MICROPROPAGATION OF SWEET POTATO (*Ipomoea batatas* L.) IN A NEW PLANT TISSUE CULTURE MEDIUM

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CERTIFICATE

*This is to certify that thesis paper entitled "**MICROPROPAGATION OF SWEET POTATO (Ipomoa batatas* **L.)** *IN A NEW PLANT TISSUE CULTURE MEDIUM**" submitted to the Faculty of Agriculture, Sher-e-Bangla Agricultural University (SAU), Dhaka in partial fulfillment of the requirements for the degree of **MASTER OF SCIENCE IN BIOTECHNOLOGY**, embodies the result of a piece of bona fide research work carried out by **TIPU SULTAN** Registration no**. 15-06621** under my supervision and guidance. No part of the dissertation has been submitted for any other degree or diploma.*

I further certify that such help or source of information, as has been availed of during the course of this investigation has duly been acknowledged.

Dated: June, 2022
Place: Dhaka, Bangladesh

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Dedicated to

My Parents and Family

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The Author

ABBREVIATIONS

Agril.	:	Agriculture
Biol.	:	Biological
cm	:	Centimeter
CRD	:	Completely Randomized Design
DMRT	:	Duncan's Multiple Range Test
Conc.	:	Concentration
DAI	:	Days After Inoculation
FAO	:	Food and Agricultural Organization
IASC	:	International Aloe Science Council
g/L	:	Gram per litre
BAP	:	6- Benzyl Amino Purine
LSD	:	Least significant difference
BA	:	Benzyladenine
KIN	:	Kinetine
IAA	:	Indole acetic acid
IBA	:	Indole butyric acid
NAA	:	*a*- Napthalene acetic acid
2, 4-D	:	2,4- Dichlorophenoxy acetic acid
Int.	:	International
J.	:	Journal
Mol.	:	Molecular
mg/L	:	Milligram per litre
µM	:	Micromole
MS	:	Murashige and Skoog
PGRs	:	Plant Growth Regulators
Res.	:	Research
Sci.	:	Science
TDZ	:	Thidiazuron
PVP	:	Polyvinylpyrrolidone
CV	:	Co-efficient of Variation
^{o}C	:	Degree Celsius
etc.	:	Etcetera

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MICROPROPAGATION OF SWEET POTATO (*Ipomoea batatas L.)* IN A NEW PLANT TISSUE CULTURE MEDIUM

BY

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ABSTRACT

The experiment was conducted at the Biotechnology Laboratory, Department of Biotechnology, Sher-e-Bangla Agricultural University, Dhaka, Bangladesh during the period of January 2021 to December 2021, to study the micro propagation of sweet potato (*Ipomoea batatas* L.) in a new plant tissue culture medium (SAU tissue culture medium). Six different culture media were compared to explore the potential of SAU Tissue culture medium for regeneration of sweet potato. Data were recorded on the percentage of regeneration, days to shoot initiation, and the number of shoots per explant at different time intervals. The SAU tissue culture medium showed best performance for all the parameter under study among the two other treatments MS (1962) medium and MS powder medium. The results showed that SAU Tissue Culture Medium supplemented with 1.00 mg/L KIN+2.00 mg/L NAA had the highest percentage of regeneration (90%), shorter days to shoot initiation (12.33 days) as compared to other treatments. The highest number of leaves per explant (11.33) and maximum length of shoot (9.2cm) were also observed in the same medium at 35 DAI. In regenerated plantlets 90% survival was found in growth chamber, 85% survival under shade house and 80% in open field condition. Micropropagation of sweet potato was successfully done in SAU tissue culture medium which can be utilized for large scale plantlet production program.

CHAPTER I

INTRODUCTION

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INTRODUCTION

Sweet potato (*Ipomoea batatas* L.) is a dicotyledonous plant that belongs to the family, Convolvulaceae. Unlike regular potatoes, which are tubers, sweet potatoes are considered to be root vegetables. The root is described as a tuberous root. Both sweet and regular potatoes grow under the soil, but they have difference on density, taste and nutrition . This starchy tuber has a sweeter taste than the potato. The sweet potato has a lovely orange color, texture and flavor that is delightful to both children and adults. It is native to south America. China is the world's biggest producer and consumer of sweet potato, where it is used for food, animal feed, and processing .

The importance of sweet potato as a food crop is growing rapidly in many parts of the world. Asia contributes for 87 percent of world sweet potato production while China alone produces 80 percent. It is a kind of major food in Japan, Philippines, Vietnam, Maldives .In 2021 Bangladesh produces 280 killoton of sweet potato that is 13.8 percent higher than the previous years according to Faostat (2021) http://faostat3.fao.org/home/index.html. Rangpur is the highest sweet potato producer district in Bangladesh .

Sweet potato is a good source of non-digestible dietary fiber, vitamins and minerals as well as starch and carbohydrates. Sweet potatoes are versatile and nutritious, consisting a good amount of vitamin A, vitamin C, and manganese. They also have anti-cancer properties and may promote immune function, gut health, brain function, and eye health. In addition, sweet potatoes especially the orange and purple varieties are rich in antioxidants that protect the body from free radicals.

There are some limitations of sweet potato production. Therefore, inadequate finance, poor storage facility and high cost of processing material greatly limit sweet potato. Again in traditional farming system, seed rate become higher as the whole sweet potato or vine is used as seed. Around 2 ton/ha of sweet potato is required which creates economic burden to the farmers and creates seed shortage also. In this case in vitro tissue culture technique can be a good alternative to the traditional farming system that higher number of seed can be produced at short area and in very short time also. Now many educational and research organizations of Bangladesh are applying this technique for large scale plantlet production. According to the Tuber Crop Research Center of Bangladesh Agricultural Research Institute (BARI) , there

are 45-50 tissue culture labs in Bangladesh which are using plant tissue techniques to solve the problem related to seed material in different crops like potato, banana, sweet potato, orchid etc. Some of the public and private organizations using tissue culture methods are- Bangladesh Agricultural Research Institute (BARI), Bangladesh Council of Scientific and Research (BCSIR), National Institute of Biotechnology (NIB), Sher-e-Bangla Agricultural University (SAU), Bangladesh Agricultural University (BAU), Bangabandhu Shiekh Mujibur Rahman Agricultural University (BSMRAU), Potuakhali Science and Technology University, Jahangirnagar University (J.U), Rajshahi University, Chittagong University, BRAC tissue culture center, Kishan Botanix Ltd, Square Agro- Biotech Division, Giant Agro Ltd., Ejab Agro Industries Ltd., North Bengal Agro Farms Ltd., Lal Teer Seed Ltd etc.

Culture media is the basic foundation for tissue culture program. Minimum , 17 different macro and micro plant nutrients are used for the preparation of culture media. Here MS media (1962) is most widely used composition of nutrients for rapid micropropagation and meristem culture technique. Ammonium Nitrate (NH_4NO_3) is an important chemical used as macro nutrient in MS media preparation. The requirement of ammonium nitrate per litre is 16.50 g/L. It is rich in nitrogen containing 35% N in it. But it has some major problem. Ammonium nitrate is an explosive chemical. It has tremendous demand on the production of bomb and in many other destructions. That's why it is not available in our country. It has already been banned for its destructive property. So both the advantages and disadvantages are seen. Finally the Department of Biotechnology of Sher-e-Bangla Agricultural University was able to identify an alternative chemical by the leadership of professor Dr. Md Ekramul Hoque which can be a substitute of NH_4NO_3. A new formulation of plant tissue culture medium was developed. Potato plantlet regeneration has been successfully done in that medium. The plantlet were healthy, vigorous and have very good morphological traits hence, the new medium is quite enough for the regeneration of plant species. The regeneration potentiality of new medium is used to validate with some other crops. Therefore, the research proposal was undertaken to observe the ability of new plant tissue culture medium for regeneration of *Ipomoea batatas* L.Taking all these circumstances into consideration , the present research program has been carried out through the following objectives:

- To study the micro propagation of sweet potato in a new plant tissue culture medium

- To study the regeneration potentiality of *Ipomoea batatas* L. in SAU tissue culture medium

 To compare the performance of different tissue culture media for *in vitro* regeneration of sweet potato

 To establish a new *in vitro* regeneration protocol of sweet potato by applying new chemical composition

CHAPTER II

REVIEW OF LITERATURE

CHAPTER II

REVIEW OF LITERATURE

Sweet Potato which is known in Bangla name as " Misty Alu " is one of the staple food and vegetable crops all over world.The people of Bangladesh has increasing demand on consumption of sweet potato. For that reason till last few decades many Bangladeshi scientists brought their research work under experiment in plant tissue culture method to establish a new practice for the better production of this crop. The literatures which are most related to this study are reviewed under the following headings below:

PROTOCOL DEVELOPMENT

In vitro regeneration process is a commercially viable method for clonal propagation of a wide range of herbaceous and woody plants (Garcia *et al.*, 2010). This technique has been proved to be very effective technique to produce high quality pathogen-free plantlets, in terms of genetic and physiological uniformities (Sathish *et al.*, 2011; Supaibulwattana *et al.*, 2011). For large scale production of uniform, identical seed material of potato, micro-propagation can be the better alternative over conventional propagation of potato. Potato virus free clones with meristem culture methods were conducted by Nagib *et al.* (2003).

Plantlets cultured on liquid media showed better growth of shoot and roots as compared to solid media. The use of growth regulators in liquid cultures also proved to be more effective and it is due to the direct contact of plant with the medium. Liquid media plantlets emerged earlier and having greater number of leaves and nodes per plantlet. Shoot and root length was significantly greater in plantlets of liquid media with mean values 11.34cm and 1.72cm respectively, while in solid media, it was 6.04cm and 1.59cm respectively. The tuber yield and weight was also higher for plantlets developed on liquid media (2.91 and 2.04g) as compared to solid media plantlets (1.76 and 1.12 g). They used (MS) medium containing 1.0 mg/L Calcium pentothenate, 0.25 mg/L Gibberellic acid (GA_3), 100 mg/L Myoinsitol and 30 gl/l sucrose at pH 5.7 was used in this for culturing nodal cuttings of potato cultivar 'Desiree' (Qureshi *et al.* 2014).

Águila *et al.* (2001) worked on Meristem culture for the elimination of the virus from the potato in plants cultivated because he found that meristem cell has little or no chance of virus contamination at early developing condition .This research has given us a tremendous idea of skipping the virus infestation . The use of single-node cuttings excised from tissue cultured plantlets is more common and avoids the influence of tuber tissue from which sprout sections originate (Mohamed and Alsadon, 2010). Nodal cuttings were also used for auxiliary shoot development and suggested to be the best explants source by several researchers (Roca et al., 1978; Hussey and Stacey, 1981) on either liquid or agar solidified medium. For large scale production of uniform, identical seed material of potato, micro-propagation can be the better alternative over conventional propagation of potato. Potato virus free clones with meristem culture methods were conducted by Nagib et al. (2003). The organ that is to serve as tissue source, depends upon the physiological or ontogenic age of the organ, the season in which the explants is obtained, the size of explants and overall quality of the parent plant from which the plant is being obtained (Murashige, 1974).

Ebad *et al.* (2015) carried out an experiment with the aim of presenting easy protocol for in vitro induction of potato plantlets stocks free of pathogens which will be used for selection under abiotic stress. In their study sprouts of four potato genotypes named Lady Rosetta, Jaerla, Cara and Hermis were used. Three concentrations of disinfectant bleach (Clorox) 15, 20, 25% with two exposure time 15 and 20 min were used for disinfecting the isolated potato sprouts. It was found that, as simplest disinfection protocol, concentration 20% Clorox was the suitable one at 20 min of exposure time giving high percentages of survived individuals with low percentage of dead and contaminated individuals. (Modarres and Moeini, 2003) conducted an experiment with solid MS medium with 0.25 mg/L of GA3 and 0.01 mg/L of NAA where he observed significant variation between different P^H levels in respect of it's ability to induction of rooting and shooting in plantlets regenerated from the single node of two potato varieties after subjecting them with thermotherapy. Overall P^H 5.5 was the best for all the traits. Low and high level of PH from that 5.5 were found to reduce the growth and rooting of single nodes. The reduction was more pronounced at low levels than high levels P^H.

Baksha *et al.* (2005) studied on micropropagation of Aloe barbadensis Mill. through in vitro culture of shoot tip explant. Multiple shoot (ten per explant) in Aloe barbadensis were

obtained from shoot tip explant cultured on MS supplemented with BAP 2.0 mg/L and NAA 0.5 mg/L. About 95% rooting was obtained from micro- shoot cultured on half strength MS supplemented with NAA 0.5 mg/L. Well- developed rooted plantlets were successfully transferred to the soil with 70% survival.

In vitro propagation of potato by the serial culture of axillary shoots on separated nodes has been reported by a number of researchers, and since 1950 has become established as an effective mean of rapidly multiplying new or existing cultivars in disease-free conditions (Hussey and Stacey, 1984).

Mohapatra and Batra (2017) presented a review work on several aspects of tissue culture of potato. In vitro regeneration process is a commercially acceptable method for somaclonal propagation of a wide range of herbaceous plants (Garcia et al., 2010).Gong et al. (1998) undertaken an experiment and established an in vitro plant regeneration system in sweet potato which had highly potential importance in sweet potato quality and quantity improvement. However, successful transformation cannot be achieved unless efficient plant regeneration has been established.

Mohamed *et al.* (2009) used potato single node as an explant for his experiment. Potato tubers were also used as an explants source (Mutasim et al., 2010). The use of single-node cuttings excised from tissue cultured plantlets is more common and avoids the influence of tuber tissue from which sprout sections originate . Mohamed et al. (2009) used potato single node as an explant for his experiment. Potato tubers were also used as an explants source (Mutasim *et al.* 2010).

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Micropropagation technique permits a huge amount of asexual multiplication of pathogen free tested potato cultivars. Considerable research has been done on the nutritional, hormonal and physical aspect of the culture media and their effects on explants growth. Murashige and Skoog medium is most widely used for potato micropropagation. Semisolid medium is used for initial nodal segment propagation; however liquid medium fosters higher growth rate of potato micro shoots (Rosell et al., 1987).

Basar, (2016) , Identified a new chemical as a substitute of Ammonium nitrate in MS media preparation for in vitro regeneration of potato (Solanum tuberosum L.) and helped to enhance the tissue culture program whereas shortage of Ammonium nitrate .

In their paper, Kikuta and Okazawa (1982) reported the results of studies on the plantlet regeneration in potato tuber tissue cultures in vitro research designed to establish culture conditions and controlling factors for shoot-bud formation in excised tuber tissue followed by plantlet regeneration.

PHYTOHORMONE USUES

Asma, *et al* (2001) experimented on the effect of growth regulators on in vitro multiplication of potato. Her founds showed that PGR are beneficiary for advanced regeneration in tissue culture technology . Vitamins according to Nitsch and Nitsch (1967), casamino acids, adenosine, mannitol, and agar. While carbohydrate was supported by self- stored starch. Zeatin and indole-3-acetic acid added to the medium induced shoot- buds in potato discs, but the other cytokinins tested did not. Gibberellic acid was effective for shoot-bud induction when discs were excised from the freshly harvested tubers. Plantlets were readily regenerated in the same medium, upon transferring to jiffy-mix and vermiculite bed where they were possible to produce small tubers as plants grew longer.

Badoni and Chauhan (2009) conducted a comparative study on the effect of different hormonal combinations of GA3: NAA and Kinetin: NAA with MS medium on in vitro shoot regeneration of potato cv. Kufri Himalini using meristem tips. In this study the shoot development was studied in terms of different parameter.The best combination of hormones with MS medium was selected and which cultures showed higher growth were further sub-cultured on its parent medium by cutting it in to small pieces in a way that each subsection have at least 1-2 nodes. They concluded that GA3 + NAA combination is best for shoot regeneration and multiplication of potato cv. Kufri Himalini in comparison to the combination Kinetin + NAA with M. S. Medium.

Rahman *et al.* (2010) studied on the regeneration through callus induction by using leaf disc of five tobacco varieties. Explants were cultured on MS medium supplement with different concentrations and combinations of plant growth regulators. Among the varieties used, the highest percentage of callus induced in 2.0 mg/L Kinetin and 2.0 mg/L IAA. Shoots were

induced from calli cultured on the same medium. Maximum shoot formation from leaf discs was on medium 2.0 mg/L Kinetin and 2.0 mg/L IAA. (Rahman *et al.* 2010).

Khadiga *et al.* (2009) reported that initially potato in vitro culture was started from nodal cuttings and maintained on a hormone free media at 23±2 ºC for 2- weeks. It is clearly evident from the data that direct shoot regeneration was remarkably influenced by type and concentrations of the auxins, cytokinins and GA3 used and no organogenesis was recorded in the basal MS media i.e., T0 media indicated that both the varieties exhibited fairly high direct plantlet 11 regeneration, when internodes explants were cultured on T2 medium i.e. MS+GA3 (1.0 mgL-1) + IAA (0.01 mgL-1)+ Zeatin (2.0 mgL-1). Maximum number of shoots per explants after 30 days were recorded in cultivar Diamant followed by Cardinal i.e. 18 and 16, respectively in comparison to control (T0) i.e.2 and1. Moreover, either increase or decrease in the concentrations of growth regulators declined number of shoots. The replacement of IAA with another auxin i.e. NAA and replacement of zeatin with another cytokinin i.e. BAP increased the number of shoots considerably in both the cultivars in comparison to control. After replacement the growth regulators maximum number of shoot was observed in T6 but the number was less than T2 treatment.

A study was conducted by Pandey *et al.* (2009) in vitro shoot regeneration by using nodal explants of potato. The regeneration medium was supplemented with 0.03 mg/L of NAA and 0.25 mg/L GA3 /L. Highest number of shoot (3.11) was obtained with the high concentration of GA3. Internodes and leaf explants of potato in combinations with different plant growth regulators specially different concentrations of Zeatin riboside (ZR) were tested when shoot induction was most successful on callus derived from internodes tissue cultured on induction medium supplemented with 2.5 mgL -1 ZR, 0.2 mgL -1 NAA, 0.02 mgL -1 of GA3 for two weeks and then transferred to shooting medium with 2.5 mgL -1 ZR (Zel et al., 1999).

Koleva *et al.* (2012) conducted a study about the potentiality and effect of cytokinins and combination of cytokinins and auxins on in vitro microtuber formation and growth of two potato cultivars i.e. Agrija and Andrea were evaluated . Gholamreza et al. (2013) worked on in vitro micropropagation of Aloe vera. They observed the effect of explant of shoot tip (with and without sheath type A and B) in Aloe vera. These explant were cultured on MS, B5 and SH media supplemented with different combination of NAA with BAP and Kn for shoot induction. The highest shoot proliferation response obtained successfully by using MS

medium containing 4 mg/L BA. The optimal rooting response was observed on B5 medium supplemented with 2 mg/L NAA, on which 100% of the regenerated shoot developed root with an average of 7.8 root per shoot within 3 weeks. The plantlets were acclimatized and transferred to greenhouse with 95% success.

Although there are several reports for the use of hormone free MS medium during potato proliferation (Yasmin et al., 2003). However, the growth of explants is slow in such hormones free, cost effective media. Otherwise, the growth rate of explant can be improved by supplementing medium with growth regulators (Hoque, 2010).

Molla *et al.* (2011) carried out an experiment to find out a suitable growth regulators and its suitable chmical concentration for direct regeneration. Seven different concentrations of BAP, six different concentrations of Thidiazuron (TDZ) and Eight different concentrations of Zeatin riboside (ZR) were tested separately for in vitro direct regeneration of potato .among them GA3 (0.2 mgL1)and IAA (0.01 mgL-1) BAP, TDZ and ZR, MS medium supplemented with 3 mgL-1 of BAP, 0.3 mgL-1 TDZ and 5 mgL-1 ZR showed very good shoot initiation.

Chaudhary and Mittal (2014) also studied on the Effects of different concentrations and Combinations of plant Growth Regulators on the micro propagation of Potato (Solanum Tubrosum). His findings emphasized on the Cytokinin, Auxin, Giberelin has positive influence on the early shooting and rooting of transplanted explant. Lee et al. (2011) completed an study on induction and proliferation of adventitious root from Aloe vera leaves tissues for in vitro production of aloe-emodin. Adventitious root induction was suitable by enrichment of 0.5 mg/L NAA and 0.2 mg/L BAP in Murashige & Skoog (MS) medium. However root proliferation was hindered by accumulation of phenolic compounds in the media that was overcome by pre-washing of the adventitious root with more than 4 g/L of polyvinylphyrollidine (PVP) analogs increasing the survival rate (up to 60 %). Inspection of aloe-emodin contents in various adventitious root grown different basal medium revealed that aloe- emodin accumulation is much higher on B5 medium (133.08 ± 0.12 μg/g) than on MS medium (3.56 ± 0.26 μg/g).

Shahab-ud-din *et al.* (2011) was conducted an experiment to investigate the effects of different concentrations of plant growth regulators and their combinations on callus induction of potato (Solanum tuberosum L.). The explants of potato tuber were cultured on Modified MS medium supplemented with different concentrations of 2,4-D, NAA, BA in combinations

with BA and NAA in combination with BA for callus induction. The concentration of sucrose was 3% W/V level and the pH of the media was adjusted to 5.7 before the addition of agar 8% W/V

The best result for number of shoot per callus (3.3 ± 0.3) and longest shoot (0.8 ± 0.1) were obtained by using TDZ at 5.0 mg/L Callus derived shoots were rooted most effectively in full-strength MS medium containing 1.0 mg/L IBA. The success of plant tissue culture for in vitro culture of potato was encouraged by acclimatization of the plantlets in the greenhouse conditions. Regenerated plants were morphologically uniform with normal leaf shape and growth pattern (Avila et al., 1996).

Yousef *et al.* (2011) and Pereira et al. (2003) reported that the data presented in represent the number of roots per explant were obtained on all tested media compositions except in T0 which was lack of plant growth hormones. The highest number of roots i.e., 28 per explant was recorded in variety on media composition in which MS media was supplemented with GA3 (1.0 mg/L) + IAA (0.01 mg/L) + Zeatin (2.0 mg/L).

DISINFESTATION

For a successful plant regeneration process, sterilization is an important step. It is done before inoculation process. All operations should be carried out in laminar airflow cabinet Different sterilization agents like $HgCl_2$ (0.1%), NaOCl (5.25% v/v approx.) and 70% ethanol etc. can be used. (Chawla, 2003).

Yasmin *et al.* (2011) used dissected segments of sprouts as the experimental plant material and were surface sterilized with 10% commercial bleach containing three drops of polyoxyethylene sorbitan monolaurate (Tween-20) for 10 minutes. Hoque (2010) has practiced sterilization treatment for Solanum tuberosum which includes the surface sterilization by dipping in 0.5g $HgCl_2$ solution for 3-5 minute and then washed 6-7 times with autoclaved distilled water. Badoni and Chauhan (2010) surface sterilized the explants of potato by treating them with sodium hypochlorite (0.1%) for 8 minutes, followed by 5 minute wash of savlon , and 30 second wash of 70% alcohol, at last 6-7 wash of distilled water followed by every treatment.

Sharifkhani *et al.* (2011) studied on an alternative safer sterilization method for explant of Aloe barbadensis Mill. This study aims at providing a new method to replace mercuric chloride. In this study sodium hypochlorite (commercial brand Clorox) with some drops of Tween 80 10%, 15%, 20%, 25% and 30% were used. Applying a Kruskal-Walis test (a nonparametric test), revealed that 5% sodium hypochlorite with 20 minutes of hard and constant shaking, gives the highest number (91.7%) of viable and sterilized explant with regeneration potential in Murashige and Skoog medium supplemented with IBA and TDZ and Zeatin.

EEFFECTIVE CHEMICALS

Sandra and Maira (2013) the effect of $AgNO_3$ on micropropagation of two potato (Solanum tuberosum) cultivars i.e. Granola and Arbolona negra was also evaluated. Different concentrations of silver nitrate for both cultivars were tested. As the AgNO3 concentration increased, leaf number diminished, stem length diminished and leaf area increased.

Hena *et al.* (2011) conducted an experiment to found regeneration capability in Ammonium Nitrate free medium composition of potato. Motallebi et al. (2011) carried out a research in order to develop a protocol for rapid shoot proliferation of potato, the node explants that were cut into pieces of 0.3- 0.5 cm, containing one axillary bud in each explant and were cultured on MS media containing three concentrations of NH_4NO_3 (800, 1900 and 2400 mg/l) and three concentrations of hydrolyzed casein (0.0, 100 and 200 mg/l), 3% sucrose, 0.8% agar and supplemented with two concentrations of BAP (0.0 and 2 mg/l). They reported the effects of different concentrations of NH_4NO_3, hydrolyzed casein and BAP on in vitro shoot proliferation in potato cv. Agria, for improving the micropropagation procedure. The most effective concentrations as regards the number of lateral shoots were media supplemented with 2400 mg/l NH_4NO_3

Qureshi *et al.* (2014) conducted an experiment on effect on media consistency in which the efficacy of liquid MS medium for potato multiplication was evaluated with the objective to find a cost effective multiplication media for potato. The data was recorded for growth parameters i.e. no of days to shoot/root initiation, no of leaves, no of leaves and nodes, intermodal distance, root and shoot length at transplantable stage. Phenotypic differences in growth were observed between the plantlets of both types of media.

Karim *et al.* (2010) conducted an experiment about Virus free seed potato production through sprout cutting technique under net-house which proved that an effective method.

CHAPTER III

MATERIALS AND METHODS

CHAPTER III

MATERIALS AND METHOD

This section discussed the necessary resources and the methodology used in this study. The units on which the data is gathered and evaluated, as well as the research materials and experimentation schedule, are explained below.

3.1 Experimental site and duration

The experiment was conducted in the Biotechnology Laboratory of The Department of Biotechnology, Sher-e-Bangla Agricultural University (SAU), Sher-e-Bangla Nagar, Dhaka-1207 from January, 2021 to december 2021.

3.2 Experimental materials and treatments used

Sweet potato sprouts were used as explant in this experiment. The sweet potato was imported from Japan with the collaboration of Naruto Japan Co. Ltd. Narayanganj, Bangladesh.

3.3 Chemicals, glassware and instruments

Necessary chemicals were collected, and required glassware and instruments were arranged prior to starting the research. A list of chemicals, glassware and instruments is given in Table,

Table: List of the chemicals, glassware and instruments used in the experiment.

Sl. No.	**Chemicals**	Sl. No.	**Glassware and Instruments**
1.	MS media powder	1.	Measuring cylinder
2.	Sterilizing chemicals (Ethanol, $HgCl_2$, Tween-20)	2.	Petridish
3.	Sucrose	3.	Culture vials
4.	Agar powder	4.	Beaker
5.	NaOH (1N)	5.	Autoclave
6.	HCl	6.	Microwave oven
7.	Distilled water (H_2O)	7.	Laminar Air Flow Cabinet
8.	Absolute Ethanol (100 percent)	8.	Refrigerator
9.	Methyl Alcohol spirit	9.	Hotplate with magnetic stirrer
10.	All chemicals for MS(1962) medium	10.	Electric balance
11.	Recommended chemicals for SAU tissue culture medium	11.	Pipettes

3.4 Sterilization of glassware

The glassware like beakers, petridish, vials etc. were washed by Trix and running clean tap water to remove dirt and remaining liquid detergent. Then these glassware were air dried and placed in the autoclave machine for sterilization at 121^0 C for 30 minutes at 115 psi.

3.5 MS(1962) media preparation

Media preparation being one of the most important work for success in tissue culture research. Murashige and Skoog medium (MS medium, 1962), the mostly used plant growth medium for the *in vitro* regeneration of plant cell culture was used. Different stock solutions were prepared with inorganic nutrients, including macro and micro salts, organic nutrients and vitamin supplements were needed. With these nutrients, four different stock solutions were made.

Stock solution-I (Macro nutrients)

The principal salts in stock solution-I were prepared at a 10x higher concentration.With the aid of the magnetic stirrer, the necessary salts were liquefied in distilled water in a one litre beaker by being weighed ten times more than they actually needed to be.Calcium chloride ($CaCl_2.2H_2O$) was separately dissolved and then added to the main solution due to a precipitation issue. The capacity was finally increased to one litre and then put in the refrigerator at 4^0 C.

Composition of stock solution-I is given below.

SL NO.	Chemical name	Amount (g/L) 10X higher concentration
1.	Potassium nitrate (KNO_3)	19.00
2.	Ammonium nitrate (NH_4NO_3)	16.50
3.	Epsom salt ($MgSO_4.7H_2O$)	3.70
4.	Potassium biphosphate (KH_2PO_4)	1.70
5.	Calcium chloride ($CaCl_2.2H_2O$)	4.40

Stock solution-II (Micro nutriuents)

They are known as minor salts due to less amount of requirement.In comparison to the concentration to be utilized in the medium, this stock solution was 100 times more concentrated. So, using a magnetic stirrer and a 1L beaker filled with purified water, the

necessary salts were weighed 100 times more than they actually needed to be. The solution was then given a label, the date was noted, and it was stored in a refrigerator at 4°C for use in later research.

Composition of stock solution-II is given below.

SL NO.	Chemical name	Amount(mg/L)
01	Potassium iodide(KI)	83
02	Boric acid(H_3BO_3)	620
03	Manganese sulohate($MnSO_4.4H_2O$)	2230
04	White vitriol($ZnSO_4.7H_2O$)	860
05	Sodium molibdate($Na_2MoO_4.2H_2O$)	25
06	Blue vitriol($CuSO_4.5H_2O$)	10
07	Cobalt Chloride ($CoCl_2.6H_2O$)	10

Stock solution-III (Iron-EDTA complex)

Stock solution III is prepared form of $FeSO_4.7H_2O$ and Na_2EDTA. The shelf life of Iron(II) stock solution can be extended by preparing fresh Iron(II) at 100 times the needed medium concentration and storing the stock solution in a dark location or covering the container with alluminium foil. A magnetic stirrer was used to prepare the solution. The container was marked with a label that included the preparation date and was kept chilled at 4°C. The required chemicals for 1000 ml solution of 100x higher concentration are given below.

SL NO.	Chemical name	Amount (g/L)100X higher concentration
01	Green vitriol ($FeSO_4 .7H_2O$)	2.78
02	$Na_2EDTA.2H_2O$	3.73

Stock solution-IV (vitamin and amino acid)

To make stock solution IV, vitamins like nicotinic acid, pyridoxine hydrochloride, thiamine hydrochloride, and glycine are needed. The concentration of these vitamins was measured at

100 times the recommended level. Using a hot plate magnetic stirrer, vitamins were added to distilled water and dissolved. After using more distilled water to increase the volume to 1L, the vial was labeled with the preparation date and stored in the freezer for later use. Composition of stock solution IV is given below.

SL NO.	Chemical name	Amount (mg/L)100X higher concentration
01	Myo-inositol	10000
02	Nicotinic Acid	50
03	Pyridoxine HCl	50
04	Thiamine HCl	20
05	Glycine	200

3.6 Stock solutions for SAU tissue culture medium (Hoque medium) preparation

Preparation of stock solution-I

The nutrient composition of SAU Tissue Culture Medium is totally different from MS (1962) medium. Ammonium nitrate was not used in SAU Tissue Culture Medium. A new chemical was identified from the Department of Biotechnology under the leadership of Professor Dr. Md. Ekramul Hoque, which was used as an alternate of Ammonium nitrate (NH_4NO_3) .Due to the privacy and patent related issue the name of the chemical was secrete for public domain. Hence it was denoted as a *- chemical. This *- chemical was used in stock solution I of SAU medium in alternate of Ammonium nitrate (NH_4NO_3). The concentration of other compounds of major salts also changed in SAU Tissue Culture Medium. The composition of Stock solution II & III was more or less similar with MS (1962) medium.

Myo-inositol is a sugar hexitol. It has several functions like sugar transport, carbohydrate metabolism, membrane structure and cell wall formation. It needs in higher concentration. The amount of Myo-inositol is 10.00 gm/l. The other component of stock solution IV of MS (1962) medium needs very small amount. For example Thiamine-HCl needs only 10.00 mg/l. The Nicotinic acid, Pyridoxine HCl, Thiamine HCl and vitamin has separate function in cell biology. For the preparation of stock solution IV in MS (1962) medium , Researchers have to mix 10,000 mg/l Myo-inositol in 10 mg/L which is quite imbalance proportion for medium preparation. Hence, considering the function and the concentration of Myo-inositol in SAU Tissue Culture Medium it was separated from stock solution IV and grouped as stock solution V.

The composition and concentration of SAU Tissue Culture Medium are given below.

SL	Chemical name	Amount (g/L)
1.	Potassium nitrate(KNO_3)	Different concentration from MS (1962) medium
2.	*Chemical	Specific dose (hidden)
3.	Ipsom salt($MgSO_4$.$7H_2O$)	Different concentration from MS (1962) medium
4.	Potassium biphosphate(KH_2PO_4)	Different concentration from MS (1962) medium
5.	Calcium chloride($CaCl_2.2H_20$)	Different concentration from MS (1962) medium

Preparation of stock solution II

To produce a 100x concentrated solution for stock solution II with a total volume of 1000 ml, the chemicals required along with their respective amounts are provided below.

SL	Chemical name	Amount(mg/L) 100X higher concentration
1.	Potassium iodide (KI)	83.00
2.	Boric acid (H_3BO_3)	620.00
3.	Manganese sulphate Tetrahydrate ($MnSO_4.4H_2O$)	2230.00
4.	White vitriol ($ZnSO_4.7H_2O$)	860.00
5.	Sodium molibdate ($Na_2MoO_4.2H_2O$)	25.00
6.	Blue vitriol ($CuSO_4.5H_2O$)	10.00
7.	Cobalt chloride ($CoCl_2.6H_2O$)	10.00

Preparation of stock solution III

The required chemicals for 1000 ml solution of 100x higher concentration are given below

SL	**Chemical name**	**Amount(g/L) 100X higher concentration**
1.	$FeSO_4 .7H_2O$	2.78
2.	$Na_2EDTA.2H_2O$	3.73

Preparation of stock solution IV

The required chemicals for 1000 ml solution of 100x higher concentration are given below

SL	**Chemical name**	**Amount (mg/L) 100X higher concentration**
1.	Nicotinic Acid	50
2.	Pyridoxine HCl	50
3.	Thiamine HCl	20
4.	Glycine	200

Preparation of stock solution-V

The required chemical for 1000ml solution of 100x concentration is given below

Chemical name	Amount(mg/L) 100X higher concentration
Myo-inositol	10000

Plate 01. Different stock solutions of SAU tissue culture medium

Experiments

Two sub experiments were conducted to fulfill the objectives.

Sub experiment-1

***In vitro* regeneration of sweet potato in different tissue culture media**

Three different tissue culture media were used to establish regeneration protocol of sweet potato. Among them, the well-established MS (1962) medium was used as a standard check medium. It was formulated on the basis of stock solution method. The MS (1962) Medium was treated as treatment-1 (T_1). The second medium was Readymade MS powder which was manufactured by Duchefa, The Netherland. It was mentioned as treatment-2 (T_2). The third medium was newly developed from the Department of Biotechnology, Sher-e-Bangla Agricultural University, Dhaka under the leadership of Professor Dr. Md. Ekramul Hoque and it was named as SAU Tissue Culture Medium. This medium was treated as treatment-3 (T_3). In brief the treatments are as follows-

Treatment-1 (T_1) = Murashige and skoog (1962) medium

Treatment-2 (T_2) = Readymade MS powder (Duchefa, The Netherland)

Treatment-3 (T_3) = SAU Tissue Culture Medium (Hoque medium)

No Phytohormone was applied in those three treatment. The objectives of the present sub experiment was to investigate the comparative performance of these three treatment for *In vitro* regeneration of sweet potato.

Detail treatment procedure of Sub Experiment-1

Media preparation procedure of each treatment

Treatment-1 (T_1) = Murashige and skoog (1962) medium

The procedure for preparing 1 liter of Murashige & Skoog (1962) medium is as follows:

1. Seven hundred ml (700ml) of sterilized distilled water was taken into a 1L glass container.
2. Hundred ml (100ml) of Stock solution I (T_1) and ten ml (10 ml) from each of the other stock solutions were carefully added to the beaker. The mixture was then gently stirred with a magnetic stirrer.
3. Thirty gram (30gm) of sucrose was added to the solution.
4. The volume was increased to 1 liter with distilled water.
5. The pH of the media was adjusted exactly 5.8 using NaOH or HCl by monitoring the pH using pH meter.
6. Five gram (5gm) of agar powder was weighted and added to the solution.
7. The solution was heated in an oven to melt the agar
8. The media was carefully transferred to clean glassware and sterilized once again before use.

Treatment-2 (T_2) = Readymade MS powder (Duchefa, The Netherland)

The process of preparing 1L Readymade MS powder (Duchefa, The Netherland) is outlined below:

1. A 1L beaker was used to hold seven hundred ml (700ml) of sterilized distilled water.
2. Thirty gram (30gm) of sucrose and five gram (5gm) of MS powder were separately weighed on an electric balance and added to the beaker to form the solution.

3. The beaker was placed on a magnetic stirrer to mix the contents.
4. Additional distilled water was added to the solution to bring the final volume up to 1L.
5. The pH of the medium was adjusted to 5.8 using either HCl or NaOH.
6. Finally, 5gm of agar powder was weighed and added to the solution. The mixture was then heated in an oven to melt the agar powder.

Treatment-3 (T_3) = SAU Tissue Culture Medium (Hoque medium)

The preparation of SAU Tissue Culture Medium is outlined as follows:

1. Seven hundred ml (700ml) of distilled water was placed in a 1L beaker.
2. Hundred ml (100ml) of stock 1 (T_3) was added to the beaker.
3. Other stock solutions were added to the beaker in 10ml portions.
4. Thirty gram (30g) of sucrose was weighed and added to the solution.
5. The volume was adjusted to thousand ml (1000ml).
6. The pH of the solution was measured and adjusted to 5.8 by the addition of an acid or base.
7. Five gram (5g) of agar was added to the solution.
8. The mixture was heated in an oven to melt the agar.
9. Finally, the prepared media was poured into glassware and sterilized once again in an autoclave before use.

Sub Experiment-2

***In vitro* culture of sweet potato in different tissue culture media supplemented with Phytohormone**

The same three media were used in this sub experiment. The major difference between sub experiments 1 and 2 was additional supplement of both Auxin and Cytokinine group of hormone. Hence the treatment was designed by the supplement of same concentration of NAA and Kinitine.

The treatments were-

Treatment-1 (T_1) = Murashige and skoog (1962) medium +1.00 mg/L Kinetin (KIN) + 2.00 mg/L α −naphthaleneacetic acid (NAA)

Treatment-2 (T_2) = Readymade MS powder (Duchefa, The Netherland) +1.00 mg/L Kinetin (KIN) + 2.00 mg/L α −naphthaleneacetic acid (NAA)

Treatment-3 (T_3) = SAU Tissue Culture Medium+1.00 mg/L Kinetin (KIN) + 2.00 mg/L α −naphthaleneacetic acid (NAA)

The objectives of this sub experiment was to investigate the potentiality of those media when supplemented with both combined hormone.

Detail treatment procedure of Sub Experiment-2

Media preparation procedure of each treatments

Treatment-1 (T_1) = Murashige and skoog (1962) medium + 1.00mg/L Kinetin (KIN) + 2.00mg/L α −naphthaleneacetic acid (NAA)

For treatment-1, the same steps were followed as the sub-experiment-1 of tratment-1, with the inclusion of two hormones: 1.00mg/L Kinetin (KIN) + 2.00mg/L α −naphthaleneacetic acid (NAA)

Treatment-2 (T_2) = Readymade MS powder (Duchefa, The Natherland) +1.00mg/L Kinetin (KIN) + 2.00mg/L α −naphthaleneacetic acid (NAA)

The preparation process for treatment-2 was the same as sub-experiment-1 of treatment-2 preparation , but with the addition of two hormones: 1.00mg/L Kinetin (KIN) + 2.00mg/L α −naphthaleneacetic acid (NAA)

Treatment-3 (T_3) = SAU Tissue Culture Medium+1.00mg/L Kinetin (KIN) + 2.00mg/L α −naphthaleneacetic acid (NAA)

The steps for preparing tratment-3 was identical to those of treatment-3 of sub-experiment-1, with the exception of adding two hormones: 1.00mg/L Kinetin (KIN) + 2.00mg/L α −naphthaleneacetic acid (NAA)

Disinfection of worker and instruments

The primary sterilizing agent used was 70% ethanol. It was discovered that washing hands with an antiviral and antifungal liquid hand wash was effective prior to spraying hands with 70% ethanol. Moreover, the Laminar airflow cabinet was sprayed with 70% ethanol to reduce contamination. All other required small metal devices were cleaned inside a laminar airflow enclosure utilizing flame sterilization and 70% ethanol soak.

Sterilization of culture media

The culture vials contained equal amounts of the culture media. The vials were then sterilized by the machine at 121^0C for 20 minutes at 15 psi. The sterilized vials were transferred to the Laminar airflow cabinet for placement of the explants.

Disinfection of culture room and laminar airflow

The tissue culture room was kept closed for an entire day after formaldehyde spraying in the whole room. The floor and rake were wiped with antibacterial detergent followed by 70% ethanol. The whole laminar airflow cabinet was wiped with 70% ethanol before starting inoculation of explant. The UV ray was kept switched on for 30 minutes without anything in it, then 20 minutes with the necessary instruments and glassware right before starting work.

Sterilization of the explants

Sweet potato sprouts were separated from the sweet potato tuber and were cleansed with tap water and washed at least four times with sterilized distilled water. After that, the explants were kept on another beaker. Explants were sterilized for one minute in 70% ethanol. The sprouts were then further surface sterilized for 2–3 minutes with 0.1% HgCl2 and a few drops of tween-20 followed by 5-6 times rinsing. To keep the sprouts alive, the surface-sterilized, disinfected sprouts were sliced into small segments and placed onto sterilized petri dishes containing distilled water. The explants were then prepared for inoculation.

Transferring of explants to the media

The explants were prepared carefully under aseptic condition inside the laminar airflow cabinet. Explants were directly inoculated to each vial containing 25 ml of MS medium. The vials were plugged crocked and total operation was done in the laminar airflow cabinet in sterile condition.

Subculturing of the regenerated shoot

After 5 weeks of plant regeneration, the established shoots taken out of vials, cut into small pieces having several nodes. These new explants with new nodal segments were then transferred in newly made culture media for further regeneration. The whole subculture was done inside laminar airflow cabinet. For proper sterilization of scalpel blade, forceps and scissors dry glass bead sterilizer was used and sterilized environment was endured.

Acclimatization

Regenerated plantlets were transplanted to plastic or earthen pots containing soil and cowdung and soil Mixture in 1:1 ratio . Occasional spray of water was done to prevent sudden desiccations and maintain proper humidity around the plantlets. Initially the plantlets were hardened in growth chamber. Then after 2 weeks, exposure to lower humidity and high light intensity . After 20 days plantlets were transferred to natural environment.

Data compilation

The data collection on 14, 21, 28, 35 days after inoculation of explant on culture media was done. The mean value of the data was calculated in case of each unit of different parameter. The parameters were-

- Days to shoot initiation
- Percent of shoot regeneration
- Length of Shoot (cm)
- Number of shoot per explant
- Number of leaves per shoot
- Days to root initiation
- Number of root per explant
- Length of root (cm)
- Survival rate

Data collection

Data on the following parameters were recorded under *in vitro* condition.

Days to shoot initiation

Days to shoot induction were calculated by counting the days from explant inoculation to the first induction of shoot.

Length of shoot (cm)

The length of shoot was recorded by using a scale placing adjacent to shoot inside a laminar airflow cabinet at 21, 28 , 35 days after sub-culture (DAS).

Number of shoots per explant

Total number of shoots per plant was recorded by visual observation at 21 ,28**,** 35

days after inoculation from each subculture.

Number of leaves per shoot

Total number of leaves per shoot was recorded by visual observation at 21 , 28 and 35

days after inoculation from each subculture.

Days to root initiation

Days to root induction were calculated by counting the days from explant inoculation to the first induction of root.

Length of root (cm)

The length of root was recorded by measuring with a plastic scale placing it adjacent to roots in laminar airflow cabinet at 28, 35 days after inoculation.

Number of roots per plant

Total number of root was recorded by visual observation at 28 and 35 days after inoculation from each subculture.

Statistical analysis:

To determine the difference between the treatments, the collected data for all parameters were statistically evaluated by ANOVA, which was carried out by the 'F' test with MSTAT-C software. Calculations were made to determine the parameters' means. The LSD test was used to determine the significance of the differences between the treatment means at a 5% level of probability. The LSD (Least Significant Difference) statistic was also used to compare the variations in the means of the three treatments.

CHAPTER IV

RESULT AND DISCUSSION

CHAPTEER IV

RESULT AND DISCUSSION

Different criteria and parameters under this experiment were observed *in vitro* propagation of sweet potato. The experiment was done to establish a method of regeneration of sweet potato using the SAU tissue culture medium. The major findings were given below.

Sub Experiment-1.In vitro culture of sweet potato in different tissue culture media

4.1 Days to shoot initiation and percent of shoot regeneration

Significant difference were observed under different treatments for days to shoot induction. The treatment-3 (SAU tissue culture medium) showed the best result as it was recorded the lowest time for shoot initiation (17.33 days showed in Table-1).Whereas treatment-1 took the highest period (20.7 days) of time for shoot regeneration. Highest percentage (77.33%) of shoot were regenerated under treatment-3 (Table-1). As least value 61.91% explant were regenerated (Table-1) under treatment-1(MS 1962).

Sourov (2020) reported that among different concentration of 2,4-D on days to callus initiation, minimum 10.66 days (V1), 3.67 days (V2) and 3.33 days (V3) were recorded for 0.5 mg/L of 2,4-D.

Wakil (2020) reported that the maximum days to shoot initiation (6.67) was recorded in treatment 4 (NH_4NO_3 free stock solution), followed by treatment1 (6.33) which was different from all other treatments. It indicates that NH_4NO_3 is an important ingredient which is needed for proper shoot initiation in potato regeneration.

Table 1. Effect of different tissue culture media on days to shoot initiation and percent of shoot regeneration in sweet potato

Treatments	Days to Shoot Initiation	(%) of shoot regeneration
T1=Murashige & Skoog (1962) medium	20. 67a	61.91b
T2= Readymade MS Powder (Duchefa The Netherland)	17. 67b	75.567a
T3=SAU Tissue Culture Medium	17.33b	77.33a
LSD(0.05)	1.15	2.374
CV (%)	3.11	1.73

4.2 Number of shoot per explant

Significant difference were found under different treatments for the number of shoot per explant at different days after shoot initiation. The treatment-3 (SAU tissue culture media) showed the highest number shoot per explant (1.84) at 35 days (Table-2).The difference of shoot per explant fall under significance range. Treatment-1 (MS 1962) showed minimum shoot per explant (1.42 at 35 days of shoot initiation).

Table 2. Potentiality of different tissue culture media on number of shoot per explant at different days after shoot initiation in sweet potato

Treatments	Number of shoot per explant at different days after initiation (DAI)		
	21 DAI	28 DAI	35 DAI
T1= Murashige& Skoog (1962) medium	1.09a	1.30b	1.42b
T2= Readymade MS Powder (Duchefa The Netherland)	1.17a	1.67b	1.73a
T3=SAU Tissue Culture Medium	1.26a	1.30a	1.84a
LSD (0.05)	0.9418	0.66	0.66
CV(%)	17.14	17.27	18.75

Plate 2: Number of shoot per explant at 35 DAI in different tratments.

T1= Murashige & Skoog (1962) medium

T2= Readymade MS Powder (Duchefa The Netherland)

T3=SAU Tissue Culture Medium

Hasna Hena (2017) revealed that maximum shoot number (2.33) was found in treatment-4 (stock solution-01 with newly developed chemical at Sher-e-bangla Agricultural University). The minimum shoot number (1.00) was recorded in treatment-2 (NH_4NO_3 free stock solution) in potato.

4.3 Length of shoot

Significant difference were found under different treatments in case of the length of shoot at different days after shoot initiation. The treatment-3 **(SAU tissue culture medium)** showed the highest length (8.15 cm) at 35 days after shoot initiation. The treatment-1 and treatment-2 showed quite similar result. While treatment-1and treatment-2 gave the length 7.1 and 7.2 cm at the same interval of time that was counted (Fig-1 and plate-2).

The effect of treatment in shoot length was presented in the study of Basar (2016). The maximum shoot length (7.52cm) was found in 5 gmL-1 of β chemical (T3) which was statistically different from all other treatments at 21 days after sub-culture in potato regeneration.

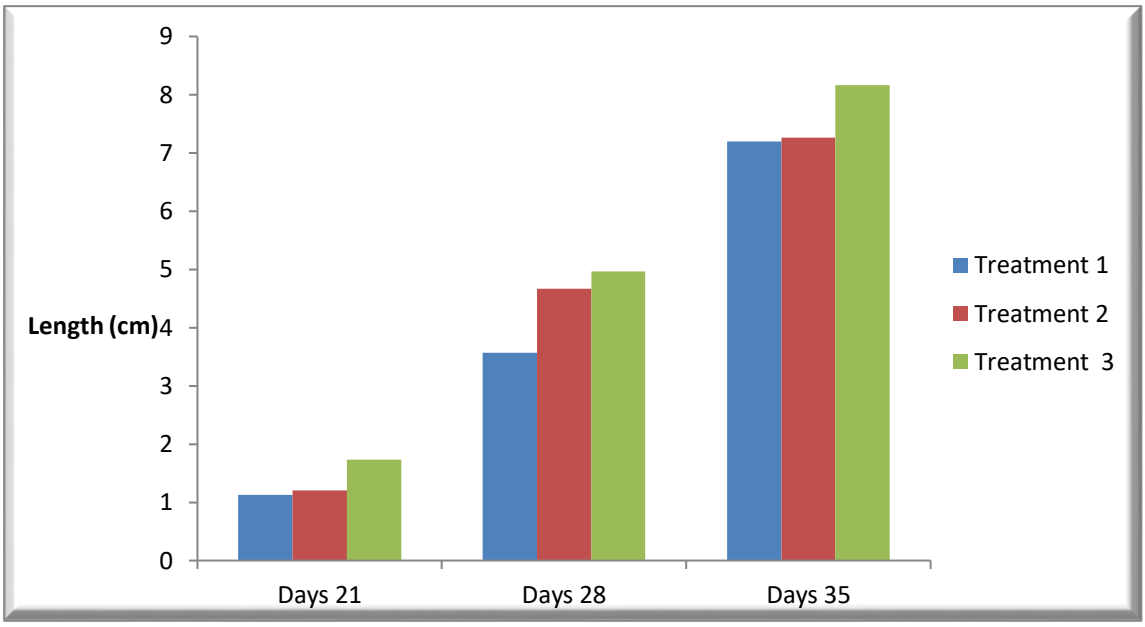

Figure 1. Potentiality of different tissue culture media on length of shoot (cm) at different days after shoot initiation .

T1= Murashige & Skoog (1962) medium

T2= Readymade MS Powder (Duchefa The Netherland)

T3=SAU Tissue Culture Medium

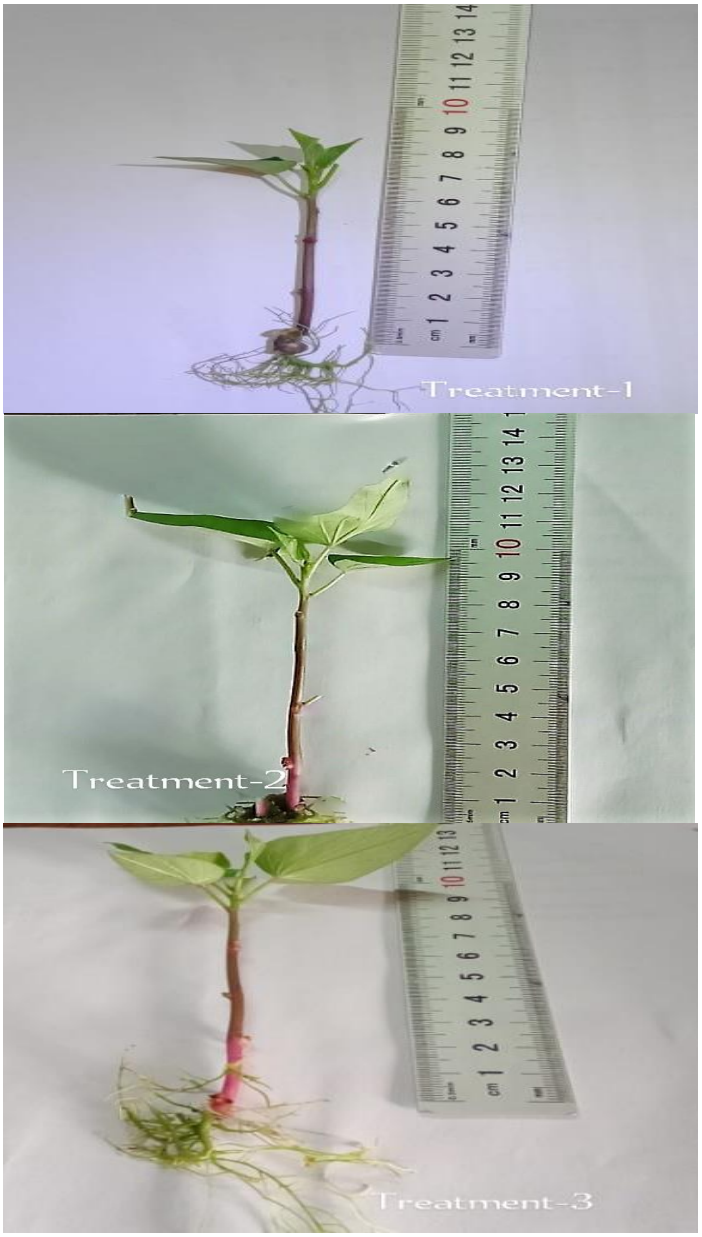

Plate 3 . The highest length of shoot at 35 days after shoot initiation under treatment-3 .

T3=SAU Tissue Culture Medium

4.4 Number of leaf per shoot

Very prominent difference with high significance were observed under different treatment .The treatment-**3 (SAU tissue culture media)** had shown highest number of leaves(9.33 cm) at the 35 days after shoot initiation .On the contrary,the treatment-1**(MS 1962)** gave the lowest number of leaves (7.7) at the same time interval. Treatment-2 was moderate .At 21 days after shoot initiation the significance was highest (Table-3) and gradually fall with the following next two weeks but chronological performance of different treatments were remained the same.

Wakil(2020) experimented that at 21 and 28 days after inoculation, the leaf number(11.17) and (12.16) were found in T_0 (MS powder) which was statistically different from all other treatments.In contrast, the least number of leaf (5.58) and (6.08) was found in 10 gm/L of α-chemical (T4) which was statistically different from all other treatments and followed by T1 in potato plantlet regeneration.

Plate 4: Highest number of leaves were found under Treatment-3 at 35 DAI

T1= Murashige & Skoog (1962) medium

T2=Readymade MS Powder (Duchefa The Netherland), T3=SAU Tissue Culture Medium

Table 3. Effect of different tissue culture media on number of leaf at different days after shoot initiation in sweet potato

Treatments	21 days	28 days	35 days
T_1= Murashige & Skoog (1962) medium	2.0d	5.0d	7.67e
T_2= Readymade MS Powder (Duchefa The Netherland)	2.33cd	5.33cd	8.33de
T_3=SAU Tissue Culture Medium	2.67bc	6.0bc	9.33cd
LSD(0.05)	0. 649	0.8386	1.0271
CV (%)	12.62	7. 64	6.08

4.5. Days to root initiation

There was a significant variation found under different treatments for days to root initiation. Highest days (20.8 days) were required in treatment-1 for root initiation while treatment-3 **(SAU tissue culture medium)** showed the fastest response for root initiation at 17.2 days.

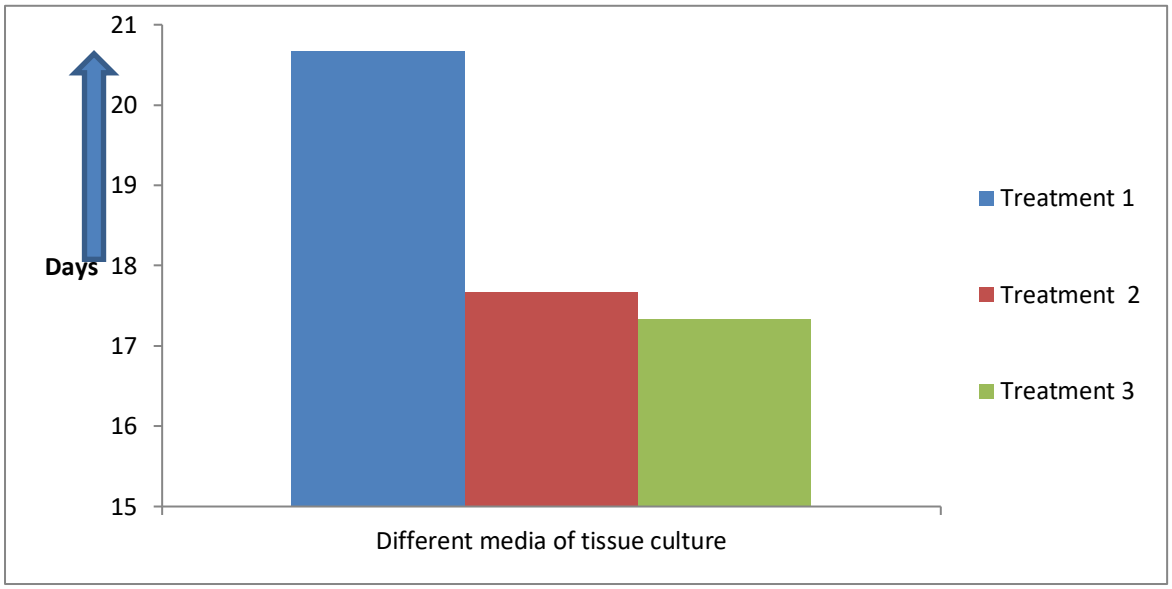

Figure 2. Days to root initiation in different treatments .

T1= Murashige & Skoog (1962) medium

T2= Readymade MS Powder (Duchefa The Netherland)

T3=SAU Tissue Culture Medium

4.6 Length of root (cm)

A significant variation was found on length of root at different days after root initiation .As root initiation took more than 20 days for treatment-1 , 28 days and 35 days after root initiation were valued to measure the root length. The treatment-3 **(SAU tissue culture medium)** showed the best result for root length (8.1 cm) .Other than treatment-1 and treatment-2 were quite similar in case of potentiality . Treatment-1 showed the lowest length (6.30 cm) . The results were presented in Table-4 and Plate-4.

Table 4. Ability of different tissue culture media on length of root at different days after root initiation in sweet potato

Treatments	Length of Root(cm) at different days after root initiation	
	28 days	**35 days**
T1= Murashige & Skoog (1962) medium	0.133b	6.30b
T2= Readymade MS Powder (Duchefa The Netherland)	0.367b	7.36a
T3=SAU Tissue Culture Medium	1.30a	8.10a
LSD(0.05)	0.3767	0.8885
CV(%)	31.43	6.13

Plate 5 : Highest length of root were observed under Tratment-3 at 35 days after root initiation in sweet potato.

T1= Murashige & Skoog (1962) medium

T2=Readymade MS Powder (Duchefa The Netherland),

T3=SAU Tissue Culture Medium

The SAU tissue culture medium was formulated without having NH_4NO_3 and the alternate ***chemical** was used in macro nutrients. Plantlet regeneration without having NH_4NO_3 was also found in some research. Bashar (2016) worked on plantlet regeneration of potato in NH_4NO_3 free stock solution-1 medium. He reported that, 14 days after sub-culture (DAS) The maximum root length (6.11 cm) was found in 5 g/L of β chemical treatment (T2) which was statistically different from all other treatments in potato.

4.7 Number of root per explant

Variation was significant while working with different tissue culture media on the number of root per explant at four and five weeks after root initiation. Among them treatment-3 (SAU tissue culture medium) showed higher result than the other treatments. Treatment-2 was moderate performer and treatment-1 (2 root per explant) showed minimum result .The results are decorated in Table-5.

Table 5. Potentiality of different tissue culture media on number of root per explant at different days after root initiation in sweet potato

Treatments	Number of root per explant at different days after Initiation (DAI)	
	28 days	35 days
T1= Murashige & Skoog (1962) medium	1.167b	2.0b
T2= Readymade MS Powder (Duchefa The Netherland)	1.23a	2.23a
T3=SAU Tissue Culture Medium	1.43a	2.39a
LSD(0.05)	0.66	0.9418
CV(%)	21.43	22.33

Sub Experiment-2: In vitro tissue culture of sweet potato in different tissue culture media supplemented with different phytohormones .

4.1 Days to shoot initiation and percent of shoot regeneration in sweet potato

Significant difference were observed under different treatment for days to shoot induction .The treatment-3 (SAU tissue culture medium with 1 mg/L KIN and 2 mg/L NAA) showed the best result as it was recorded the lowest time for shoot initiation (12.33 days).Whereas treatment-1 took the highest period (14.33 days) of time (Table-6) for shoot regeneration. Highest percentage (86%) of shoot were regenerated under treatment-3. As least value 61.91 % explant were regenerated under treatment-1 (Murashige & Skoog 1962 Medium+1.00 mg/L KIN+2.00 mg/L NAA) which was given in Table-6

Table-6 Effect of different tissue culture media applied with hormone on days to shoot initiation and percent of shoot regeneration in sweet potato

Treatments	Days to shoot initiation	percent(%) of shoot regeneration
T_1= Murashige & Skoog (1962) Medium+1.00 mg/L KIN+2.00 mg/L NAA	14.33b	76.33b
T_2= Readymade MS Powder +1.00 mg/L KIN+2.00 mg/L NAA	13. 67ab	85.26a
T_3= SAU Tissue Culture Medium+1.00 mg/L KIN+2.00 mg/L NAA	12.33a	86.1a
LSD(0.05)	1.0271	2.374
CV (%)	4.29	1.73

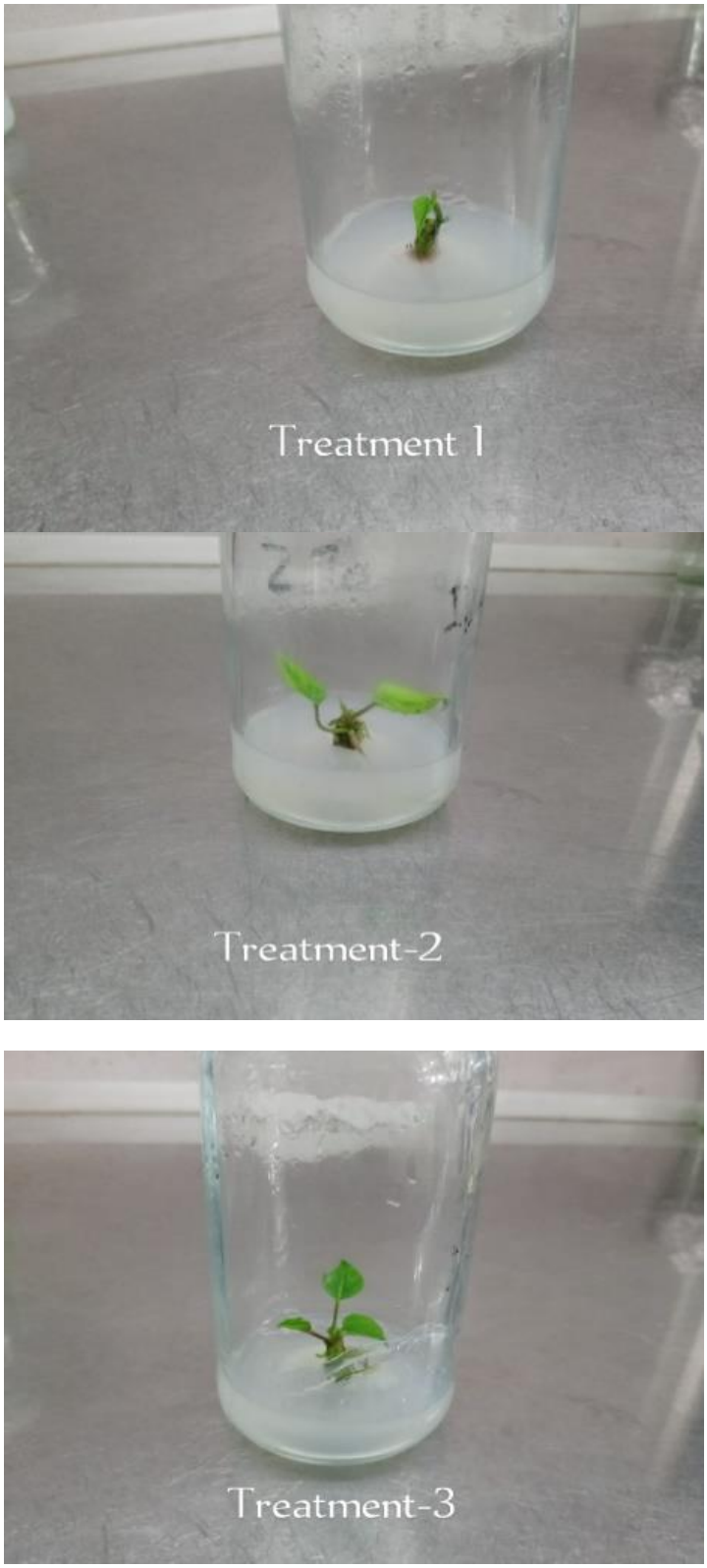

Plate 6: Shoot regeneration at 14 days in different treatments (T1= Murashige & Skoog (1962) Medium+1.00 mg/L KIN+2.00 mg/L NAA), (T2= Readymade MS Powder (Duchefa, The Netherland)+1.00 mg/L KIN+2.00 mg/L NAA), (T3= SAU Tissue Culture Medium+1.00 mg/L KIN+2.00 mg/L NAA).

4.2 Number of shoot per explant

Significant difference were found under different treatments for the number of shoot per explant at different days after shoot initiation. The treatment-3 **(SAU tissue culture media 1 +1mg/L KIN + 2 mg/L NAA)** showed the highest number shoot per explant (2.13) at 35 days . The difference of shoot per explant fall under significance range. Treatment-1 **(MS 1962 Medium+1.00 mg/L KIN+2.00 mg/L NAA)** showed minimum shoot per explant (1.2 at 35 days of shoot initiation).The treatment-2 and treatment-3 had considerable similarities on value (Plate-7 and Table-7).

Plate 7: Highest number of shoot was found under Treatment-3 **(SAU tissue culture media 1 +1mg/L KIN + 2 mg/L NAA)** applied with hormone at 35 days of shoot initiation in sweet potato.

Table 7. Potentiality of different tissue culture media on number of shoot per explant at different days after shoot initiation in sweet potato

Treatments	Number of shoot per explant at different days after initiation (DAI)		
	21 DAI	**28 DAI**	**35 DAI**
T_1= Murashige & Skoog (1962) medium +1.00 mg/L KIN+2.00 mg/L NAA	1.19b	1.10b	1.2b
T_2= Readymade MS Powder (Duchefa, The Netherland)+1.00 mg/L KIN+2.00 mg/L NAA	1.82b	1.73ab	1.73ab
T_3= SAU Tissue Culture Medium+1.00 mg/L KIN+2.00 mg/L NAA	1.90a	1.90a	2.13a
LSD (0.05)	0.66	1.15	1.48
CV (%)	7.27	8.57	7.15

Hasna Hena (2017) revealed that maximum shoot number (2.33) was found in treatment-4 (stock solution-01 with newly developed chemical at Sher-e-bangla Agricultural University). The minimum shoot number (1.00) was recorded in treatment-2 (NH_4NO_3 free stock solution) in potato.

4.3 Length of shoot (cm)

Significant difference were found under different treatments in case of the length of shoot at different days after shoot initiation. The treatment-3 **(SAU tissue culture media 1 +1mg/L KIN + 2 mg/L NAA)** showed the highest length (9.15 cm) at 35 days after shoot initiation. The treatment-1 and treatment-2 showed quite similar result while treatment-1(Murashige & Skoog (1962) Medium+1.00 mg/L KIN+2.00 mg/L NAA showed minimum length(8.4 cm) at the same interval of time that was counted Fig-3 and Plate-8.

The effect of treatment in shoot length was presented in the study of Basar (2016). The maximum shoot length (7.52cm) was found in 5 gmL-1 of β chemical (T3) which was statistically different from all other treatments at 21 days after sub-culture in potato regeneration.

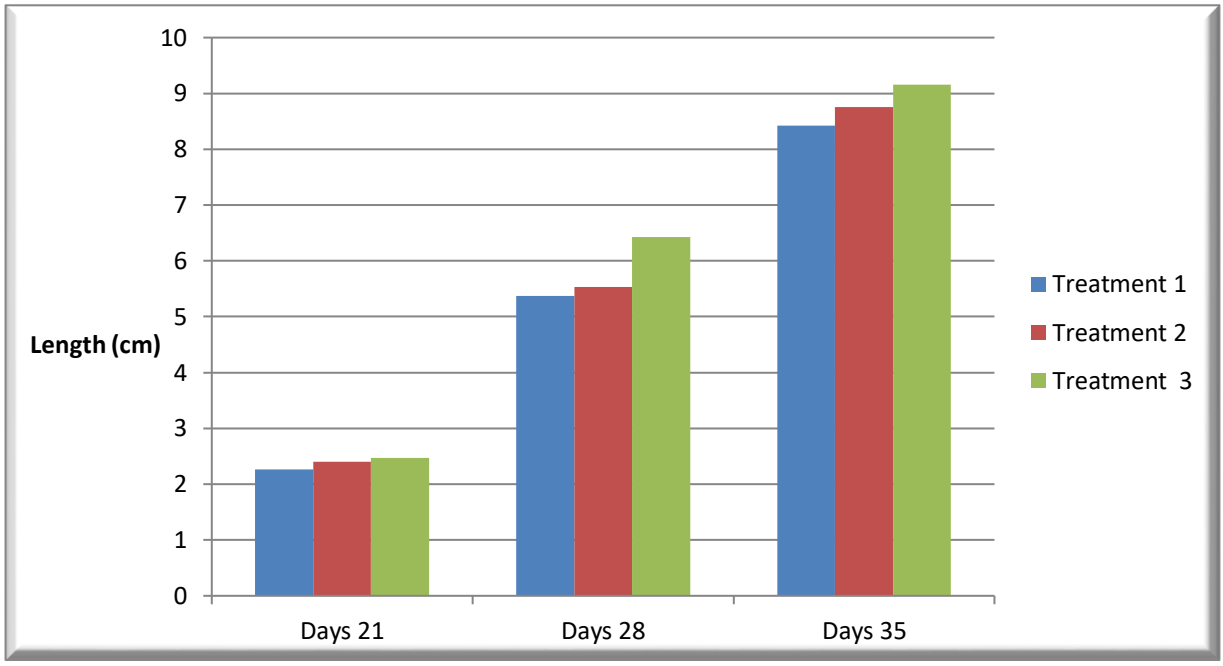

Fig-3. Length of shoot (cm) at different days after shoot initiation in different treatments in sweet potato.

T1= Murashige & Skoog (1962) medium +1.00 mg/L KIN+2.00 mg/L NAA

T2= Readymade MS Powder (Duchefa, The Netherland)+1.00 mg/L KIN+2.00 mg/L NAA

T3= SAU Tissue Culture Medium+1.00 mg/L KIN+2.00 mg/L NAA

Plate 8: Length of shoot at 35 days after shoot initiation.

T1= Murashige & Skoog (1962) medium +1.00 mg/L KIN+2.00 mg/L NAA

T2= Readymade MS Powder (Duchefa, The Netherland)+1.00 mg/L KIN+2.00 mg/L NAA

T3= SAU Tissue Culture Medium+1.00 mg/L KIN+2.00 mg/L NAA

Plate 9. The highest length of shoot at 35 days after shoot initiation under treatment-3**(SAU tissue culture media 1 +1mg/L KIN + 2 mg/L NAA)** .

4.4 Number of leaf

Very prominent difference with high significance were observed under different treatment .The treatment-3 **(SAU tissue culture media 1 +1mg/L KIN + 2 mg/L NAA)** had shown highest number of leaves(11.33 cm) at the 35 days after shoot initiation .On the contrary,the treatment-1 **(**Murashige & Skoog (1962) Medium+1.00 mg/L KIN+2.00 mg/L NAA **1962)** gave the lowest number of leaves (9.7) at the same time interval. Treatment-2 was moderate .At 21 days after shoot initiation the significance was highest and gradually fall with the following next two weeks but chronological performance of different treatments were remained the same as more than 11 leaves per shoot were found on an average in treatment-3 after 35days after shoot initiation (Table-8).

Plate 10: Highest number of leaves were found under Treatment-3 Applied with hormone

T3= SAU Tissue Culture Medium+1.00 mg/L KIN+2.00 mg/L NAA

Table-8. Effect of different tissue culture media on number of leaf at different days after shoot initiation in sweet potato

Treatments	21 days	28 days	35 days
T_1= Murashige&Skoog (1962) Medium+1.00 mg/L KIN+2.00 mg/L NAA	3.0b	6.33b	9.67bc
T_2= Readymade MS Powder (Duchefa, The Natherland)+1.00 mg/L KIN+2.00 mg/L NAA	3.67a	6. 67b	10.67ab
T_3= SAU Tissue Culture Medium+1.00 mg/L KIN+2.00 mg/L NAA	3.70a	7.67a	11.33a
LSD(0.05)	0. 649	0.8386	1.0271
CV (%)	12.62	7. 64	6.08

Wakil(2020) experimented that at 21 and 28 days after inoculation, the leaf number(11.17) and (12.16) were found in T_0 (MS powder) which was statistically different from all other treatments and followed by T6 also followed by T5 and T3 .In contrast, the least number of leaf (5.58) and (6.08) was found in 10 gmL-1 of α- chemical (T4) which was statistically different from all other treatments and followed by T1 in potato plantlet regeneration.

4.5. Days to root initiation

There was a significant variation found under different treatments for days to root initiation. Highest days (17 days) were required in treatment-1 (Murashige & Skoog (1962) Medium+1.00 mg/L KIN+2.00 mg/L NAA) for root initiation while treatment-3**(SAU tissue culture media 1 +1mg/L KIN + 2 mg/L NAA)** showed the fastest response for root initiation at 13 days. With hormone treatments among the treatment-1 , treatment-2 and treatment-3 , the treatment-3 showed the fastest response (13 days). Gradually treatment-2 and treatment-1 had less time chronologically(Fig-4) .

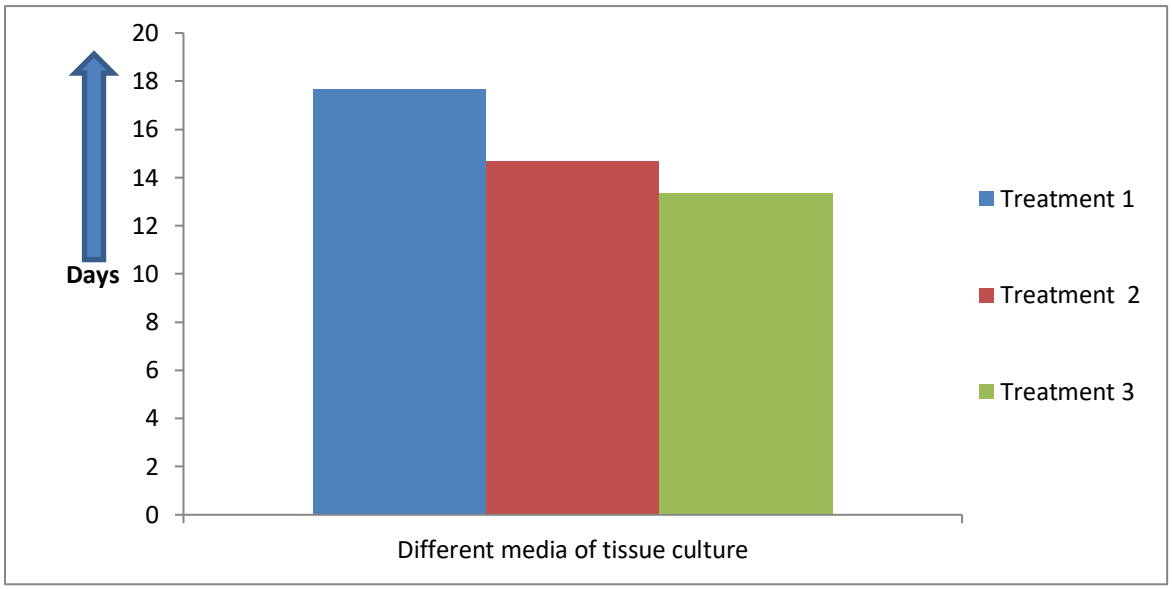

Fig-4. Days to root initiation in different tissue culture media applied with phytohormone .

T1= Murashige & Skoog (1962) Medium+1.00 mg/L KIN+2.00 mg/L NAA

T2= Readymade MS Powder (Duchefa, The Netherland)+1.00 mg/L KIN+2.00 mg/L NAA

T3= SAU Tissue Culture Medium+1.00 mg/L KIN+2.00 mg/L NAA

4.6 Length of root

A significant variation was found on length of root at different days after root initiation .As root initiation took more than 17 days for treatment-1 (Murashige & Skoog (1962) Medium+1.00 mg/L KIN+2.00 mg/L NAA) , 28 days and 35 days after root initiation were valued to measure the root length. The treatment-3 **(SAU tissue culture medium + 1mg/L KIN + 2 mg/L NAA)** showed the best result for root length(10.73 cm) .Other than treatment-1 and treatment-2were quite similar in case of potentiality . The results were presented in Table-9 and Plate-11.

Plate 11: Longest length was observed under Treatment-3 at 35 days after root initiation

T1= Murashige & Skoog (1962) medium +1.00 mg/L KIN+2.00 mg/L NAA

T2= Readymade MS Powder (Duchefa, The Netherland)+1.00 mg/L KIN+2.00 mg/L NAA

T3= SAU Tissue Culture Medium+1.00 mg/L KIN+2.00 mg/L NAA

Table-9. Ability of different tissue culture media on length of root at different days after root initiation in sweet potato

Treatments	**Length of Root(cm) at different days after root initiation**	
	28 days	**35 days**
T_1= Murashige&Skoog (1962) Medium+1.00 mg/L KIN+2.00 mg/L NAA	1.143b	9.30b
T_2= Readymade MS Powder (Duchefa, The Natherland)+1.00 mg/L KIN+2.00 mg/L NAA	1.397b	9.36a
T_3= SAU Tissue Culture Medium+1.00 mg/L KIN+2.00 mg/L NAA	1.49a	10.73a
LSD(0.05)	0.3767	0.8885
CV(%)	21.43	6.7

Plate 12 : The highest length of root at 35 days after root initiation found under treatment-3

(T3= SAU Tissue Culture Medium+1.00 mg/L KIN+2.00 mg/L NAA)

4.7 Number of root per explant

Variation was significant while working with different tissue culture media on the number of root per explant at four and five weeks after root initiation. Among them treatment-2 (MS Powder +1 mg/L KIN + 2 mg/L NAA) and treatment-3 (SAU tissue culture media 1 +1mg/L KIN + 2 mg/L NAA) showed the quite similar and higher result than treatment-1(Murashige & Skoog (1962) Medium+1.00 mg/L KIN+2.00 mg/L NAA).Treatment-1 (2.40 root per explant) showed minimum result .The results are decorated on the Table-10

Table-10. Potentiality of different tissue culture media on number of root per explant at different days after root initiation in sweet potato

Treatments	Number of root per explant at different days after Initiation (DAI)	
	28 days	**35 days**
T_1= Murashige & Skoog (1962) Medium+1.00 mg/L KIN+2.00 mg/L NAA	1.67b	2.4b
T_2= Readymade MS Powder (Duchefa, The Netherland)+1.00 mg/L KIN+2.00 mg/L NAA	1.43a	2.43a
T_3= SAU Tissue Culture Medium+1.00 mg/L KIN+2.00 mg/L NAA	1.73a	2.79a
LSD(0.05)	0.66	0.918
CV(%)	19.43	12.33

4.8 : Acclimatization of plantlets

The acclimatization of *Ipomoea batatas* L. plantlets was evaluated based on the survival rate in different conditions. The plantlets were initially grown in a growth chamber for 7 days and then transferred to a shade house with less humidity and indirect sunlight for 14 days and in field condition for 30 days. In regenerated plantlets 90% survival was found in growth chamber, 85% survival under shade house and 80% in open field condition. Micropropagation of sweet potato was successfully done in SAU tissue culture medium which can be utilized for large scale plantlet production program.

Table 11. Survival rate of *in vitro* regenerated plants of sweet potato

In growth chamber (07 Days)		
Plantlets transferred	Seedling established	Survival rate (%)
20	19	95

In shade house (14 Days)		
plantlets transferred	seedlings established	Survival rate (%)
20	18	90

In field Condition (30 Days)		
plantlets transferred	seedlings established	Survival rate (%)
10	8	80

Plate 13.Hardening of plantlet at 21 days in shade house condition

CHAPTER V

SUMMARY AND CONCLUSION

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Two sub experiment were conducted to evaluate the regeneration ability of sweet potato in newly developed SAU tissue culture medium (Hoque medium), the standard MS (1962) Medium and MS powder (Duchefa The Netherland) were used as check media for regeneration of sweet potato .

The sub experiment 2 was designed with the same previous media but the each medium was supplemented with same dose of phytohormone. The treatment combination of sub experiment 2 were as follows T1= Murashige & Skoog (1962) Medium+1.00 mg/L KIN+2.00 mg/L NAA, T2= Readymade MS Powder (Duchefa, The Netherland)+1.00 mg/L KIN+2.00 mg/L NAA, T3= SAU Tissue Culture Medium+1.00 mg/L KIN+2.00 mg/L NAA. The key findings of both sub experiments were given below.

The researchers evaluated the percentage of regeneration and days to shoot initiation for each treatment. The findings gave an output that the SAU tissue culture medium and the same medium supplemented with 1.00 mg/L KIN+2.00 mg/L NAA had the highest percentage of regeneration (90%) and shorter days to shoot initiation compared to other treatments.

The effect of different tissue culture media were examined in case of some countable parameters. The number of shoots was recorded at different time intervals, including 21, 28, 35 days after initiation. The results showed that SAU tissue culture medium with hormones gave 1.90, 1.90, 2.13 shoots per explant respectively and MS Powder (Duchefa The Netherland) with hormone treatments gave 1.82, 1.73 ,1.73 shoots per explant . This two treatments produced higher numbers of shoots as compared to other treatments at different days after shoot initiation.

The highest number of leaves (11.33) per explant was found in SAU Tissue Culture medium + 1.00 mg/L KIN+2.00 mg/L NAA. The same treatment showed maximum length of shoot (9.33 cm) at 35 days after inoculation. MS (1962) medium showed lowest response for both the parameter under investigation .

The maximum length of root (10.73cm) was obtained from SAU Tissue Culture medium + 1.00 mg/L KIN+2.00 mg/L NAA and it was minimum in (6.30 cm) in simple MS(1962)

medium . In regenerated plantlets 90% survival in growth chamber , 85% survival in shade house and 80% in open field condition.

The results of this study suggested that SAU Tissue Culture medium and the same medium supplemented with 1.00 mg/L KIN and 2.00 mg/L NAA have the highest potentiality for *in vitro* plant production of *Ipomoea batatas* L. The findings of this study is a signature of development of more efficient and cost-effective technique for the regeneration of *Ipomoea batatas* L. *in vitro.*

The results also suggest that the optimization of media composition and growth regulator concentrations could lead to further improvements in shoot initiation and regeneration in tissue culture.

RECOMENDATION

SAU Tissue Culture Medium and the same medium with supplement of 1.00 mg/L KIN+2.00 mg/L NAA showed the highest percentage of regeneration and shorter days to shoot initiation compared to other treatments. Therefore, these media could be used for commercial plant production for tissue culture of *Ipomoea batatas* L.

i . Further study can be done with more genotypes of sweet potato.

ii. Some other growth hormones like BAP,IAA, 2,4-D can be used for validation of present findings .

iii. Regeneration potentiality of SAU tissue culture medium can be checked in some other crops like zerbera, orchid etc.

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APPENDICES

a. Analysis of variance on days to shoot initiation

	Degrees of Freedom	Sum of squares	Mean Square	F-value	Probability
Between	5	88.500	17.7000	17.70	0.0000
Within	12	12.000	1.0000		
Total	17	100.500			
CV (%)	6.85				
LSD	1.0271				

b. Analysis of variance on number of shoots at 21 days after initiation

	Degrees of Freedom	Sum of squares	Mean Square	F-value	Probability
Between	5	3.76176	0.75235	10.52	0.0005
Within	12	0.85840	0.07153		
Total	17	4.6201			
CV (%)	6.97				
LSD	0.1919				

c. Analysis of variance on number of shoots at 28 days after initiation

	Degrees of Freedom	Sum of squares	Mean Square	F-value	Probability
Treat	5	9.7963	1.95926	19.18	0.0000
Error	12	1.2256	0.10213		
Total	17	11.0219			
LSD	0.1875				
CV (%)	7.19				

d. Analysis of variance on number of shoots at 35 days after initiation

	Degrees of Freedom	Sum of squares	Mean Square	F-value	Probability
Treat	5	10.8610	2.17221	25.60	0.0000
Error	12	1.0184	0.08487		
Total	17	11.8794			
LSD	0.1251				
CV (%)	4.47				

e. Analysis of variance on length of shoot at 21 days after initiation

	Degrees of Freedom	Sum of squares	Mean Square	F-value	Probability
Treat	5	2.58944	0.51789	155.37	0.0000
Error	12	0.04000	0.00333		
Total	17	2.62944			
LSD	0.40				
CV (%)	12. 5				

f. Analysis of variance on length of shoot at 28 days after initiation

	Degrees of Freedom	Sum of squares	Mean Square	F-value	Probability
Treat	5	3.09833	0.61967	19.23	0.0000
Error	12	0.38667	0.03222		
Total	17	3.48500			
LSD	0.9608				
CV (%)	10. 61				

g. Analysis of variance on length of shoot at 35 days after initiation

	Degrees of Freedom	Sum of squares	Mean Square	F-value	Probability
Treat	5	7.96944	1.59389	168.76	0.0000
Error	12	0.11333	0.00944		
Total	17	8.08278			
LSD	0.459				
CV (%)	3. 16				

h. Analysis of variance on number of leaves at 21 days after initiation

	Degrees of Freedom	Sum of squares	Mean Square	F-value	Probability
Treat	5	14.5854	2.91708	24.85	0.0000
Error	12	1.4089	0.11741		
Total	17	15.9943			
LSD	0. 649				
CV (%)	12. 62				

i. Analysis of variance on number of leaves at 28 Days after initiation

	Degrees of Freedom	Sum of squares	Mean Square	F-value	Probability
Treat	5	15.3633	3.07266	25.16	0.0000
Error	12	1.4654	0.12212		
Total	17	16.8287			
LSD	0.8386				
CV (%)	7. 64				

j. Analysis of variance on number of leaves at 35 Days after initiation

	Degrees of Freedom	Sum of squares	Mean Square	F-value	Probability
Treat	5	7.71138	1.54228	32.48	0.0000
Error	12	0.56987	0.04749		
Total	17	8.28124			
LSD	1.0271				
CV (%)	6.08				

k . Analysis of variance on days to root initiation

	Degrees of Freedom	Sum of squares	Mean Square	F-value	Probability
Treat	5	7.71138	1.54228	32.48	0.0000
Error	12	0.56987	0.04749		
Total	17	8.28124			
LSD	1.2579				
CV (%)	2.87				

l. Analysis of variance on length of root at 28 days after initiation

	Degrees of Freedom	Sum of squares	Mean Square	F-value	Probability
Treat	5	8.16444	1.63289	226.09	0.0000
Error	12	0.08667	0.00722		
Total	17	8.25111			
LSD	0.3381				
CV (%)	12.96				

m. Analysis of variance on length of root at 35 days after initiation

	Degrees of Freedom	Sum of squares	Mean Square	F-value	Probability
Treat	5	6.31111	1.26222	206.55	0.0000
Error	12	0.07333	0.00611		
Total	17	6.38444			
LSD	1.0037				
CV (%)	6.89				

n. Analysis of variance on number of root at 28 days after initiation

	Degrees of Freedom	Sum of squares	Mean Square	F-value	Probability
Treat	5	6.31111	1.26222	206.55	0.0000
Error	12	0.07333	0.00611		
Total	17	6.38444			
LSD	0.327				
CV (%)	9.55				

o. Analysis of variance on number of root at 35 days after initiation

	Degrees of Freedom	Sum of squares	Mean Square	F-value	Probability
Treat	5	6.31111	1.26222	206.55	0.0000
Error	12	0.07333	0.00611		
Total	17	6.38444			
LSD	0.273				
CV (%)	5.75				