3.5.1 Number of Collaborative activities for research, Faculty exchange, Student exchange/ internship during the year

Memorandum of Understanding between Bankura Sammilani College and Ramananda College

MEMORANDUM OF UNDERSTANDING

BETWEEN

Bankura Sammilani College

Kenduadihi, Bankura, West Bengal

&

Ramananda College

Bishnupur, Bankura, West Bengal

Bankura Sammilani College Kenduadihi, Bankura and **Ramananda College** Bishnupur, Bankura both affiliated to Bankura University, are linked by common academic interest and seek to develop collaborations and exchanges in fields of shared interest and expertise. The activities undertaken pursuant to this **Memorandum of Understanding (MoU)** are based on a spirit of cooperation and reciprocity, that is intended to be of **mutual benefit to both the institutions.**

1. Purpose*

This Memorandum of Understanding (MoU) serves as a written understanding of agreed upon principles between **Bankura Sammilani College** and **Ramananda College** concerning a set of general academic objectives.
This is a non-binding agreement and is intended to clarify the nature and extent of the harmonizing activities that might be undertaken for the mutual benefit of the two institutions. Each institution will be responsible for managing its own expenses and also share as and when necessary. Commitments of specific institutional resources, personnel, space, facilities, or any other academic or intellectual activities considered hereunder may or may not be beyond the scope of this MoU. To the extent that the implementation of any agreed upon activity requires a commitment of resources, personnel, credit-bearing coursework, or intellectual property, a supplementary agreement will be negotiated and approved by the two parties before work on any of the projects can commence.

2. Objectives, Scope, and Major Activities*
Both institutions agree to encourage the development of the following types of activities:

- Visits and formal exchanges of faculty, scholars and administrators in specific areas of education, research and outreach.
- Cooperate in postgraduate education and training through library as well as laboratory resources.
- Organize joint conferences, symposia, or other scientific meetings on subjects of mutual interest.
- Exchange of academic information and materials.
- Pursue avenues for undergraduate and post graduate student exchange during the academic year or through summer / winter internship programs.
- Explore the possibilities for developing joint research programs and collaborations.
- Other exchange and cooperation programs such as extension activities to which both institutions agree.

3. Responsibilities of the Institutions*

The two institutions identify that the execution of any agreed upon activity will depend upon the interest and expertise of the individuals involved and the availability of financial resources, space and other resources. Accordingly, the functioning of any exchange and cooperative program based on this MoU shall be conferred and determined between the two institutions. It is further expected that both institutions will be compliant with all applicable Government of India and State legislations and University policies.

4. Duration and Option to Amend, Extend or Terminate*

This MoU will become effective when signed by authorities of both institutions. The agreement will remain in effect for five years from the date of signature given below, and may be renewed or amended by mutual agreement of the institutions. The institutions should agree to periodically review the activities undertaken and the progress made and to consult concerning amendments, renewal or termination of this MoU. Either authority may terminate this MoU at any time by providing written notice of such termination to the other authority.

5. General Terms*

This MoU is not intended to, and does not create any right, benefit, or trust responsibility, substantive or procedural, enforceable at law or equity, by either party, its officers, employees, or agents against the other party, its officers, employees, or agents. Nothing in this MoU obligates either party to commit or transfer any funds, assets, or other resources in support of projects or activities between the two parties. Neither party will use the name of the other, either expressly or by implication, in any publicity, solicitation or advertisement without the express written approval of the other party to this MoU.

6. Signatures*

This MoU shall enter into force on the date of the signing by qualified representatives of both institutions.

(Dr. Samir Kumar Mukherjee)
Principal
Bankura Sammilani College, Bankura

PRINCIPAL
Bankura Sammilani College
Kenduadihi, Bankura

Date 01-03-2022

(Dr. Swapna Ghorai)
Principal
Ramananda College, Bishnupur, Bankura

Principal
Ramananda College,
Bishnupur, Bankura

RAMANANDA COLLEGE ESTD.-1945 (BANKURA) BISHNUPUR

Date 01-03-2022

Cultivation of disease free *Fragaria vesca* through plant tissue culture and study of its bioactive compounds funded by DHESTBT, Govt. of West Bengal

Dr. Sabyasachi Chatterjee

GOVERNMENT OF WEST BENGAL
Science & Technology and Biotechnology DEPARTMENT

Tel:
Fax:

Memo No : 679/(Sanc.)/BT/ST/P/S&T/2G-30/2017 Date: 04/01/2021

Sanction Order for Grant-In-Aid in Cash

Demand No. : 76 Department Code : BS Financial Year : 2020 - 2021

1. Sanctioning Authority: ASSISTANT SECRETARY,Science & Technology and Biotechnology
2. Recipient of Grant: The University of Burdwan
3. Category of the recipient of Grant: Grantee Institution
4. Amount Sanctioned: Rs.329559/-
   Rupees Three Lakh Twenty Nine Thousand Five Hundred Fifty Nine Only.
5. DDO Code :- CAFSTA003
6. DDO Designation: Sec. Officer, Science & Technology & Biotechnology Dept.
7. Department Code: BS-Science & Technology and Biotechnology
8. Head of Account Code :76-3425-60-004-043-31-02-V
9. Scheme Name : Scientific Research in Biotechnology
10. Name of the Treasury/PAO & Accounts office: Pay & Accounts Officer-III, Calcutta PAO-III
11. Type of Grant:- Recurring
12. Utilization Certificate Required or Not: Yes
13. Purpose of Grant : 2nd year of on-going project of The University of Burdwan
14. Applicable T.R Form No:- TR Form No.31
15. An amount of Rs.329559/-(Rupees Three Lakh Twenty Nine Thousand Five Hundred Fifty Nine Only.) is hereby sanctioned for payment of Grant to the recipients as per Sl.No.2 from the Head of Account as stated in Sl.No.8 above against the Budget Provision of the Financial Year 2020 - 2021. The sanctioned amount will be payable through Transfer Credit into the LF/PL/Other Deposit Account/ECS/Cheque, as the case may be following the order issued by Finance Department in this regard.
16. Total released amount is within the Budget Provision of the Financial Year. 2020 - 2021
17. This order issues in exercise of the power delegated under Finance Department Memo. No. nullwith the concurrence of Finance Deptt.vide Gr. F.A. Branch U.O. No. 30 Date 27/11/2020
18. The Principal Accountant General (A&E), West Bengal and Pay & Accounts Officer/Treasury Officer and other concerned are being informed.
19. Remarks: The fund has been sanctioned as the 2nd year installment of the project. Total project cost is Rs. 14,99,240/-. Now Rs. 3,29,559/- as 2nd year amount will be transferred to Registrar, The University of Burdwan, Kolkata, through e-Pradan system to BANK OF INDIA A/C No. 420110100019490-(SB), IFSC CODE-BKID0004201, Mobile Number of the PI 9433193779. All the expenditure must be incurred following the WBFR and UC to be submitted.

Sd/-
ASSISTANT SECRETARY
Science & Technology and Biotechnology

DSTBT PROJECT REPORT (2ND YEAR)

Title of the project: Cultivation of Disease Free *Fragaria vesca* Through Plant Tissue Culture and Study of Its Bioactive Compounds.

Project memo no: 679/(Sanc.)/BT/ST/P/S&T/2G-30/2017 dated on 04/01/2021

Principal investigator: Dr. Indrani Chandra.

Implementing institution: Department of Biotechnology, The University of Burdwan, Burdwan- 713104.

Date of Project Commencement (2nd Year): 06/09/2019.

Project Objective (2nd year):

- Acclimatization of *in vitro* raised Fragaria vesca L. plantlets.
- Establishment of genetic homogeneity between in vivo germinated plants and in vitro raised plantlets using different DNA markers (RAPD and ISSR markers).

Abstract: This is the 2nd year project report granted by DSTBT(Govt. of West Bengal) is mainly aiming towards a successful and cost effective acclimatization protocol for *in vitro* regenerated *Fragaria vesca* L. plantlets through plant tissue culture. Preliminarily a suitable potting mixture, (i.e- sand:soil=3:1) with a plastic supporting material to the stem was selected among 5 different soil mixtures. In search for an ideal light source and intensity for hardening of plantlets we observed that 50% shade provides 98% of survival percentage of *in vitro* plantlets in *in vivo* condition. There are experiments which provide evidences in support of the new protocol of hardening. Here leftover plastic bottles, jars and bowls are used as pots for plantlets which made this process cost effective. There are experiments which provide evidences in support of the new protocol of hardening. Use of RAPD and ISSR DNA markers confirmed genetic uniformity and stability of *in vitro* raised *Fragaria vesca L*. Thus, the newly established protocol can be applied for large scale propagation.

Introduction: Vegetative parts of *Fragaria vesca* L. is a rich source of different commercially important bioactive compounds. i.e- Phenolic acids, Tannins, Anthrocyanins etc(Buendia et al.,2010). Some of these bioactive compounds or secondary metabolites having antioxidant activity are used as therapeutic agents in drug industry.

Campus of Burdwan University is located in a climate which is transitional between CW_{g3} and AW_1, where 'C' stands for warm temperate rainy climates with mild winter, 'W' for dry winter not compensated for by total rain in the rest for the year, 'g3' for eastern Ganges type of temperature trend and AW_1 for tropical savanna climates. This environmental condition is completely different from alpine region, which is native place for *Fragaria vesca* L. (Alpine or woodland strawberry). So, survival of Alpine strawberry in Indian climatic condition is a tough task.

Acclamation of tissue culture regenerated plants requires many precautionary measures. There are several issues (Excess transpiration, microbial infection, inability to use soil nutrient directly etc.) which must be countered to obtain a hardened plant. Initially higher moisture and low light intensity is required for new transplanted plantlets to avoid extra transpiration and to maintain water content inside plant system. Then microbial infection is another threat for hardening. Even a mild infection can cause death of not only a single plant but can kill most of the plants nearby. To prevent that kind of disaster, fungicides are used. Even after taking these measures sometime acclamation percentage in lower than expectation. There is some other protocol which allows in vitro regenerated plantlets to harden directly in modified natural environments to shorten the time period of hardening. But after all of those higher expenses is serious issue for plant tissue culture and hardening.

On the other hand, long term *in vitro* propagation may induce somaclonal variation due to different concentrations of plant growth regulators and other environmental factors. So it is important to ensure genetic homogeneity by using various genetic markers like-RAPD, ISSR, AFLP etc.(Amiri et al., 2020; Butiuc-Keul et al., 2016).

Materials and Methods:

Hardening: Acclimatization of *in vitro* grown plantlets were done in two step process, where primarily plantlet were hardened in 5 different potting mixtures divided in two experimental sets, one with plastic material support and another without plastic material support to the stem system. Here newly regenerated plants were kept under low light intensity and covered with ventilated plastic bags for 3days. Then they were transferred to natural environmental conditions and plastic cover was removed after 10 days. This kind of acclimatization can be called conventional type hardening method.
After obtaining best potting mixture from the aforesaid experiment, further study of acclimatization was done under different light intensities. These are :(A) natural environment with 100% light intensity and (B) natural environment with 50% light intensity using UV stabilized shade net.

DNA extraction and RAPD analysis: The genomic DNA was extracted by the cetyl trimethyl ammonium bromide (CTAB) method(Coen et al., 1990) from fresh leaves of conventionally acclimatized *Fragaria vesca* L., new method oriented acclimatized Fragaria vesca and of the mother plant. The quality and concentration of DNA were measured by NanoDrop spectrophotometer (Nano Drop 1000, Termo Scientifc, USA). For RAPD analysis, a total of 18 arbitrary primers were used.
DNA amplification for RAPD and ISSR maker analysis was performed according to pre established method (Cui et al., 2019). The size of the amplification products were estimated by 1.5Kbp DNA ladder. The gels were photographed using the gel documentation system (Bio-Rad, USA), only clear and scorable DNA bands were considered.

Results and Discussion:

Acclimatization of in vitro grown plantlets: The whole experiment for acclamation of in vitro regenerated plantlets was conducted in between November to March, 2019 and 2020. At that time, temperature was at its lower side. In a two step acclamation process, primarily a suitable potting mixture was selected from 5 different combinations. During acclimatization process several problems were observed like- Fungal infection, rotting of stem, wrinkling of leaves etc. it was necessary to avoid the contact between stem and the soil mixture. So here, a plastic support material was introduced for that purpose. Mostly potting mixture or soil mixture containing cocopeat showed least survival support to these newly grown plantlets. Potting mixtures containing cocopeat caused higher water retention. Extra moisture is harmful for roots but suitable for plant pathogen. On the other hand higher volume soil compared to sand also unable to provide sufficient rate of hardening. Whereas, sand: soil= 3:1 with plastic material support showed highest survival rate (68%) with best morphological responses (Table1). So, excess amount of sand is required to encounter these particular issues.
The table below is showing the survival capacity of in vitro regenerated plantlets in different potting mixture with plastic material support to stem system and without plastic material support to stem system. All the data were taken after 21 days of transplantation.

Table1: *In vitro* acclamation of *Fragaria vesca* L. using different potting mixtures with(S) and without material(WS) support to the stem system

Potting mixture		Survival(%)	No. of leaf	Response
Sand:Soil=1:1	WS	22%	6±0.01	Expaned small size leaf, mild fungal infection
	S	46%	9±0.32	**
Sand:Soil=3:1	WS	52%	9±0.22	Expaned large size leaf, mild fungal infection
	S	68%	13±0.01	No fungal infection
Sand:Soil=1:3	WS	14%	5±0.01	Expaned small size leaf, mild fungal infection.
	S	48%	5±0.50	**
Soil:Sand:Cocopeat=2:1:1	WS	5%	4±0.32	Fungal infection, wrinkled yellowish leaf
	S	18%	5±0.32	**
Soil:Sand:Cocopeat=1:2:1	WS	14%	4±0.01	Fungal infection, wrinkled yellowish leaf
	S	36%	4±0.01	**

Even after selecting an ideal potting mixture for hardening the survival rate was not satisfactory in terms of expenses. Here other environmental factors like- light source and intensity can be a key factor for successful hardening(Ali et al., 2005; Faisal & Anis, 2010). In the next step, plantlets with optimized potting mixture were allowed to acclimatize under different light intensities. Among them 100% exposure to sunlight caused excess transpiration from leaf and drying of plantlets. On the other hand in laboratory condition light intensity in good for photosynthesis but plants were died due to mild infection and other environmental related issues. 50% shade under open air became ideal environment for hardening where puckering of leaf was initially a problem due to excess transpiration. But adequate irrigation could revive plant condition within 7 days of transplantation. After 30 days of experiment 50% shaded condition showed least number of plant death which was nearly 2% (Table2, Figure:1A,1C). After standardizing a protocol of hardening we used leftover plastic bottles, jar and bowls as a pot to acclimatize in vitro grown *Fragaria vesca* L.(Fig:1E & 1F) and satisfactorily the results were same as the main experimental sets.

After obtaining an ideal potting mixture another experiment was performed. Here plantlets were submitted to different light intensities and the data were given below in consequent two tables-

Table4: Acclimatization of *Fragaria vesca* L. plantlets submitted to different light intensities

Light source	**Survival rate**	**No. of leaf**	**Leaf morphology**	**Length of shoot(cm)**	**Length of root**	**Freah weight(g)**	**Dry weight(g)**
100% sun light	0%	0	-----	0	0	0	0
50% shade net	98	17±0.25	Dark green and expanded	12.55±0.99	9.20±0.66	8.75±0.25	2.02±0.50
Green house(2200lux with 16/8h photoperiod)	68	13±0.01	Green and expanded	10.61±1.25	6.95±0.25	6.20±0.25	1.25±0.25

A

B

C

D

E

F

G

H

Figure1- A&C: **Hardened under 50% shade net; B: Hardened under lab; Condition; D: Hardened under 100% sunlight; E&F: Hardening of *Fragaria vesca* L. Using leftover pots, water bottles and softdrink bottles. G: Flowering in acclimatized *Fragaria vesca* L. plant; H: Fruiting in acclimatized *Fragaria vesca* L. plant;**

Assessment of genetic stability: Long term *in vitro* culture and environmental factors during acclimatization may cause genetic variability. As Fragaria vesca is diploid in nature, it is a suitable choice for genomic study (Dyduch-siemi & Gawro, 2018). 10 different Random Amplified Polymorphic DNA (RAPD) were used to test genetic uniformity. Here total 120 bands were generated, ranging from 250 to 2500bp with an average of 12 bands per primer. The number of bands generated by a single RAPD primer varied from 8 to 17. That RAPD analysis detected no polymorphic bands between mother plant and regenerated plants.

Table3: List of RAPD primers and banding pattern

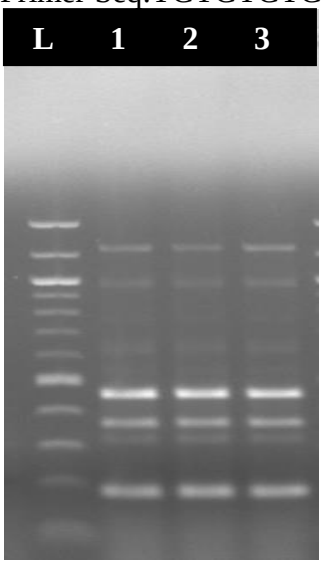
Primer code	Primer sequence(5'-3')	No. of scorable bands	Approximate range of amplification(bp)
OPV-14	AGATCCCGCC	**8**	**450-1800**
OPD-16	AGGGCGTAAG	**14**	**280-2000**
OPP-13	GGAGTGCCTC	**11**	**250-1500**
OPL-12	GGGCGGTACT	**11**	**320-1600**
OPA-15	TGCCGAGCTA	**17**	**280-1900**
OPC-5	GATGACCGCC	**10**	**320-1600**
OPAB-04	GGCACGCGTT	**11**	**400-1600**
OPV-5	TCCGAGAGGG	**12**	**350-2500**
OPD-1	ACCGCGAAGG	**12**	**320-1800**
OPD-3	GTCGCCGTCA	**14**	**400-1600**
Total number of scorable bands= 120			

Another 5 Inter simple sequence repeat (ISSR) DNA markers generated 47 scorable bands ranging from 150-1250bp with an average of 9.4bands/marker. UBC819 produced highest number of bands (12). All the ISSR markers showed no polymorphic bands with 100% genetic stability. Both RAPD and ISSR markers both are denoted as Dominant DNA markers and they are easy to handle(Butiuc-Keul et al., 2016; Jena & Chand, 2021; Rathore et al., 2014). Fragaria vesca acclimatized both in conventional way and by newly established protocol showed genetic uniformity and stability. There are many reports of genetic homogeneity assessment of other species of Fragaria sp. Submitted to different environmental conditions(Biolo, 2002; Gustavo et al., 2011; Naing et al., 2019).

Table4:List of ISSR primers and banding pattern

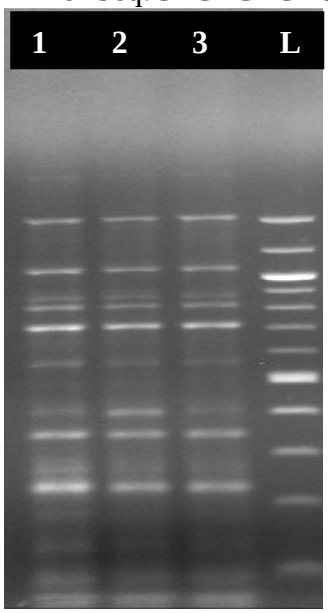
Primer code	Primer sequence(5'-3')	No. of scorable bands	Approximate range of amplification(bp)
UBC-829	TGTGTGTGTGTGTGTGC	**6**	**190-1250**
UBC-813	CTCTCTCTCTCTCTCTT	**9**	**250-1250**
UBC-836	AGAGAGAGAGAGAGAGTA	**11**	**210-1000**
UBC-819	GTGTGTGTGTGTGTGTA	**12**	**150-1100**
UBC-847	CACACACACACACACATC	**9**	**150-780**
Total number of scorable bands= 47			

Marker Used- **UBC** 829 **(ISSR Marker)**
Primer Seq:TGTGTGTGTGTGTGTGC



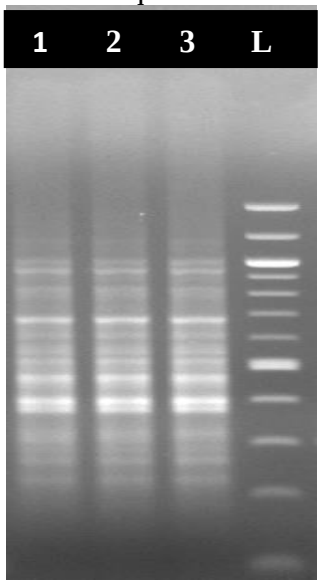
Report: Banding pattern of ISSR primer UBC 829 for *Fragaria vesca* L., Lane 1 represent mother plant, Lane 2 represent acclimatization through conventional method, Lane 3 rep
resent acclimatization through newly generated protocol, the band sizes observed 1250,970,480,350,330,190 bp.

Marker Used- UBC 813**(ISSR Marker)**
Primer Seq:CTCTCTCTCTCTCTCTT



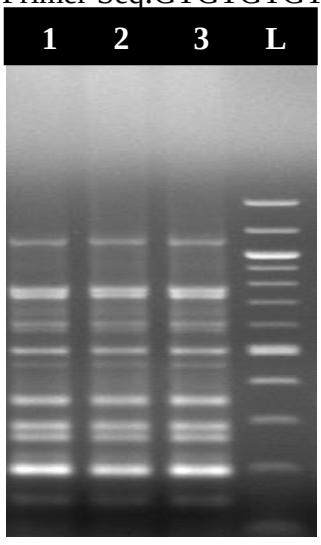
Report: Banding pattern of ISSR UBC 813 primer for *Fragaria vesca* L., Lane 1 represent mother plant, Lane 2 represent acclimatization through conventional method, Lane 3 represent acclimatization through newly generated protocol, the band sizes observed 1500, 1050, 850, 810, 700, 550,400,350,250bp.

Marker Used- **UBC** 836**(ISSR Marker)**
Primer Seq:AGAGAGAGAGAGAGAGYA



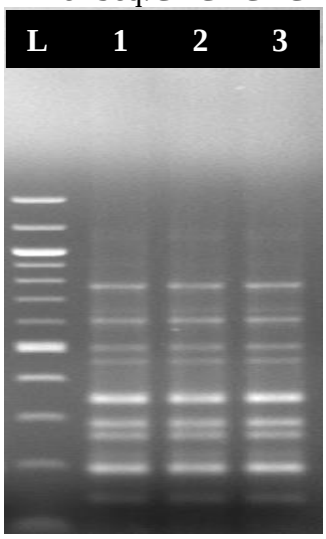
Report: Banding pattern of ISSR primer UBC 836 for *Fragaria vesca* lines. Lane 1 represents mother plant, Lane 2 represents acclimatization through conventional method, Lane 3 represents acclimatization through newly generated protocol, the band sizes observed 1000, 950,670,600,520,450,390,370,300,250,210 bp.

Marker Used- **UBC 819(ISSR Marker)**
Primer Seq:GTGTGTGTGTGTGTGTA



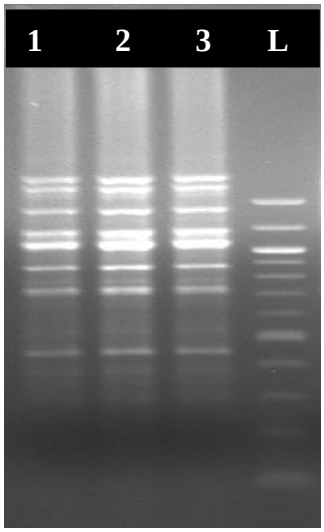
Report: Banding pattern of ISSR primer UBC 819 for *Fragaria vesca* lines. Lane 1 represent mother plant, Lane 2 represent acclimatization through conventional method, Lane 3 represent acclimatization through newly generated protocol, the bandsizesobserved1100,750, 730, 600, 580,500,480,350,270,250,200,150 bp.

Marker Used- **UBC** 847**(ISSR Marker)**
Primer Seq:CACACACACACACACARC



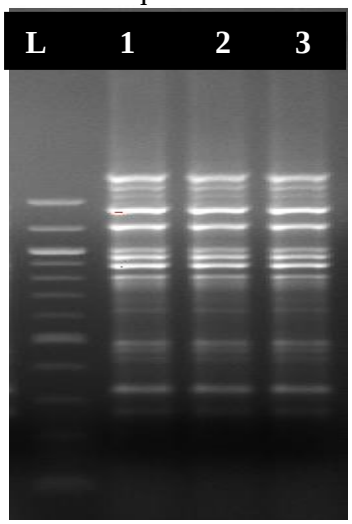
Report: Banding pattern of ISSR primer UBC 847 for *Fragaria vesca* L., Lane 1 represents mother plant, Lane 2 represents acclimatization through conventional method, Lane 3 represents acclimatization through newly generated protocol, the band sizes observed 780, 600, 500, 450, 350, 270,250,200,150bp.

Marker Used- **OPV14 (RAPD Marker)**
Primer Seq: AGATCCCGCC



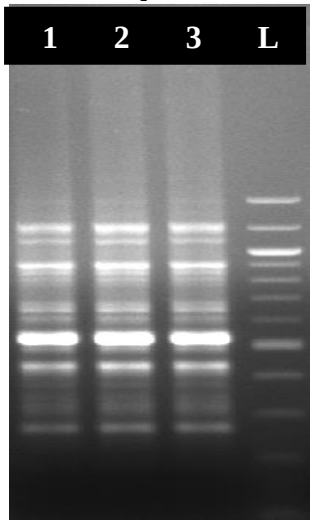
Report: Banding pattern of RAPD primer OPV14 for *Fragaria vesca* L., Lane 1 represent mother plant, Lane 2 represent acclimatization through conventional method, Lane 3 represent acclimatization through newly generated protocol, the band sizes observed 1800, 1700, 1400, 1100, 1150, 850, 750, 450bp.

Marker Used- OPD16 **(RAPD Marker)**
Primer Seq:AGGGCGTAAG



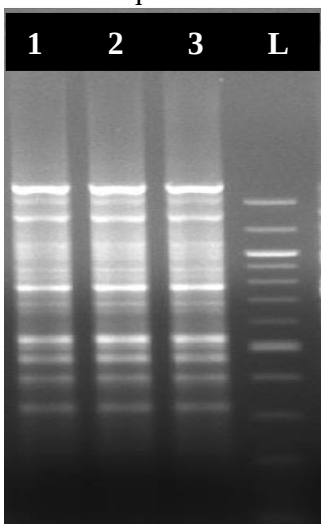
Report: Banding pattern of RAPD primer OPD16 for *Fragaria vesca* L., Lane 1 represent mother plant, Lane 2 represent acclimatization through conventional method, Lane 3 represent acclimatization through newly generated protocol, the band sizes observed 2000, 1900, 1500, 1300, 1200, 1000, 950, 850,800,620,480,450,350,280 bp.

Marker Used- **OPP 13(RAPD Marker)**
Primer Seq: GGAGTGCCTC



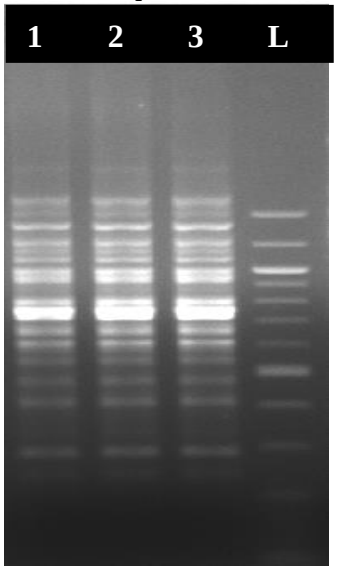
Report: Banding pattern of RAPD primer for OPP 13 *Fragaria vesca* L., Lane 1 represent mother plant, Lane 2 represent acclimatization through conventional method, Lane 3 represent acclimatization through newly generated protocol, the band sizes observed 1500, 1200, 1100, 880, 850, 650, 600, 520, 450,380,250bp.

Marker Used- **OPL12 (RAPD Marker)**
Primer Seq: GGGCGGTACT



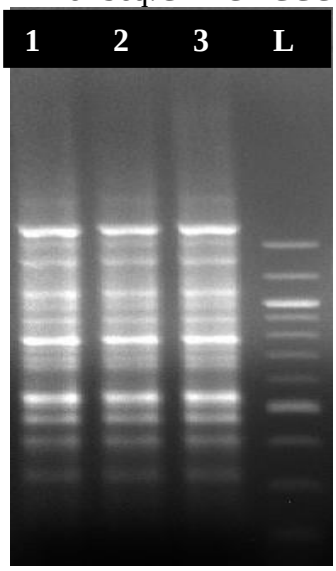
Report: Banding pattern of RAPD primer OPL 12 for *Fragaria vesca* L., Lane 1 represent mother plant, Lane 2 represent acclimatization through conventional method, Lane 3 represent acclimatization through newly generated protocol, the band sizes observed 1600, 1500, 1300, 850, 770, 680, 650, 520, 450,400,320 bp.

Marker Used- **OPA15(RAPD Marker)**
Primer Seq:TGCCGAGCTA



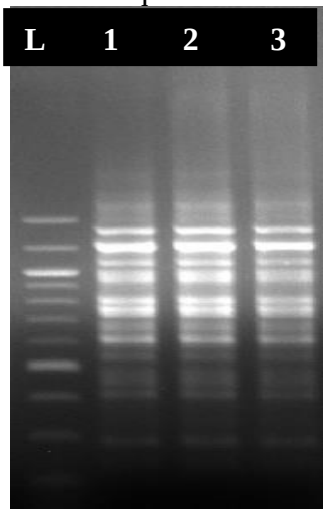
Report: Banding pattern of RAPD primer OPA15for *Fragaria vesca* L., Lane 1 represent mother plant, Lane 2 represent acclimatization through conventional method, Lane 3 represent acclimatization through newly generated protocol, the band sizes observed1900, 1600, 1500, 1300, 1200, 1150, 1100, 1000,950,780,750,650,600,550,450,400,280bp.

Marker Used- **OPC 5(RAPD Marker)**
Primer Seq:GATGACCGCC



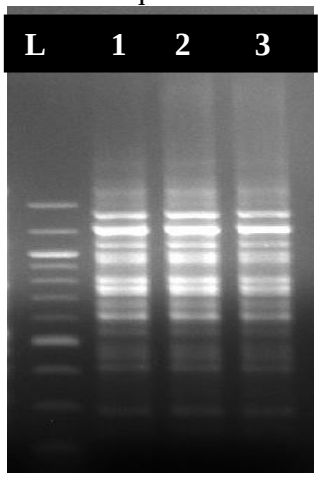
Report: Banding pattern of RAPD primer OPC 5 for *Fragaria vesca* L., Lane 1 represent mother plant, Lane 2 represent acclimatization through conventional method, Lane 3 represent acclimatization through newly generated protocol, the band sizes observed1600, 1500, 1400, 1050, 880, 780, 550, 450, 400,320,bp.

Marker Used- **OPAB04 (RAPD Marker)**
Primer Seq:GGCACGCGTT



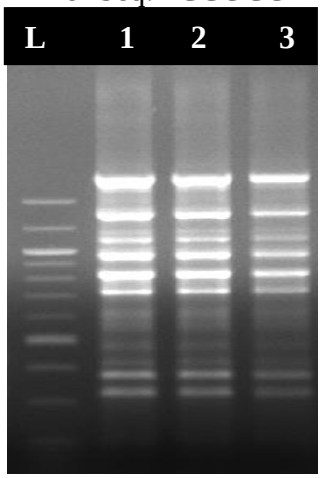
Report: Banding pattern of RAPD primer OPAB04 for *Fragaria vesca* L., Lane 1 represent mother plant, Lane 2 represent acclimatization through conventional method, Lane 3 represent acclimatization through newly generated protocol, the band sizes observed1600, 1300, 1200, 1100, 1000, 780, 750, 720,600,520,400 bp.

Marker Used- **OPV5(RAPD Marker)**
Primer Seq:TCCGAGAGGG



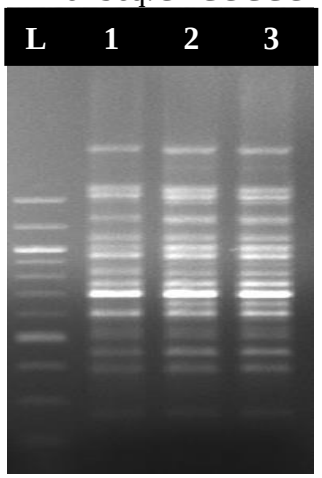
Report: Banding pattern of RAPD primer OPV5 for *Fragaria vesca* L., Lane 1 represent mother plant, Lane 2 represent acclimatization through conventional method, Lane 3 represent acclimatization through newly generated protocol, the band sizes observed 2500, 2000, 1800, 1470, 1300, 1200, 1150, 1000,980,600,450,350 bp.

Marker Used- **OPD-1(RAPD Marker)**
Primer Seq:ACCGCGAAGG

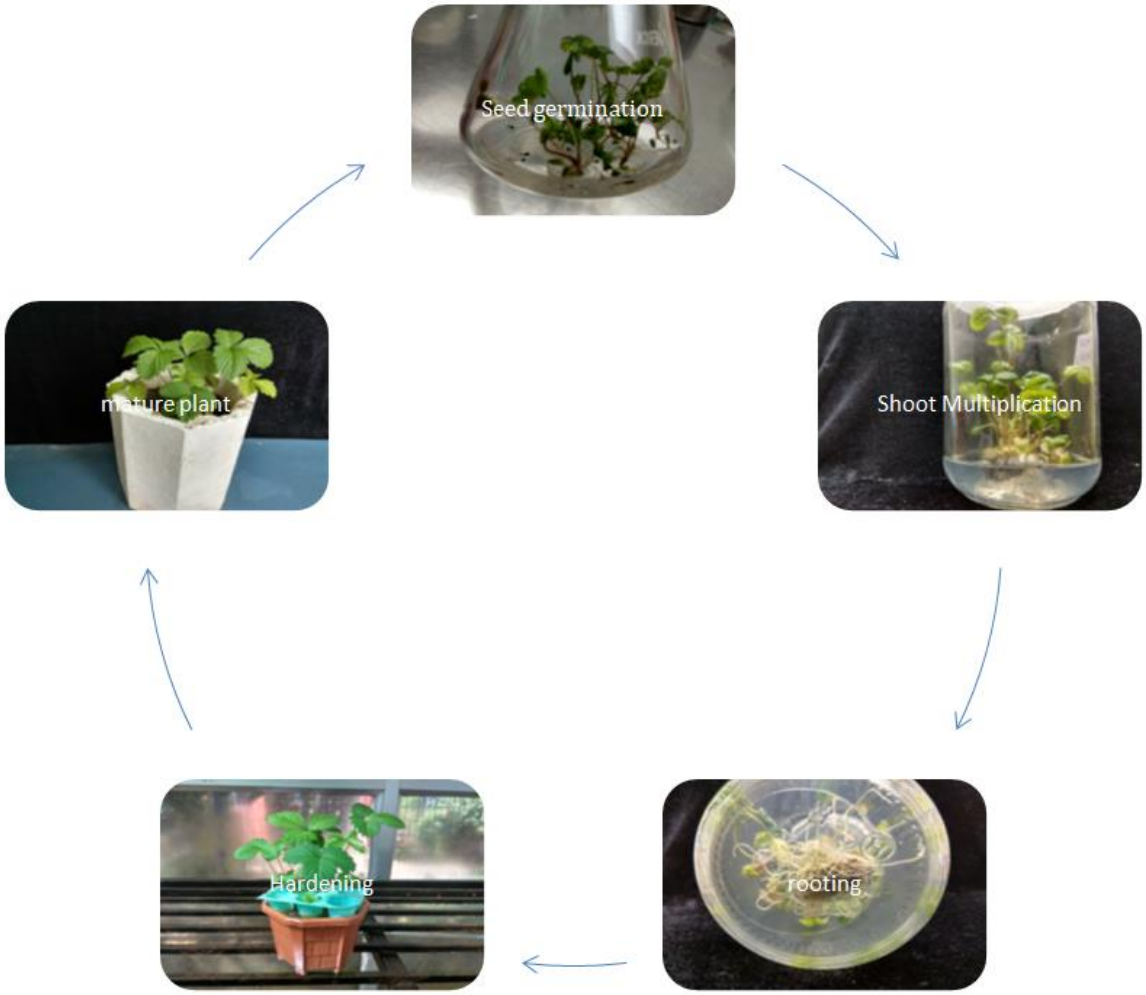


Report: Banding pattern of RAPD primer OPD1 for *Fragaria vesca* L., Lane 1 represent mother plant, Lane 2 represent acclimatization through conventional method, Lane 3 represent acclimatization through newly generated protocol, the band sizes observed1800, 1300, 1100, 1050, 980, 800, 700, 600, 480,400,370,320 bp.

Marker Used- OPD 3**(RAPD Marker)**
Primer Seq:GTCGCCGTCA



Report: Banding pattern of RAPD primer OPD 3for *Fragaria vesca* L., Lane 1 represent mother plant, Lane 2 represent acclimatization through conventional method, Lane 3 represent acclimatization through newly generated protocol, the band sizes observed2000, 1600, 1530, 1300, 1050, 1000, 950, 800,750,700,600,500,450,400 bp.



Complete cycle of direct regeneration of Fragaria vesca

Conclusion: Complete Micropropagation process upto hardening is expensive, laborious and risky. But here a least expensive protocol is established by not using any controlled environment and chemical fungicides. Leftover plastic materials were used here as pots for plants and applied only sand-soil for potting mixture. The only expense of that experiment was a shadenet. If the cost of shadenet kept aside then we can call this protocol as "**zero budget in vivo acclamation process of in vitro regenerated *Fragaria vesca***".

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Miscellaneous:

Apart from Fragaria Vesca L. , partial gene sequence of Fragaria×ananassa was submitted to NCBI database.

GenBank

Fragaria x ananassa cultivar Chandler internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence

GenBank: MZ726403.1

FASTA Graphics

```
LOCUS       MZ726403                590 bp    DNA     linear   PLN 14-AUG-2021
DEFINITION  Fragaria x ananassa cultivar Chandler internal transcribed spacer
            1, partial sequence; 5.8S ribosomal RNA gene, complete sequence;
            and internal transcribed spacer 2, partial sequence.
ACCESSION   MZ726403
VERSION     MZ726403.1
KEYWORDS    .
SOURCE      Fragaria x ananassa (strawberry)
  ORGANISM  Fragaria x ananassa
            Eukaryota; Viridiplantae; Streptophyta; Embryophyta; Tracheophyta;
            Spermatophyta; Magnoliopsida; eudicotyledons; Gunneridae;
            Pentapetalae; rosids; fabids; Rosales; Rosaceae; Rosoideae;
            Potentilleae; Fragariinae; Fragaria.
REFERENCE   1  (bases 1 to 590)
  AUTHORS   Sen,S., Chandra,I. and Das,P.
  TITLE     Direct Submission
  JOURNAL   Submitted (09-AUG-2021) Department of Biotechnology, The University
            of Burdwan, Golapbag, Barddhaman, West Bengal 713104, India
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            Sequencing Technology :: Sanger dideoxy sequencing
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//
```

https://www.ncbi.nlm.nih.gov/nuccore/MZ726403.1

Proposed Research Work For 3rd Year of this Project:

1. Spectrophotometric determination of phenolic content and antioxidant activity
2. LC-MS and HPLC of phenolic acids.
3. In vitro and in vivo determination of Antidiabetic activity.
4. Antimicrobial activity.

Dr. INDRANI CHANDRA (PI)
Assistant Professor
&
Teacher-in-Charge
Dept. of Biotechnology
The University of Burdwan

Indrani Chandra

Signature of PI

Effect of Gold and Silver Nanoparticles on Fish Endocrinology and Reproductive biology

Dr. Nilanjana Chatterjee

Collaborative project Report of Dr. Nilanjana Chatterjee

Name of the Institute: Ramananda College, Bishnupur, Bankura (Mother Institute) and Viddyasagar University, West Midnapur, WB

Name of the faculty: Dr. Nilanjana Chatterjee (Ramananda College, Bishnupur, Bankura), Assistant Professor of Zoology

Name of the Collaborator: Dr. Priyanka Halder Mallick, Assosiate Professor of Zoology, VU with registered research scholar Mr. Subir Mal.

Period of collaboration: 19/08/2017 till date

Area of research: Effect of Gold and Silver Nanoparticles on Fish Endocrinology and Reproductive Biology.

Progress of work: The gold and silver nanoparticles have been extracted using bile of *Labeo rohita* as the reducing agent in order to ensure a cleaner synthesis of the nanoparticles. The matured fish exposed to different quantities of nanoparticles over various time periods have shown several remarkable changes in the histological as well as histochemical structures of several vital organs such as the liver, kidney, testes and ovary.

Different responses to the nanoparticles have shown remarkable alteration in the seasonal reproductive cycle of the fish. The effect of silver nanoparticles on the seasonal reproductive cycle have been studied elaborately and the observation of the effects of gold nanoparticles on the metabolic organ (liver), excretory organ (Kidney) and reproductive organs (testes and ovary) are being studied. The elaborate work with gold nanoparticles is being conducted now a days.

Achievements: So far, few parts of our work have been presented in the form of paper in three reputed International Seminars conducted in different parts of the state i.e., SKBU, Purulia (West Bengal Science Congress), Midnapur City College and Science City, Kolkata (2nd World Clean Summit, 2018). One of our papers presented by Mr. Subir Mal has also received the "Best Paper Award" from Midnapur College.

****

Nilanjana Chatterjee