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Innovation of a New Plant Tissue Culture Medium Without Utilizing Explosive Chemical Ammonium Nitrate (NH_4 NO_3)

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ABSTRACT

The growth and development of explants is governed by the composition of culture medium. Ammonium nitrate (NH_4NO_3) is a major salt of stock solution-01 for the preparation of Murashige and Skoog (MS 1962) medium. But, it is not available in our country due to many destructive activities. A series of experient was conducted to identified any alternate of ammonium nitrate. The MS (1962) medium and MS powder (Duchefa Biocheme, The Netherland) were used as check treatment-1 and check treatment-2. Two potato varieties viz. Diamant and Asterix were used as experimental materials. Finally, an inorganic salt was indetified as a substitute of ammonium nitrate, which is suitable and excellent performer in the preparation of stock solution-01. The concentrations of the other ingredients of major and minor salt were also modified from the MS 1962 medium. Hence, it is totally different from the MS (1962) medium. The regeneration potentiality of potato onto the new medium was best as compare with the two check treatments. The traits like- node number, leaf number, shoot length, root lengths were highest in new medium. The plantlets were healthy, robust and strong as compare to plantlets regenerated from

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check treatments-1. The regeneration and plantlet production potentiality of the new tissue culture medium was validated with two other crops *viz.* Sweet potato and *Aloe vera*. The new medium showed excellent performance in respect of all the trails under studied in those two crops. Hence, the innovation of the new plant tissue culture medium can be utilized for regeneration and large scale plantlet production of any crops.

Keywords: New medium, Potato, Sweet potato, *Aloe vera* and regeneration.

INTRODUCTION

Cell or tissue culture technology offers unlimited benefit to the mankind. *In vitro* propagation for large scale cultivation of econmically important cropy like potato, banana, gerbera, orchid has been commercially practiced in all over the world. Disease free and quality plantlet production of potato through tissue culture technology has been adopted in our country in last 5 decades. Preparation of culture media is the foundamental step for any tissue culture work. At least, 20 different macro, micro nutrients and organic compound are used for the preparation of culture media. Murashige and Skoog (1962) media composition of nutrients is mostly used for rapid micropropagation and meristem culture technique. Ammonium Nitrate (NH_4NO_3) is an important chemical used as a major salt in MS (1962) media preparation. The amount of ammonium nitrate per litre is 16.50 gm. It is rich in nitrogen. It is a good source of nitrogen in culture media. But extremely sorry to say that, it has a great disadvantage in human civilization. Ammonium nitrate is an explosive chemical. As an oxidizing agent, it is used for the production of bomb and many other destractive activities. Hence, it is totally ban in many countries like Bangladesh, India, Pakintan and etc.. The supplier or importer are not selling a single gram of ammonium nitrate in our country. Therefore, the tissue culture works all over the country is seriously hampered. Different research Institutes and private tissue culture companies adopted alternate approach for their on going tissue culture program. They used ready made MS powder which is manufactured by different companies- like Duchefa (Netherland), Sigma (USA, Germany), SRL (India) etc. Those ready made MS media is expensive as compared to stock solution method of media preparation. Strock solution method is cheap and user friendly. Generally the students, teachers, researchers, lab technicians are familiar with this method. It has been practicsed for last 40-50 years in our country. This long time adopted technology is tremendously hampared due to non availability of ammonium nitrate. The justification of present research was to identify any alternate chemical of ammonium nitrate (NH_4NO_3) which will be cheap, non destructive, environmental friendly and easily available in our country. So, the experiment was disigned to identifiy any inorganic nitrogenous salt which has the ability to released nitrogen in the medium and the estimation of accurate concentration of that chemical which can be use for the preparation of new plant tissue culture medium.

METHODOLOGY

Different experiments were conducted to find out the substitute of ammonium nitrate for the preparation of tissue culture medium. A series of nitrogenous inorganic salt were used for the source of nitrogen. Some of them were urea, ammonium sulphate (NH_4SO_4), ammonium hydoxide (NH_4OH), ammonium phosphate (NH_4PO_4), ammonium di-phosphate, ammonium

carbinate $(NH_4)_2CO_3$ ammonium chloride (NH_4Cl) etc. Among the different nitrogenous sources the ammonium chloride (NH_4Cl) showed excellent result for *in vitro* regeneration of potato. The concentrations of other ingredients of macro elements also change for the preparation of new medium. A comparative table was given for the clarification of nutrient composition and concentration of stock solution-01. Some other modification also done in organic nutrients. Hence, new tissue culture medium is quite different from the MS (1962) tissue culture medium. The new formulation, concentration and composition of culture medium is denoted as "SAU Plant Tissue Culture Medium.

Table 1: Composition and concentration of macro nutrients of the MS (1962) medium and new formulation of medium

Sl. No.	Name of the chemicals	Concentration in MS (1962) medium	Concentration in new medium
01.	NH_4NO_3 (Ammonium nitrate)	Present- 1650 mg/L	Absent
02.	NH_4Cl (Ammonium chloride)	Absent	Present- 1000 mg/L
03.	KNO_3 (Potassium nitrate)	1900 mg/L	3800 mg/L
04.	$MgSO_4$.$7H_2O$ (Magnesium sulphate)	370 mg/L	740 mg/L
05.	KH_2PO_4 (Potassium di-hydrogen phosphate)	170 mg/L	340 mg/L
06.	$CaCl_2.2H_20$ (Calcium chloride)	440 mg/L	880 mg/L

Modification of MS (1962) Medium by Ammonium Chloride (NH_4Cl)

Stock solution-01 of MS (1962) medium was modified by using ammonium chloride (NH_4Cl). Different concentrations of ammonium chloride (NH_4Cl) were used in stock solutions-01 and marked as different treatments. Three different concentrations of ammonium chloride (NH_4Cl) were used for this purpose. The modification of stock solution-1 was given below:

- **Modification –1 of stock solution- 01**: The modification -1 of stock solution-01 was done by using 500 mg/L of ammonium chloride (NH_4Cl). This modification was treated as treatment-2. Concentration of other component also changed and it was mentioned in the Table 1.
- **Modification –2 of stock solution- 01:** The modification-2 of stock solution-01 was same as modification-1 but the concentration of ammonium chloride (NH_4Cl) was 1000 mg/L. It was treated as treatment-3.
- **Modification –3 of stock solution- 01**: The modification -3 of stock solution- 01 is same as modification-1 but the concentration of ammonium chloride (NH_4Cl) was 1500 mg/L. It was treated as treatment-4.

Treatments

Murashige & Skoog (1962) recommended dose of ammonium nitrate (1650 mg/L) was used as standard check treatment-0 (T_0). Ready made MS powder is internationally accepted tissue culture media composition. MS powder (Duchefa, The Netherlands) was used as another standard check treatment-1 (T_1). The three concentration of ammonium chloride was used as treatment-2 (T_2), treatment-3 (T_3). and treatment-4 (T_4).

Nutrient Composition and Stock Solution Preparation

The preparation of stock solutions of macronutrients, micronutrients, Iron-EDTA, vitamins and amino acids were made as per Table 2.

Table 2: Nutrient compositions and concentration of three treatment where NH_4Cl used as a substitute of NH_4NO_3

Sl no.	Components	Concentrations	
		Original concentration	**Stock solution conc.**
	Major salts	**mg/L**	**Stock solution-01 g/L (10x)**
01	KNO_3	3800	38.00
02	NH_4Cl	500 (T_2), 1000 (T_3), 1500 (T_4)	5.0 (T_2), 10.0 (T_3), 15.0 (T_4)
03	$MgSO_4.7H_2O$	740	7.40
04	KH_2PO_4	340	3.40
05	$CaCl_2.2H_2O$	880	8.80
	Minor Salts	**mg/L**	**Stock solution-02 mg/L (100x)**
01	KI	0.83	83
02	H_3BO_3	6.20	62
03	$MnSO_4H_2O$.	22.30	2230
04	$ZnSO_4.7H_2O$	8.60	860
05	$Na_2MoO_4.2H_2O$	0.25	25
06	$CuSO_4.5H_2O$	0.10	10
07	$CoCl_2.6H_2O$	0.10	10
	Iron-EDTA complex	**mg/L**	**Stock solution-03 g/L (100x)**
01	$FeSO_4.7H_2O$	27.80	2.78
02	$Na_2EDTA.2H_2O$	37.30	3.73
	Organics-1	**mg/L**	**Stock solution-04 mg/L (100x)**
02	Nicotinic acid	0.50	50
03	Pyridoxin HCl	0.50	50
04	Thiamine HCl	0.10	10
05	Glycine	2.00	200
	Organics-2	**mg/L**	**Stock solution-05 g/L**
01	Myo-Inositol	100	10.00
	Energy source and pH		
01	Sucrose	30.00 gm	30.00 gm
02	pH	5.8	5.8

Sub-experiment I: *In vitro* plantlets regeneration in Asterix variety of potato on a new tissue culture medium using ammonium chloride (NH_4Cl) as a substitute of ammonium nitrate (NH_4NO_3)

The treatment combinations of experiment -0I was given below.

- T_0 = MS (1962) standard dose, where ammonium nitrate used as conc. of 1650 mg/L
- T_1 = Ready made MS powder (Duchefa, The Netherlands) , 4.5 gm/L
- T_2 = 500 mg of ammonium chloride /L
- T_3 =1000 mg of ammonium chloride /L
- T_4 =1500 mg of ammonium chloride /L

Sub-experiment II : *In vitro* plantlets regeneration in Diamant variety of potato on a new tissue culture medium using ammonium chloride (NH_4Cl) as a substitute of ammonium nitrate (NH_4NO_3) The same treatments were used for the regeneration potentiality study in Diamant variety of Potato.

Experimental Materials:

Potato is a model plant for tissue culture research. We used potato plant as experimental material for *in vitro* regeneration to discover the effect of ammonium chloride. Two potato varieties *viz* Diamant and Asterix were used for this experiment. All the materials were collected from Tuber Crop Research Center (TCRC), Bangladesh Agricultural Research Institute (BARI), Gazipur. Data were recorded on the following parameter: Days to shoot regeneration, percent of shoot regeneration, length of shoot (cm), number of node, number of leaf, days to root regeneration, number of root and length of root (cm) per plantlet. Observation was done at 2^{nd}, 3^{rd} and 4^{th} week after inoculation of explant onto culture media. The standard autoclaving procedure was applied for sterilization of small instrument, media and other essential materials. Sprout and shoot tip were used as explant which were surface sterilized by 0.2% $HgCl_2$ chemical.

Design of Experiment and Data Analysis

The experiments were conducted by CRD design with 3 replication and data were analysis by Statistix-10 program showing LSD and CV value. The ± sign in tables denotes the standard deviation or possible errors associated with the values.

RESULTS AND INTERPRETATION

A series of experiments were conducted to find out any substitute of ammonium nitrate for the formulation of new plant tissue culture medium. Different nitrogenous sources of major salt were used for the purpose. Among them ammonium chloride (NH_4Cl) was identified as a good alternate of ammonium nitrate. Variety wise two sub-experiments was conducted to observed the regeneration potentially and the morphological appearance of plantlet grown onto the in the new medium. The key findings were given below:

Sub-Experiment I

In vitro plantlets regeneration in Asterix variety of potato on a new tissue culture medium using ammonium chloride (NH_4Cl) as a substitute of ammonium nitrate (NH_4NO_3). The major findings of sub-experiment were given below

Days to Shoot Regeneration and Percent of Shoot Regeneration:

There was no remarkable difference among the treatments on days to shoot regeneration and percent of regeneration. Days to shoot regeneration ranges from 6.12 to 6.98 days and hundred percent (100%) shoot regeneration was occurred in all the treatments (Table 3). It indicated that, the new formulation of culture medium has equal potentiality as compared to standard check treatments {MS (1962) and MS powder (Duchefa, The Netherlands)}.

Table 3: Days & percent of shoot regeneration, shoot length and node number at different week in Asterix variety of potato

Treatments	Days and percent of shoot regeneration		Shoot length at different week after regeneration (cm)			Node number at different week after regeneration		
	Days	Percent	2nd	3rd	4th	2nd	3rd	4th
T0 = MS (1962) standard dose, where ammonium nitrate used as conc. of 1650 mg/L	6.25	100%	7.06 b ± 0.12	8.16 c ± 0.09	9.20 b ± 0.21	5.33 cd ± 0.33	8.33 ab ± 0.33	9.33 b ± 0.33
T1 = Ready made MS powder (Duchefa, The Netherlands) , 4.5 gm/L	6.12	100%	7.20 b ± 0.12	8.96 b ± 0.26	9.63 b ± 0.22	6.33 bc ± 0.33	8.66 ab ± 0.33	9.66 ab ± 0.33
T2 = 500 mg of ammonium chloride /L	6.9	100%	7.56 b ± 0.15	9.00 b ± 0.12	9.26 b ± 0.32	6.66 ab ± 0.33	8.00 b ± 0.58	9.33 b ± 0.33
T3 =1000 mg of ammonium chloride /L	6.3	100%	9.90 a ± 0.15	11.66 a ± 0.18	13.20 a ± 0.46	7.66 a ± 0.33	9.33 a ± 0.33	10.66 a ± 0.33
T4 =1500 mg of ammonium chloride /L	6.98	100%	5.50 c ± 0.29	6.83 d ± 0.24	7.86 c ± 0.07	4.33 d ± 0.33	4.67c ± 0.33	7.00 c ± 0.58
CV (%)	-	-	4.1	3.66	5.04	9.52	8.76	7.43
LSD (0.05)	-	-	0.55	0.59	0.9	1.05	1.24	1.24
S. error	-	-	0.25	0.27	0.41	0.47	0.56	0.56

Shoot Length (cm) of Plantlet in Asterix Variety of Potato:

Maximum length of shoot viz. 11.66 cm and 13.20 cm were recorded in the treatment-3 at 3rd & 4th week of regeneration, respectively. The 2nd highest shoot length was in the check treatment-1 {MS powder (Duchefa, The Netherland)}(Table 3, Fig.1b). The minimum shoot length was reported in T4. The T4 contained high dose of NH_4Cl. The treatment-3 was prepared by the composition of ammonium chloride. It proved that, ammonium chloride can be easily replace on ammonium nitrate. The growth, physical appearance and overall morphology of plantlet in the treatment-3 was best among the treatments (Fig.1a & 1b). Hence, it recommended that, 10.00gm/L of ammonium chloride was the appropriate concentration for media preparation. Hoque et al., (2022) reported that, shoot length was highest when readymade MS powder (Duchefa, the Netherland used as a medium in compare to stock solution-01 made without NH_4NO_3.

Node Number of Plantlet in Asterix Variety of Potato:

Highest number of nodes *viz.* 9.33 and 10.66 was observed in the treatment-3 (T3) at 3rd and 4th week of explants inoculation (Table 3; Fig. 1a & 1b). It was statistically similar with the check treatment-1. The lowest number of nodes was recorded in the treatment-4 at 4th week of regeneration. It indicated that, the higher dose of ammonium chloride has some sort of negative effect on potato plantlet regeneration. Hena, (2017) reported that, shoot length and node number per explant was highest in the treatment-4 (stock solution-01, developed from the Department of Biotechnology, Sher-e-Bangla Agricultural University, Dhaka).

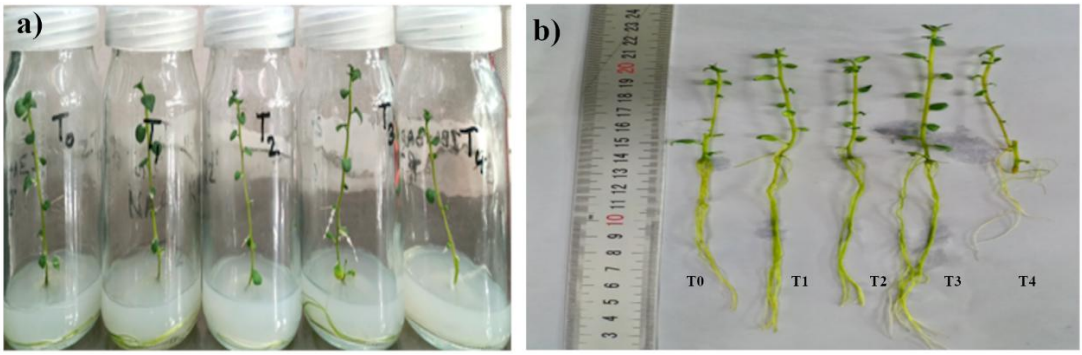

Fig 1: (a) Node number and (b) Shoot length (cm) in Asterix variety of potato in different treatment at 4th week of regeneration

Leaf number of plantlet in Asterix variety of potato:

The maximum number of leaf 9.10, 11.50 and 12.00 were recorded in the treatment-3 at 2nd, 3rd and 4th week of regeneration, respectively. The two check treatments {MS (1962) and MS powder (Duchefa, The Netherlands)} produced 2nd highest number of leaf (Fig. 2). The treatment-4 generated the lowest number (6.66) of leaf at 4th week of inoculation. It proved that; 10.00 gm/L of ammonium chloride has the excellent effect on leaf formation in Asterix variety of potato.

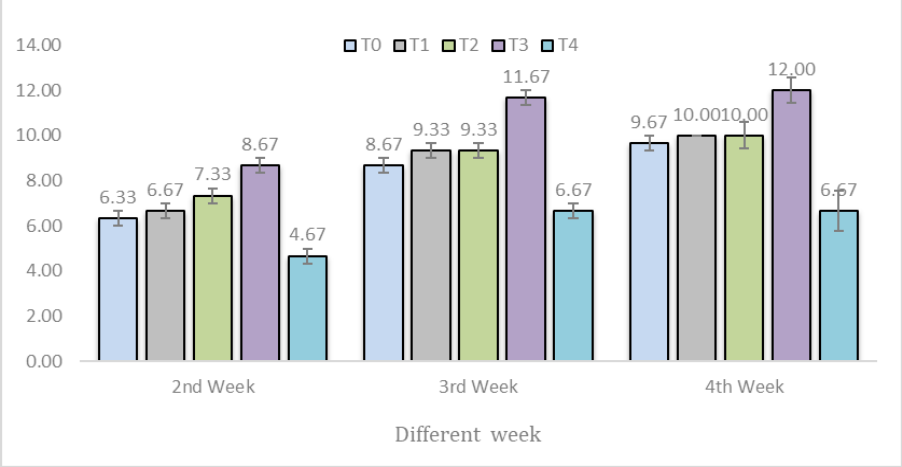

Fig 2: Leaf number of Asterix variety at different weeks in different treatment

Bashar *et al.* (2021) showed that *In vitro* regeneration of potato can be possible by using urea as a substitute of NH_4NO_3 in stock solution-01. They reported that highest number of leaf produced when 5.00 mg/L of urea used in stock solution-01.

Days to Root Initiation, Number of Roots and Root Length (cm) in Asterix Variety of Potato:

It was varied from 8.33 to 11.00 days. The lowest time was required in the treatment-3 and it was highest in the treatment-5. The highest number of roots (8.00) was noticed in the treatments-3 at 4th week of regeneration and the length of root (15.13 cm) was also highest in the same treatment at 4th week of regeneration (Table 4, Fig.1b). It revealed that, the new

chemical ammonium chloride has very good positive effect on root regeneration in potato. Bashar *et al.* (2021) reported that healthy and robust plantlet can be regenerated when urea used in the stock solution-01.

Table 4: Days to root initiation, root number and length from the treatments at different week after regeneration in Asterix variety of potato

Treatment	Days to root initiation	Root number at different week after regeneration		Root length at different week after regeneration	
		3rd	4th	3rd	4th
T0 = MS (1962) standard dose, where ammonium nitrate used as conc. of 1650 mg/L	6.66 bc ± 0.33	4.00 bc ± 0.58	6.00 b ± 0.58	8.86 b ± 0.32	9.86 c ± 0.09
T1 = Readymade MS powder (Duchefa, The Netherlands) , 4.5 gm/L	7.33 bc ± 0.33	5.00 ab ± 0.58	6.66 ab ± 0.33	10.06 b ± 0.41	10.80 bc ± 0.36
T2 = 500 mg of ammonium chloride /L	8.00 ab ± 0.58	4.66 bc ± 0.67	7.66 a ± 0.33	9.80 b ± 0.47	11.00 b ± 0.38
T3 =1000 mg of ammonium chloride /L	6.33 c ± 0.33	6.66 a ± 0.33	8.00 a ± 0.58	11.93 a ± 0.09	15.13 a ± 0.17
T4 =1500 mg of ammonium chloride /L	9.00 a ± 0.58	3.00 c ± 0.58	4.33 c ± 0.33	5.36 c ± 0.67	8.73d ± 0.43
CV (%)	10.37	20.7	11.86	8.22	4.98
LSD (0.05)	1.4	1.75	1.4	1.37	1
S.error	0.63	0.79	0.63	0.62	0.45

Sub-experiment-II

In vitro plantlets regeneration in Diamant variety of potato on a new tissue culture medium using ammonium chloride (NH_4Cl) as a substitute of ammonium nitrate (NH_4NO_3)

Days to Shoot Regeneration and Percent of Shoot Regeneration:

Days to shoot regeneration were ranges from 6.20 to 8.30 days among all the treatments. The minimum time (6.20 days) required in the treatment-3 and it was maximum (8.30 days) in the treatment-4. Hundred percent (100%) shoot regeneration was observed in all the treatments (Table 5). Its indicated that, the new formulation of medium was suitable for regeneration of Diamant variety of potato.

Node Number of Plantlet in Diamant Variety of Potato:

The highest number of nodes *viz.* 8.33 and 9.66 was found in the treatment-3 at 3rd and 4th week of regeneration, respectively. It was lowest in the treatment-4 (Table 5) The check treatment-1 {MS powder (Duchefa, The Netherlands)} showed the second highest (8.00) data at 4th week of regeneration. It proved that, ammonium chloride has the potentiality to regenerate plantlet in Diamant variety of potato. Bashar *et al*. (2021) reported that highest shoot length and leaf number found in Diamant variety of potato in the treatment when 5.00 mg/L Urea used in stock solution-01.

Leaf Number of Plantlet in Diamant Variety of Potato:

Maximum number of leaf (11.66) was mentioned in the treatment-3 at 4th week of regeneration. The second highest value (10.66) was recorded in the check treatment-0 (MS (1962) and and treatment-1 MS powder (Duchefa, The Netherlands). It was statistically similar with treatment-3 (Table 5; Fig.3). Hence, it is clear that, the ammonium chloride shown very good performance on potato plantlet production in potato

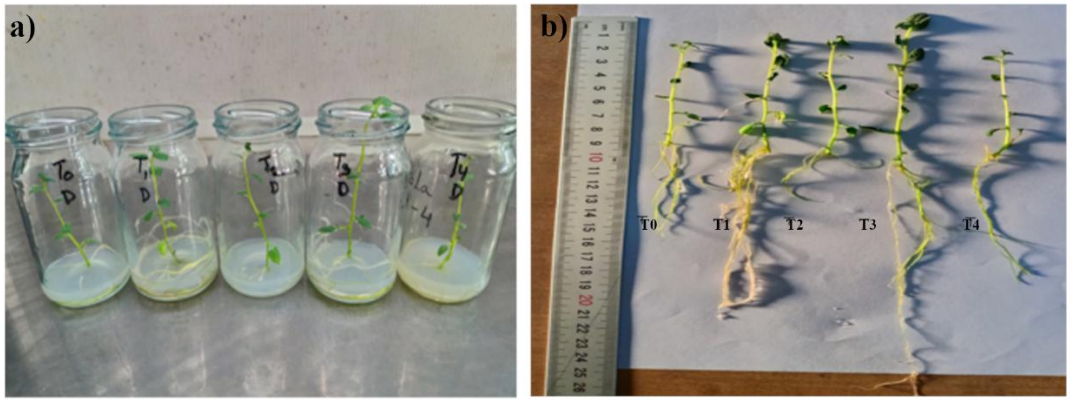

Fig 3: (a) Shoot and (b) root length (cm) of Diamant variety of potato in different treatment at different weeks of regeneration.

Table 5: days & percent of shoot regeneration, node and leaf number at different week after regeneration of different treatments in diamant variety of potato

Treatments	Days and Percent of shoot regeneration		Node number at different week after regeneration			Leaf number at different week of regeneration		
	Days	Percent	2nd	3rd	4th	2nd	3rd	4th
T0 = MS (1962) standard dose, where ammonium nitrate used as conc. of 1650 mg/L	7.12	100%	4.00b ± 0	6.00b ± 0	7.66b ± 0.33	4.33 b ± 0.33	7.33 b ± 0.33	10.66 ab ± 0.33
T1 = Ready made MS powder (Duchefa, The Netherlands) , 4.5 gm/L	6.34	100%	4.66b ± 0.33	5.66b ± 0.33	8.00b ± 0.33	4.66 b ± 0.33	7.66 b ± 0.33	10.33 b ± 0.33
T2 = 500 mg of ammonium chloride /L	6.25	100%	4.33b ± 0.33	5.33b ± 0.33	7.33b ± 0.58	4.667b ± 0.33	8.33 ab ± 0.33	10.66 ab ± 0.33
T3 =1000 mg of ammonium chloride /L	6.2	100%	7.00a ± 0.58	8.33a ± 0.33	9.66a ± 0.33	8.00 a ± 0.58	9.33 a ± 0.33	11.66 a ± 0.33
T4 =1500 mg of ammonium chloride /L	8.3	100%	2.33c ± 0.33	3.66c ± 0.33	5.66c ± 0.33	3.66 b ± 0.33	5.00 c ± 0.58	6.33 c ± 0.33
CV (%)	-	-	14.16	8.9	8.91	13.48	9.07	5.81
LSD (0.05)	-	-	1.15	0.93	1.24	1.24	1.24	1.05
S. error	-	-	0.52	0.42	0.56	0.56	0.56	0.47

Shoot Length (cm) of Plantlet in Diamant Variety of Potato:

The longest shoot length (11.76 cm) was noticed in the treatment-3 and it was shortest (6.66 cm) in the treatment-4 at 4th week of regeneration. The two check treatments showed lower performance than the treatment-3. (Fig. 3a & 3b & 4). Rahman *et al.* (2011)) reported that, low nitrate media produced better shoot in Shepody and Dimant variety of Potato whereas higher nitrate media resulted maximum shoot fresh weight.

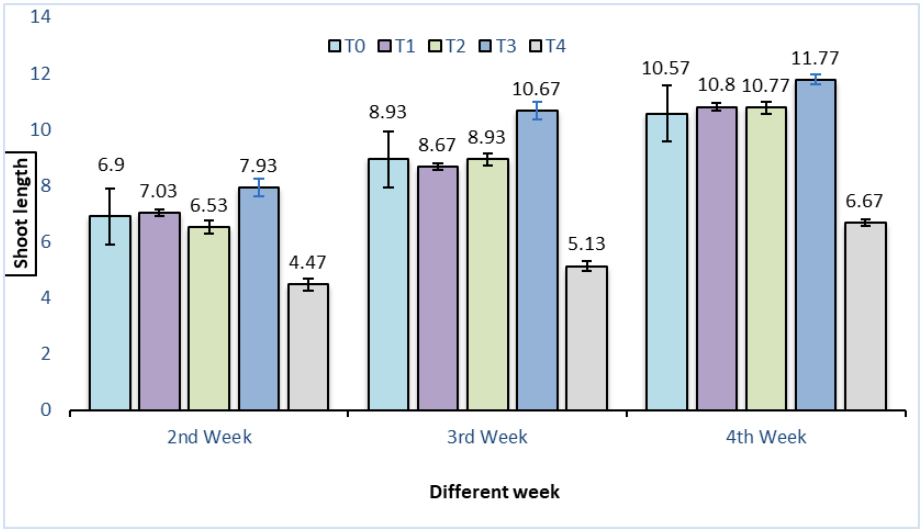

Fig 4: Shoot length (cm) of plantlet in Diamant variety of potato

Number of Root and Root Length (cm) of Plantlet in Diamant Variety:

Highest number of root (8.00) and longest length (17.73 cm) of root was recorded in treatment-3 which was statistically significant with two standard check treatments {MS (1962) and MS powder (Duchefa, The Netherland)} (Table 6; Fig. 3b).

Table 6: Root number and root length at different week after regeneration in Diamant variety of potato

Treatments	Root number at different week after regeneration		Root length at different week after regeneration	
	3rd	4th	3rd	4th
T0 = MS (1962) standard dose, where ammonium nitrate used as conc. of 1650 mg/L	4.66 a ± 0.33	7.33 a ± 0.33	9.73 b ± 0.19	14.56 c ± 0.18
T1 = Ready made MS powder (Duchefa, The Netherlands) , 4.5 gm/L	5.33 a ± 0.33	7.33 a ± 0.33	9.56 b ± 0.13	15.53 b ± 0.15
T2 = 500 mg of ammonium chloride /L	5.66 a ± 0.33	7.33 a ± 0.33	10.83 b ± 0.88	15.23 bc ± 0.18
T3 =1000 mg of ammonium chloride /L	5.66 a ± 0.33	8.00 a ± 0.58	13.53 a ± 0.62	17.73 a ± 0.15
T4 =1500 mg of ammonium chloride /L	3.33 b ± 0.33	4.33 b ± 0.33	4.23 c ± 0.15	6.96 d ± 0.37
CV (%)	11.7	9.95	9	2.72
LSD (0.05)	1.05	1.24	1.56	0.69
S. error	0.47	0.56	0.7	0.31

The two sub-experiments revealed that, different modifications of stock solution-01 by ammonium chloride has significant effect on plantlet regeneration and its development. The

new formulation of medium is as equal as the two check treatments. The objective of the present research is to find out any alternate chemical of ammonium nitrate and formulation of a new tissue culture medium which can be showed similar performance as the two standard check treatments {MS (1962) and MS powder (Duchefa, The Netherland)}. It was successfully achieved by the using of ammonium chloride and changing the concentration of major and minor salt of culture medium. Hence, it is a new innovation in the field of tissue culture technology. The new formulation and composition medium is marked as "SAU Plant Tissue Culture Medium (Hoque Media)". It has several adventages as compare to MS (1962). It is cheap, available, required less amount, no harmful effect and finally plantlet regenerated from this chemical is robust, healthy and vigorous.

Validation for Plantlet Regneration Potentiality of the SAU Tissue Culture Medium (Hoque Medium, 2023):

The newly developed SAU plant tissue culture medium showed excellent results on *in vitro* regeneration of potato. The growth and morphology of plantlets were healthy and robust as compare to MS (1962) medium. Hence, it is necessary to check the validation for the regeneration ability of SAU medium in some other crops. Therefore, sweet Potato and *Aloe vera* regeneration potentiality was studied onto the SAU medium including MS (1962) medium and MS powder (Duchefa) medium as two standard check.

Table 7: Effect of different tissue culture media on percent of shoot regeneration and number of leaf per explant at different days after initiation in Sweet Potato.

Treatment	Percentage of shoot regeneration	Number of leaf per explant at different days after initiation		
		3 Week	4 Week	5 Week
T1= Murashige and Skoog (1962) medium	61.9	2.0 d	5.0 d	7.67 e
T2= Readymade MS powder (Duchefa, the Netherlad)	75.57	2.33 cd	5.33 cd	8.33 de
T3= SAU Tissue culture medium	77.33	2.67 bc	6.0 bc	9.33 cd
LSD (0.05)	-	0.65	0.84	1.02
CV (%)	-	12.62	7.64	6.08

The highest regeneration percentage (77.33%) was found in SAU Tissue Culture Medium (Hoque medium). Healthy and excellent quality plantlet was notice at 3rd and 5th week of regeneration in the treatment SAU Tissue Culture. Medium (Table 7, Fig. 5a & 5b). In case of *Aloe vera* tissue culture, the highest percent (90%) of shoot regeneration was found in the SAU Tissue Culture Medium. The same treatment showed maximum number of shoots in all the study period (Fig. 6) Longest length of root (6.917.00 cm) was observed in SAU Tissue Culture Medium and it was shortage (5.91cm) in MS (1962) Medium (Table 8). The study highlighted the potentiality of SAU Tissue Culture Medium on *In vitro* regeneration of sweet potato and *Aloe vera.* It has indicated that the newly developed SAU Tissue Culture Medium (Hoque Medium) has excellent result on plantlet regeneration in both the crops. Hence, the formulation of SAU

Tissue Culture Medium is a successful innovation in the field of tissue culture technology which can be applied for any other plant species.

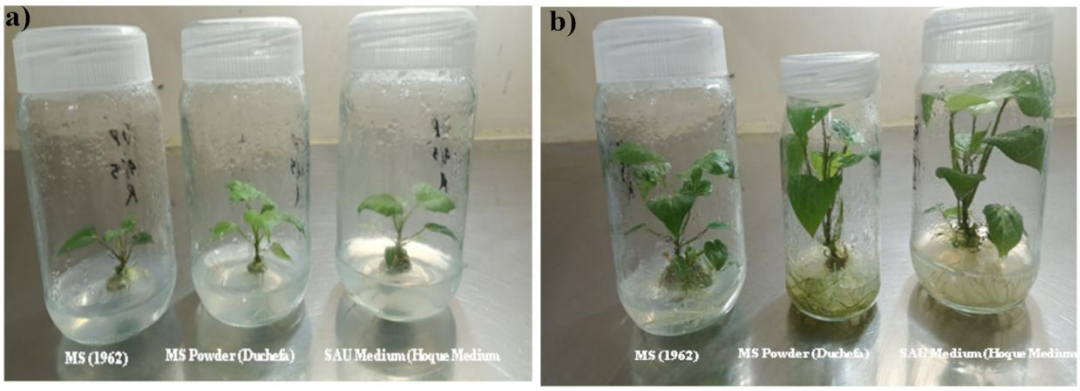

Fig 5: Sweet Potato plantlet regeneration in SAU Tissue culture medium (Hoque medium, 2023) including two standard check MS (1962) medium and MS powder (Duchefa, The Netherlands) at (a) 3rd week and (b) 5th of explants inoculation.

Table 8: Effect of different tissue culture media on number of shoots per explant at different days after initiation in *Aloe vera*

Treatment	Percentage of shoot regeneration	Number of shoots per explant at different days after initiation			
		3 Week	4Week	5 Week	6 Week
T1= Murashige and Skoog (1962) medium	75	1.93 d	3.02 d	4.58 d	5.91 c
T2= Readymade MS powder (Duchefa, the Netherlands)	85	2.37 cd	3.63 bc	5.64 bc	6.80 b
T3= SAU Tissue culture medium	90	2.70 bc	3.7 b	5.83 b	6.91 b
LSD (0.05)	-	0.86	0.75	0.88	0.50
CV (%)	-	14.76	8.72	6.56	3.09

Fig 6: *Aloe vera* plantlet regeneration in SAU Tissue culture medium (Hoque, 2023) including two standard check MS (1962) medium and MS powder (Duchefa, The Netherlands) at 5th week of explants inoculation.

CONCLUSION

Plant tissue culture media preparation from stock solution is a regular work in all the tissue culture laboratory. It was seriously hampered due to lacking of ammonium nitrate. The present investigation was able to identify a alternate chemical of ammonium nitrate, which perform excellent result on media preparation and subsequent plantlet regeneration on the crops like Potato, Sweet potato and *Aloe vera.* The newly identified chemical-ammonium chloride (NH_4Cl) and the changed concentration of macro and micro nutrients is a new innovation for the culture media. The new formulation of tissue culture medium has commercial application on tissue culture base seed industry under Bangladesh condition. The present new tissue culture medium is denoted as "SAU Plant Tissue Culture medium (Hoque Medium,2023).

Statements & Declarations

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Competing Interests:

Author Md. Ekramul Hoque and others has no financial interest. The author Md. Ekramul Hoque is the supervisor and the other authors are MS student of the Department of Biotechnology, Sher-e-Bangla Agricultural University, Dhaka, Bangladesh.

Author Contributions:

Md. Ekramul Hoque contributed to the study conception and design. Material preparation and regeneration work done by all authors. Data collection and analysis were performed by Md. Ekramul Hoque and Mokaram Hanifa Koly. The first draft of the manuscript was written by Md. Ekramul Hoque and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Compliance with Ethical Standards:

No conflicts of interest. Research has not involved human participants or animal kingdom.

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