bacterial Blight, Gall Midge, Saltol and IRBB 57 showed presence of multiple resistance traits for Blast, Bacterial Blight, Gall Midge, and Drought. Moreover genotypes; IR 11 A 546, IR 11 A 581, IR 11 N 169, IRBB 2, IRBB 4, IRBB 5, IRBB 13, IRBB 64, IRBB 12 DS-GMEI-22, IR 64, IRBB 55, IRBB 62, IRBB 14, IRBB 50, IR 11 N 223 showed presence of multiple resistance for three traits. Hence these genotypes appear to be promising genotypes for presence of multiple resistance traits.

Genotype IRBB 10 showed presence of resistance Linked Alleles for two Gall Midge resistance genes Gm4 (270bp) and Gm8 (170bp).Hence it appears to be promising for Gall Midge Resistance trait. Genotypes IRBB 61 and IRBB 62 showed presence of resistance Linked Alleles for two Saltol genes. Hence it appears to be promising for Saltol Resistance trait.

All the nineteen SSR primers used in this study amplified and showed the polymorphism in rice genotypes. A total of 103 alleles were detected with an average of 5.42 alleles per locus. The polymorphism information content (PIC) values ranged from 0.40 to 0.90 with an average PIC value of 0.65 per primer. UPGMA grouped 50 rice genotypes into two main clusters which were further divided into two sub-clusters.

This study will be helpful for selection of parental lines and development of new breeding population tolerant to specific traits through Marker Assisted Selection (MAS).

Name of the candidate: S. M. Borade

Degree for which the thesis/project report submitted: M. Sc. (Ag. Biotechnology)

Year of submission: 2018

Name of the Guide/ Co guide: Dr. S. V. Sawardekar

Abstract:

Diversity Analysis of promising mutant lines of finger millet through ISSR marker

ABSTRACT

The molecular marker technology has a great potential for assessing genetic variability and relationship among the selected mutant lines. In the present study, promising mutants of finger millet along with 3 check varieties showing distinct morphological differences were screened using 18 ISSR markers.

The DNA was extracted from the green leaf samples collected from 15 days old seedlings of finger millet from 10 mutant lines along with 3 check varieties by rapid DNA extraction method. The most suitable combination of extraction buffer was found to be 200 mM Tris-HCl having pH 8.0, 25 mM EDTA, 250 mM NaCl which showed clear and specific banding pattern when subjected to PCR.

Initially the PCR master mix was standardized by changing the quantity of each component and the optimum concentration of each component in master mix was used for further ISSR analysis. In which 10 mM (1µl) dNTPs concentration and Taq polymerase 3 U/µl (0.5 µl) gave better amplification. The annealing temperature ranging from 45.40C to 54.80C for 1 minute yielded good results.

The finger millet DNA showed better amplification with 18 ISSR primers studied. A total of 760 bands were amplified and out of which 747 were polymorphic which showed 98.83 % polymorphism. The primer UBC-834 showed 79.03 minimum per cent polymorphism while the average bands per primer were 42.22.

The ISSR profile generated by each of the primer was analyzed using standard DNA ladder (1353-310bp and 1800-200bp) and compared with their respective banding pattern. The average size of amplified fragment ranged from 388.8 bp to 1627.78bp. The primer UBC-814 and UBC-891(0.82) recorded minimum PIC value (0.25), whereas primer UBC-881 gave maximum PIC value 0.91 and average polymorphic information content is 0.50 among the all 10 mutants. It indicates that ISSR markers have a great potential to show the polymorphism among the finger millet mutants.

The data of 10 mutants along with 3 check varieties of finger millet were used to generate pair-wise matrix based on the Jaccard's co-efficient. The genetic distance was calculated on the basis of pooled data and the dendrogram was constructed. The similarity coefficient ranged from 0.112 (between the mutant lines M-3 and M- 21) to 0.49 (between mutant lines M-1 and Dapoli-2) indicating the similarities and distinctness of these mutants, respectively.

Cluster analysis was carried out based on UPGMA analysis and it divided 10 mutants along with 3 check varieties into two main clusters and each having two sub-clusters. The first sub-clusters of first major cluster comprised of 6 mutants and the second sub-clusters also comprised of 6 mutants. The first sub-clusters of second major cluster had 8 mutants. While the second sub-clusters of second major cluster consisted of 6mutant lines.

Name of the candidate: Patil Vinayak Babanrao

Degree for which the thesis/project report submitted: M. Sc. (Ag. Biotechnology)

Year of submission: 2019

Name of the Guide/ Co guide: Dr. S. V. Sawardekar

Abstract:

Molecular analysis of Mango (Mangifera indica L.) Cv. Alphonso from different locations of South Konkan

ABSTRACT

The present study was carried out with an objectives to establish the molecular profile of Alphonso from different locations of south konkan and to analyze the variation between them. The DNA was extracted from tender leaf samples collected from ten different selected locations (5 samples from each location). The standardization of buffer constituents for DNA isolation was carried out. Five components of the extraction buffer were standardized by using rapid method. The results obtained using 0.900 g glucose, 0.100 g PVP, 0.040 g sodium bisulphite, 0.050 g sodium lauryl sulphate, 500 µl sarcosyl were most suitable. Modifications in extraction procedure resulted into better and clear banding pattern when subjected to PCR analysis. Modification in PCR parameters like PCR master mixture and thermo profile showed clear and specific banding pattern. The average per cent polymorphism showed by 16 ISSR primers in between ten locations were 46.62 per cent. In ISSR marker, the overall range of similarity ranged from 0.559 to 0.733. The polymorphic information content (PIC) values ranged from 0.00 to 0.884 with an average PIC value of 0.733 per primer. UPGMA grouped 5 alphonso samples of each location into two main clusters. Substantial average polymorphism was detected by ISSR primers among five samples from Achara (42.83 %), Adivare (42.39 %), Rameshwar-Girye (42.73 %), Nadan-Jamsande (46.77 %), Katta (44.22%), Ambivali-Kelshi (44.68 %), Murud (46.29 %), Padel (30.45 %), Pawas (47.05 %) and Vengurle (42.67 %).

The ISSR analysis revealed the moderate polymorphism in Alphonso. Study indicates that genetic variation was found between selected ten locations and also within five samples of Alphonso of each location. Such study is important for detecting the distinctness of the same variety from different geographical locations and also for identification of desirable samples and it's utilization for further breeding program.

Keywords: Mango, Polymorphism, ISSR, Marker.

Name of the candidate: Patil Vishal Naval

Degree for which the thesis/project report submitted: M. Sc. (Ag. Biotechnology)

Year of submission: 2019

Name of the Guide/ Co guide: Dr. N.B. Gokhale

Abstract:

Molecular analysis of Rice (Oryza sativa L.) germplasm

ABSTRACT

The present study was carried out to screen the rice germplasm for biotic (Blast, Bacterial Blight, Brown Plant Hopper, Gall Midge) and abiotic (Drought, Salt) stress tolerance in 46 genotypes using 18 SSR markers. Blast resistance linked alleles, for gene Pi-1 detected by primer RM 5926 were observed in the genotypes viz., RP Bio 189, IET-8580, IR-1544-238-2-3, IR 665575-56-1-3-19, IR-60819-34-2-1, IR-35, IR-663879-195-2-2,

Kon-23, Kasturi, IET-23537-1351, IR-60919- 150-3-3. Bacterial Blight resistance linked alleles, for gene Xa5 detected by primer RM 122 were observed in almost all the genotypes. Brown Plant Hopper resistance linked alleles, for gene Bph25 detected by primer RM 6775 were observed in the genotypes; KJT-1- 1-21-3-19. Gall midge resistance linked alleles, for gene Gm8 detected by primer RM 22709 were observed in the genotypes; KJT 11-1-26-25- 23, IR-22896-225. Saltol QTL detected by primer RM 140 was observed in the genotypes; RP Bio 189, IET-8866, DBS-13-3-47-A9, SLR 51214, Gujrat-102, CR-57 MP-1523. Saltol QTL detected by the marker RM 3412 was observed in the genotypes; IET 13840-RP-66-67, HKR 2002-81, IR 664, KJT 11-1-26-25-23, IR 61614-38-19-3-2. Drought tolerance linked alleles detected by the marker RM 302 were observed in the genotypes; IR 56381-139-2-2. And by the marker RM 3825 were observed in the genotypes; IR 56381-139-2-2, Paras Sona, IR-50, IR-293-41-41-1, KJT 11-1-26-25-23. Among all the screened genotypes; KJT 11-1-26-25-23 showed presence of multiple resistance traits for the Bacterial Blight, Gall Midge, Saltol, Drought. Moreover genotype; RP Bio 189 showed presence of multiple resistance for three traits. Hence these genotypes appear to be promising genotypes for presence of multiple resistance traits. Genotype IR 56381-139-2-2 showed presence of resistance Linked Alleles for two Drought Tolerance genes Dr(140bp) and Dr(147bp). Hence it appears to be promising for Drought Tolerance trait. All the eighteen SSR primers used in this study amplified and showed the polymorphism in rice genotypes. A total of 88 alleles were detected with an average of 4.88 alleles per locus. The polymorphism information content (PIC) values ranged from 0.13 to 0.82 with an average PIC value of 0.47 per primer. UPGMA grouped 46 rice genotypes into two main clusters which were further divided into two sub-clusters. This study will be helpful for selection of parental lines and development of new breeding population tolerant to specific traits through Marker Assisted Selection (MAS).

Keywords: Abiotic & Biotic stress, Germplasm, MAS, Marker, Polymorphism, SSR

Name of the candidate: Sartape Sumitra Satish

Degree for which the thesis/project report submitted: M. Sc. (Ag. Biotechnology)

Year of submission: 2019

Name of the Guide/ Co guide: Dr. S. V. Sawardekar

Abstract:

Study of in vitro regeneration regeneration in Mango (Mangifera indica L.)

ABSTRACT

The present investigation entitled, “Study of In Vitro regeneration technique in mango (Mangifera indica L.)” was aimed to establish axenic culture, to standardize explant type, size for callus induction and to explore the possibility of morphogenesis through regenerated calli. The study was undertaken in factorial randomized block design with 3 replications. Among the different surface sterilizing treatments deployed, treatment T5 (71.81%) for mango leaves, T3, T4 and T5 (100%) for mango inflorescence, T5 (63.33%) for apical bud, T4 (63.40%) for Villai Kolamban nucellar embryo, (85.33%) for Alphonso nucellar embryo was found to be best

combination to achieve highest percentage of aseptic cultures and showing highest per cent survival. Leaf disc and inflorescence explants transferred to MS medium containing different combinations of PGRs. Among the various combinations of 2, 4-D (0.5-4.0mgl-1), BAP (1.0mgl-1), NAA (0.5mgl-1) and PVP the frequency of callus induction was highest (92.85%) on MS + 3.0mgl-1 2,4 –D + 1mgl-1 BAP + 0.5 mgl-1 NAA+ 0.1gml-1 PVP when inflorescence used as explant. Among the various combinations of the frequency of callus induction were significantly found highest (67.30%) on MS + 2.5mgl-1 2,4 –D + 1mgl-1 BAP + 0.5 mgl-1 NAA+ 0.1gml-1 PVP when leaf disc used as explant. During organogenic callus formation, different types of calli with variation in color and texture were noticed but none of them responded for shooting. In apical meristem and nucellar embryo explants with different compositions of MS and B5 media were found only axenic culture further morphogenesis was not observed. Monoembryonic var. Alphonso showed very high axenic culture in vitro as compared to polyembryonic var. Villai Kolamban used in the investigation.

Key word: Mango, surface sterilization, axenic culture, callus

Name of the candidate: Mr. Gajare Tushar

Degree for which the thesis/project report submitted: M. Sc. (Ag. Biotechnology)

Year of submission: 2020

Name of the Guide/ Co guide: Prof. R.S.Deshpande

Abstract:

Standardization of sex discrimination technique in Kokum (Garcinia indica Choisy) using molecular markers

ABSTRACT

In the present investigation it was attempted to standardize the sex discrimination technique in Kokum by considering hermaphrodites along with male and female by using ISSR markers. The study utilized five samples each of male, female (cv. Konkan Amruta) and hermaphrodite of Kokum which were identified on the basis of their flowering and fruiting behavior. DNA isolated using rapid method was used for PCR amplification using 60 ISSR primers. Out of the 60 primers used for the study, 29 primers did not amplify, 3 primers showed intangible amplification, 23 primers were monomorphic and five were polymorphic. Out of these five polymorphic primers, UBC 807(Male specific), UBC 844 (Hermaphrodite specific) and UBC 845(Female specific) were not reproducible. Only two primers namely, UBC 825 and UBC 878 were reproducible with polymorphism of 20 and 63.63 percent respectively. The polymorphic bands UBC 8252000,2100 (Female specific), UBC 8781600 (female specific), UBC 878780,980 (hermaphrodite specific) were faint but reproducible. UBC 878 also produced two bands, UBC 878990,1900 in female and hermaphrodite but absent in males, three bands UBC 8781480, UBC 8781000 and 980 only in male and females and absent in hermaphrodites. The similarity matrix and cluster analysis revealed that hermaphrodites

are closely related to males than the females. Thus these markers successfully standardized the sex discrimination technique in Kokum. They can further be utilized for SCAR development and understanding the evolution of dioeciousness and various pathways and processes associated with it.

Name of the candidate: Mhatre Dhanashree Prashant

Degree for which the thesis/project report submitted: M. Sc. (Ag. Biotechnology)

Year of submission: 2020

Name of the Guide/ Co guide: Dr. S. V. Sawardekar

Abstract:

Dna barcoding for identification of rice (oryza sativa l.) varieties developed by Dr. Balasaheb sawant konkan krishi vidyapeeth, dapoli.

ABSTRACT

The present study was conducted with an objective to characterize and add a molecular tag i.e. the DNA barcode for the 20 varieties developed by Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth, Dapoli. The study involved analysis of these varieties by using 10 SSR markers and 10 barcoding loci markers. The evolutionary analysis and phylogenetic relationship study was done using MEGA software. CLUSTAL Omega online tool detected the conserved region in the varieties.

DNA barcodes derived from the chloroplast genome were used to identify varieties and in the conservation of breeding resources. The gene regions are chosen because they have less intraspecific (within species) variations than interspecific (between species) variations, which are known as the "Barcoding Gap". DNA barcoding has wider applications in the fields of taxonomy, conservation and identifications of different crops, trees and plants.

Two major types of seed grains were observed as bold and slender and categorized as short and long studied on the basis of morphological data. Amplification efficiency observed was about 100 % in 3 primers to 81.25 % in remaining primers. Fragment size range was 600-2900 bp in 10 loci. Sequencing efficiency of loci ranged from 100% to 18.75%. The estimated evolutionary divergence between sequences ranged from 0.000-2.170 (average 0.001-0.405).

The Maximum Likelihood values of maximum transitional and transversional rate were observed. The parsimony informative sites was estimated with maximum 161 sites recorded in *psbA-trnH,* followed by 159 sites *trnK* and 33 *rbcL,* 21 in *matK* and number of variable sites reported highest in *psbA-trnH* 568, *trnK* 457 and *matK-1M-matK3RIM* 285.While nucleotide diversity (per site pi) reported maximum in *trnK* 0.1455.

Phylogenetic relationship established using Neighbor Joining Method for all the 10 loci distinctly separated out Ratnagiri-2 for *matK,* Palghar-2 for *psbA-trnH* , Karjat-1 And Karjat-2 for *trnH-psbA* , Ratnagiri-4 for *atpH-atpI* , Panvel-2 for *petA-psbJ* , Karjat-7 for *trnK* and Ratnagiri-7 for *matK-1M-matK3RIM* . Both strand of *psbA-trnH* and *trnH-psbA* showed good discrimation power, also *petA-psbJ* showed higher discrimination. *trnK* distinctly separated four of the 16 varieties in alignments. *rbcL* did not

show discrimination but more of conserved region was seen whereas *matK* could give an average discrimination among the varieties. Barcodes were generated using online tool for the different loci considering the variations in sequence at nucleotide level.

Comparative analysis of SSR markers was done with RM 338 highly informative with PIC value of 0.631 and observing the most frequent band size among 20 varieties for the microsatellites markers. Jaccard's similarity coefficient ranged from 0.190 to 0.750. Cluster analysis was established by UPGMA and similarity index by Jaccard's coefficient.

In conclusion, this study provides a precursive assessment data that will be useful for extensive application of DNA Barcoding in not only rice varieties but other fruit crops, ornamental and forestal plants of Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth, Dapoli.

Name of the candidate: Miss. Bhosale Pooja

Degree for which the thesis/project report submitted: M. Sc. (Ag. Biotechnology)

Year of submission: 2020

Name of the Guide/ Co guide: R. S. Deshpande

Abstract:

Molecular Profiling of Mango (Mangifera indica L.) hybrids and their parents using molecular markers

ABSTRACT

The present study was carried out with an objective to study the band sharing pattern in mango (Mangifera indica L.) hybrids and their parents using molecular markers and to analyse the similarity and distinctiveness between them.

For the present study, the hybrids developed by Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth, Dapoli were used. These included Ratna, Sindhu, Konkan Ruchi, Suvarna and Konkan Samrat. The parent varieties comprised of Alphonso, Neelum, Ratna and Tommy Atkins. DNA was extracted from tender leaf samples collected from Regional Fruit Research Station, Vengurle. Using the rapid method, the five components of the extraction buffer were standardized. The results obtained using 0.900 g glucose, 0.100 g PVP, 0.040 g sodium bisulphite, 0.050 g sodium lauryl sulphate, 500 µl sarcosyl were most suitable. Modification in PCR parameters like PCR master mixture and thermo profile resulted in clear and specific banding pattern.

The average per cent polymorphism showed by 13 ISSR primers amongst four parent varieties and

five hybrid varieties was 75.64 & 79.41 per cent, respectively. The average per cent polymorphism across the 13 primers amongst eight mango varieties was 83.92 per cent. The polymorphic information content (PIC) values ranged from 0.575 to 0.913 with an average PIC value of 0.825 per primer. UPGMA followed the similar pattern of grouping the individuals; i.e. it separated all the varieties into two main clusters.

The results of present study indicated the efficiency of ISSR markers in examining the genetic variation in the crop at molecular level. The study helps in getting the molecular profile of each hybrid which will assist the mango growers to identify the planting material at the juvenile stage itself. The findings of this molecular study can establish the identity and creditability of the variety at molecular level and substantiate its claim in PPVFRA registration at national and international level.

Name of the candidate : Pawar Pravin Bhausaheb

Degree for which the thesis/project report submitted: M. Sc. (Ag. Biotechnology)

Year of submission: 2020

Name of the Guide/ Co guide: Dr. S. V. Sawardekar

Abstract:

Study of in vitro regeneration technique in elephant foot yam (Amorphophallus paeoniifolius L.)

ABSTRACT

The present investigation entitled, “Study of in vitro regeneration technique in elephant foot yam (Amorphophallus paeoniifolius L.)” was aimed to standardize surface sterilization technique and to standardize culture establishment technique for in vitro regeneration of elephant foot yam. The study was conducted in completely randomized design with 3 replications.

The surface sterilization treatment of 750 mg/L Carbendazim (overnight) before cut, 12.5 ml/L Sodium hypochlorite (20 min), 10 ml/L Tween 20 (15 min), 10 ml/L Dettol and 50 ml/L Savlon (30 min), 1 g/L Carbendazim (10 min), under running warm water (1 hr), 2 g/L Carbendazim (15 min), 70 % Ethanol (2 min), 0.12 g/L Mercuric Chloride (15 min), 50 ml/L Sodium hypochlorite (5 min), 16 ml/L GA3 (10 min), + 3 ml/L Streptomycin (20 min), 750 mg/L Cefotaxime (I) (20 min) and 750 mg/L Cefotaxime (II) (40 min) was found to be best combination to achieve highest percentage of aseptic cultures (88.57%).

Corm bud explants transferred to MS medium containing different combinations of plant growth regulators for culture establishment. Among the various combinations, MS medium supplemented with 6 mg/L BAP + 0.5 mg/L NAA showed highest percentage of culture establishment (60%). The media combination of MS + 6 mg/L BAP + 0.5 mg/L NAA + 0.1 g/L Ascorbic acid was found effective for highest percentage (88.89%) of shoot induction.

The number of days required for first shoot initiation ranged from 15.33 to 23.37 days. The media combination comprised of MS + 6 mg/L BAP + 0.5 mg/L NAA + 0.1 g/L Ascorbic acid showed minimum (15.33) days for shoot induction. Same media combination was also found effective for

highest average number of multiple shoot bud induction (5.33). However, all the hormonal combinations failed to induce in vitro rooting.

Name of the candidate: Karanje Pranit Dhananjay

Degree for which the thesis/project report submitted: M. Sc. (Ag. Biotechnology)

Year of submission: 2021

Name of the Guide/ Co guide: Dr. S. V. Sawardekar

Abstract:

Standardization of In vitro culture establishment technique in Mango (Mangifera indica L.)

ABSTRACT

The research was aimed to standardize the sterilization technique and to optimize the in vitro culture establishment in mango using apical meristem, axillary bud and leaf of three different varieties of mango viz. Alphonso, Kesar and Ratna. The maximum aseptic culture was in treatment T8 (86.81%) consisting Wiping with Ethanol + Running Water + Tween 20 + Dettol + Savlon + Polyvinylpyrrolidone + Activated Charcoal + Carbendazim + Mercuric Chloride + Cefotaxime. Similarly, media combination M9 consisting WPM + 5 mgL-1 BAP + 0.5 mgL-1 NAA was found best as it recorded 84.04% culture establishment.

Name of the candidate: Patil Shubham Dadasaheb

Degree for which the thesis/project report submitted: M. Sc. (Ag. Biotechnology)

Year of submission: 2021

Name of the Guide/ Co guide: R. S. Deshpande

Abstract: **Screening of Rice Genotypes for Biotic and Abiotic Stress Using Specific Markers**

ABSTRACT

Present study, aimed to identify tolerant rice varieties for biotic and abiotic stresses and to establish rice genotype profiles through SSR markers. Amongst the 33 rice varieties evaluated 2 varieties were tolerant to Blast, 4 genotypes were tolerant to BB, 1 genotype was tolerant to BPH, 3 varieties were tolerant to Gall midge, 3 genotypes were salt tolerant and 2 varieties were tolerant to drought.

All 19 primers amplified and showed 100% polymorphism. Total 100 alleles were observed with average of 5.26 alleles per primer. PIC values ranged from 0.27 to 0.894 with an average PIC value of 0.65 per primer. The genetic distance ranged from 0.022 to 0.92 and disclosed wide variability. All 33 rice genotypes divided into two clusters, I cluster containing 19 genotypes and II cluster containing 14 genotypes. This concludes that the molecular screenings of rice varieties using SSR markers provided sufficient knowledge on traits of biotic and abiotic tolerance at molecular level.

Name of the candidate: Mr. Govind Bhaurao Pawde

Degree for which the thesis/project report submitted: M. Sc. (Ag. Biotechnology)

Year of submission: 2022

Name of the Guide/ Co guide: Dr. S. V. Sawardekar

Abstract:

Study of in vitro regeneration through floral organ of banana, cv. Grand naine (g-9)

ABSTRACT

Present investigation aimed to banana (*cv*. Grand Naine) optimize medium combination for callus induction and *in vitro* regeneration from male floral organ of banana (*cv.* Grand Naine). The study was conducted in Plant Biotechnology Centre, Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth, Dapoli, Dist. Ratnagiri (M.S) during the academic years 2020-2022.

The various sized explants from male flower bud were transferred to MS medium containing different combination of PGRs. Among the various combination, highest frequency of callus induction (86%) was observed in MS medium supplemented with MS + 2.5 mg/l 2,4-D+0.5mg/l^{-1} + BAP + NAA. During development of callus different types of calli with variation in colour and texture were noted.

Highest frequency of shoot regeneration from callus was reported in MS medium supplemented with 4.5 mg/l BAP. *In vitro* regenerated shoots successfully rooted (80%) in full strength MS medium fortified with 0.5 mg/l NAA + 1.5 mg/l IBA. *In vitro* regenerated plantlets were transferred to the pots with 80% success rate in best suitable harding media i.e., soil + FYM + Sand (1:1:1) mixture.

Name of the candidate: Mr. Hemant Santosh Sawant

Degree for which the thesis/project report submitted: M. Sc. (Ag. Biotechnology)

Year of submission: 2022

Name of the Guide/ Co guide: R. S. Deshpande

Abstract:

Screening of Rice (Oryza sativa L.) cultures : for biotic and abiotic stress using SSR markers.

ABSTRACT

The current research employed 18 simple sequence repeat (SSR) markers to screen 27 rice cultures for biotic (bacterial blight, blast, gall midge, brown plant hopper) and abiotic stress tolerance (salinity, drought) and to develop rice culture profiles using SSR markers. DNA was extracted from twenty-seven rice cultures and molecular profiling was performed using 18 SSR markers. MVSP software was used to determine genetic profiles. Blast tolerant linked alleles were not found in any of the 27 rice genotypes investigated. Bacterial Blight tolerant linked alleles were discovered in cultures Nom-2102, Nom-2104,

Nom-2107, Nom-2108, Nom-2110, Nom-2111, Nom-2118, and Nom-2119. Gall midge tolerant linked alleles were found in the cultures Nom-2112, Nom-2114, Nom-2115, Nom-2116, Nom-2117, Nom-2118, Nom-2119, Nom-2121, Nom-2122, Nom-2124, Nom-2127, and Nom-2132. Brown Plant Hopper tolerant linked alleles were found in Nom-2117, Nom-2118, Nom-2119, and Nom-2123 cultures. There were no drought-tolerant cultures found. Salt tolerance linked allele were found in Nom-2102, Nom-2103, Nom-2104, Nom-2105, Nom-2108, Nom-2109, Nom-2110, Nom-2111, Nom-2112, Nom-2116, Nom-2117, Nom-2132, and Nom-2133. All 18 primers amplified and exhibited 100% polymorphism. There were a total of 75 alleles detected, with an average of 4.16 alleles per primer. The polymorphism information content (PIC) values ranged from 0.314 to 0.927, with an average PIC value of 0.619 per primer. The genetic distance varied from 0.053 to 0.818 and indicated a wide range of variation. All 27 rice cultures were categorized into two clusters: I, which had 10 cultures, and II, which contained 17 cultures by UPGMA. This leads to the conclusion that molecular screenings of rice crops employing SSR markers gave sufficient knowledge on biotic and abiotic tolerance traits at the molecular level. This research will be used in future strategic crop enhancement breeding programs.

Name of the candidate : Mr. Mahendra Kumar Chouksey

Degree for which the thesis/project report submitted: M. Sc. (Ag. Biotechnology)

Year of submission: 2022

Name of the Guide/ Co guide: Dr. T. J. Bedse

Abstract:

Molecular screening of alphonso mother plant (mangifera indica l.) and it's seedlings through molecular markers.

ABSTRACT

The most valued table cultivar of mango (*Mangifera indica* L.), known as "Alphonso," has been grown in India for more than a century. In order to explore intracultivar heterogeneity based on microsatellite markers, 15 seedlings of Alphonso mango were grown by the stones taken from identified mother plant from Agriculture Research Station, Shirgaon, District-Ratnagiri (Maharashtra). It was investigated that whether there was genetic heterogeneity or relatedness between the chosen seedlings and their mother plant. The all fourteen verified mango-specific simple sequence repeats (SSRs) were found polymorphic. In the result we found total 224 alleles were produced by polymorphic microsatellites, they showed the 100 % polymorphism. The Jaccard's similarity coefficient ranged from 0.037 to 0.867. The polymorphic information content (PIC) values were observed between 0.218 (SSR-19) to 0.964 (SSR-26). The Microsatellites with a high degree of polymorphism expressed by primer SSR-26, SSR-24, SSR-16 and MngSSR-26, were more effective in differentiating the chosen alphonso samples.

Name of the candidate : Ms. Samiksha Rameshwar Chavhan

Degree for which the thesis/project report submitted: M. Sc. (Ag. Biotechnology)

Year of submission: 2022

Name of the Guide/ Co guide: Dr. S. V. Sawardekar

Abstract:

Standardization of *in vitro* organogenesis technique in bamboo

ABSTRACT

Sterilization of nodal explant with 0.2% Carbendazim for 60 min + 1.250mg/l Cefotaxime for 15 min + 14% Sodium hypochlorite for 15 min + 0.15% $HgCl_2$ for 11 min + 70% alcohol for 1 min were did. And sterilized explants were inoculated on MS medium containing different concentration and combinations of cytokinins (BAP, BAP+TDZ), 100% bud break was obtained in the MS medium supplemented with 1.0 mg/l BAP and 0.5 mg/l TDZ. While MS medium with 1.5 mg/l BAP and TDZ 1.0 mg/l had the highest average of 10 shoots/explant and an average length of shoot 3 cm. Highest shoot multiplication was observed with clump of 6 shoots on MS medium containing 1.0 mg/l BAP and shown 28.12 shoots/clump with highest average shoot length 4.1 cm. A clump of 4-5 shoots were subcultured for root induction on MS medium containing different concentration of IBA and NAA. MS Medium with IBA 1.0 mg/l + NAA 1.0 mg/l showed 87.66% rooting and 8.90 roots/clump. Medium with 35 gm/l sucrose had 6.83 cm average length of shoots, 4.5 cm length of roots, and 8 roots. So, it’s considered the optimum concentration of sucrose. Rooted plants were transferred to mixture of soil: cocopeat: FYM (1:1:1) showed survived plants (56.77%).

Key words: Nodal explant, *Dendrocalamus stocksii*, MS medium, BAP, TDZ, IBA, NAA

Extension Activities

a. The training programmes organized : Nil

b. Seminar/Symposia/Conference/workshop Organized : Nil

c. Farmer Melawa Organized : Nil

d. Radio/ TV Talks delivered by the staff members of the Department/Section:

Sr. No.	Name of the person	Topic	Where	Date of delivered
1.	Dr. N. B. Gokhale	Jaiwatantradnyanadware Poshan Muley Vrudhi	All India Radio, Ratnagiri	2006
2.		Jaiwatantradnyanadware Jywik, Ajywik Wanachi Nirmitte	All India Radio, Ratnagiri	2006

3.		Jaiwatantradnyanadware Kadhani Pachayat tantranancha Vikas.	All India Radio, Ratnagiri	2007
4.		Jaiwatantradnyanadware kadhani paschhat tantradnyanachi vikas warta.	All India Radio, Ratnagiri	2009
5.		Jaiwatantradnyan ani krishividyappethach yogdan Bhetwarta	All India Radio, Ratnagiri	2010
6.		B.T. Tantradnyan- samaj-Gairsamaj	All India Radio, Ratnagiri	2011
7.		Haritagruhatil Bhagipala Lagavad	All India Radio, Ratnagiri	2011
8.	Dr. S. V. Sawardekar	Kharif Bhendichi lagwad	All India Radio, Ratnagiri	1997
9.		Khar Jaminit Bhatachi Lagwad	All India Radio, Mumbai	2002
10.		Khar Jaminit Bhagipala pike	All India Radio, Mumbai	2002
11.		Paryavaran Sanrkshansathi Jaivatantradnyan	All India Radio, Ratnagiri	2008
12.		Bhajipala Utpadanat Bt Tantradnyan	All India Radio, Ratnagiri	2009
13.		Tissue culture Keli bagechi	All India Radio,	2010
		niga	Ratnagiri	
14.		Jaivatantradwara falbag vikas	All India Radio, Ratnagiri	2011
15.		Jaivatantradnyantil pragati ani samsya	All India Radio, Ratnagiri	2012
16.		Jaivatantradnyanadware kokanatil shetichi sudharna	All India Radio, Ratnagiri	2022
17.		Utisanvardhit lal keli lagvad – kokanatil shetkaryana vardan	All India Radio, Ratnagiri	2023

18.	Mrs. S. S. Sawant	Deliver the Radio talk on Bhatachi Navin Jat Phondaghat	All India Radio, Ratnagiri	1998
19.		Delived the Radio talk on ‘Jaivatantradnyan ani Krushi Vikas’	All India Radio, Ratnagiri	2006
20.		Delived the Radio talk on ‘Uti savardhanatun Aushadhi Vanaspati Vikas’	All India Radio, Ratnagiri	2008
21		Delived the Radio talk on ‘Jaivatantradnyanatil Pragati Ani Samasya’	All India Radio, Ratnagiri	2010

e. Farmer-Scientist Forum:- Nil

f. Other Extension Activities:- Nil

g. Publications:

Journal Research Papers

Sr. No.	Title of Publication	Author(s)	Year	Name of Journal	Vol issue	ISSN	NAAS rating
1	Weed management in Okra	**S.V. Sawardekar** and P.A. Fugro	1998	Pestology	XXII No.9. 34-35.	0970-3012	2.5
2	Panvel –3, Promising rice variety for coastal saline soils	**S.V. Sawardekar,** S.S. Dhane, V.G. Sodaye and V.N. Deshpande	2002	J. Indian Soc. Coastal agric. Res.,	18 (2), 155-160.	0972-1584	3.7
3	Limits of varietal tolerances of salinity in rice.	**S.V. Sawardekar** S.S. Dhane and B.B. Jadhav	2002	Indian Soc. Coastal, agric. Res.,	21 (1), 63-65, 2003.	0972-1584	3.7
4	Feasibility of rice – cum – fish culture in coastal saline land of Maharashtra	Meshram S.J., **S.V. Sawardekar**, S.S. Dhane and D.M. Mahale	2002	J. Indian Soc. Coastal, agric. Res.,	. 21 (1), 75-78,	0972-1584	3.7

Sr. No.	Title of Publication	Author(s)	Year	Name of Journal	Vol issue	ISSN	NAAS rating
5	Evaluation of direct paddy seeder for coastal saline soils	Mahale, D.M., **S.V. Sawardekar** and S.S.Dhane	2003	J. Mah. Agril. Uni.	28 (2) : 199-200.	0378-2395	3.2
6	Screening of rice germplasm lines against coastal salinity.	**S.V. Sawardekar** and S.S.Dhane	2004	J. Indian soc. Coastal agric. Res.,	22 (1& 2), 244-245.	0972-1584	3.7
7	Integrated effect of UB - DAP placement and glyricidia green manuaring on grain yield of sahyadri hybrid rice in partially reclaimed coastal saline soils..	S.S.Dhane and **S.V. Sawardekar**	2004	J. Indian soc. Coastal agric. Res	22 (1& 2), 226-227.	0972-1584	3.7
8	Variability for Flower traits in Rice.	**S.V. Sawardekar**	2007.	J Indian soc. Coastal agric. Res.	25 (2), 121-124	0972-1584	3.7
9	Effect of Seed Colouring and Bavistin Treatment on Stroability of Cocumber.	**S.V. Sawardekar**, V.K. Despande and S.M. Mantri	2008	J. Mah. Agril. Uni.	33(3):200-202.	0378-2395	3.2
10	Transformation studies in Chickpea (*Cicer arietinum* L.)	**S.V. Sawardekar** and I.S. Katageri	2007	*Karnataka J. of Agri. Science.*	20(4): 890	0972-1061	3.3
11	Development of somaclones and their genetic diversity analysis through RAPD in Finger millet (*Elensine coracana* L. Gaertn.)	Patil, S.M., **Sawardekar, S.V**., Bhave S.G., Sawant, S.S. Jambhale, N.D. and Gokhale, N.B.	2009	*Indian Journal of Genetics and Plant Breeding*	*69 (2) : 132-139*	0019-5200	**6.6**
12	Assessment of Lablab been hybrids for biomass partitioning under coastal zone of Maharashtra.	Sawant, S.S., Bhave, S.G., Bendale, V.W. and **Savardekar S.V.**	2009	*J. Indian Soc. Coastal Agric. Res.*	27 (2) : 12-4.	0972-1584	3.7

Sr. No.	Title of Publication	Author(s)	Year	Name of Journal	Vol issue	ISSN	NAAS rating
13	Sex detection of kokum (*Garcinia indica* Choisy) by RAPD markers.	**Sawardekar S.V.**, N.B. Gokhale, M.S. Mote, S.N. Joshi and S.S. Sawant	2010	*Indian J. Gent.*,	**70**(4): 325-327.	0019-5200	**6.6**
14	Variability studies for weight of callus in Finger Millet (*Eleusine coracana* (L.)).	Bhave S.G., Patil, S.M., **Sawardekar, S.V.,** Sawant S.S. and Gokhale, N.B	2010	*J. Mah. agric. Univ.*	35 (1): 72-76.	0378-2395	3.2
15	Regeneration from hypocotyls derived callus of chickpea (*Cicer arietinu* L.)	**Sawardekar S.V.** I.S. Katageri	2011	J. Agric. Res. Technol.	36(2):212-217	0378-2395	3.2
16	Efficient In-vitro generation of Pigeon pea (*Cajanns cajan* (L.)Mill sp.) through somatic embryogenesis for genetic transformation	**Sawardekar, S.V**., Sarode, S.N., Sawant, S.S., Bhave, S.G. and Gokhale, N.B.	2011	*J. Arid Legumes.*	*8 (1) : 58-64*	0973-0907	2.5
17	Assessement of nature and weight of callus in diverse genotypes of Pigeonpea (*Cajanus cajan* (L) Mill sp.)	Sarode, S.N., **Sawardekar, S.V**., Bhave, S.G. Sawant, S.S., and Gokhale, N.B.	2011	*J. Agric. Res. Technol*	*37 (1) : 161-163*	0378-2395	3.2
18	Integrated management of coastal saline soils of Maharashtra	Dhane S.S.,**S.V. Sawardekar,** D.M. Mahale and V.N. Khade	2011	J. Indian Soc. Coastal agric. Res.,	29(1):26-34	0972-1584	3.7
19	Genetic Engineering for Salt Tolerance	Gokhale, N.B. ,S. S. Sawant, **Sawardekar S.V.,** and S. N. Joshi	2011	J. Indian Soc. Coastal agric. Res	29(1), 78-81	0972-1584	3.7
20	Per se performance of line, testers and hybrids for yield and yield contributing characters in Tomato	Gaikwad K.J., S.G. Bhave, S.S. Sawant and **S.V. Sawardekar**	2011	J. Indian Soc. Coastal agric. Res.,	29(1):82-92	0972-1584	3.7

Sr. No.	Title of Publication	Author(s)	Year	Name of Journal	Vol issue	ISSN	NAAS rating
21	Agrobacterium-mediated genetic transformation of pigeonpea (*Cajanus cajan*(L.) Millisp) for pod borer resistance: Optimization of protocol	**Sawardekar, S. V.,** Mhatre, N. K., Sawant, S. S., Bhave, S. G., Gokhale, N. B., Narangalkar, A. L., Katageri, I. S. and Kumar, P. A.	2012	*Indian Journal of Genetics and Plant Breeding*	72(3):380-383	0019-5200	**6.6**
22	Genetic variability in *porphyra* C. Ag. Along the coast of Maharashtra.	Kovale M. G., B. B. Chaugule, **S. V. Sawardekar** and N. B. Gokhale	2013	Indian Journal of Biotechnology	**12**:277-280.	0972-5849	**7.0**
23	Standardization of Callus Derived *In Vitro* Screening Technique for Salt Tolerance in Rice (*Oryza sativa* L.).	S. R. Kamble, **S. V. Sawardekar***, N. B. Gokhale, S. S. Sawant and A. H. Bankar	2014	Indian Soc. Coastal, agric. Res.,	32(2): 59-63	0972-1584	3.7
24	Studies on mutagenic effectiveness and efficiency of gamma rays and its effect on quantitative traits in finger millet (*Eleusine coracana* L. Gaertn)	Ambavane A. R., **Sawardekar S. V.,** Sawantdesai S. A., and Gokhale N. B.	2014	*Journal of Radiation Research and Applied sciences*	xxx(2014)I -6	1687-8507	**7.6**
25	Analysis of genetic variability in fingermillet mutant lines using ISSR markers	Gilande H. G.,N. B. Gokhale, **S.V. Sawardekar** and D. M. Patil	2015	J. Agric. Res.Technol.	40 (2) : 233-238.	0378-2395	3.2
26	Studies on mutagenic effectiveness and efficiency of finger millet (*Eleusine coracana* L. Gaertn) in M_1 generation and effect of gamma rays its on quantitative traits	Ambavane A. R., **S. V. Sawardekar,** S. A. Sawantdesai, N. B Gokhale,S. S. Sawant, S. G. Bhave, and J. P. Devmore	2014	Internat. J. agric. Sci.	10 (2): 603-607	0976-5670	2.37

Sr. No.	Title of Publication	Author(s)	Year	Name of Journal	Vol issue	ISSN	NAAS rating
	during M_2 generation.						
27	Elimination of bacterial contamination by using antibiotics in micropropagtion of banana (*Musa Spp.*) Cv. Grand Naine.	Sable S. N. D. M. Patil, N. B. Gokhale, **S.V. Sawardekar** and Sawant S.S.	2015	*Journal of Cell and Tissue Research*	15(2) 5111-5115	0973-0028	**4.3**
28	Analysis of genetic variability in finger millet mutant lines by using ISSR markers.	Gilande H. G., N. B. Gokhale, and **S.V. Sawardekar** D. M. Patil	2015	*Journal of Agriculture Research and Technology*	40 (2): 233-238	0378-2395	**3.4**
29	Molecular screening of rice (*oryza sativa*) Germplasm for biotic and abiotic stresses and their diversity study by using SSR markers	Chungada A. S, N. B. Gokhale, D. M. Patil, and **S.V. Sawardekar**	2015	*Indian Society of Coastal Agriculture.*	34(2):7-14	0972-1584	**3.7**
30	DNA fingerprinting of rice varieties using SSR Markers	Nikam D. P, Bhave SG , **Sawardekar SV***, Gokhale NB, Sawant SS. Patil DM, Mhatre N. K.	2016	*Journal of Agriculture Research and Technology*	41 (1) 142-147	0378-2395	**3.4**
31	Molecular characterization of coconut (*Cocos nucifera* L.) varieties by using ISSR and SSR markers	Rasam D. V., N. B. Gokhale, **S.V. Sawardekar** and D. M. Patil	2016.	*Journal of Horticultural Science & Biotechnology*	1160544	1462-0316	**6.48**
32	Study of M5 Generation of Finger Millet (*Eleusine coracana* ***L. Gaertn.) Promising Mutants with***	S. S. Zuge, **S. V. Sawardekar** D. M. Patil S. G. Bhave N. B. Gokhale J. S. Dhekale S. G. Mahadik	*2016*	*Journal of Agriculture Research and Technology*	***·41 (2) : 324-328***	-2395	3.4

Sr. No.	Title of Publication	Author(s)	Year	Name of Journal	Vol issue	ISSN	NAAS rating
	Quality Parameters	J. P. Devmore					
33	Analysis of Callus Generated Somoclonal Variation in Proso Millet (L.) Through Molecular Markers	S. G. Mhatre, **S. V. Sawardekar,** D. M. Patil And N. B. Gokhale	2016	J. Indian Soc. Coastal agric. Res.	34(1): 81-87	0972-1584	3.7
34	Genetic variability for yield and yield attributing traits in F4 generation of Lablab bean (Lablab purpureus L. Sweet)	S.S. Kambale, J.P. Devmore, S.G. Bhave, **S.V. Sawardekar** and J.S. Dhekale	2016	Electronic Journal of Plant Breeding	7(3): 809-813	0975-928X	4.97
35	Genetic analysis for fruit yield and its component traits in brinjal (Solanum melongena L.)	J.P. Devmore*, S.G. Bhave, M.M. Burondkar, J.S. Dhekale and **S.V. Sawardekar**	2016	Electronic Journal of Plant Breeding	7(4): 1040-1045	0975-928X	4.97
36	In Vitro propagation Technique for Bamboo Species of Western Ghats	S. S. Sawant*, N. B. Gokhale, **S.V. Sawardekar,** and S. G. Bhave	2016	Journal of Tree Sciences	*31 (1) : 53-58*	0970-7662	3.51
37	Study of M4 and M5 Generations of Finger Millet (Eleusine coracana L. gaertn) and Quality Analysis of	S. S. Zuge, **S. V. Sawardekar,** S. G. Bhave, N. B. Gokhale, J. S. Dhekale, S. G. Mahadik, J. P. Devmore, D. M. Patil	2016	Environment & Ecology	35 (3C) 2266—2270	0970-0420	4.18

Sr. No.	Title of Publication	Author(s)	Year	Name of Journal	Vol issue	ISSN	NAAS rating
	Promising Mutants						
38	Analysis of Genetic Variability among the Finger Millet Germplasm by using ISSR Markers	V. G. Kelkar, **S. V. Sawardekar,** S. G. Bhave, N. B. Gokhale D. V. Rasam, S. S. Sawant	2017	Environment & Ecology	35 (2C) : 1233—1237	0970-0420	4.18
39.	Effect of gamma radiation on germination and seedling parameter of finger millet (*Eleusine coracana* L. Gaertn.)	Ambavane Ajinkya Rajendra, Patil Dagdu Magan and **Dr. Savardekar Santosh Vishnu**	2017	International Journal of Chemical Studies	5(4): 1978-1982	2349–8528	5.31
40.	Standardization of protocol and detection Of *bbtv* for tissue cultured plantlets of Banana cv. *Safed velchi* through sandwich Elisa technique	**S. S. KADAM*, N. B. GOKHALE, S. V. SAWARDEKAR, M. S. JOSHI AND V. G. KELKAR**	2017	Plant Cell Biotechnology and Molecular Biology	18(7&8):570-574	0972-2025	4.31
41	Evaluation of rice (Oryza sativa L.) germplasm for biotic and abiotic stresses and their genetic diversity using SSR markers	Supriya Bhagwat , NB Gokhale , **SV Sawardekar** , VG Kelkar1 , SR Kambale1 and RL Kunkarker2	2017	Oryza	Vol. 54 No. 3, (258-265)	0474 – 7615	4.4
42	Genetic behaviour bacterial wilt resistance in F_2 population of brinjal (*Solanum melongena L)*	G. M. Kurhade, S. G. Bhave, **S. V. Sawardekar,** M. M. and V. V. Dalvi	2017	J. Res. ANGRAU	45 (2) 1-5	0970-0226	3.60

Sr. No.	Title of Publication	Author(s)	Year	Name of Journal	Vol issue	ISSN	NAAS rating
43.	Standardization of DNA Isolation Protocol for Banana using Rapid Method and Fidelity Testing in Tissue Culture Developed Banana Plantlets of *Safed velchi*	S. S. Kadam, N. B. Gokhale, **S. V. Sawardekar,** M. M. Burondkar, V. G. Rasam, D. V. Rasam.	2018	Environment and Ecology:	36 (1A) 192—196,	0970-0420	4.18
44	Regeneration potential of scutellum-derived calli from different Indica rice (Oryza Sativa L.) varieties	**GB Sawant, SG Bhave, SV Sawardekar and SM Jadhav**	2018	Journal of Pharmacognosy and Phytochemistry	7(1): 2683-2690	2349-8234	5.21
45	Effect of acetosyringone and age of callus on Agrobacterium-mediated transformation of rice (Oryza sativa L.) calli	**GB Sawant, SV Sawardekar, SG Bhave and JK Kshirsagar**	2018	International Journal of Chemical Studies 2018;	6(3): 82-88	2349–8528	5.31
46	In vitro regeneration study in lablab bean and dolichos bean (Lablab purpureus (L). Sweet) Genotypes	**JK Kshirsagar, SV Sawardekar, GB Sawant, JP Devmore and SM Jadhav**	2018	Journal of Pharmacognosy and Phytochemistry 2018;	7(1): 2782-2789	2349-8234	5.21
47	Diversity Analysis of Cashewnut (Anacardium occidentale L.)Varieties Grown in Coastal Region of Maharashtra using ISSR Marker	S. R. Kambale, N. B. Gokhale, **S. V. Sawardekar***, V. G. kelkar, B. R. salvi, S. S. Desai and S. S. Bhagwat	2018	J. Indian Soc. Coastal Agric. Res.	*36(1): 93-96 (2018)*	0972-1584	4.0
48	Study of Genetic Variability Parameters in F2 Generation of Interspecific Hybrids in Cowpea	Nair, K.R., Desai , S. S, **Sawardekar , S. V**. and Burondkar , M. M.,	2018	Int. J. Pure App. Biosci	*6 (1): 954 - 958*	2320 – 7051	4.74

Sr. No.	Title of Publication	Author(s)	Year	Name of Journal	Vol issue	ISSN	NAAS rating
49	Genetic Diversity Analysis in CMS Lines of Chilli (Capsicum annuum L.)	M. Vinodhini*, V.V. Dalvi, S.S. Desai and **S.V. Sawardekar**	2019	International Journal of Current Microbiology and Applied Sciences	*8(6): 649-654*	2319-7706	5.38
50	Molecular analysis of Mango (Mangifera indica L.) Cv. Alphonso from different locations of South Konkan.	Vinayak Babanrao Patil, **Santosh Vishnu Sawardekar,** Ravindra Sadashiv Deshpande.	2019	Int. J. Adv. Res. Biol. Sci..	6(12): 1-15	2348-8069	3.33
51	Molecular screening and diversity analysis of rice (Oryza sativa L.) genotypes for biotic and abiotic stresses using SSR markers	Pragati Randive1*, Gokhale NB1, **Sawardekar SV 1**, Kunkerkar RL2, Bhagwat SS1, Kelkar VG1	2019	Journal of Rice Research	12:1 PP 1-13	2319-3670	3.22
52	Eco-friendly and sustainable asian seabass culture system :an alternate candidate species other than shrimp forbrackishwater aquaculture	P. E. Shingare*, A. U. Pagarkar, K. J. Chaudhari, H. S. Dhaker, S. J. Meshram, N. H. Sawant, S. B. Satam, S. P. Shingare, B. T. Sawant, P. H. Sapkale, R. D. Bondre, S. D. Patil, **S. V. Sawardekar** and A. N. Narangalkar	2020	***J. Exp. Zool. India***	Vol. 23, Supplement 1, pp. 983-985	0972-0030	
53	Assessment of Genetic Diversity and to Study the Relationship in Selected Green Gram Germplasm by ISSR Marker	S. S. Mangave1*, N. B. Gokhale1, C. B. Kuchekar2**, S. V. Sawardeka**r1 and J. P. Devmore3	2020	***Int.J.Curr.Microbiol.App.Sci***	9(2): 2752-2760	2319-7706	
54	Dapoli-2: A Mineral Rich Variety of Finger millet (Eleusine coracana L. Gaertn) developed	**S. V. Sawardekar**, S. G. Bhave2, S. S. Sawant3, N. B. Gokhale4 and V. G. Kelkar5	2017	***J. Agric. Res. Technol***	42 (1) : 008-014	2230-9705	4.0

Sr. No.	Title of Publication	Author(s)	Year	Name of Journal	Vol issue	ISSN	NAAS rating
	through Biotechnological Intervention						
55	Optimization of genetic transformation method for an indica rice (Oryza sativa L.) variety Ratnagiri-711	G. B. Sawant*, S. G. Bhave**, S. V. Sawardekar**, M. M. Burondkar, N. B. Gokhale and V. S. Desai	2020	***Indian J. Genet.,***	***80(2) 147-153***	0019-5200	6.6
56	Population structure of Dendrocalamus stocksii along its Geographical distribution	Rane A.D1*.Viswanath S2. Sheshshayee M.S3. **Sawardekar S.V1**	2019	***J. Bamboo and Rattan,***	18;3 pp 44-54		
57	In vitro regeneration technique in Rauwolfia serpentina and quantification of reserpine	SM Mahadik, **SV Sawardekar**, VG Kelkar and NB Gokhale	2020	***International Journal of Chemical Studies***	8(6): 520-525	2349–8528	5.0
58	RAPD Analysis for Genetic Diversity and Verification of Hybridity in Cowpea [*Vigna unguiculata* (L.) Walp.]	U. B. Pethe1*, N. S. Dodiya2, S. G. Bhave3 and **S. V. Sawardekar4**	2020	***International Journal of Current Microbiology and Applied Sciences***	9(08): 2442-2449.	319-7706	5.0
59.	Effect of Moisture Stress Conditions on Yield and Yield Attributing Characters of Forty Lablab Bean (Lablab purpureous L. Sweet) Genotypes	S.S. Chavan*, A.K. Shinde, , V.J. Gimhavnekar, **S.V. Sawardekar,** S.G. Mahadik and M.M. Burondkar.	2020				
60	Standardization of in vitro Regeneration Technique in Elephant Foot Yam (Amorphophallus	Pravin B. Pawar1 *, **S. V. Sawardekar1** , R. S. Deshpande1 , M. G. Palshetkar2 and R. G. Khandekar3	2021	Int.J.Curr.Microbio l.App.Sci (2021)	10(03): 1246-1256	2319-7706	

Sr. No.	Title of Publication	Author(s)	Year	Name of Journal	Vol issue	ISSN	NAAS rating
	paeoniifolius L.)						
61	Identifying drought tolerant genotypes of lablab bean (Lablab purpureus L. Sweet) grown under residual moisture	SS Chavan, AK Shinde, MM Burondkar, **SV Sawardekar** and V Gimhavnekar	2021	Journal of Pharmacognosy and Phytochemistry	10(1): 2598-2601	2349-8234	5.21
62	Screening of rice genotypes for biotic and abiotic stress using specific markers	Shubham D Patil, RS Deshpande, SV Sawardekar, PD Karanje, PS Bhosale and RS Shinge	2022	The Pharma Innovation Journal 2022;	11(2): 2841-2850	2349-8242	5.23
63	Genetic Transformation for Pod Borer Resistance in Dolichos Bean [Lablab purpureus (L.) Sweet]	J.K. Kshirsagar, S.V. Sawardekar, S.G. Bhave, N.B. Gokhale, A.L. Narangalkar, M.M. Burondkar, G.B. Sawant	2021	Legume Research- An International Journal	10.18805/LR-4471		
64	Surface sterilization and callus induction potential of Various indica rice (*Oryza sativa* l.) Varieties	G.B. Sawant1*, S.V. Sawardekar2, M.Y. Patil3 and S.M. Jadhav4	2021	*Applied Biological Research*	23(2): 147-156	0.5958/0974-4517.2021.00020.3	
65	CRISPR/Cas9: A versatile tool for genome editing in crop improvement	BS Thorat, SG Bhave, SG Shinde, RL Kunkerkar, BD Waghmode, SS Desai, VV Dalvi, UB Pethe and SV Sawardekar	2021	*The Pharma Innovation Journal 2021*	*10(11): 105-120*	2349-8242	5.23
66	Effect of mutagenic treatments on seed germination, seedling growth and survival of pigeon pea [Cajanus	Palshetkar MG, Sawardekar SV, Dalvi VV, Narangalkar AL and JS Dhekale	2022	*The Pharma Innovation Journal 2022;*	*11(2): 1160-1164*	2349-8242	5.23

Sr. No.	Title of Publication	Author(s)	Year	Name of Journal	Vol issue	ISSN	NAAS rating
	cajan (L.) Mill. Sp.]						
67.	Study of the Mode of Reproduction and Fruit Development in Garcinia Indica	Mamata S. Dike, Surendr K. Malik, **Santosh V. Sawardekar**, and Manjushri A. Deodhar	2020	International Journal of Fruit Science	20 (1)	1553-8362	7.5 (1.35 IF)
68	Combining ability and gene action analysis in red kernel rice (Oryza sativa L.)	Siddharth R Kadam, Sanjay G Bhave**,** **Santosh V Sawardekar**, Arun V Mane and Nitin B Gokhale	2022	The Pharma Innovation Journal	11(4): 1983-1989	ISSN (P): 2349-8242	5.23
69	Analysis of combining ability for yield and its attributing characters in red cowpea (Vigna unguiculata (L.) Walp)	Joshi SN, SS Desai, RL Kunkerkar, SV Sawardekar and AV Mane	2022	The Pharma Innovation Journal;	11(4): 1131-1138	ISSN (P): 2349-8242	5.23
70	Seasonal incidence of fall armyworm Spodoptera frugiperda (J. E. Smith) infesting maize in Konkan region of Maharashtra	JJ Dubale, BD Shinde, SK Mehendale, PS Bodake and SV Sawardekar	2022	The Pharma Innovation Journal;	11(12): 1837-1841	2349-8242	5.23
71	Biology of fall armyworm Spodoptera frugiperda (J. E. Smith) (Lepidoptera; Noctuidae) on maize in Konkan region of Maharashtra	JJ Dubale, BD Shinde, SK Mehendale, PS Bodake and SV Sawardekar	2022	The Pharma Innovation Journal 2022;	11(12): 1842-1845	2349-8242	5.23
72	Genetic variability studies in the indigenous and exotic accessions of okra (*Abelmoschus sps.)* under Konkan conditions	Jasti Srivarsha, VV Dalvi, SG Bhave, SS Desai, MS Joshi, AV Mane and **SV Sawardekar**	2022	The Pharma Innovation Journal;	11(4): 1876-1880	2349-8242	5.23

Sr. No.	Title of Publication	Author(s)	Year	Name of Journal	Vol issue	ISSN	NAAS rating
73	Screening of rice (Oryza sativa L.) cultures for biotic and abiotic stress using SSR markers	Hemant S Sawant, RS Deshpande, SV Sawardekar, SG Mahadik, Samiksha C, Mahendra KC and Govind P	2023	The Pharma Innovation Journal	12(1): 3007-3018	2277-7695	5.23
74	An efficient protocol for aseptic shoot induction coupled with phenol alleviation in teak (*Tectona grandis* Linn. f.)	V. P. Namala , R. S. Deshpande , A. V. Mane , **S. V. Sawardekar** , A. D. Rane	2023	International Journal of All Research Education and Scientific Methods (IJARESM),	Volume 11, Issue 1, January-2023	2455-6211	Impact Factor: 7.429
75	Genetic evaluation of M3 population of pigeon pea (*Cajanus Cajan* (L.) Millispaugh) through molecular markers	MA Bhosale, **SV Sawardekar,** UB Pethe, SG Mahadik, MG Palshetkar, SD Patil and SB Rupannavar	2023	The Pharma Innovation Journal;	; 12(2): 1143-1146	2277-7695	5.23
76	Variability studies in M3 generation of pigeon pea (Cajanus cajan L. Millsp.)	SB Rupannavar, MG Palshetkar, UB Pethe, **SV Sawardekar**, RS Deshpande and MA Bhosale	2023	The Pharma Innovation Journal;	12(2): 973-976	2277-7695	5.23
77	Standardization of in vitro organogenesis technique in bamboo (*Dendrocalamus stocksii*)	Samiksha R Chavhan, **Santosh V Sawardekar**, RS Deshpande, Uday B Pethe, Hemant S Sawant, Mahendra K Chouksey, Govind B Pawde and Sandip H Sherkar	2023	The Pharma Innovation Journal;	12(2): 46-51	2277-7695	5.23
78	In Vitro Reviews of Teak	Nidhi Purav1 , **Santosh Sawardekar2** , Sandip Sherkar3 , Rohit Shinge4 , Revanth Reddy5	2023	International Journal of All Research Education and Scientific Methods (IJARESM),	Volume 11, Issue 2, February-2023,	2455-6211	Impact Factor: 7.429,
79	Significance of standard evaluation score to assess salinity tolerance in rice	Madhavi Sonone, Arun Mane, **Santosh Sawardekar** and Ramesh Kunkerkar	2023	The Pharma Innovation Journal 2023;	12(6): 3276-3278	2277-7695	5.23

Sr. No.	Title of Publication	Author(s)	Year	Name of Journal	Vol issue	ISSN	NAAS rating
80	Consequences of salt stress on chlorophyll pigments of rice genotypes	Madhavi Sonone, Arun Mane, **Santosh Sawardekar** and Ramesh Kunkerkar	2023	The Pharma Innovation Journal 2023;	12(6): 3272-3275	2277-7695	5.23
81	Assessment of genetic variability in gamma rays induced mutants of rice (Oryza sativa L.)	AH Jadhav, SS Desai, SG Bhave, AV Mane, **SV Sawardekar**, VV Dalvi and RL Kunkerkar	2023	The Pharma Innovation Journal 2023;	12(6): 4417-4422	2277-7695	5.23
82	Optimization of LD50, frequency and spectrum of chlorophyll mutation, efficiency and effectiveness of gamma rays in different cultivars of rice (Oryza sativa L.)	AH Jadhav, SS Desai, SG Bhave, AV Mane, **SV Sawardekar**, VV Dalvi and CP Bal	2023	The Pharma Innovation Journal;	12(6): 4327-4333	2277-7695	5.23

Articles published in other than NAAS rated journal

Sr. No.	Title of Publication	Author(s)	Year	Name of Journal	Vol issue	ISSN	NAAS rating
1	Front- line demonstration performance of salt tolerant rice varieties in coastal saline soils.	**S.V. Sawardekar** S. S. Dhane and B.B. Jadhav	2003	IRRN	28.1 / 2003 : 73	0117-4185	-
2	In vitro regeneration technique in pigeonpea (*Cajanus cajan* (L.) Millisp) CV Konkan Tur-1 through direct organogenesis	Mhatre, N. K., **Sawardekar, S. V.,** Bhave, S. G., Gokhale, N. B., Sawant, S. S., Deomore, J. P.	2012	*International Journal of Biotechnology and Biosciences*	2(2):163-166	2231-0304	-
3	Genotypic differences for callus induction and plantlet regeneration in cowpea (*Vigna*	**Sawardekar, S. V**., Jagdale, V. R., Bhave, S. G., Gokhale, N. B., Sawantdesai, S. A. and Lipne, K. A.	2013	*International Journal of Applied Biosciences*	1(1): 01-08	-	-

Sr. No.	Title of Publication	Author(s)	Year	Name of Journal	Vol issue	ISSN	NAAS rating
	unguiculata (L.) Walp)						
4	Genetic diversity analysis in cowpea (*Vigna unguiculata* (L.) Walp) by using RAPD markers	Patil, D. M., **Sawardekar, S. V.,** Gokhale, N. B., Bhave, S. G., Sawant, S. S., Sawantdesai, S. A. and Lipne, K. A., Sabale, S. N., Joshi, S. N.	2013	*International Journal of Innovative Biotechnology and Biochemistry*	1(1): 15-23	-	-
5	Standardization of DNA isolation protocol in kokum (*Garcinia indica* choicy) and identification of sex linked PCR based markers for gender identification.	Bagkar, T. A. **Sawardekar* S. V.,** Gokhale N. B. Sawant S. S. And D. M. Patil	2014	International Journal of Biotechnology and Biosciences	4(3), 218-233	2231-0304	-
6	Optimization of *in vitro* sterilization technique in local cv. safed velchi Along with grand naine of banana	Koli M N, **Sawardekar S V,** Gokhale N B and Patil D M	2014	International Journal of Biotechnology and Biosciences	4(3), 2224-230	2231-0304	-
7	Development of genetic variability through biotechnological intervention in proso millet (*panicum miliaceum* l.)	Bankar AH, **Sawardekar SV***, Bhave SG , Gokhale NB, Sawant SS Mahadik SG & Devmore JP	2014	International Journal of Biotechnology and Biosciences	4(3), 210-217	2231-0304	-
8	Correlation and path coefficient analysis for yield and yield components in segregating (f 4) generation of lablab bean (lababperpureus l. Sweet)	S S Kamble · J P Devmore · **S V Sawardekar** · · M G Palshetkar	2015	International Journal of Applied Biology and Pharmaceutical Technology	6(3):237-239	0976-4550	

Sr. No.	Title of Publication	Author(s)	Year	Name of Journal	Vol issue	ISSN	NAAS rating
9	Molecular Characterization of rice germplasm (*Oryza sativa L.)* using Simple Sequence Repeats (SSR) Markers	Okello Moses, **S. V. Sawardekar,** N. B. Gokhale, B. D. Waghmode and D. M. Patil	2017	Advanced Agricultural Research & Technology Journal	Vol. I Issue 1, 92-97	-	-
10	Effect on media combination on In Vitro callus induction in sandalwood (*Santalum album* L.)	B. P. Khande, M. M. Burondkar, **S. V. Sawardekar,** S. S. Chavan, N. B. Gokhale S. G. Bhave, A. K. Shinde and J. P. Devmore	2017	Advanced Agricultural Research & Technology Journal	Vol. I Issue 1,98-105		
11.	*Standardization of* In-vitro *Genetic Transformation Technique in Chickpea (Cicer arietinum L.) for Pod-borer Resistance*	**S. V. Sawardekar*1, I. S. Katageri2, P. M. Salimath2, P. A. Kumar2 and V. G. Kelkar 1**	2017	Advanced Agricultural Research & Technology Journal	Vol. I Issue 2		

10. Details of other activities (for e.g. seed production, production of other commodities etc.) -Nil.

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12. News and Events:- -Nil.