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STUDIES ON THE USE AND EFFECT OF ALTERNATIVE MATRICES AND CARBON SOURCE IN PLANT *IN VITRO* SYSTEM

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ABSTRACT

The present study mainly focused on the use and effect of alternative cost-effective matrices and carbon sources in plant in vitro systems. An efficient reproducible protocol for the in vitro induction of Pineapple and Vanilla plants using shoot tips and nodal segments in MS media supplemented with different combinations and concentrations of plant growth regulators has been established in this study. It is concluded from the above observations and graphical representations that MS media with different combinations and concentrations of growth regulators and also with the alternative cost-effective matrices instead of conventional matrix agar and commercial sugar instead of tissue culture grade sucrose showed a good positive result compared to the conventional method. MS basal media with combination of hormone and sucrose – PA2CS (BA- 1.0mg/l, Kin- 0.2mg/l, IAA- 0.4mg/l and Commercial Sucrose- 30gm/l) and PA3LS (BA- 1.5mg/l, Kin- 0.3mg/l, IAA- 0.6mg/l and Tissue culture grade Sucrose- 30gm/l) are the best media so far tested for optimum micropropagation of pineapple in in vitro system with the convention gelling matrix, agar. In the case of using alternative matrices with the same combinations and concentrations used in the agar matrix, bagasse (PA2LS) showed a better comparative result than the coir and luffa sponge.

KEYWORDS: Pineapple and Vanilla, MS Basal Media, PA2CS, PA3LS

Commercial application of tissue culture technology is restricted due to high production costs (Babbar and Jain, 2006). Hence, the most challenging aspect at present is to reduce the production cost, thereby improving production efficiency. During the past couple of decades, there has been an increased interest in problems related to large-scale plant production as wellas in its cost reduction for commercial micropropagation.

Pineapple (*Ananas comosus* L Merr.) is the third most crucial tropical fruit in the world, afterthe banana and citrus. The fruits are a vital supply of vitamin A and B1 and contain the protein- digesting enzyme bromelain. Pineapple is consumed fresh or processed into canned fruit, juice, or jam. Conventionally, pineapple is propagated by the usage of slips springing up from the stalk beneath the fruit, suckers originating from leaf axils or leaves, and crowns of the fruits that get up from the underground part of the stems.

Pineapple is the most important vegetatively propagated crop in tropical countries. Reproduction *in vivo* is not difficult, but the reproduction rate is as low as 11 to 17 per 5 months. In contrast, micropropagation of sprouts is quite expensive. To reduce the prices of micropropagation, scientists have attempted to broaden different methods, as an example: automated subculturing, use of natural daylight to sell photoautotrophic boom (George, 1993), huge scale production using a temporary immersion machine, periodic immersion bioreactor, an alternative for synthetic mild, tubular skylight, and herbal mild situations of a net house.

Plant tissue culture has emerged as crucial biotechnology and commercially viable tool to generate this excessive fine, sickness free and excessive-yielding planting fabric hastily insidethe laboratory irrespective of the season. At present, there are around two hundred laboratories in India with a gross production ability of about 500 million plantlets consistent with annum *Vanilla planifolia* var. Fragrans (Salib) Ames grows in Mexico and is distributed in the tropics of America, Africa (Madagascar) and Asia. In India, it is grown in Nilgiris Coorg and Mysore. *Vanilla planifolia* Andrews is a plant belonging to the Orchidaceae family. It is the most valuable and colourful orchid as an edible crop. The genus Vanilla consists of about 100 species, all distributed between 27°N and 27°S on all continents except Australia. In natural conditions, vanilla helps and protects itself as a plant when it reaches the top.

Vanilla is a unique form of orchid that, unlike its spouses and children, no longer grows on thebranches and trunks of trees, but curls out of the ground like a creeper. Its tendrils attach to trees, posts, or walls, creating new roots. It grows in warm, humid tropical climates up to 2,500feet above sea level. It is sheltered from the wind and receives about 75 to 100 inches of annualrainfall. Vanilla has a lifespan of 12 to 14 years. *Vanilla planifolia*

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Andrews, the most healthful spicy orchid in the tropics, is highly regarded for its dried fragrant beans, making it one of the second most expensive spices after saffron. Native to Mexico and highly valued in the United States, this plant adds to all the elements of the tropics and grows heavily in Madagascar, Reunion and Comoros. India is one of the largest international growing regions of vanilla andcan be grown as a catch crop on coconut and areca nut plantations and as a trade crop on cardamom and coffee plantations. The vanilla plant clings to support as a vine with long succulent green stems and greenish-white stalks growing on the other side of the leaf. Plants are propagated commercially by stem cuttings, which are reported to be uneconomical as the entire plant must be sacrificed.

Therefore, to overcome the limitations, several cost reduction strategies have now been developed. Low-cost tissue culture is very useful not only for farmers but also for routine large large-scale commercial multiplication. Different plant tissue culturing components are, namely, nutrients/media chemicals (plant growth hormones, vitamins and minerals nutrients), plant materials, equipment (culture containers, autoclave, laminar flow, instruments used for micropropagation, pH meter etc) and the infrastructures (media preparation, inoculation, growth and hardening rooms) and all these factors are subjected to play important roles in cost reduction as noted by Ganapath *et al.* (1995). Research institutions and universities have played a very important role in developing and refining tissue culture technology. The contribution of the Department of Biotechnology (DBT), the Government of India is highly appreciable. DBT has developed a complete technology package for more than 65 plant species of economic importance through a network programme among nearly 80 research institutes/universities/laboratories. This package of information would provide valuable data and also be a reference material for future research activities in this area (http://dbtmicropropagation.nic.in/).

The present investigation involves approaches to reduce the cost of micropropagation by replacing some of the costly components with readily available and much cheaper substitutes. Two attempts had been taken. Plant tissue culture grade Sucrose, a major and yet costly ingredient of the media was attempted to be replaced by commercial sugar. Again, liquid media is commercially cheaper than solid media as it eliminates the cost of agar, though the major problem there is vitrification (Bhojwani and Razdan, 2005). In earlier attempts, an enhanced rate of multiplication and rooting were obtained through the use of Coir and Luffa sponges in Gladiolus and Philodendron respectively (Roy *et al.*, 2006; Gangopadhyay *et al.*, 2004). In the current project both 'Luffa', 'Coir' and 'Bagasse' had been tested as supporting matrices in liquid media during the shooting stage. They are mainly used because of their low cost, eco-friendly and biodegradable nature and those matrixes are very much suitable for profuse rooting and easier for the hardening process. That's why in present days, it is one of the most searchable research/project topics among researchers.

From the perusal of the literature, it has been revealed that few or scanty reports have been made on this aspect. For the present investigation aseptic culture of Pineapple and Vanilla has been used for explants *in vitro* system.

MATERIALS AND METHODS

Materials: Aseptic culture of *Ananas comosus* and *Vanilla planifolia* procured from **OIST** tissue culture laboratory facility as the explants under the present study.

Media: MS basal media (with different nutrient combinations).

MS [Murashige and Skoog, 1962] basal media with different combinations and concentrations of growth regulators were used.

Materials required for preparation of nutrient media:

The nutrient media used in the present study was the Murashige and Skoog media. To prepare this media we prepared some stock solutions. The composition of these stock solutions is given below:

Table 1.1: Composition of stock I chemicals (Sulphates stock)

Chemicals	For 1 litre	For 500 ml	For 250 ml
MgSO4,7H2O	37 gm	18.5 gm	9.25 gm
MnSO4,4H2O	1.69 gm	0.845 gm	0.4225 gm
ZnSO4,7H2O	0.86 gm	0.43 gm	0.215 gm
CuSO4,5H2O	2.5 gm	1.25 gm	0.625 gm
dH2O	Up to 1000 ml	Up to 500 ml	Up to 250 ml

Add 10 ml for 1 Litre of media

Table 1.2: Composition of stock II chemicals (Halides stock)

Chemicals	For 1 litre	For 500 ml	For 250 ml
CaCl2,7H2O	44 gm	22 gm	11 gm
KI	83 gm	41.5 gm	20.75 gm
CoCl2,6H2O	2.5 gm	1.25 gm	0.625 gm
dH2O	Up to 1000 ml	Up to 500 ml	Up to 250 ml

Add 10 ml for 1 Litre of media

Table 1.3: Composition of stock III chemicals (Nitrates stock)

Chemicals	For 1 litre	For 500 ml	For 250 ml
*NH4NO3	165 gm	82.5 gm	41.25 gm
*KNO3	190 gm	95 gm	47.5 gm
dH2O	Up to 1000 ml	Up to 500 ml	Up to 250 ml

***First dissolve in 500 ml of water then adds 20 ml for 1 litre of culture media**

Table 1.4: Composition of stock IV chemicals (Phosphates and other stock)

Chemicals	For 1 litre	For 500 ml	For 250 ml
KH2PO4	17 gm	8.5 gm	4.25 gm
H3BO3	0.62 gm	0.31 gm	0.155 gm
NaMO4,2H2O	25 mg	12.5 mg	6.25 mg
dH2O	Up to 1000 ml	Up to 500 ml	Up to 250 ml

Add 10 ml for 1 Litre of media

Table 1.5: Composition of stock V chemicals (Iron EDTA stock)

Chemicals	For 1 litre	For 500 ml	For 250 ml
FeSO4,7H2O	2.78 gm	1.39 gm	0.695 gm
Na2 EDTA	3.37 gm	1.685 gm	0.8425 gm
dH2O	Up to 1000 ml	Up to 500 ml	Up to 250 ml

Add 10 ml for 1 Litre of media

Table 1.6: Composition of Amino acid stock solution

Amino Acid	Stock Solutions	The amount required for 1 Litre
Glycine	25 mg in 25 ml	2 ml

Table 1.7: Composition of Vitamins used in the medium

Vitamins	Stock Solutions	The amount requiredfor 1 Litre
Nicotinic Acid	25 mg in 50 ml	1 ml
Pyridoxine HCl	25 mg in 50 ml	1 ml
Thiamine HCl	25 mg in 25 ml	0.1 ml

Table 1.8: Composition of other chemicals used in the medium

Others Chemicals	The amount required for 1 Litre
Nicotinic Acid	30 gm (3 %)
Pyridoxine HCl	8 gm (0.8 %)
Thiamine HCl	100 mg

pH Should be Maintained Between 5.6 to 5.8.

Preparation of Nutrient Media

For preparing the nutrient media, different stock solutions were prepared along with the stocks of growth regulators. During the preparation of the individual stock solution, all the ingredients listed were measured and the required quantity of each was added one after one in ddH_2O. Each ingredient was dissolved completely before adding the next ingredient. All the stocks were made up to the volume by using ddH_2O and were stored in the refrigerator at 4ºC.

While preparing MS media, using different nutrients and stocks, the required amount of stock solution was added along with lab grade sucrose and also commercial grade sucrose and made up the volume by adding ddH_2O. The pH was then checked and adjusted to 5.6 with the help of 0.1(N) HCl and 0.1(N) NaOH. The volume was finally made up and the required amount of agar was added into the medium to solidify. Agar in the medium was completely melted with gentle heating up to 90ºC. The medium was then poured into a culture tube (12-15 ml respectively) before autoclave.

Another side in the case of low-cost supportive matrices became ready by the following processes-

- Collected the sample (Coir, Bagasse and Luffa Sponge) from local shops.
- Boiled the sample individually 3 to 4 times to get the fibrous part.
- Gently removed the fibres and kept them in jam bottles.
- Before using those matrices, those jam bottles were autoclaved 7 to 8 times to avoidcontamination.

After adjusting the pH 5.6, the liquid MS media was autoclaved in conical flasks. The fibres were also autoclaved 3 to 4 times after pouring into individual culture tubes.

Inoculation: - After that, the inoculation was done in an aseptic condition using MS basal media. Inoculates were put into the culture room at 27ºC. The intensity of the illumination is assured by neon lighting of the intensity of 20,000 – 25,000 lux (Nazif *et al.*, 1999).

Observation: - The responses of the explants were observed after 45 days of the inoculation.

RESULTS AND DISCUSSION

***In Vitro plants* Tissue Culture**

MS basal media containing different concentrations and combinations of nutrients plays a key role in shoot regeneration as well as the proliferation of shoot in *in vitro* culture of *Ananas comosus* and *Vanilla planifolia* plant. *In vitro* investigation of *Ananas comosus* and *Vanilla planifolia* plant using MS basal media with different concentrations of plant growth regulators (BA, Kinetin and IAA) and different combinations of supportive matrices (Agar, Coir, Bagasseand Luffa Sponge) has been studied very carefully. Observations were taken after about 45 days of inoculation. Data of the investigations are listed in the below table (Table 2).

Table 2: Studies on the effect of different concentrations of nutrients in *in vitro* culture of Pineapple and Vanilla using MS basal media

S. N.	Media Name	Date of inoculation	Date of observation	No of Explants	Response (%)
MS media with conventional gelling agent (Agar)					
1	PA1LS (MS basal media with hormone BA- 0.5mg/l, Kin- 0.1mg/l, IAA- 0.2mg/l and Tissue culture grade Sucrose- 30gm/l)	25/03/2022	09/05/2022	8	62
2	PA1CS (MS basal media with hormone BA- 0.5mg/l, Kin- 0.1mg/l, IAA- 0.2mg/l and Commercial Sucrose- 30gm/l)	25/03/2022	09/05/2022	8	25
3	PA2LS (MS basal media with hormone BA- 1.0mg/l, Kin- 0.2mg/l, IAA- 0.4mg/l and Tissue culture grade Sucrose- 30gm/l)	31/03/2022	16/05/2022	8	37
4	PA2CS (MS basal media with hormone BA- 1.0mg/l, Kin- 0.2mg/l, IAA- 0.4mg/l and Commercial Sucrose- 30gm/l)	31/03/2022	16/05/2022	8	87
5	PA3LS (MS basal media with hormone BA- 1.5mg/l, Kin- 0.3mg/l, IAA- 0.6mg/l and Tissue culture grade Sucrose- 30gm/l)	05/04/2022	23/05/2022	8	87
	PA3CS (MS basal media with hormone BA- 1.5mg/l,				

6	Kin- 0.3mg/l, IAA- 0.6mg/l and Commercial Sucrose- 30gm/l)	05/04/2022	23/05/2022	8	75
7	PA4LS (MS basal media with hormone BA- 2.0mg/l, Kin- 0.4mg/l, IAA- 0.8mg/l and Tissue culture grade Sucrose- 30gm/l)	11/04/2022	30/05/2022	8	75
8	PA4CS (MS basal media with hormone BA- 2.0mg/l, Kin- 0.4mg/l, IAA- 0.8mg/l and Commercial Sucrose- 30gm/l)	11/04/2022	30/05/2022	8	25
MS media with low-cost alternative matrices (Coir, Bagasse and Luffa Sponge)					
9	PA1LS (MS basal media with hormone BA- 0.5mg/l, Kin- 0.1mg/l, IAA- 0.2mg/l and Tissue culture grade Sucrose- 30gm/l) + Coir	18/04/2022	03/06/2022	1	45
10	PA1CS (MS basal media with hormone BA- 0.5mg/l, Kin- 0.1mg/l, IAA- 0.2mg/l and Commercial Sucrose- 30gm/l) + Coir	18/04/2022	03/06/2022	1	40
11	PA1LS (MS basal media with hormone BA- 0.5mg/l, Kin- 0.1mg/l, IAA- 0.2mg/l and Tissue culture grade Sucrose- 30gm/l) + Bagasse	18/04/2022	03/06/2022	1	45
12	PA1CS (MS basal media with hormone BA- 0.5mg/l, Kin- 0.1mg/l, IAA- 0.2mg/l and Commercial Sucrose- 30gm/l) + Bagasse	18/04/2022	03/06/2022	1	0
13	PA1LS (MS basal media with hormone BA- 0.5mg/l, Kin- 0.1mg/l, IAA- 0.2mg/l and Tissue culture grade Sucrose- 30gm/l) + Luffa Sponge	18/04/2022	03/06/2022	1	48
14	PA1CS (MS basal media with hormone BA- 0.5mg/l, Kin- 0.1mg/l, IAA- 0.2mg/l and Commercial Sucrose- 30gm/l) + Luffa Sponge	18/04/2022	03/06/2022	1	47
15	PA2LS (MS basal media with hormone BA- 1.0mg/l, Kin- 0.2mg/l, IAA- 0.4mg/l and Tissue culture grade Sucrose- 30gm/l) + Coir	18/04/2022	03/06/2022	1	40
16	PA2CS (MS basal media with hormone BA- 1.0mg/l, Kin- 0.2mg/l, IAA- 0.4mg/l and Commercial Sucrose- 30gm/l) + Coir	18/04/2022	03/06/2022	1	42
17	PA2LS (MS basal media with hormone BA- 1.0mg/l, Kin- 0.2mg/l, IAA- 0.4mg/l and Tissue culture grade Sucrose- 30gm/l) + Bagasse	18/04/2022	03/06/2022	1	100
18	PA2CS (MS basal media with hormone BA- 1.0mg/l, Kin- 0.2mg/l, IAA- 0.4mg/l and Commercial Sucrose- 30gm/l) + Bagasse	18/04/2022	03/06/2022	1	45
19	PA2LS (MS basal media with hormone BA- 1.0mg/l, Kin- 0.2mg/l, IAA- 0.4mg/l and Tissue culture grade Sucrose- 30gm/l) + Luffa Sponge	18/04/2022	03/06/2022	1	47
20	PA2CS (MS basal media with hormone BA- 1.0mg/l, Kin- 0.2mg/l, IAA- 0.4mg/l and Commercial Sucrose- 30gm/l) + Luffa Sponge	18/04/2022	03/06/2022	1	48
21	PA3LS (MS basal media with hormone BA- 1.5mg/l, Kin- 0.3mg/l, IAA- 0.6mg/l and Tissue culture grade Sucrose- 30gm/l) + Coir	26/04/2022	10/06/2022	1	54
22	PA3CS (MS basal media with hormone BA- 1.5mg/l, Kin- 0.3mg/l, IAA- 0.6mg/l and Commercial Sucrose- 30gm/l) + Coir	26/04/2022	10/06/2022	1	49
23	PA3LS (MS basal media with hormone BA- 1.5mg/l, Kin- 0.3mg/l, IAA- 0.6mg/l and Tissue culture grade Sucrose- 30gm/l) + Bagasse	26/04/2022	10/06/2022	1	46
24	PA3CS (MS basal media with hormone BA- 1.5mg/l, Kin- 0.3mg/l, IAA- 0.6mg/l and Commercial Sucrose- 30gm/l) + Bagasse	26/04/2022	10/06/2022	1	38
25	PA3LS (MS basal media with hormone BA- 1.5mg/l, Kin- 0.3mg/l, IAA- 0.6mg/l and Tissue culture grade Sucrose- 30gm/l) + Luffa Sponge	26/04/2022	10/06/2022	1	29

26	PA3CS (MS basal media with hormone BA- 1.5mg/l, Kin- 0.3mg/l, IAA- 0.6mg/l and Commercial Sucrose- 30gm/l) + Luffa Sponge	26/04/2022	10/06/2022	1	0
27	PA4LS (MS basal media with hormone BA- 2.0mg/l, Kin- 0.4mg/l, IAA- 0.8mg/l and Tissue culture grade Sucrose- 30gm/l) + Coir	26/04/2022	10/06/2022	1	0
28	PA4CS (MS basal media with hormone BA- 2.0mg/l, Kin- 0.4mg/l, IAA- 0.8mg/l and Commercial Sucrose- 30gm/l) + Coir	26/04/2022	10/06/2022	1	30
29	PA4LS (MS basal media with hormone BA- 2.0mg/l, Kin- 0.4mg/l, IAA- 0.8mg/l and Tissue culture grade Sucrose- 30gm/l) + Bagasse	26/04/2022	10/06/2022	1	51
30	PA4CS (MS basal media with hormone BA- 2.0mg/l, Kin- 0.4mg/l, IAA- 0.8mg/l and Commercial Sucrose- 30gm/l) + Bagasse	26/04/2022	10/06/2022	1	0
31	PA4LS (MS basal media with hormone BA- 2.0mg/l, Kin- 0.4mg/l, IAA- 0.8mg/l and Tissue culture grade Sucrose- 30gm/l) + Luffa Sponge	26/04/2022	10/06/2022	1	38
32	PA4CS (MS basal media with hormone BA- 2.0mg/l, Kin- 0.4mg/l, IAA- 0.8mg/l and Commercial Sucrose- 30gm/l) + Luffa Sponge	26/04/2022	10/06/2022	1	35

MS media with conventional gelling agent (Agar)

According to the observations, it has been revealed that the PA1CS media (BA- 0.5mg/l, Kin- 0.1mg/l, IAA- 0.2mg/l and Commercial Sucrose- 30gm/l) and PA4CS media (BA- 2.0mg/l, Kin- 0.4mg/l, IAA- 0.8mg/l and Commercial Sucrose- 30gm/l) render poor response with the percentage of 25 both (Fig.1 and Fig. 2).

Better response with the percentage of 87 was obtained by the PA2CS (BA- 1.0mg/l, Kin- 0.2mg/l, IAA- 0.4mg/l and Commercial Sucrose- 30gm/l) and PA3LS (BA- 1.5mg/l, Kin- 0.3mg/l, IAA- 0.6mg/l and Tissue culture grade Sucrose- 30gm/l) supplemented MS (Murashige and Skoog, 1962) basal media (Fig 1 and Fig 2).

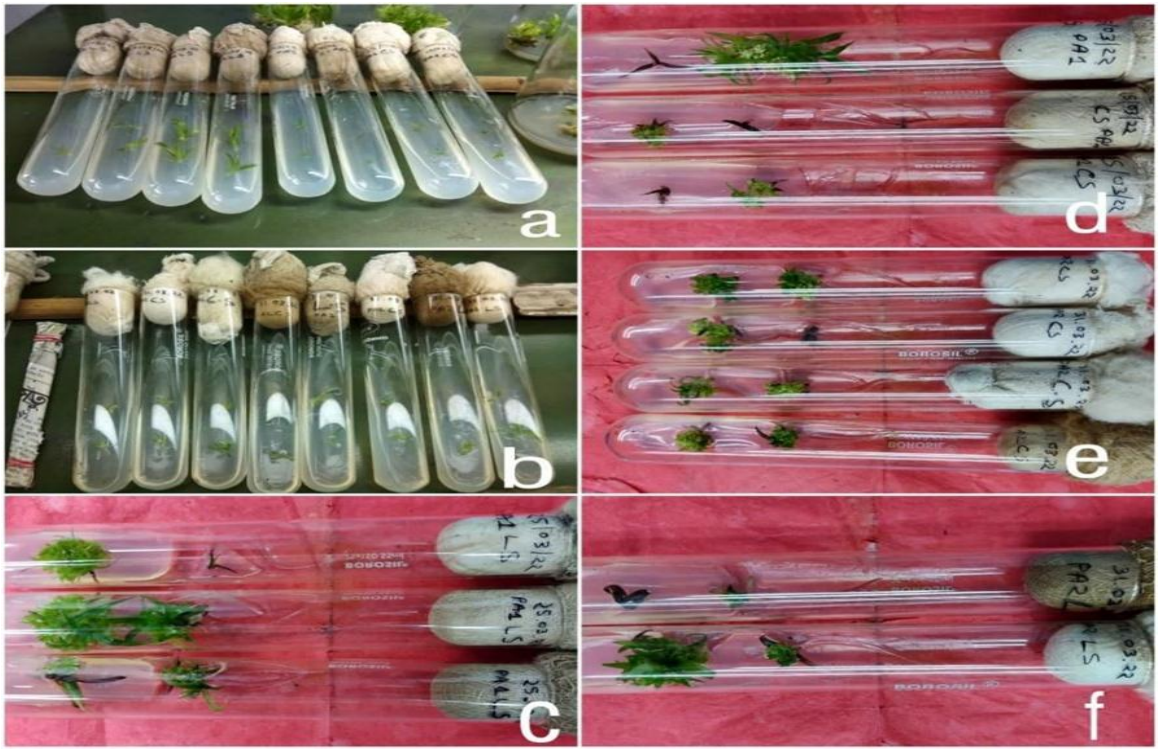

Figure 1: *In vitro* micropropagation of Pineapple (*Ananas comosus* L Merr.). Inoculation of explants: (a) PA1LS and PA1CS media, (b) PA2LS and PA2CS media. Establishment and proliferation ofexplants: (c) PA1LS media, (d) PA1CS media, (e) PA2CS media and (f) PA2LS media.

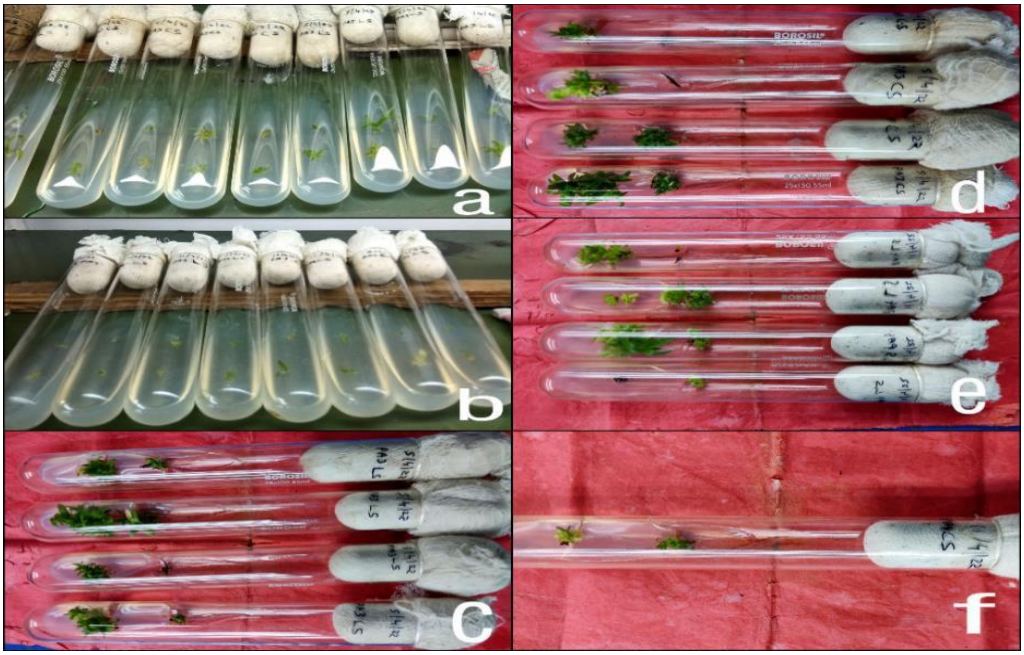

Figure 2: *In vitro* micropropagation of Pineapple (*Ananas comosus* L Merr.). Inoculation of explants: (a) PA3LS and PA3CS media, (b) PA4LS and PA4CS media. Establishment and proliferation ofexplants: (c) PA3LS media, (d) PA3CS media, (e) PA4LS media and (f) PA4CS media.

MS media with low-cost alternative matrices (Coir, Bagasse and LuffaSponge):

According to the observations, all the alternative matrices were showing good positive responses except PA2LS media (BA- 1.0mg/l, Kin- 0.2mg/l, IAA- 0.4mg/l and Tissue culturegrade Sucrose- 30gm/l), which shown a very established positive result with the percentage of100 with the help of the alternative matrix bagasse (Fig 3, Fig 4, Fig 5).

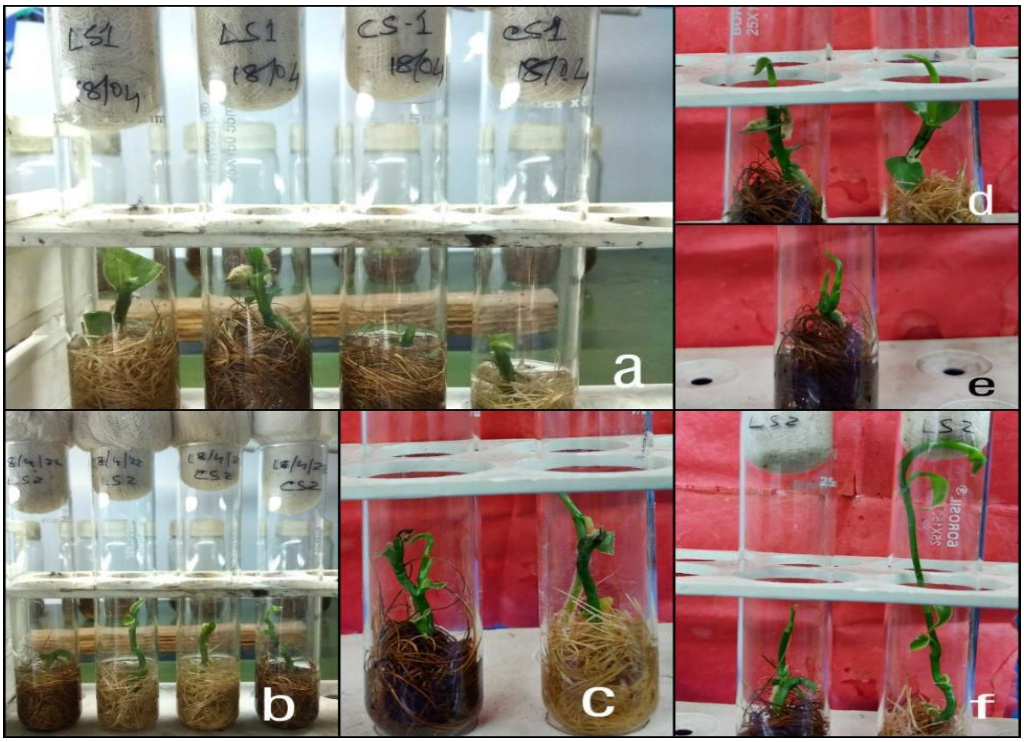

Figure 3: *In vitro* micropropagation of Vanilla (*Vanilla planifolia* Andrews). Inoculation of explants incost-effective gelling media (Coir and Bagasse): (a) PA1LS and PA1CS media, (b) PA2LS and PA2CS media. Establishment and proliferation of explants in cost-effective gelling media (Coir and Bagasse): (c) PA2CS media, (d) PA1LS media, (e) PA1CS media and (f) PA2LS media.

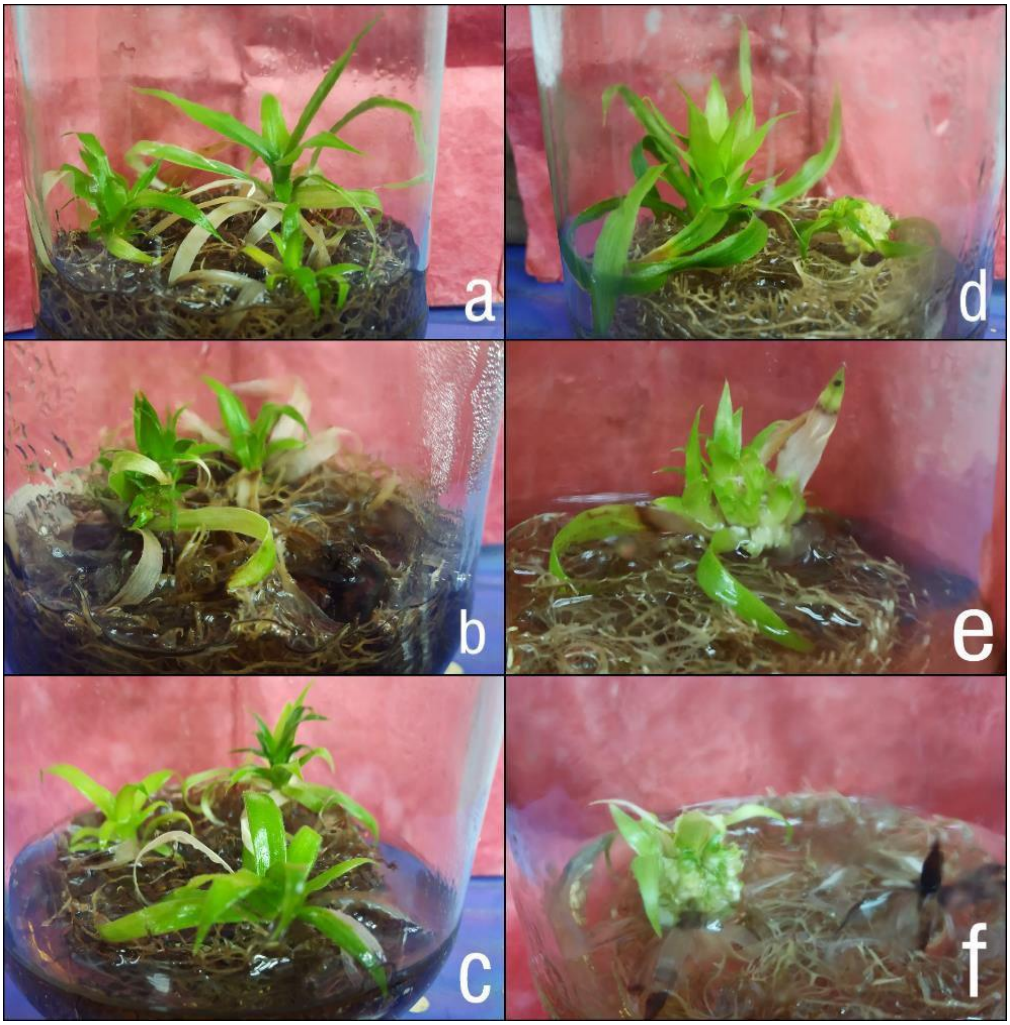

Figure 4: *In vitro* micropropagation of Pineapple (*Ananas comosus* L Merr.). Establishment andproliferation of explants in cost-effective gelling media (Luffa Sponge): (a) PA1CS media, (b) PA2CS media, (c) PA2LS media, (d) PA4CS media, (e) PA4LS media, (f) PA3LS media.

Figure 5: *In vitro* micropropagation of Vanilla (*Vanilla planifolia* Andrews). Inoculation of explants incost-effective gelling media (Coir and Bagasse): (a) PA3CS and PA4CS media, (b) PA3LS and PA4LS media and (c) Establishment and proliferation of explants.

DISCUSSION

During the establishment and proliferation stage, maximum responses were observed in liquid media using bagasse as an alternative cost-effective matrix, followed by coir and luffa sponge.Cost reduction was maximum in coir matrix followed by bagasse and luffa sponge concerning conventional agar media. Liquid media has many advantages over solid media such as efficient nutrient uptake, lower cost and dilution of excreted material (Smith and Spoomer, 1995; Aitcken-Christie *et al.* 1995). When the liquid media is supported by solid, biodegradable, fibrous matrices, the nutrients can diffuse easily through it and vitrification can be prevented (Gangopadhyay *et al.*, 2002). Among the three alternatives, cost-effective gelling agents tested, minimum greening was observed in media with luffa sponge.

This cost reduction was attributed to the replacement of tissue culture grade sucrose (3%) withtable sugar (3%) which reduced the cost of carbon source by almost 97%. The carbon source such as tissue culture grade sucrose that is often used in the micropropagation of plants in the laboratory contributes about 34% of the production cost (Demo *et al.*, 2008). Sucrose has been reported as a source of both carbon and energy (Bridgen, 1994). There is reported success in reducing the 90% cost of tissue culture banana plants by replacing sucrose. In the plant propagation medium Kaur *et al.*, (2005) substituted sucrose with table sugar which reduced thecost of the medium considerably by 96.8% similar to the present study and Prakash *et al.*, (2002) reported the reduction in the cost of the medium by 78 to 87% using common sugar.

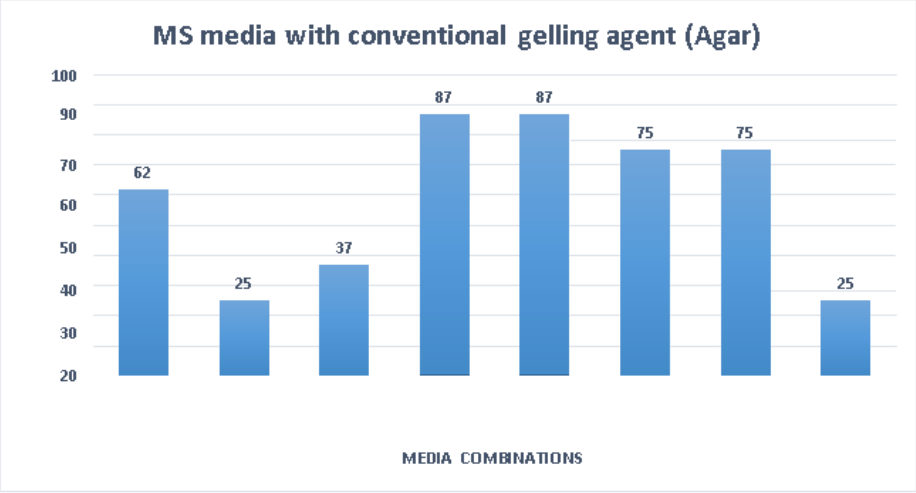

Figure 6: Graphical representation of several responses (%) of *in vitro* culture of *Ananas comosus* in different media supplements (PA1LS - PA4CS).

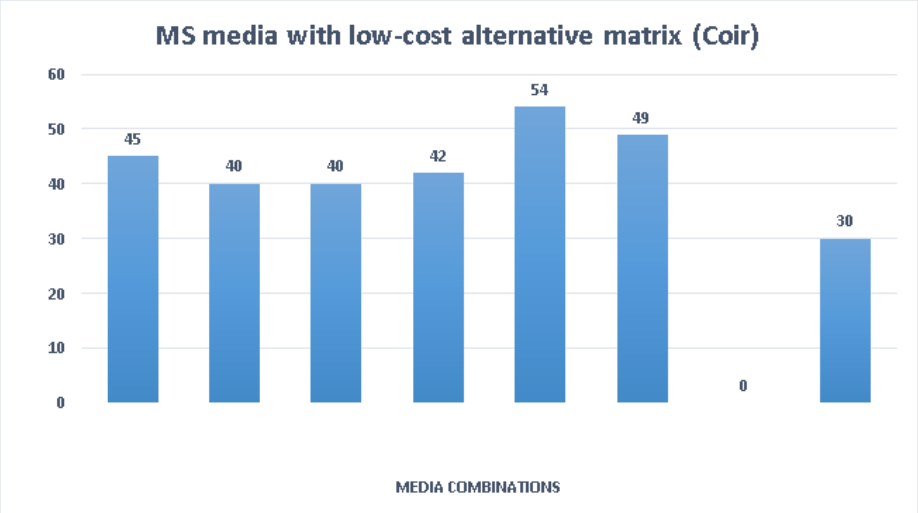

Figure 7: Graphical representation of several responses (%) of *in vitro* culture of *Vanilla planifolia* in different media supplements (PA1LS - PA4CS) and with the alternative matrix Coir.

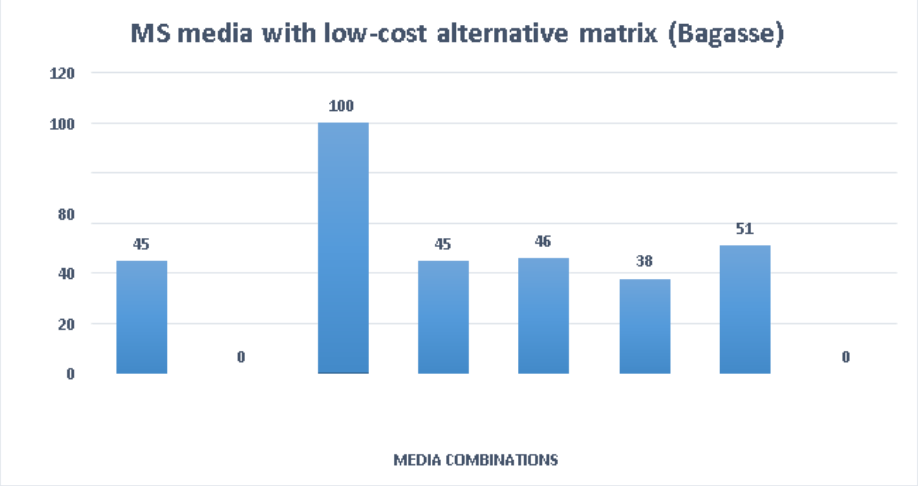

Figure 8: Graphical representation of several responses (%) of *in vitro* culture of *Vanilla planifolia* in different media supplements (PA1LS - PA4CS) and with the alternative matrix Bagasse.

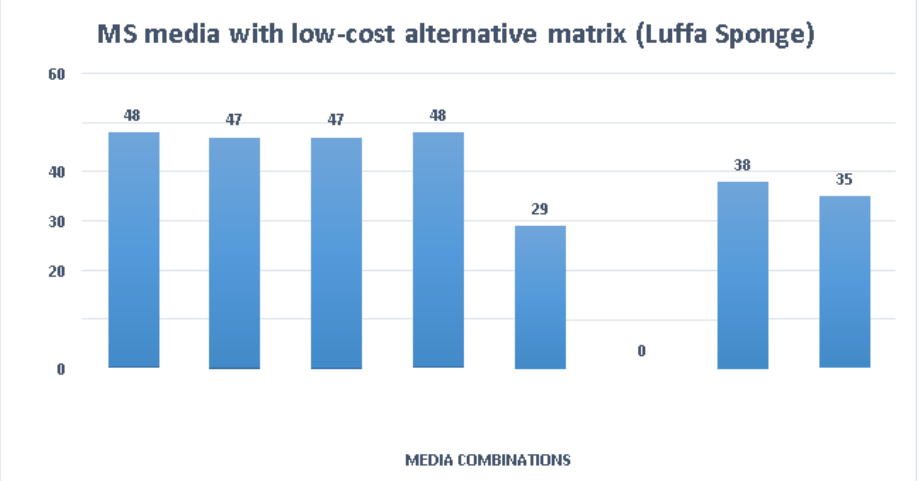

Figure 9: Graphical representation of several responses (%) of *in vitro* culture of *Ananas comosus* in different media supplements (PA1LS - PA4CS) and with the alternative matrix Luffa Sponge.

CONCLUSION

The present study mainly focused on the use and effect of alternative cost-effective matrices and carbon sources in plant *in vitro* systems. An efficient reproducible protocol for the *in vitro* induction of Pineapple and Vanilla plants using shoot tips and nodal segments in MS media supplemented with different combinations and concentrations of plant growth regulators has been established in this study. It is concluded from the above observations and graphical representations that MS media with different combinations and concentrations of growth regulators and also with the alternative cost-effective matrices instead of conventional matrix agar and commercial sugar instead of tissue culture grade sucrose showed a good positive result compared to the conventional method. MS basal media with combination of hormone and sucrose – PA2CS (BA- 1.0mg/l, Kin- 0.2mg/l, IAA- 0.4mg/l and Commercial Sucrose- 30gm/l) and PA3LS (BA- 1.5mg/l, Kin- 0.3mg/l, IAA- 0.6mg/l and Tissue culture grade Sucrose- 30gm/l) are the best media so far tested for optimum micropropagation of pineapple in *in vitro* system with the convention gelling matrix, agar. In the case of using alternative matrices with the same combinations and concentrations used in the agar matrix, bagasse (PA2LS) showed a better comparative result than the coir and luffa sponge.

I Hope, this investigation will provide an emphasis on the technical knowhow of tissue culture methods of pineapple and vanilla plants especially the use of commercially available sucrose over tissue culture grade sucrose and also the using of alternative low-cost biodegradable matrices such as coir, bagasse, luffa sponge etc. over conventional gelling matrix agar for the researchers and industrialists in future. If I get another opportunity that would be appropriate, I will make more attempts to describe future investigations.

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