Final Report
Centre of Excellence in Agricultural Biotechnology at CPBMB (2013-2014)

Submitted to
Director of Research, KAU

Period of project (01.04.2014 to 31.10.2016)

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Professor & Head (Rtd.)

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Gok Plan project - Centre of Excellence in Agricultural Biotechnology at CPBMB, College of Horticulture, Vellanikkara

1. Project title : Centre of Excellence in Agricultural Biotechnology at CPBMB.

2. Location : CPBMB, College of Horticulture , KAU

3. PI : Dr. P.A. Valsala, Proffesor and Head, CPBMB, CoH,Vellanikkara.

4. Co PI : Dr.P.A Nazeem,Professor, CPBMB and co-ordinator DIC

Associates : Dr. Deepu Mathew, Assistant Professor, CoH Vellanikkara

Dr. Girija, Professor & Head, Microbiology , CoH Vellanikkara

Dr. P.S Abida, Associate Professor, CPBMB, CoH Vellanikkara

Dr. M.R. Shylaja, Professor, CPBMB, CoH Vellanikkara

5. AS & TS details : No.R8/61043/14 dtd 31.04.2014

6. Financial sanction details of the comptroller – No. EP-B1/6219/14 dated 26/05/2014

Year of Start- 01.04.2014 (The sanction order for the project came on 31-3-2013.So fund was re-validated for 2014-2015)

7. Date of commencement – 01/04/2014

8. Date of completion – 31/10/2016

9. Total budget and total expenditure of the project

a. Total budget – Rs.25.00 Lakhs

b. Total expenditure – Rs.24,80,998/- (Rupees Twenty four lakhs eighty thousand nine hundred ninety eight only)

10. Year-wise budget and expenditure –

Details given as annexure – 1

11.Background of the project :-

The centre for Plant Biotechnology and Molecular Biology functioning at College of Horticulture has put forth significant achievements since its inception in 1996. Twenty externally aided R&D projects worth Rs.591.0 lakhs were implemented at the centre. The centre has facility for Plant tissue culture, DNA ,RNA and Protein work. Bioinformatic centre also present for *in silico* analysis for assisting in product development. The centre has made remarkable achievements in the field of Biotechnology. Standardised technologies for exploitation of somaclonal variability in Black Pepper and Zingiberacaeous crops . Developed in vitro

regeneration protocol in 30 economic crop plants. Mass production of TC plants of Banana, Orchids, Anthurium and Curry leaf and sale of the same to public is done here. Mass production and sale of Biocontrol agents ie Psuedomonas and Trichoderma also done . Considering the developments in infrastructure and research out put DBT has recogonized CPBMB as a PG centre for Plant Biotechnology. Considering the achievements Govt. Of kerala sanctioned the project **Center of Excellence in Agricultural Biotechnology** during 2013-14

12. Objectives:

To strengthen research, technology transfer and infra-structure build up at CPBMB so as to cater the needs of the farming community, entrepreneurs, academicians and biotech scholars.

Specific objectives:

1. Validation of RAPD primer OPK-0 I and SCAR primers for female - gender identification in nutmeg.

2. DNA finger printing of all released varieties of black pepper and banana.

3. Strengthening of TC training center .

13. Technical program:

Attempts will be made to validate the suitability of RAPD primer OPKO1 from Operon Technologies USA and SCAR primer SP1 designed by Sudhamayee (2010) in female gender identification in nutmeg. Positive results will lead to the sale of gender identified nutmeg seedlings.

DNA finger printing for all released varieties of black pepper and banana with ISSR markers.

TC training center will be upgraded.

14. Results and Discussion

Validation of RAPD primer OPK-0 I and SCAR primers SP I for female —gender identification in Nutmeg.

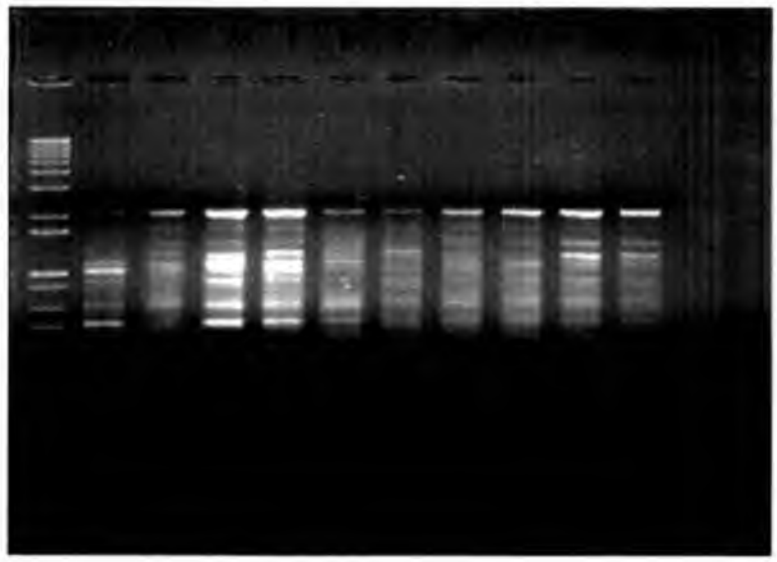
Molecular marker study was conducted with mature identified male and female nutmeg plants. DNA was isolated from 4 identified males and female nutmeg plants. DNA was amplified using PCR techniques with RAPD primer OPK 01 and SCAR primers. The DNA sequence of the primers is given below.

OPK 01- 5^1 TGG CGA CCT-3^1

The primer Sp1 sequence is given below: F- TGG CGA CCT TAA GTT AAC TTA TGG

R- ACC GCT GGA ACT TGA CAA TAT ATC

No polymorphism was observed with male and female plants.



Amplification observed for male and female samples, but no polymorphism observed

New SCAR primers were designed from OPK01 amplicon of size 1100 bp

New SCAR primers were designed using primer 3 programme

Sequence data of OPK-01 amplicon

TGGCGACCTT**AAGTTAACTTATGC**AAGGTAGCTTTTAGCCTGGACTGATCGGGGCATTTAAGGCCGATGCATGCGTAGAAATCA
GTCGACTAGAGTCATCATATATACAAAAGTCATCCATCGGGACTGAGGCTAGAGTACCAGTCCCAGTACAAGATACCGACATAG
CTGCGAGATTATTTAACCGTACTGTCAGCATGGGACTTAGCAGGTCCCCATACGGACTATGATCGATCGGACCTAGTAGGAACC
TAGCTACGAGGCCAATTCCGGGTGCGACGTAACAGTCTTTTAAAATCGGAAGCCCCCGTACCGGGTGGGAAGTTAGCCTAAGCT
ACGTACTAGAAGGTTAATGGTCACCCTAAGCAATTGGACTAGCTGGAATCTCTTGGAAAGTGTTACCAATGCAGTACCTGATCG
TACTAGGGAATATCTACGTATAGCTATTGCTGATAGTGAGTCGGATCATCGCATATACGTGGCGACCTTGGATGTCGATTCTAG
TATTAAGCAACCGGAACCTATACGGACGAAGCCGATTAGCAATGCATACAGCTAGAGAATTCCCGTACAGGTACAAACACCCTA
GTAGTGCGATCGAGCTTGGGAAACTAGCTGGATCGATCTGGGGTACCCTGACTGATCGATCGGTAGTCCGATCGATCGTAGCTA
GCTAGCTCGCTCTGGAAGGGTCCAGATCCGAGTCTCGGAGATCGATCGGATCGATCATGCTAGTACGTAGGATCGGATCGATCG
ATCACCGCGGATTAACCGTGAACCTTAGTTAGCGGGGTAAGCGAATGGGCAAGCGGTTAACTGCAGACGCGTTACGTATCGGTC
AGAAGTAAGTTGGCCGCAGTGTTATCACTCATGGTTATGGTGCACTGCATAATTCTCTTACTGTCATGCCATCCGTAAGATGCT
TTTCTGTGACTGGTGAGTACTCAACCAAGTCATTCTGAGATAGTGTATGCGGCGACCGAGTTGCTCTTGCCCGGCGTCAATACG
GGATAATACCGCGCCACATAGCAGAACTTTAAAAGTGCTCAGTGCCCAACGTGTGGTCTAGAGCTAGCCTAGGCTCGAGAAGCT
TGTCGACGAATTCAGATCAATTTCATGGCCGTCG

Results of PCR analysis with newly designed primers

No.	PRIMER	SEQUENCE	RESULT
1	SP3	F 5[1]TGG CGA CCT TAAGTT AAC TTA TTA TG3[1] R 5[11] ACC GCT GGA ACT TGA CAA TAT AT 3[1]	Single band at about 200bp in male and female sample
2	SP4	F1 5[1] TGG CGA CCT TAA GTT AAC TTA T 3[1] R 5 [1] ACC GCT GGA ACT TGA CAA TAT A3[1]	Single band at about 200bp in male and female sample
3	SP5	F1 5 [1] TGG CGA CCT TAA GTT AAC TTA3[1] R1 5[1]ACC GCT GGA ACT TGA CAA TAT3[1]	Single band at about 200bp in male and female sample
4	SP6	F1 5 [1]TGG CGA CCT TAA GTT AAC TT3[1] R1 5[1]ACC GCT GGA ACT TGA CAA TA3[1]	Single band at about 200bp in male and female sample
5	SP7	F1 5[1]TGG CGA CCT TAA GTT AAC T3[1] R1 5[1] ACC GCT GGA ACT TGA CAA T3[1]	Single band at about 200bp in male and female sample
6	SP8	F1 5[1] TGG CGA CCT TAA GTT AAC3[1] R1 5 [1]ACC GCT GGA ACT TGA CAA3[1]	Single band at about 200bp in male and female sample
7	SP9	F1 5 [1] TGG CGA CCT TAA GTT AA3[1] R1 5[1] ACC GCT GGA ACT TGA CA3[1]	Single band at about 200bp in male and female sample
8	SP10	F1 5[1] TGG CGA CCT TAA GTT A3[1] R1 5[1]ACC GCT GGA ACT TGA C3[1]	Multiple bands observed in male as well as female samples

The earlier identified molecular marker OPK01 didn't amplify a female specific polymorphic band of 1.1kB. The SCAR primer SP1 amplified 200bp amplicon in both male and female. Scar primers designed from OPK 01 amplicon were not given polymorphisam for gender identification in nutmeg. **So eleven SCAR primers reported in other crops were tested.**

Results of Scar primers reported in other crops for gender identification

SCAR primer	Sequence 5' – 3'	PLANT	Ref.	Reported result	Result obtained
MOR-634F MOR-634R	CAGCGACTGTTGGCCGAATG AAAACTATGTATGTCAGCGAC	Garcinia morella	K. S. Joseph.,H.N. Murthy and K. V. Ravishankar(2014):Development of male-specific SCAR marker in *Garcinia morella* (Gaertn.) Desr. *Genet. 93, xx–xx]*	Male specific band observed at 634bp	Multiple bands were observed
SCAR – 23 F5' SCAR – 23 R5'	GACAGACAGACACCAAGTTC AAGC ATATATTTAGTGGTGTCTGTC TGTCA	Hemp	Ott´o Törj´ek1., N´andor Bucherna1., Erzs´ebet Kiss1, Hajnalka Homoki1, Zsuzsanna Finta-Korpelov´a (2002): Novel male-specific molecular markers (MADC5, MADC6) in hemp. *Euphytica 127: 209–218, 2002.*	Male specific band observed at 634bp	No amplification was obtained
RnivY-F RnivY-R	GTTAGAATAATCTATTTCATT TGCC TTCACCTATATCGATGACC	Rumix nivalis	Stehlik F., R,Blattner (2003):Sex-specific SCAR markers in the dioecious plant Rumex nivalis (Polygonaceae) and implications for the evolution of sex chromosomes.*Springer-Verlag 2003*	Male specific band observed at 500bp	Male specific band observed at 500bp Not able to reproduce

S281-1 S281-2	CCTGGTTGCTTGTGTTGATTAG GAGTGTCATCAAGCCATCTGTC	Pistacia chinensis bunge	Q. Sun, X., Yang and R. Li. (2011): Scar marker for sex identification in Pistacia chinensis Bunge. *Springer Science+Business Media B.V. 2011*	Male specific band observed at 1241bp	No amplification was obtained
MEP -1 MEP-2	TTCCACGGTGCCGATATCCC AAGGTGCCACGGCTATAGGG	Eucommia ulmoides	Wen-Jie Xu., Bing-Wu Wang & Ke-Ming Cui(2004):RAPD and SCAR markers linked to sex determination in Eucommia ulmoides Oliv. *2004 Kluwer Academic Publishers.*	569bp pistilate specific polymorphic band were observed	No amplification was obtained
GBA F GBA R	CTGCTGGGACACAGTACAGAGTTTG GGGTTGTCGCCAAGGTTAT	Gingo biloba	Liqin Liao., Jun Liu ., Yanxia Dai. , Qian Li ., Ming Xie .,Qijiong Chen .,Huaqun Yin , Guanzhou Qiu .,Xueduan Liu .(2009):Development and application of SCAR markers for sex identification in the dioecious species Ginkgo biloba L. *Springer Science+Business Media B.V. 2009*	Male specific band observed at 571bp	No amplification was obtained
GBB F GBB R	CTGCTGGGACTTATAGGTCTTACTG	Gingo biloba	Liqin Liao., Jun Liu. , Yanxia Dai ., Qian Li. ,	Female specific band observed at	No amplification was

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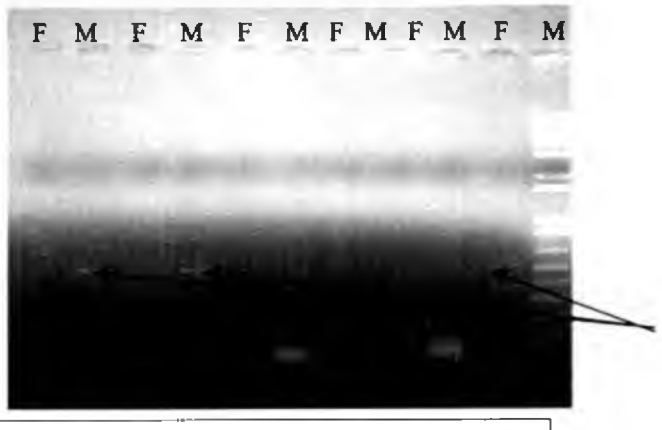
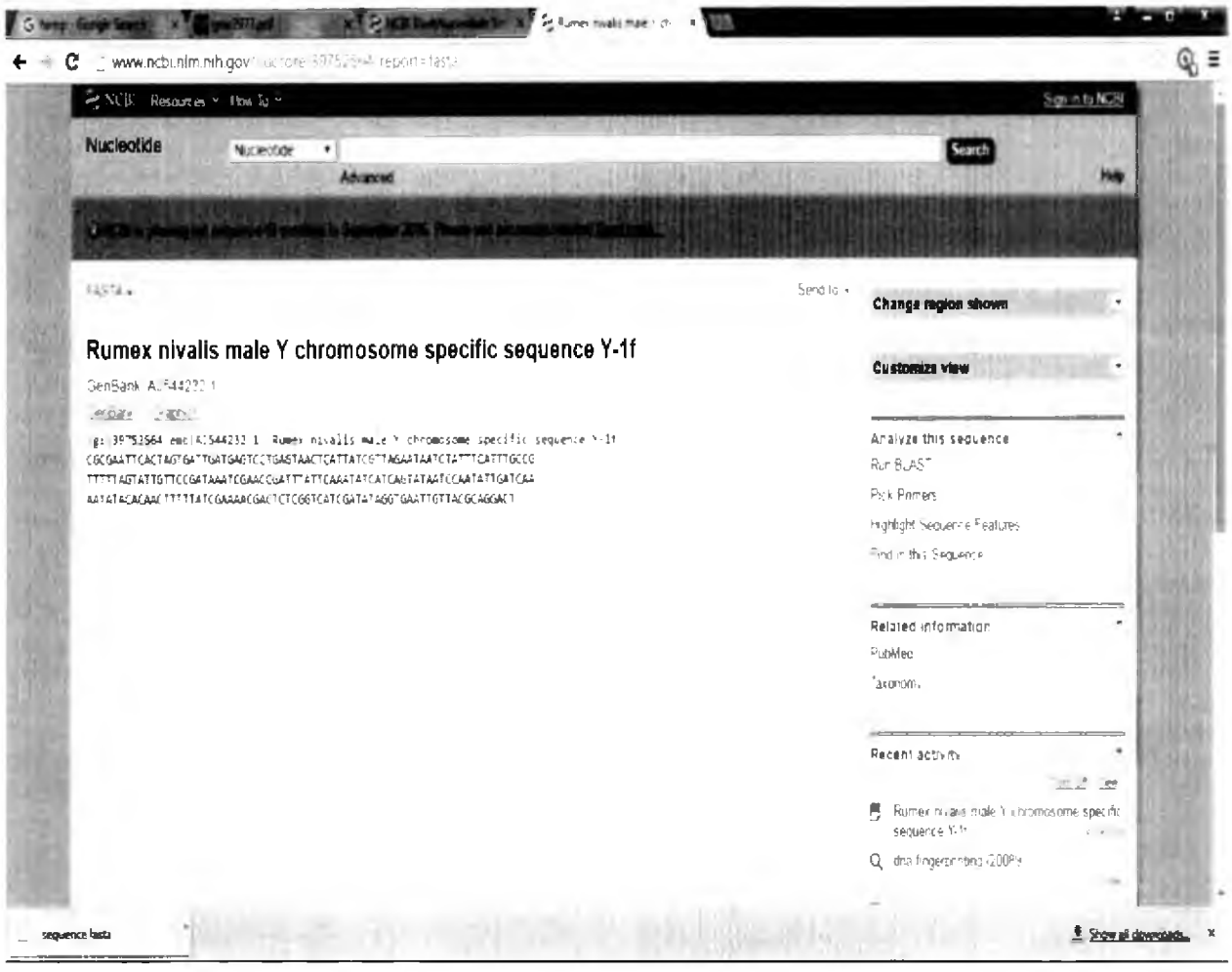
	AGATCCTATCACTGATCCGAA ACAA		Ming Xie .,Qijiong Chen ,Huaqun Yin ., Guanzhou Qiu .,Xueduan Liu (2009):Develop ment and application of SCAR markers for sex identification in the dioecious species Ginkgo biloba L. *Springer Science+Busines s Media B.V.* 2009	688bp	obtained
UBC 354520 F UBC 354520 R	GAGAGGGAGGGAGATTTAAG CGCCGTAGCAGATTGTTAATC AC	Salix viminalis	L. E. Gunter., G. T. Roberts., K. Lee., F. W. Larimer., and G. A. Tuskan (2003): The Development of Two Flanking SCAR Markers Linked to a Sex Determination Locus in Salix viminalis. *2003 The American Genetic Association*	Male specific band observed	No amplificati on was obtained

SCAR primer RnivY showed polymorphism. Male-specific SCAR marker in *Rumex nivalis amplified* 500bp in male nutmeg.



F-Female, M-Male, L- 1 Kb plus Ladder

But the results were not reproduciable. Male specific Rniv-Y sequence retrieved from NCBI nucleotide database, using the BLAST tool as given below and new primers were designed for further research.



New scar primers designed from the sequence of rumix nivalis using primer 3plus.

Sl. No.	Primers	Sequence 5'-3'	Result
1	SCAR1 F AJ544232.1	AATCTATTTCATTTGCCGTTTTT	Multiple bands were obtained
	SCAR1 R AJ544232.1	TCCTGCGTAACAATTCACCT	
2	SCAR2 F AJ544231.1	CACTAGTGATTGATGAGTCCTGAGTAA	Multiple bands were obtained
	SCAR2R AJ544231.1	GACCGTGAGTCGTTTTCGAT	
3	SCAR3F AJ544230.1	TTCGATTGATGAGTCCTGAGT	Multiple bands were obtained

	SCAR3 R	CGATGACCGAGAGTCGTTTT	
3	SCAR4F AJ544229.1 SCAR4R AJ544229.1	CACTAGTGATTGATGAGTCCTGAGTAA CGATGACCGAGAGTCGTTTT	Multiple bands were obtained
4	SCAR5 F AJ544228.1 SCAR 5 R AJ544228.1	CACTAGTGATTGATGAGTCCTGAGTAA ATGACCGAGAGTCGTTTTCG	Multiple bands were obtained
5	SCAR6F LM384063.1 SCAR6R LM384063.1	TGCTCTCCTCCGTTTGTTCT CCGCTGAGTCTATCGCTACC	Multiple bands were obtained

2. Black pepper - DNA fingerprinting of 8 varieties completed.

The Protocol was perfected for DNA fingerprinting of seven varieties of black pepper (Panniyur 1,Panniyur 2,Panniyur 3,Panniyur 4,Panniyur 5. Panniyur 6 and Panniyur 7) . A PCR based ISSR assay was conducted with UBC 825 primer (5'- AC AC ACAC AC AC AC ACT -3')and amplification patterns were analyzed. Polymorphic bands were identified for each variety which could be used to differentiate one variable from the other. UBC 825 is found to be a good primer for the genetic analysis of seven panniyur varieties of black pepper.

Lane 1 – panniyur 1

Lane 2– panniyur 2

Lane 3– panniyur 3

Lane 4 – panniyur 4

Lane 5 – panniyur 5

Lane 6 – panniyur 6

Lane 7 – panniyur 7

Lane 8 – 1 Kb Plus Ladder

PCR assay of seven different Black pepper varieties using specific ISSR primer UBC 825

For the identification of panniyur 7 and vijay varieties a new ISSR primer was identified. PCR assay with primer ISSR 3 (5'-CTCTCTCTCTCTCTCTTG-3') is giving a polymorphic band in vijay which is helpful to differentiate it from panniyur 7.

PCR assay of panniyur 7 and vijay Black pepper varieties using specific ISSR primer ISSR 3

Lane 1-4 Panniyur 7,Lane 5to 8 vijay and Lane 9- 1 kb plus ladder

ISSR 3 showing polymorphic band at 650bp

8C 1C 66

Stengthening of TC training unit

3. Conducted one Farmers Seminar on "Biotechnology for Development of Agriculture and Entrepreneurship Development" on 05-03-2015. A total of 150 farmers participated in the seminar. KAU experts took classes on the following aspects (Plate -1).

   a. Management of TC Banana: Dr.K.Aravindakshan, Proffesor Central nursery Vellanikkara
   b. TC based entrepreneurship: Sri.Sherin Ashraf, Hafi tissue culture world Cochin.
   c. Precision farming: Dr. Abdhul Hakim, Associate professor, Precision farming development center, Thavanoor
   d. Waste management: Dr.Girija, Professor and Head, Microbiology Department Vellanikkara.

Farmers wished to have such seminars every year.

4. Conducted a sponsored training of one week from 23-03-2015 to 29- 03-2015 on "Micropropagation of Banana for entrepreneurship development to 14 biotech graduates and post graduate scholars (plate-2).

Honorable vice chancellor of KAU inaugurating Farmers Seminar along with Dierctor of Research and Director of Extension (Plate -1)

Training "Micropropagation of Banana for entrepreneurship development"- Trainees along with resource persons

Objectives to be met - DNA fingerprinting of KAU released banana varieties.

15. Major equipments purchased:

Laminar air flow cabinet

16. Major infrastructure created:

a. Solar paneling was done to reduce the electricity bill of CPBMB.

b. Repaired glass house and is used for explants maintenance of black pepper for mass production of TC plants

17. **Major outcome of the project (Not more than one paragraph).** – Validation of RAPD primer OPK-01 and scar primers designed from OPK-01amplicon didn't give polymorphisam for gender identification in nutmeg. Other SCAR primers reported in other crops also were tested. RnivY-F & R primer Rumex nivalis (Polygonaceae) amplified 500 bp band in male nutmeg plants. Atteptms can be made to get repeatable results by modifying PCR procedure. DNA finger printing of all released varities of black pepper was done. ISSR primer UBC 825 and ISSR 3 is good for differentiating varities from one another. A farmers seminar was conducted to popularize TC banana, precsion farming and waste management among farmers. Biotech graduates and post graduates were given training on Micropropagation of banana for enterpunership development. TC lab was strengthened with the addition of a laminar air flow chamber. Solar paneling was done to generate electricity for running TC lab of the centre. Glass house was repaired for explants maintenance of black pepper for mass production of virus free TC plants.

18. Major technological outcome *i.e*, useful for the farming community as a whole (Not more than three sentences).- Identified molecular markers for gender identification in nutmeg was not reproduceable. ISSR primers UBC 825 and ISSR -3 is good for distingusing released varities of black pepper .

Date: 01.04.2017

Place: Vellanikkara

Name and Signature of PI

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Signature of Head of Station

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Annexure - 1

Centre of Excellence in Agricultural Biotechnology

Head	Fund sanctioned 1st year	Total budget	Total amount released	Expenditure			Total expenditure	Balance
				1st year	2nd year	3rd year		
	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(3-7)
Non recurring								
Equipment	60000	60000	60000	59387	-	-	59387	613
Civil works	1240000	1240000	1240000	28352	1211483	-	1239835	165
Recurring								
Contractual staff	504000	504000	504000	335859	287021	23200	646080	-142080
Consumables	246000	246000	246000	148855	66802	--	215657	30343
Cost of labour	50000	50000	50000	48975	52800	--	101775	-51775
Travel	50000	50000	50000	650	6427	--	7077	42923
Hiring of vehicle	100000	100000	100000	6000	--	--	6000	94000
Training/ workshop	250000	250000	250000	205187	--	--	205187	44813
Total	**2500000**	**2500000**	**2500000**	**833265**	**1624533**	**23200**	**2480998**	**19002**

Dr. P. A. Valsala
Professor & Head (Retired)