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Micropropagation of Banana Using Coconut Water as Growth Hormone

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ABSTRACT

The term "tissue culture" refers to the practice of propagating plants by employing plant parts in a test tube under strict control. The degree of success in any technology based on plant cell, tissue, or organ culture is determined by a small number of factors. For optimum yield, uniformity, disease-free planting material, and plants that are true to type, tissue culture technique is superior to the traditional method of producing bananas. The selection of nutritional components and growth regulators is an important factor. Murashige and Skoog (1962) (MS) media compositions are commonly utilised, particularly in operations requiring plant regeneration. Plant tissue culture media contain a relatively small number of mineral salts. Sucrose, glucose, and to a lesser extent, fructose are the only carbon sources that will support plant cell growth in culture. There are five recognized major kinds of substances that have the ability to control plant development. These are ethylene, cytokinins, gibberellins, auxins. This study reveals the alternate growth medium can also bring growth in the tissue culture of Banana plant. Here, the alternative plant growth regulator (PGR) in the banana tissue culture is coconut water. This study aimed to examine the ability of coconut water to substitute the growth hormone. Coconut water serves as the main source of cytokine. Cytokines are a class of plant growth regulators that play a key role in cell division in the roots and shoot systems of plants. Due to the presence of natural cytokinins in coconut water, mixing it with medium frequently produces the same results as mixing in a known cytokinins. The coconut water with four levels of concentration like. 50, 100, 150 and 200 ml per liter are added. The parameters such as leaf length, shoot length are observed. The coconut water concentration of 150 ml per liter had the best results, giving the plantlets a significant shoot length.

Key word: MS Media, Coconut Water, Elongation, Banana species, Cytokines, Tissue culture.

INTRODUCTION

The science of cultivating isolated plant cells, tissues, or organs on artificial media is known as plant tissue culture. It offers various practical goals as well as methodologies and approaches suitable for several botanical disciplines of research (Nayar 2010).

THE EARLY TISSUE CULTURE

Attempts to cultivate isolated root tips were successful by Haberlandt pupil Kotte and Robbins (1922). Gottlieb Haberlandt's lecture to the German Academy of Science in 1902 on his findings on the culture of single cells served as the theoretical foundation for plant tissue culture (Mardhikasari *et. al*., 2020). By employing explants with meristematic cells, white was able to successfully cultivate tomato root tips for an extended period of time.

A fully defined medium for root culture was made possible by additional work. These root cultures were initially employed for virus research and afterwards as a crucial tool for physiological research (Hanumantharaya *et al*., 2008).

Bud cultures also had success (Debabandya *et al*., 2010) & (Strosse *et al*., 2015). Embryo culture also had its start with barley embryos early in the first decade of the previous century (George *et al*., 1993). Gautheret obtained the first actual plant tissue cultures from the cambial tissue of Acer pseudoplatanus.

TERMS IN TISSUE CULTURE

A list of terms used in the plant tissue culture are provided below:

EXPLANT:

An explant is a piece of differentiated tissue or organ that has been excised. Any part of the plant body may be used for the explant. i.e., Stem, root, leaf. For rapid in vitro multiplication of banana, shoot tips from young suckers of 40-100 cm height are most commonly used as explants. The plant can be exactly cloned like its mother plant without any variations.

CALLUS:

The term "callus" refers to the disordered, undifferentiated mass of plant cells. A plant callus is a mass of undifferentiated cells that grow over an explant as a result of wounding or when growth factors are added to culture media.

DEDIFFERENTIATION:

Dedifferentiation is the process through which mature cells go back to their meristematic stage to create callus. Differentiation leads to the formation of permanent tissues which have specialized structures for specific functions.

REDIFFERENTIATION:

Redifferentiation is the process through which callus cells might change into an individual plant cell or an entire plant. The phenomenon by which cells divide and produce cells that once again lose their dividing capacity but mature to perform specific functions.

TOTIPOTENCY:

Totipotency refers to a cell's capacity to develop into a whole plant. It can ability of a cell to give rise to a whole new plant which allows the meristematic tissues of a banana plant to grow in a culture medium. The meristematic tissues don't get infected by virus. The totipotency allows them to form the callus.

Totipotent cells are the most potent of all stem cells, and dying them is important for research and the field of regenerative medicine.

It is the genetic potential of a plant cell to produce the entire plant. It is the cell characteristic that has the potential for forming all the cell types in the adult.

MATERIALS AND METHOD

PLANT COLLECTION

The healthy young plants were collected from the Tamil Nadu News Print and papers Limited (TNPL), Kagithapuram, Karur, Tamilnadu.

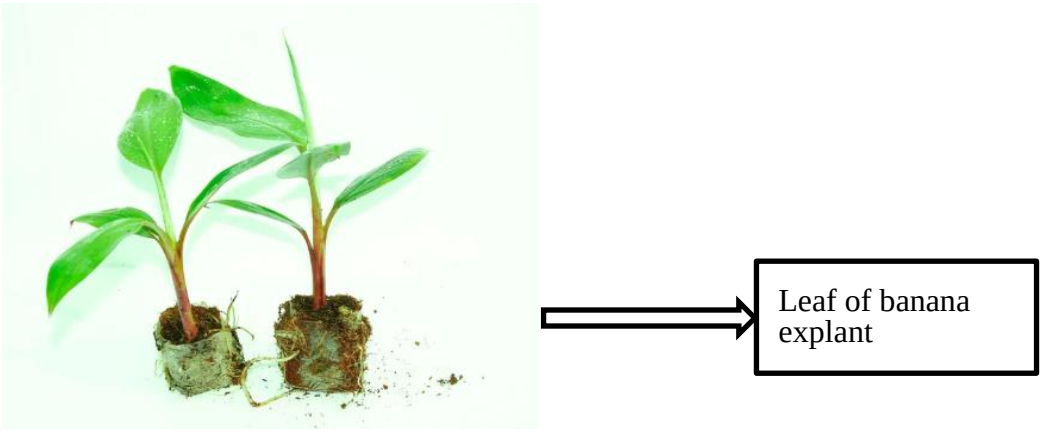

FIGURE 1: BANANA EXPLANT

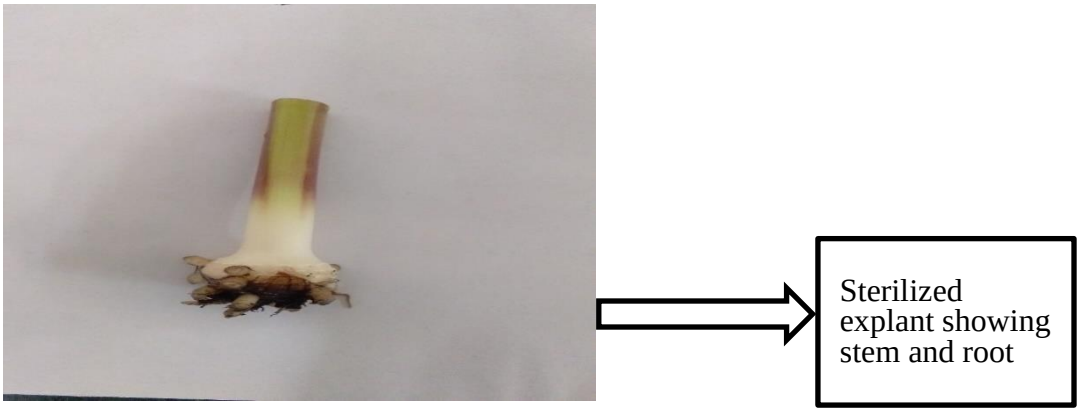

FIGURE 2: COLLECTION OF CLEANED EXPLANT

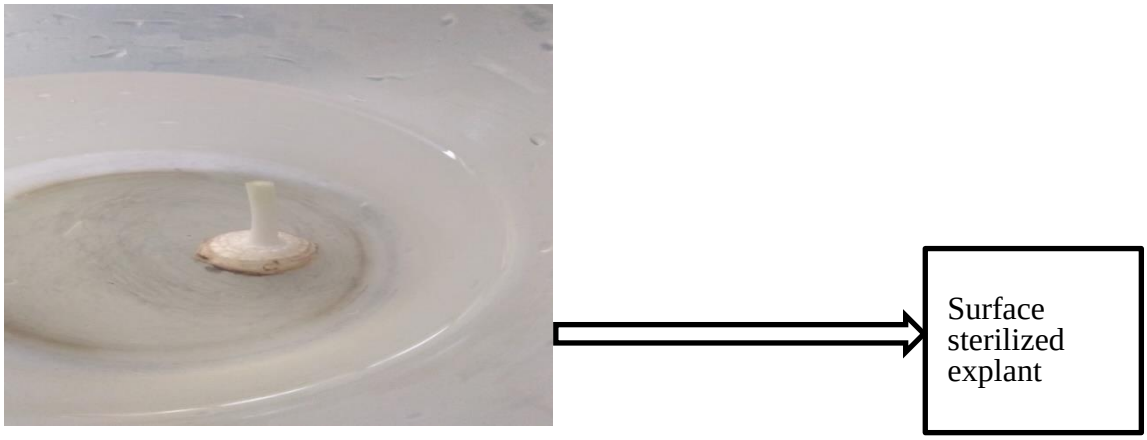

FIGURE 3: EXPLANT BANANA SUCKER WASHED OUT USING DISTILLED WATER

Figure.3 shows the explants were washed thoroughly in running tap water for 15-20 min with detergent solution to remove adherent soils. After rinsing three times *with* sterile *distilled water*, the *explants* were excised into final size under aseptic conditions.

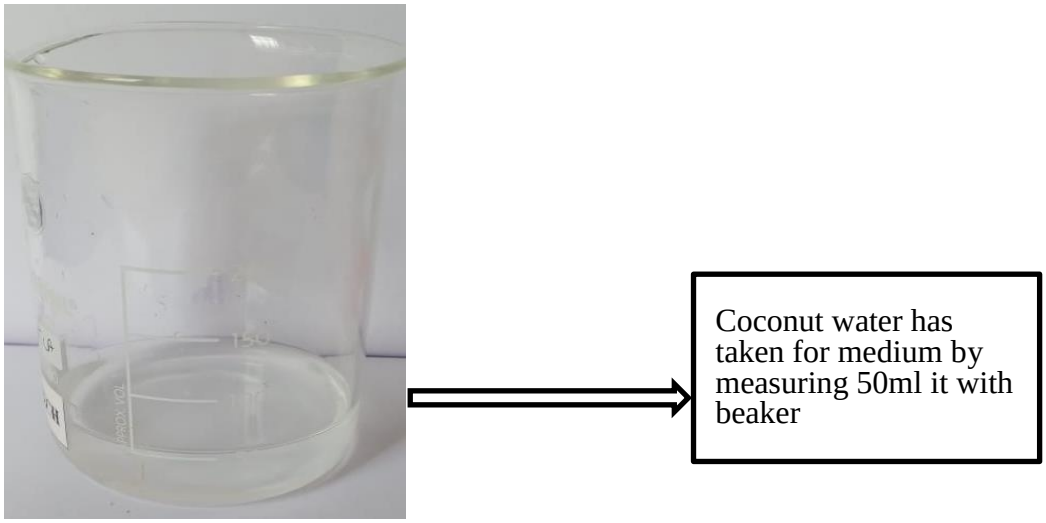

FIGURE 4: 50 ml COCONUT WATER

Figure 4 shows the coconut is measured by a measuring flask and checked for contamination. The 50 ml of coconut water is added to the medium. The medium with 50ml coconut water is sterilized in the autoclave. The sterilized Medium is then used for the culture.

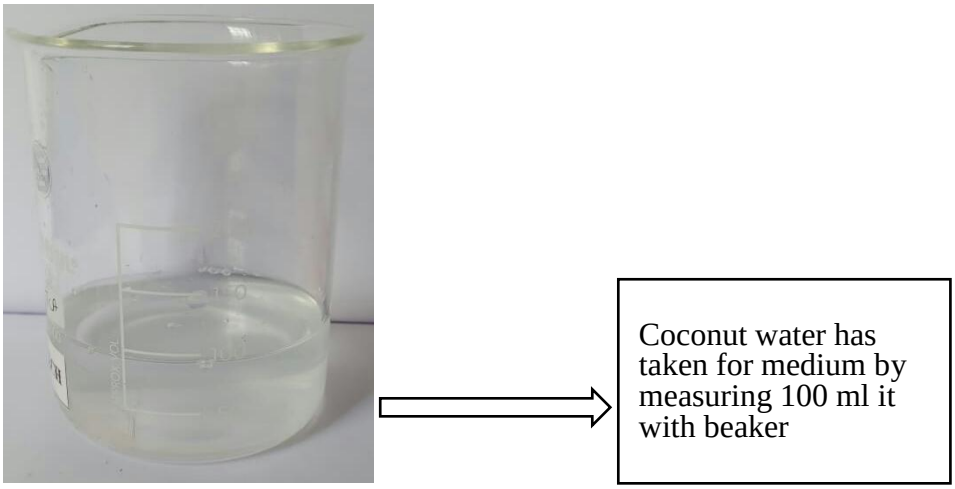

FIGURE 5: 100 ml COCONUT WATER

Figure.5 shows the coconut is measured by a measuring flask and checked for contamination. The 100 ml of coconut water is added to the medium. The medium with 100ml coconut water is sterilized in the autoclave. The sterilized Medium is then used for the culture.

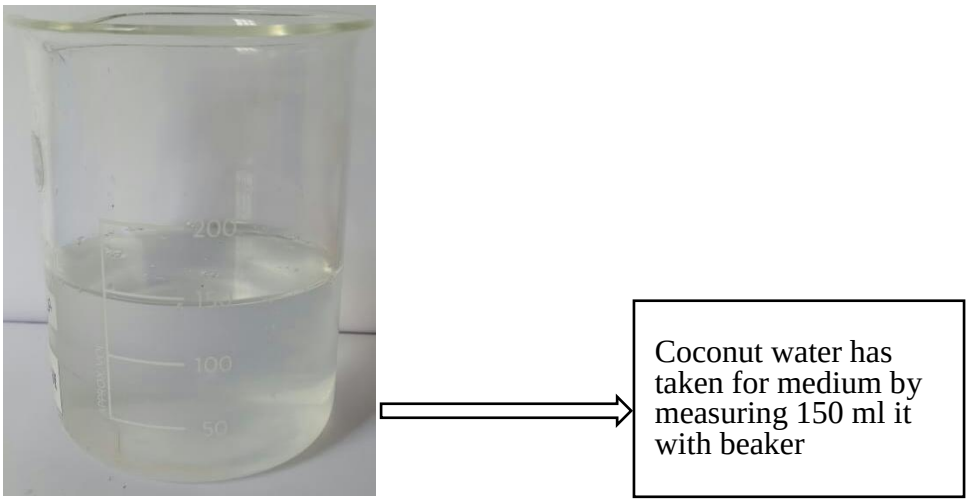

FIGURE.6: 150 ml COCONUT WATER

Figure 6 shows the coconut is measured by a measuring flask and checked for contamination. The 150 ml of coconut water is added to the medium. The medium with 150ml coconut water is sterilized in the autoclave. The sterilized Medium is then used for the culture.

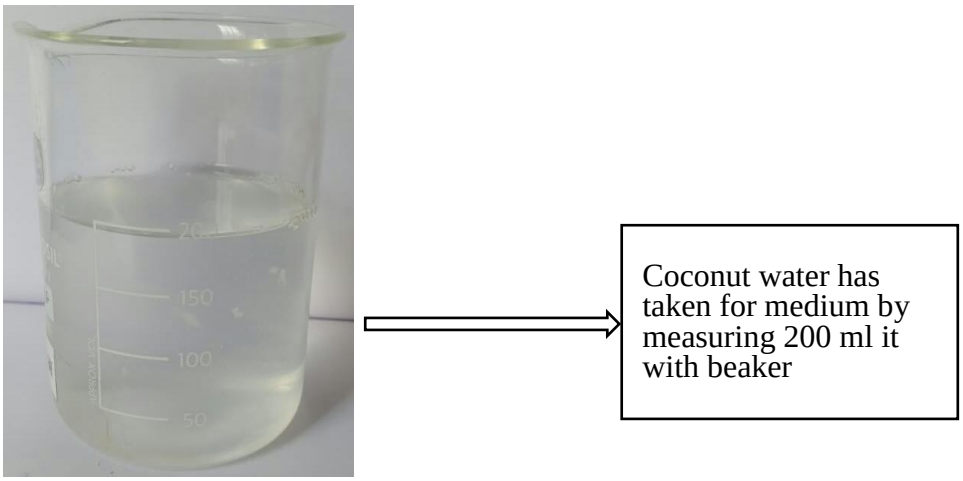

FIGURE 7: 200 ml COCONUT WATER

Figure.7 shows the coconut water which is the main nutrient source for the elongation stage are collected**.** The coconut is measured by a measuring flask and checked for contamination. The 200 ml of coconut water is added to the medium. The medium with 200 ml coconut water is sterilized in the autoclave. The sterilized Medium is then used for the culture.

MEDIA PREPARATION

MS I PREPARATION

The standard MS Media is prepared as a stock solution with the composition of micro nutrients, macro nutrients, vitamins, growth regulators, etc.

The following table will provide the necessary information needed for the preparation of Medium. For the initiation MS I, MS II, MS III, MS IV, MS V are added. The MS I consists of KNO_3, NH_4NO_3, $MgSO_4.7H_2O$, KH_2PO_4. The above nutrients are added to prepare MS I. The 40 ml of MS I is taken for the initiation medium.

TABLE 1: TABULATION OF (MURASHIGEE AND SKOOG) MS I 25X (5 LITRES)

S.NO	Chemical Name	Media mg/liter	Mg/stock	g/stock
1	KNO_3	1900	237500	237.5
2	NH_4NO_3	16.500	206.250	62.5
3	$MgSO_4.7H_2O$	370	46250	46.25
4	KH_2PO_4	170	21250	21.25

Table 1 shows the composition (MURASHIGEE AND SKOOG) MS I Medium for tissue culture from the desired variety of plant can be produced.

MS II PREPARATION

The following table will provide the necessary information needed for the preparation of Medium. For the initiation MS I, MS II, MS III, MS IV, MS V are added.

The MS II consists of $MnSO_4.H_2O$, $ZnSO_4.7H_2O$, H_3BO_3, KI, $CuSO_4.5H_2O$, $Na_2MoO_4.2H_2O$, and CaCl2.6H2O. The above nutrients are added to prepare MS II. The 40 ml of MS II is taken for the initiation medium.

TABLE 2: TABULATION OF (MURASHIGEE AND SKOOG) MS II 100 X (2.5 LITRES)

S.NO	Chemical Name	Media mg/liter	Mg/stock	g/stock
1	$MnSO_4.H_2O$	17	4250	4.25
2	$ZnSO_4.7H_2O$	8.6	2150	2.15
3	H_3BO_3	6.2	1550	1.55
4	KI	0.83	207.5	-
5	$CuSO_4.5H_2O$	0.025	6.25	-
6	$Na_2MoO_4.2H_2O$	0.25	62.5	-
7	$CaCl_2.6H_2O$	0.025	6.25	-

Table 2 shows the composition (MURASHIGEE AND SKOOG) MS II Medium for tissue culture from the desired variety of plant can be produced.

MS III PREPARATION

The following table will provide the necessary information needed for the preparation of Medium. For the initiation MS I, MS II, MS III, MS IV, MS V are added. The MS III consists of $CaCl_2$. The above nutrients are added to prepare MS III. The 10 ml of MS III is taken for the initiation medium.

TABLE 3: TABULATION OF (MURASHIGEE AND SKOOG) MS III 100X (4 LITRES)

S.NO	Chemical Name	Media mg/liter	Mg/stock	g/stock
1	$CaCl_2$	332	33200	33.2

Table 3 shows the composition (MURASHIGEE AND SKOOG) MS III Medium for tissue culture from the desired variety of plant can be produced.

3.3.4 MS IV PREPARATION

The following table will provide the necessary information needed for the preparation of Medium. For the initiation MS I, MS II, MS III, MS IV, MS V are added. The MS IV consists of $FeSo_4.7H_2O$, $Na_2EDTA.2H_2O$. The above nutrients are added to prepare MS IV. The 5ml of MS IV is taken for the initiation medium.

TABLE 4: TABULATION OF (MURASHIGEE AND SKOOG) MS IV (200X) (2 LITRES)

S.NO	Chemical Name	Media mg/liter	Mg/stock	g/stock
1	$FeSo_4.7H_2O$	27.85	11140	11.4
2	$Na_2EDTA.2H_2O$	37.25	14900	14.9

Table 3.4 shows the composition (MURASHIGEE AND SKOOG) MS IV Medium for tissue culture from the desired variety of plant can be produced.

MS V PREPARATION

The following table will provide the necessary information needed for the preparation of Medium. For the initiation MS I, MS II, MS III, MS IV, MS V are added. The MS V consists of Inositol, Thiamine Hcl, Nicotinic Acid, Pyridine Hcl, and Glycine. The above nutrients are added to prepare MS IV. The 5 ml of MS V is taken for the initiation medium

TABLE 5: TABULATION OF MS V 200X (2LITRES)

S.NO	Chemical Name	Media mg/liter	Mg/stock	g/stock
1	Inositol	100	40000	40
2	Thiamine Hcl	0.3	120	-
3	Nicotinic Acid	0.5	200	-

4	Pyridine Hcl	0.5	200	-
5	Glycine	2	800	-

Table 5 shows the composition (MURASHIGEE AND SKOOG) MS V Medium for tissue culture from the desired variety of plant can be produced.

STERILIZATION OF EXPLANT

The banana suckers were trimmed to a height of 7 to 8 cm, rinsed under running water to remove any dirt particles, and then treated for 30 minutes with 0.1% carbendazim (a fungicide) and 0.1% streptocycline (a bactericide). The suckers were then rinsed with autoclaved distilled water after being immersed in antiseptic solution (Dettol) for 10 minutes at a concentration of 5 ml/L. Tween 20 was applied for 20 minutes, followed by treatments with ethanol (70%) for 5 minutes and HgCl2 (0.1%) for 30 minutes. The suckers were thoroughly cleaned with distilled water that had been disinfected three to four times before being cut to a height of 3 to 4 cm.

The explants were then introduced into the culture medium under aseptic conditions after being surface sterilized (Figure 3.6). The aforementioned surface sterilization procedure was carried out in a laminar hood under sterile circumstances.

INITIATION

The initial stage in tissue culture is initiation. For the initiation media the following nutrients are added like MS I, MSII, Calcium, Vitamin, Iron, BAP Hormone, Sucrose, PVP, Gel rite. The MS I of 40 ml, MS II of 10ml, calcium of 10ml,Vitamin 5ml, Iron 5ml, BAP Hormone 5ml, sucrose 30g, PVP 1g, Gel rite 2.5g are added to prepare initiation medium. The prepared medium is sterilized in autoclave for 1-2 days.

The Sterilized medium is then taken out to the laminar air flow chamber where the explants are cultured. Before culturing, the air flow chamber is fully sterilized with ethanol. The surgical blades and forceps were also sterilized with the ethanol. Along with these the hands of the culturing person also sterilized with ethanol. The explants were cut and cultured into the medium and placed in growth room and the growth is checked regularly. The Constituents and its proportion are given in the table.

TABLE 6: TABULATION FOR MEDIA COMPOSITION

S.NO	**Nutrient**	**Composition**
1	MS I	40 ml/liter
2	MS II	10 ml/liter
3	Calcium	10 ml/liter
4	Vitamin	5 ml/liter
5	Iron	5 ml/liter
6	BAP Hormone	5 ml/liter
7	Sucrose	30 g/liter
8	PVP	1 g/liter
9	Gel rite	2.5 g/liter

Table 6 shows the comprises the micro and macro nutrient and hormone needed for the preparation of initiation medium. The prepared medium should contain the pH around 6 to maintain the culture condition. The explants are inoculated in to the media for initiation.

ELONGATION

After Initiation, the next step in tissue culture is subculturing or elongation. For the elongation media the following nutrients are added like MS I, MS II, Calcium, Vitamin, Iron, BAP Hormone, Sucrose, PVP, Gel rite. The MS I of 40 ml, MS II of 10ml, calcium of 10ml, Vitamin 5ml, Iron 5ml, BAP Hormone 5ml, sucrose 30g, PVP 1g, Gel rite 2.5g, IAA 10ml are added to prepare elongation medium. The prepared medium is sterilized in autoclave for 1-2 days. With the elongation medium the four different concentrations of coconut water are added like 50ml, 100ml, 150ml and 200ml. The Sterilized medium is then taken out to the laminar air flow chamber where the explants are cultured. Before culturing, the air flow chamber is fully sterilized with ethanol. The surgical blades and forceps were also sterilized with the ethanol. Along with these the hands of the culturing person also sterilized with ethanol. The explants were cut and cultured into the medium and placed in growth room and the growth is checked regularly. The Constituents and its proportion are given in the table.

TABLE 7: TABULATION FOR ELONGATION MEDIA WITH HORMONE

S.NO	Nutrient	Composition
1	MS I	40 ml/litre
2	MS II	10 ml/litre
3	Calcium	10 ml/litre
4	Vitamin	5 ml/litre
5	Iron	5 ml/litre
6	Gel rite	2.5 g/litre
7	PVP	1 g/litre
8	Sucrose	30 g/litre
9	IAA	10 ml/litre

Table 7 shows the comprises the micro and macro nutrient and hormone needed for the preparation of elongation medium. The prepared medium should contain the pH around 6 to maintain the culture condition. The explants are inoculated in to the media for initiation.

TABLE 8: TABULATION FOR ELONGATION MEDIUM

S.NO	Nutrient	Composition
1	MS I	40 ml/litre
2	MS II	10 ml/litre
3	Calcium	10 ml/litre
4	Vitamin	5 ml/litre
5	Iron	5 ml/litre
6	Gel rite	2.5 g/litre
7	PVP	1 g/litre
8	Sucrose	30 g/litre

Table 8 shows The elongation medium without hormone is prepared as per the above composition. The above table comprises the micro and macro nutrient and hormone needed for the preparation of elongation medium. The prepared medium should contain the p^H around 6 to maintain the culture condition. The explants are inoculated in to the media for Elongation.

The different concentrations of the coconut water is added to the elongation medium.

Thus, the elongation medium without hormone is prepared with different concentrations of coconut water. The initiation cultivated plant is inoculated into the elongation medium with coconut water and the elongation medium with hormone.

Elongation medium without hormone +50 ml coconut water
Elongation medium without hormone +100 ml coconut water
Elongation medium without hormone +150 ml coconut water
Elongation medium without hormone +200 ml coconut water

RESULT AND DISCUSSION

MEDIA PREPARATION WITH COCONUT WATER

The coconut water added to the (MURASHIGEE AND SKOOG) MS media are shown below.

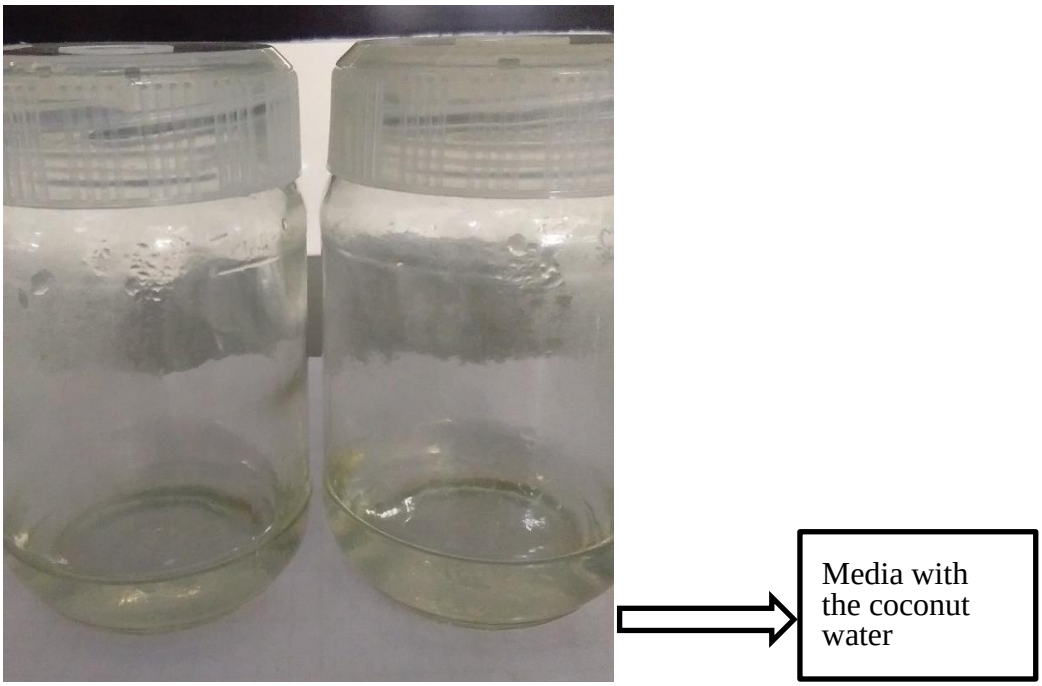

FIGURE 8: MEDIA WITH COCONUT WATER

Figure **8** shows the above medium consists of coconut water and the necessary nutrients needed for the culture. After the preparation of the media with coconut water, the media is checked for the bacteria and fungi. If there is no contaminant like bacteria and fungi then it is used for the culture (Figure.4.1). Similarly, the media is utilized to promote the subculture. Coconut water is one of the natural growth regulating hormones that contain phyto-hormone, especially cytokines and indole-3-acetic acid (IAA). The coconut water provide nutrients needed by plantlets to grow and develop during in vitro culture. Coconut water has high levels of zeatin in its composition it is frequently used in micropropagation protocols of economically important crops. The elongation of the culture are tabulated as follows:

ELONGATION MEDIA WITH HORMONE

TABLE 9: TABULATION FOR CALCULATE THE PLANTS SHOOT LEANTH IN ELONGATION MEDIA WITH HORMONE

BOTTLE NO	**INITIAL STAGE**		**FINAL STAGE**	
	No. of shoots	**Shoot length(cm)**	**No. of shoots**	**Shoot length(cm)**
1	4	1	5	3
2	3	1	6	2.8
3	3	0.5	5	2.6
4	4	0.8	5	4*
5	3	0.65	5	3*
6	3	1	5	3*
7	4	1.2	5	2.8
8	3	0.6	3	2.5
9	4	0.98	6	2
10	3	0.7	8	2

*****Indicates the most elongated shoot

Table 9 shows the elongation of banana shoot and it showed the medium result by successfully elongating 4, 5 and 6 bottles.

ELONGATION MEDIA WITH HORMONE

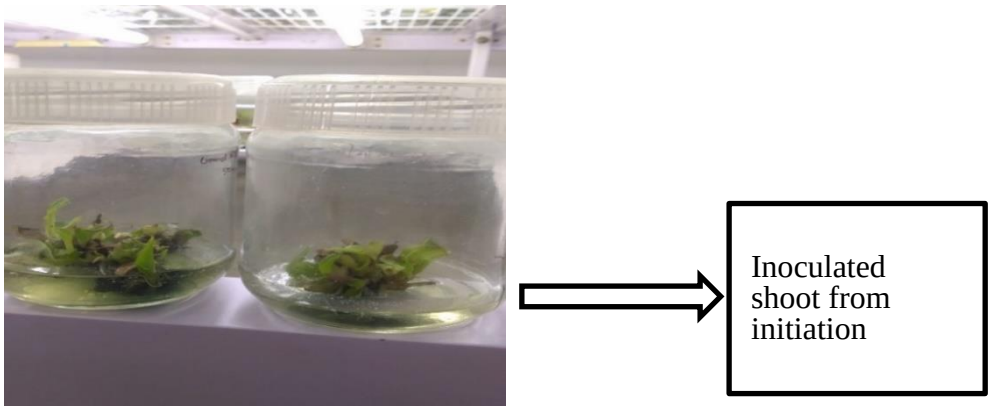

FIGURE 8 : INITIAL STAGE OF ELONGATION MEDIA WITH HORMONE

Figure 8 shows the initial stage of bud in the elongation media without coconut water.The buds were collected from the initiation stage and are cultured in the elongation medium with hormone.The buds were inoculated in the elongation medium with surgical blades and forceps.Then the iniculated medium are transferred to the growth room.The growth of shoot, shoot length were observed regularly.

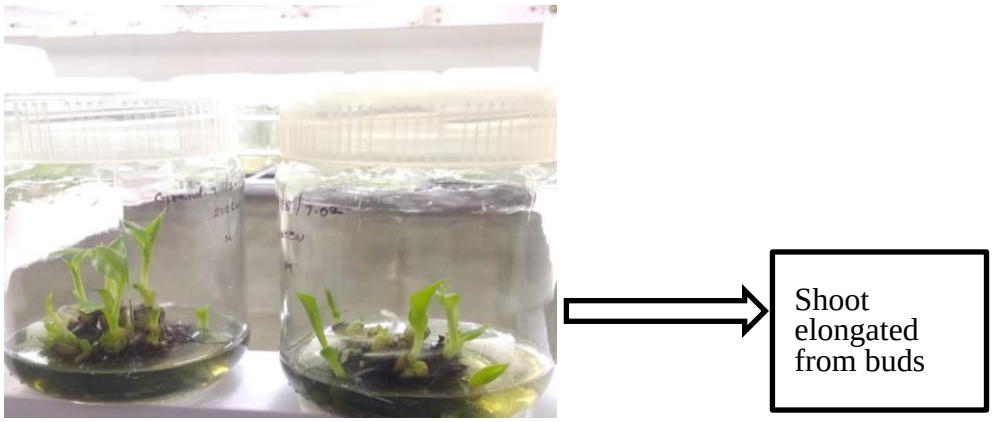

FIGURE 9: FINAL STAGE OF ELONGATION MEDIA WITH HORMONE

Figure 9 shows the final stage of bud in the elongation media without coconut water. In this media without hormone, there will be few elongation of buds and also the media gets contaminated easily. There is no faster growth and only less number of shoots were observed. Sometimes, the buds become dry on contamination. Fewer shoots were grown and the length of the shoot were also low.

4.3 ELONGATION MEDIA +50 ml COCONUT WATER

TABLE 10: TABULATION FOR CALCULATE THE PLANTS SHOOT LENGTH IN ELONGATION MEDIA +50 ml COCONUT WATER

*****indicates the most elongated shoot

Table 10 shows the elongation of banana shoot in the medium which consists of 50 ml coconut water instead of hormone and it showed the good result by successfully elongating 1, 5 and 6 bottles.

BOTTLE NO	**INITIAL STAGE**		**FINAL STAGE**	
	No. of shoots	**Shoot length(cm)**	**No. of shoots**	**Shoot length(cm)**
1	4	1	5	3*
2	3	1	6	2.8
3	3	0.5	5	2.6
4	4	0.8	5	4
5	3	0.65	5	3*
6	3	1	5	3*

7	4	1.2	5	2.8
8	3	0.6	3	2.5
9	4	0.98	6	2
10	3	0.7	8	2

ELONGATION MEDIUM+50 ml COCONUT WATER

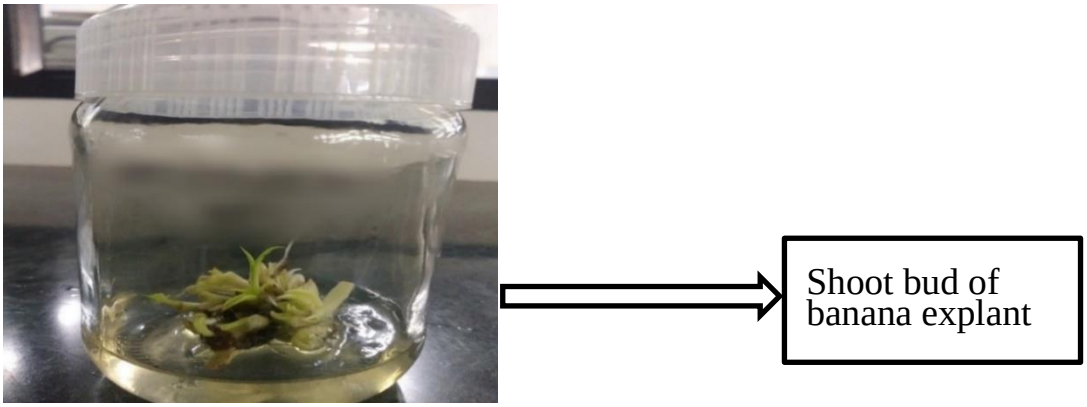

FIGURE 10: INITIAL STAGE OF ELONGATION MEDIUM+50 ml COCONUT WATER

Figure 10 shows the initial stage of bud in elongation medium with 50ml concentration of coconut water. In this media the 50 ml concentration of coconut water is added as hormone. The coconut water is added to reduce the cost of the media as the cost of the growth hormone is high. The buds were collected from the initiation stage and are cultured in the elongation medium with coconut water.The buds were inoculated in the elongation medium with surgical blades and forceps.Then the inoculated medium are transferred to the growth room.The growth of shoot, shoot length were observed regularly.

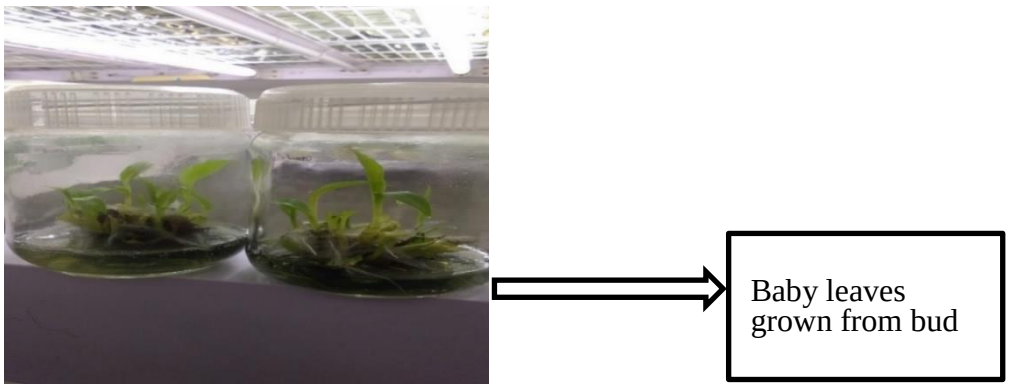

FIGURE 11: FINAL STAGE OF ELONGATION MEDIUM+50 ml COCONUT WATER

Figure 11 shows the final stage of bud which has grown in elongation medium+50ml coconut water. In this media with coconut water showed better results than media with hormone. The contamination media is also low. But the shoot length stops at 3-4cm of growth in the short time. Simultaneously, the number of shoots have increased regularly. More than 5-6 shoots produced in the short time in significant number of bottles. It showed the better result in both shoot number and shoot length.

ELONGATION MEDIA +100ml COCONUT WATER

TABLE 11: TABULATION FOR CALCULATE THE PLANTS SHOOT LENGTH IN ELONGATION MEDIA +100 ml COCONUT WATER

BOTTLE NO	**INITIAL STAGE**		**FINAL STAGE**	
	No. of shoots	**Shoot length(cm)**	**No. of shoots**	**Shoot length(cm)**
1	3	0.7	5	1.5
2	4	0.98	5	2

3	4	0.6	5	2.5
4	3	1.2	4	2.8
5	4	1	5	2.6
6	4	0.65	6	3*
7	3	0.8	5	3*
8	3	1	5	4*
9	3	1	6	3*
10	3	0.5	4	2.8

*****indicates the most elongated shoot

Table 11 shows the elongation of banana shoot in the medium which consists of 100 ml coconut water instead of hormone and it showed the good result by successfully elongating 6, 7, 8 and 9 bottles.

ELONGATION MEDIUM+100 ml COCONUT WATER

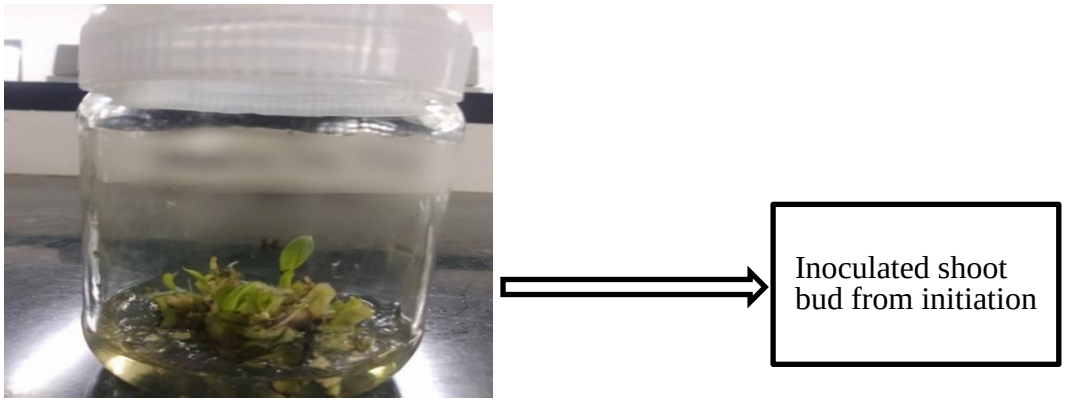

FIGURE 12 : INITIAL STAGE OF ELONGATION MEDIUM+100 ml COCONUT WATER

Figure 12 shows the initial stage of bud in elongation medium with 100ml concentration of coconut water. In this media the 100 ml concentration of coconut water is added as hormone. The coconut water is added to reduce the cost of the media as the cost of the growth hormone is high. The buds were collected from the initiation stage and are cultured in the elongation medium with coconut water.The buds were inoculated in the elongation medium with surgical blades and forceps.Then the inoculated medium are transferred to the growth room.The growth of shoot, shoot length were observed regularly.

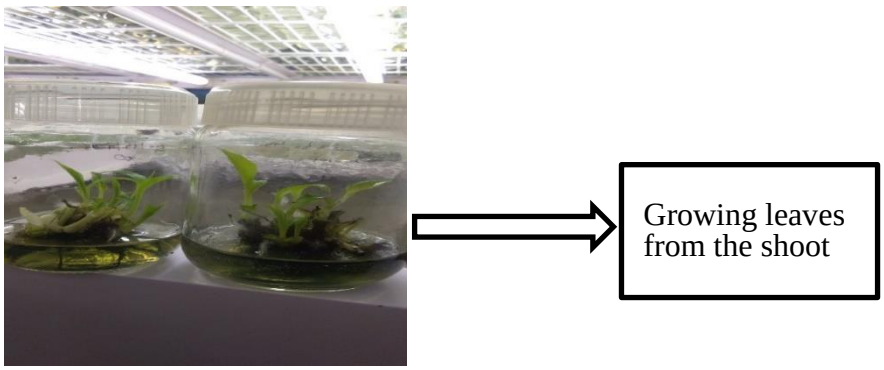

FIGURE 13 : FINAL STAGE OF ELONGATION MEDIUM+100 ml COCONUT WATER

Figure 13 shows the final stage of bud which has grown in elongation medium+100ml coconut water. In this media with coconut water also showed better results than media with hormone. The contamination media is also low. But the shoot length stops at 3-4cm of growth in the short time. Simultaneously, the number of shoots have increased regularly. More than 5-6 shoots produced in the short time in significant number of bottles. It showed the better result in both shoot number and shoot length. The medium with 100ml of coconut water have showed less contamination than the 50ml concentration.

ELONGATION MEDIA +150ml COCONUT WATER

TABLE 12: TABULATION FOR CALCULATE THE PLANTS SHOOT LENGTH IN ELONGATION MEDIA +150 ml COCONUT WATER

BOTTLE NO	INITIAL STAGE		FINAL STAGE	
	No. of shoots	Shoot length(cm)	No. of shoots	Shoot length(cm)
1	3	0.98	8	6
2	4	1.2	9	8*
3	4	0.65	10	7*
4	3	0.5	9	6.5
5	4	1	8	5.8
6	4	0.8	8	8*
7	3	1	8	8*
8	3	0.7	9	8*
9	3	1	8	8*
10	3	0.6	9	8*

*****Indicates the most elongated shoot

Table 12 shows the elongation of banana shoot in the medium which consists of 150 ml coconut water instead of hormone and it showed the good result by successfully elongating 2, 3, 6, 7, 8, 9 and 10 bottles.

ELONGATION MEDIUM+150 ml COCONUT WATER

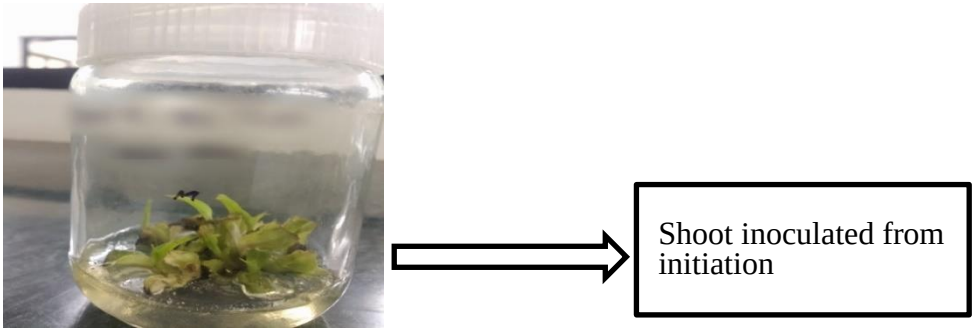

FIGURE 14: INITIAL STAGE OF ELONGATION MEDIUM+150 ml COCONUT WATER

Figure 14 shows the initial stage of bud in elongation medium with 150ml concentration of coconut water. In this media the 150 ml concentration of coconut water is added as hormone. The coconut water is added to reduce the cost of the media as the cost of the growth hormone is high. The buds were collected from the initiation stage and are cultured in the elongation medium with coconut water.The buds were inoculated in the elongation medium with surgical blades and forceps.Then the inoculated medium are transferred to the growth room.The growth of shoot, shoot length were observed regularly.

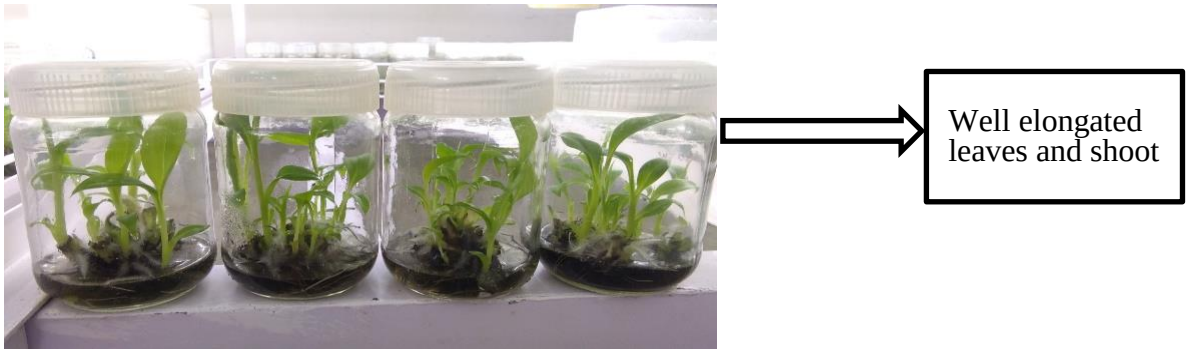

FIGURE 15: FINAL STAGE OF ELONGATION MEDIUM+150 ml COCONUT WATER

Figure 15 shows the final stage of bud which has grown in elongation medium+150ml coconut water. In this media with coconut water also showed better results than media with hormone. The contamination media is also low. It showed the highest shoot length of 8 cm in almost the bottles and also the shoot number also increased tremendously. More than 9-10 shoots were produced in the short time in significant number of bottles. It showed the

better result in both shoot number and shoot length. The medium with 150ml of coconut water have showed less contamination than all the concentrations. The 150ml concentration have showed the best result among all the concentration.

4.6 ELONGATION MEDIA +200 ml COCONUT WATER

TABLE 13: TABULATION FOR CALCULATE THE PLANTS SHOOT LENGTH IN ELONGATION MEDIA +200 ml COCONUT WATER

BOTTLE NO	INITIAL STAGE		FINAL STAGE	
	No. of shoots	Shoot length(cm)	No. of shoots	Shoot length(cm)
1	3	0.7	5	5
2	4	0.98	6	6.5*
3	4	0.6	5	5*
4	3	1.2	5	3
5	4	1	5	4
6	4	0.65	5	5*
7	3	0.8	4	4.85
8	3	1	4	4
9	3	1	5	4.5*
10	3	0.75	5	5.5*

*****Indicates the most elongated shoot

Table 13 shows the elongation of banana shoot in the medium which consists of 200 ml coconut water instead of hormone and it showed the good result by successfully elongating 2,3,6,9 and 10 bottles.

ELONGATION MEDIUM+200 ml COCONUT WATER

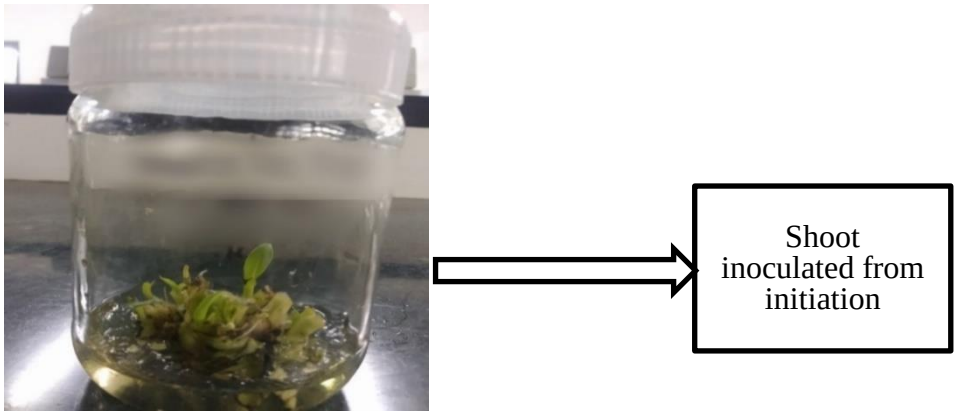

FIGURE 16: INITIAL STAGE OF ELONGATION MEDIUM+200 ml COCONUT WATER

Figure 16 shows the initial stage of bud in elongation medium with 200ml concentration of coconut water. In this media the 200 ml concentration of coconut water is added as hormone. The coconut water is added to reduce the cost of the media as the cost of the growth hormone is high. The buds were collected from the initiation stage and are cultured in the elongation medium with coconut water. The buds were inoculated in the elongation medium with surgical blades and forceps. Then the inoculated medium are transferred to the growth room.The growth of shoot, shoot length were observed regularly.

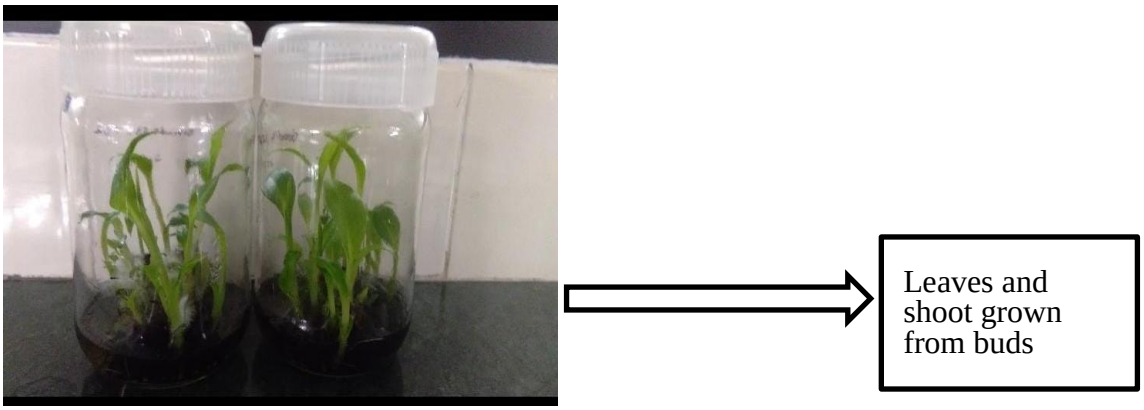

FIGURE 17: FINAL STAGE OF ELONGATION MEDIUM+200 ml

COCONUT WATER

Figure 17 shows the final stage of bud which has grown in elongation medium+200ml coconut water. In this media with coconut water also showed better results than media with hormone. The contamination media is also low. It showed the highest shoot length of 8 cm in almost the bottles and also the shoot number also increased tremendously. More than 9-10 shoots were produced in the short time in significant number of bottles. Observing the 200ml concentration of coconut water medium, it is clear that 150 ml is the optimum concentration for the bud to grow. Because increasing the concentration of coconut water than 150ml showed the same result as in the medium with hormone. The shoot length of the buds were decreased to 4-5 cm. simultaneously the shoot number were also decreased. Thus, it showed that the Medium with 150ml of coconut water is optimum for the buds to grow in the tissue culture

CONCLUSION

Banana is one among the earliest crop plant that has been domesticated by humans and is being cultivated under a broad range of climatic conditions for dessert and culinary purpose. Banana is a popular fruit in the world market and it is a staple food in many countries. It is also one of the healthiest fruit which contains potassium, calcium, magnesium, iron, proteins, fibers, carbohydrates and an array of vitamins. India is one of the largest global banana producers with a current production of about 26 million tones. Banana planting material obtained through tissue culture have many advantages such as it can be utilized for high density planting, yields higher per unit area of land and can result in disease free and true to type plants. From the results, it is observed that the elongation media with different concentration of coconut water have shown the best results in shoot number and shoot length as compared to the standard elongation medium with the hormone. Hence, we can use the coconut water to get the elongated shoots in short time as compared to the normal media with hormone. All concentrations of coconut water have showed the expected result but the concentration 150ml has the well elongated and differentiated plantlets. The normal media with hormone have the plantlets which are small in size when compared to the coconut concentrated media. Hence, this study concluded that the optimum concentration of coconut water for the elongation is 150ml/liter (Bottle No: 2, 3, 6, 7, 8, 9 and 10). Cost effective plant tissue culture techniques and reduction of media cost can be practiced to lower the production cost without compromising on the quality as effectively proved through the study. Such media cost reduction studies carried out for tissue culture dependent crops can be of service to the farming community. The shoot proliferation rate plays a vital role in bringing down the production cost of plantlets.

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