RESEARCH ARTICLE

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Potato (*Solanum tuberosum* L.) Plantlet Regeneration in Ammonium Nitrate Free Stock Solution-1 of Murashige & Skoog (MS, 1962) Plant Tissue Culture Medium

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ABSTRACT

Potato (*Solanum tuberosum* L.) plantlet regeneration potentiality was studied in ammonium nitrate (NH_4NO_3) free tissue culture medium. Four different treatments were designed from the compositions of the stock solution-01 of plant tissue culture medium. Those were -Treatment-1 (Stock solution -01 as recommended by Murashige & Skoog (1962), Treatment-2 (MS stock solution-01 without having NH_4NO_3), Treatment-3 (MS stock solution-01without NH_4NO_3 but other component had double concentration), Treatment-4 (Readymade MS powder, Duchefa Biocheme, The Netherland). Shoot length, shoot diameter, node number and leaf number per plantlet were highest in Treatment-4 at 14, 21 and 28 days after inoculation (DAI). Shoot regeneration parameters were statistically similar with the treatment-3 and the check treatment-1. But the check treatment-1 showed better result in root number and root length (cm) as compared to treatment-3 and treatment-4. The treatment-2 showed lowest result in each of the said parameter. The stock solution-01 which was formulated without ammonium nitrate and has double dose of other ingredient has the potentiality for potato plantlet regeneration, but it was not as suitable as Readymade MS powder (Duchafa, The Netherland).

Keywords: Ammonium nitrate, potato regeneration, stock solution-1, tissue culture medium.

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I. Introduction

Potato (*Solanum tuberosum L.)* is one of the important vegetables of tuberous plant under the Solanaceae family. It is a popular vegetable for most of the world's population and has different health benefits. These health benefits include their ability to improve digestion, reduce cholesterol levels, protect from polyps, prevent cancer and manage diabetes (Englyst *et al.,* 1992.; Cummings, *et al.,*1996; Hylla *el al.,* 1998). Bangladesh ranked 7th position in potato production among the world contest and more than 95 lac ton potato production occurred in every year at Bangladesh (BBS, 2020). Accumulation of several diseases in seed tuber is one of the limiting factors for its yield and quality (Kolev*aet al.*, 2012; Qureshi *et al.*, 2014). Healthy planting material has important role in potato production. Disease and virus free plantlet can be produced through merister culture. Tissue culture techniques are used worldwide for production of minituber and disease-free planting materials (Wakil 2020; Molla *et al.*, 2011). In addition, *in vitro* methods can be used for conservation, storage, and easy distribution of potato germplasm from one place to another (Badoni and Chauhan, 2010; Motallebi-Azar *et al.*, 2011; Khadiga *et al.*, 2015). Media compositions are the governing factor for successful plantlets production of any crop. There were many reported media compositions which were used for *in vitro* propagation of potato. Some of them are – MS medium (Murashige and Skoog, 1962), LS medium (Linsmeir and Skoog 1965), B5 medium (Gamborg's *et al.,* 1968), NN medium (Nitch & Nitch, 1969) etc. Among them MS (1962) medium is most widely used for rapid *in vitro* regeneration of many crops. Generally, the plant tissue culture media are made by macro and micronutrients, plant hormone, amino acid, and vitamins. The nutrient media is prepared by the mixture of stock solutions of various chemical ingredients. Among the major salts, very important one is ammonium nitrate (NH_4NO_3) and used in stock solution-1 preparation (Murashige and Skoog, 1962). It supplies ammonium and nitrate ion in the medium for the explants which is essential for proper growth and development of plantlet. But unfortunately, it is an oxidizing agent and use for the preparation of bomb. It is an important tool of terrorist people for doing different anti-social activities. Ammonium nitrate is a weapon for bomb blasting and other destructive activities. Hence, it is totally ban in our country. Its import, purchase, and sale it fully restricted in Bangladesh. So not a single amount of ammonium nitrate is available in Bangladesh. Therefore, our hypothesis is to develop *in vitro* regeneration protocol of potato without having ammonium nitrate in stock solution-1.

TABLEI: DIFFERENT TREATMENTS AND THE COMPOSITION OF STOCK SOLUTION-1

Serial number	Treatments	Ingredients of each treatment	Chemical formula	Amount (gm/litre)
01	Check Treatment 1 (T1)= Stock solution-1 as recommended by Murashige & Skoog (1962)	Potassium nitrate	KNO_3	19.00
		Ammonium nitrate	NH_4NO_3	16.50
		Magnesium sulphate	$MgSO_4.7H_2O$	3.70
		Calcium chloride	$CaCl_2.2H_2O$	4.40
		Potassium dihydrogen phosphate	KH_2PO_4	1.70
02	Treatment 2 (T2)= MS stock solution-1 without having NH_4NO_3	Ammonium nitrate	NH_4NO_3	Nill
		Potassium nitrate	KNO_3	19.00
		Magnesium sulphate	$MgSO_4.7H_2O$	3.70
		Calcium chloride	$CaCl_2.2H_2O$	4.40
		Potassium dihydrogen phosphate	KH_2PO_4	1.70
03	Treatment 3 (T3)= MS stock solution-1without NH_4NO_3 but other component had double concentration	Ammonium nitrate	NH_4NO_3	Nill
		Potassium nitrate	KNO_3	38.0
		Magnesium sulphate	$MgSO_4.7H_2O$	7.40
		Calcium chloride	$CaCl_2.2H_2O$	8.80
		Potassium dihydrogen phosphate	KH_2PO_4	3.40
04	Treatment 4 (T4)= Readymade MS powder (Manufacturer: Duchefa,, The Netherland	Powder of different ingredients	Unknown	4.50

II. MATERIALS AND METHODS

The research work was carried out at Biotechnology Laboratory, Sher-e-Bangla Agricultural University, Bangladesh. Asterix variety of potato was used as experimental material and nodal segment was used as explants. Growth and development of plant cell under *in vitro* condition are largely governed by the composition of the culture media. The principal components of most of the plant tissue culture media are inorganic and organic nutrients, growth regulators and vitamins etc. Media preparation is one of the primary and most essential steps in tissue culture (Hena, 2017). Murashige and Skoog (1962) medium is a most commonly used medium in the laboratories for regeneration of plant. Ammonium Nitrate (NH_4NO_3) is an important salt in stock solution-1 for Murashige and Skoog (1962) media preparation. The hypothesis of the research work is to develop a stock solution-1 composition without ammonium nitrate and to study the potentiality for the regeneration of potato. Different concentration and combination of stock solution-1were treated as the treatment of this experiment. Four different treatments including check treatment (T1) were designed to conduct the experiment. The ingredients and its concentration of each treatment were given in Table I.

Stock solutions for the micronutrients, Fe-EDTA (Iron stock), vitamins and growth regulators were also prepared for final medium preparation. All the chemicals used for stock solution is highly purified and labeled as plant tissue culture tested grade.

One liter (1000 ml) of tissue culture medium was made by added 100ml from each treatment of stock solution-1 and 10 ml from rest of the stock solution-II to IV. Water was added up to 1000 ml volume. One liter of treatment-4 (T4) was made on the basis of manufacture instruction. Thirty (30) sucrose was added in each treatment and pH was adjusted at 5.8. Then 8gm agar was added for solidifying the media. It was sterilized in 121 °C for 25 minute with 15 psi. The nodal segment of regenerated potato plantlet was used as explants. It was inoculated in a culture vial containing 25 ml of medium and the mouth of culture vial was covered tightly. The total operation was done in the laminar hood in sterile condition. The culture bottles were placed in a growth chamber. The physical environment of the growth room was maintained at 21±1 °C with light intensity varied from 4000–5000 Lux (23 W white bulbs). The photoperiod was generally 16 hours light and 8 hours dark having 60-70% relative humidity (RH). The one factors experiment was laid out in Completely Randomized Design (CRD) with three replications. Ten culture vials were used as one replication for each treatment. Data were collected on shoot length, shoot diameter number of node, number of leaves, number of root and length of root per plantlet. It was recorded on 14, 21 and 28 days after inoculation (DAI) of explants. Different parameters were statistically analyzed for further interpretation of result. The mean values of all the recorded characteristics were evaluated and analysis of variance was performed by the 'F' (variance ratio) test using MSTAT-C software. Duncan's Multiple Range Test (DMRT) at 5% level of probability was done for identify the significance of the difference among the treatments. LSD was also calculated to compare the differences between two treatment means.

III. RESULT AND DISCUSSION

The present research was carried out to evaluate the regeneration capability of potato (*Solanum tuberosum* L.) in ammonium nitrate free culture medium. The results and feasible explanation has been given under the following parameters.

A. Days to Shoot Initiation, Shoot Length and Shoot Diameter of Plantlet

The result of days to shoot regeneration was presented in Table II. The maximum days to shoot initiation (8.67) was recorded in treatment-2 (NH_4NO_3 free stock solution), followed by treatment-3 which was different from all other treatments. In contrast, the minimum data (5.00) was recorded in treatment-4 (Readymade MS powder) followed by check treatment-1 (5.67). The treatment-3 taken 7.30 days for shoot initiation. Statistical variations were observed among different treatments on days to shoot initiation.

TABLE II: DAYS TO SHOOT INITIATION, SHOOT LENGTH AND SHOOT DIAMETER OF REGENERATED PLANTLET

Sl no.	Treatments	Days to shoot initiation	Shoot length (cm)			Shoot diameter(mm)
			14 DAI	21 DAI	28 DAI	28 DAI
01	$T_{1=}$ Stock solution -1 as recommended by Murashige& Skoog (MS) (1962)	5.67c	4.00 ab	6.83 ab	8.57a	1.62a
02	$T_{2=}$ MS stock solution-1 without having NH_4NO_3	8.67a	2.33 b	4.67 c	6.60b	0.81c
03	$T_{3=}$ MS stock solution-1without NH_4NO_3 but other component had double concentration	7.30b	4.00 ab	6.67 ab	7.90b	1.17b
04	$T_{4=}$ Readymade MS powder (Manufacturer: Duchafa, The Netherland)	5.00c	5.33 a	7.33 a	9.00 a	1.97a
-	LSD (0.05)	0.91	1.79	1.22	1.27	0.29
-	CV (%)	7.63	12.95	9.83	8.21	10.56

Shoot length of different the treatments was presented in Table II. The highest shoot length (5.33cm) was observed in the treatment-4 at 14 DAI (Days After Inoculation). The second highest shoot length (4.00 cm) was noticed in both the treatment T1 and T3 at 14 DAI. The lowest shoot length (2.33 cm) was found in the treatment-2. The maximum shoot length (7.33 cm) was observed in the treatment-4 (Readymade MS powder) at 21 days after inoculation (DAI). Statistically similar data 6.83 cm and 6.67 cm were recorded in the check treatment-1 and treatment-3. The treatment-2 showed the lowest (4.67 cm) result. Highest shoot length (9.00 cm) was noticed at 28 DAI from the treatment-4 and the second highest shoot length (8.57 cm) was found in check treatment-1. It was statistically insignificant with the treatment-4. The third highest shoot length (7.90 cm) was found in the treatment-3. The lowest shoot length (6.60 cm) was found in treatment-2 at 28 DAI, respectively.

The minimum shoot diameter (0.81 mm) was recorded in the treatment-2 (NH_4NO_3 free stock solution) at 28 DAI (Table II) which was statistically different from all other treatments. The maximum shoot diameter (1.97 mm) was found in treatment-4 which was statistically similar with Treatment-1 but different from all other treatments at 28 days after inoculation (DAI). The treatment effect of shoot length were presented in Fig.1 The treatment-2 which has no NH_4NO_3 showed lowest performance among the three parameter under studied. It may be due to absence of NH_4^+ ion in the medium. The treatment -3 which also has no NH_4NO_3 but the other ingredients have double concentration for the preparation of stock solution-01 showed better respond among the parameter under investigation. The observed data of treatment-3 was very closer to the check treatment-1 and also treatment -4 which was consist of Readymade MS powder. Its indicated that, the double dose of potassium nitrate (KNO_3) may supply necessary nitrogen (N) in the medium for the growth and development of potato explants.

Rahman *et al.* (2011) reported that zero nitrate media gave poor performances on different morphological traits among the different varieties of potato under investigation. Bashar *et. al.* (2021) indicated that the treatment having 5 gmL-1 and 1 gmL-1 of β chemical showed better result in respect of shoot length than ammonium nitrate free medium.

Hena (2017) reported that *In vitro* regeneration of a potato was successfully achieved in ammonium nitrate free medium by changing the concentration of other ingredients of stock solution -1.

Fig. 1. Shoot length and number of node of regenerated plantlet in treatment-1 (T1), treatment-3 (T3) and treatment-4 (T4).

Fig.2. Root length of regenerated plantlet in treatment-1 (T1), treatment-3 (T3) and treatment-4 (T4).

TABLE III: NUMBER OF NODE AND LEAVES PER PLANTLET AT DIFFERENT DAYS AFTER REGENERATION

Sl No.	Treatments	Number of node			Number of leaves		
		14DAI	21 DAI	28DAI	14 DAI	21DAI	28 DAI
01	$T_{1=}$ Stock solution -1 as recommended by Murashige & Skoog (MS) (1962)	2.33ab	6.87a	7.33a	2.67b	8.00 ab	11.00 a
02	$T_{2=}$ MS stock solution-1 without having NH_4NO_3	1.33 b	4.00 b	5.00 b	1.33 d	5.33 c	6.00 b
03	$T_{3=}$ MS stock solution-1without NH_4NO_3 but other component had double concentration	2.33 ab	6.67 a	.67ab	1.67 c	7.00 bc	9.00 a
04	$T_{4=}$ Readymade MS powder (Manufacturer: Duchafa, The Netherland)	2.67 a	7.00 a	8.00 a	3.67 a	8.67ab	11.00a
-	LSD (0.05)	0.97	1.79	1.75	0.53	1.75	2.06
-	CV (%)	21.52	5.14	13.30	10.04	12.25	11.91

TABLE IV: ROOT LENGTH AND NUMBER OF ROOT PER PLANTLET AT DIFFERENT DAYS AFTER REGENERATION

Sl no.	Treatments	Length of root (cm)			Number of root
		14 DAI	21 DAI	28 DAI	28DAI
01.	$T_{1=}$ Stock solution -1 as recommended by Murashige & Skoog (MS) (1962)	2.67 a	9.90a	13.50a	13.67a
02.	$T_{2=}$ MS stock solution-1 without having NH_4NO_3	1.17 b	5.00 b	7.10 b	6.67c
03.	$T_{3=}$ MS stock solution-1without NH_4NO_3 but other component had double concentration	1.83 ab	8.60 a	11.40 a	10.33b
04.	$T_{4=}$ Readymade MS powder (Manufacturer: Duchafa, The Netherland)	2.67a	8.60a	9.67b	7.00c
-	LSD (0.05)	1.01	2.02	2.72	2.50
-	CV (%)	7.73	13.60	13.47	12.95

A. Number of Node and Leaves per Plantlet

Number of node in the treatment-1 and treatment-3 was equal (2.33) at 14 days after inoculation (DAI). The minimum node number (1.33) was found in treatment-2 (NH_4NO_3 free stock solution) which was statistically different from all other treatments. The highest node number (8.00) was recorded in the treatment-4 and it was statistically insignificant with the result (7.33) found in the treatment-1 at 28 DAI. The lowest number of node was recorded in the treatment-2. Among the four treatments, the maximum leaf number (3.67) was found in T4 which was statistically different from all other treatments at 14 DAI. While minimum leaf number (1.33) was found in T2 (NH_4NO_3 free stock solution). This poor leaf formation in treatment-2 may be due to the absence of NH_4NO_3. At 21 days after inoculation (DAI), the maximum leaf number (8.67) was found in treatment-4 which was statistically similar with treatment-1 (8.00). Minimum leaf number (5.33) was found in treatment-2 which was statistically different from all other treatments. The treatment-03 (NH_4NO_3 free stock solution with double dose of other ingredients) performed better (7.00) than Treatment-2 and it may be due to the double dose of all ingredients specially the amount of KNO_3. The maximum leaf number (11.00) was found in both treatment-4 (readymade MS powder) and the check treatment-1 which was statistically different from treatment-2 (6.00) at 28 days after inoculation (DAI) (Table III). The second highest node number and leaf number was recorded in treatment-3. The check treatment-1 which was formulated on the principle of MS (1962) tissue culture medium composition showed very closer result with Readymade MS powder treatment and statistically both the treatment showed similar result. The treatment -3 which has no NH_4NO_3 but other component has double concentration gave second highest result for this two parameter. It proved that, plantlet can be regenerate by NH_4NO_3 free medium but the growth and development of plantlet is little weaker than Readymade MS powder and MS (1962) tissue culture medium composition.

B. Root Length and Number of Root per Plantlet

The maximum root length (2.67 cm) was found in T4 (readymade MS powder) and ckeck treatment-1 which was statistically different from all other treatments at 14 DAI. Minimum root length (1.17 cm) was found in T2 (NH_4NO_3 free stock solution) (Table IV and Plate 1). The maximum root length (9.90 cm and 13.50 cm) was found in check treatment-1 which was statistically similar with T3 (8.60 cm and 11.40 cm) at 21 and 28 DAI, respectively. The treatment-4 showed 8.60 cm and 9.67 cm root length at 21 and 28 DAI, respectively. The treatment-1 gave the best result among the four treatments for root length in potato plantlet. The maximum root number (13.67) was found in T1 which was statistically different from all other treatments at 28 days after inoculation (DAI). The treatment-3 (NH_4NO_3 free stock solution with double dose of other ingredients) performed second highest number (10.33) at 28 DAI. Minimum result (6.67) was recorded in the treatment-2 at 28 DAI which was statistically different from all other treatments (Table IV).

Plantlet regeneration of potato is 50-60 years back technology. Unlimited literature is available in this regard. Different plant tissue culture media also developed by many scientists. But the objective of the present study is to develop a new composition of tissue culture medium where ammonium nitrate will be absent. The finding of the research gave an information to the scientist community regarding the alternate of ammonium nitrate for the preparation of plant tissue culture medium.

IV. Conclusion

Potato plantlet regeneration ability was studied in different concentration of stock solution-01 of tissue culture media. MS 1962 medium composition of stock solution-01 showed satisfactory level of plantlet regeneration in potato. Absence of NH_4NO_3 along with standard dose of other ingredients in MS 1962 medium of stock solution-1 has negative effect and showed weak performance on plantlet production in potato. It may be due to shortage of nitrogenous salt in the medium. All the morphological parameters *viz* shoot length, node number, leaf number, root length etc. showed lowest performance in this treatment. The treatment-3 which was prepared by the absence of ammonium nitrate but other ingredient has double concentration gave better respond on *in vitro* regeneration of potato. It may be due to higher concentration of potassium nitrate (KNO_3) which gave additional supplement of nitrogen in the medium. Readymade MS powder (Duchefa, The Netherland) showed best respond on *in vitro* regeneration in potato. Finally, it concluded that, potato plantlet regeneration is possiblewithout NH_4NO_3 by modifying the other ingredients stock solution-01 of Murashige and Skoog (1962) medium.

Conflict of Interest

Authors declare that they do not have any conflict of interest.

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