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Single and combined effects of synthetic auxin and cytokinin on *in vitro* microplants of sweet potato (*Ipomoea batatas* L.)

Abdur Rahim, Shakil Rahman Shoagh, Anika Tasnim Khan Mim, Baishakhi Roy, M Nasiruddin*
Department of Botany, University of Rajshahi, Rajshahi, Bangladesh

Abstract
The sweet potato (*Ipomea batatas* L.) is vegetatively propagated crop rich in vitamins and sugar. Producers avoid it because vegetative development favors virus-infected materials, lowers yields, and increases input needs. Virus-free microplants are possible but difficult through tissue culture. This work evaluated a highly effective disease-free *in vitro* microplant production protocol using MS media with BA, KIN alone, and together with NAA. The result showed that all the hormonal tests have exhibited a consistent and proportional increase over time (15 and 30 days respectively). The character shoot initiation was early responsive in the T3 (2.00 mg^{-l}) of single treatment and T12 (3.00 mg^{-l} BA with 0.5 mg^{-l} NAA), T14 (1.00 mg^{-l} KIN with 0.5 mg^{-l} NAA), T15 (2.00 mg^{-l} KIN with 0.5 mg^{-l} NAA) of combined treatments and less responsive was T6 (1.00 mg^{-l} KIN) of single treatment. Among the combined treatments optimum effect was observed in most of the studied characters with a notable root growth in T7 (2.00 mg^{-l} KIN), T8 (3.00 mg^{-l} KIN), T11 (2.00 mg^{-l} BA with 0.5 mg^{-l} NAA), T13 (4.00 mg^{-l} BA with 0.5 mg^{-l} NAA) and T15 (2.00 mg^{-l} KIN with 0.5 mg^{-l} NAA). Interestingly, the treatment T1 (control) was observed good quality of every agronomic characters but root growth were absent. Thus, it should be concluded that hormonal effect was affected by growth and development of the sweet potato microplants.

Keywords: Sweet potato, microplant, 6-benzylaminopurine, kinetin, naphthaleneacetic acid

Introduction
The sweet potato (*Ipomea batatas*) is a succulent tuberous crop that is rich in both vitamins and sugar. Sweet potato tubers are utilised in the production of alcohol, acetic acid, yeast, and for extracting starch [1, 2]. This plant is a dicotyledonous member of the Convolvulaceae family. The tuberous root crop is crucial for ensuring food security in tropical, subtropical, and temperate climates. It is a crop that thrives in warm seasons and is grown in poor nations. It is highly valued as a source of food for humans, feed for animals, and raw material for industries. According to Jarret and Florkowski (1990) [3], it is considered one of the top five most important food crops in over 50 countries (FAOSTAT, 2012) [4]. Faostat reported that the sweet potato production in Bangladesh reached 304 kt in 2022. People have known about sweet potatoes for a very long time. In ancient times, people in Central America and the tropical parts of South America ate it (Bovell-Benjamin, 2007) [5]. A lot of people in Southeast Asia, Africa, and Latin America eat this dish every day. Tubers, young shoots, and leaves are the parts of the plant that can be eaten (Islam, 2014) [6].

It is very hard to grow and develop in tissue culture. It is also spread a lot through vegetative growth, which is detrimental because it favours virus-infected materials and leads to low yields and high input needs, which makes producers less likely to use it (Aloufa, 2002; El-Afifi *et al.*, 2012) [7, 8]. The use of vegetative planting materials is often difficult to come by, especially when working with larger regions. Tissue culture is used to grow healthy, homogenous plants (Dolinski and Olek, 2013) [9]. Bud explants, according to Dolisnki and Olek, (2013) [9], are the most successful way for renewing large planting materials in sweet potatoes. A variety of characteristics, including genotypes and the type and dosage of numerous growth regulators, have been discovered to influence the rate and type of sweet potato regeneration (Shaibu *et al.*, 2016) [10]. *In vitro* micropropagation of potatoes is commonly used to increase the number of novel cultivars and breeding lines, store germplasm, transport, and produce small tubers that are easy to store, transfer, and distribute disease-free plants quickly and year-round (Jones, 1988) [11]. Several studies have found that *in vitro* potato propagation through serial culture of axillary shoots on divided nodes is a dependable strategy for rapidly developing innovative or existing cultivars in disease-free settings (Hussey and Stacey, 1984) [12]. These approaches, which employ *in vitro* plantlet, micro tubers, or tiny tubers (Bizarri and Ranalli, 1995; Hussey and Stacey, 1981) [13, 14], have hastened the first stages of multiplication in seed production programmes in a number of countries.

Thus, the main aim of the present research was to screen out a very efficient protocol among different hormonal treatments for the production of disease-free seed tubers and the large-scale reproduction of healthy plant material.

Materials and methods
Plant materials
The economically important sweet potato genotype (*Ipomoea batatas* L.) were collected from 'Aka-Fuji Agro Technology Laboratory', Katakhali, Rajshahi, Bangladesh; and *in vitro* cultural operation were performed in 'Plant Breeding and Gene Engineering Laboratory' at the Department of Botany, University of Rajshahi, Rajshahi 6205, Bangladesh.

Sterilization and inoculation of explant
Collected tubers were surface sterilised by washing them in running tap water and laundry bleach for 20 minutes, then spraying with 10% alcohol and washing multiple times with sterile distill water. The sterilised tubers were kept at 4 °C in the dark for sprouting and *in vivo* culture. The germinating tubers were grown in containers and maintained in the greenhouse; the matured plants followed standard potato

farming practices including maintenance, watering, fertilisation, and management of weeds and pests. After three to four weeks, when the plants were 25 to 35 cm tall with 6 to 8 nodes, they cut into single nodes and eliminated enormous leaves. Before use, single node cuttings (1-3 cm in length) were cleaned under running tap water for 20 minutes.

Culture condition

Explants were cultured in culture bottles containing MS (Murashige and Skoog 1962) [15] basal medium supplemented with 30 g/l sucrose and 6-benzyl aminopurine (BAP), kinetin (KIN) singly, and combination with naphthalene acetic acid (NAA) (Table 1). Every culture vessel contains 20 ml of medium solidified with 7 g/l Nobel agar (Merck, India) and PH were adjusted at 5.7. Culture bottles were closed with polypropylene caps and sealed with parafilm M (Laboratory Film) (Chicago, II. 60631, USA). After being autoclaved (121 °C, 15 psi for 20 minutes), they were incubated at 16 h photoperiod (from cool white fluorescent lamps, approx. 20 µmol/m2/s light intensity) at 24 ± 1 °C in the tissue culture growth chamber.

Table 1: Media composition of different treatments

Treatment code	MS media with			
	MS (g/l)	BA (mg/l)	KIN (mg/l)	NAA (mg/l)
T-1 (Control)	30	0	0	0
T-2	30	1.00	0	0
T-3	30	2.00	0	0
T-4	30	3.00	0	0
T-5	30	4.00	0	0
T-6	30	0	1.00	0
T-7	30	0	2.00	0
T-8	30	0	3.00	0
T-9	30	0	4.00	0
T-10	30	1.00	0	0.5
T-11	30	2.00	0	0.5
T-12	30	3.00	0	0.5
T-13	30	4.00	0	0.5
T-14	30	0	1.00	0.5
T-15	30	0	2.00	0.5
T-16	30	0	3.00	0.5
T-17	30	0	4.00	0.5

Experimental design

The experiment was conducted using a factorial design, namely a randomised completely block design (17 medium × 1 cultivar). There were 6 replicate culture vessels employed in this investigation. During the hardening process, plantlets were directly transferred from the laboratory to the field and transplanted into a net house. Another batch of plantlets were transferred to a plastic pot containing topsoil, while the third batch was transferred to a mixture of 3:1 sand and biochar.

Statistical analysis

Phenotypic record were taken for the shoot number, shoot height (cm), root number, root length (cm), and number of leaves at 15 and 30 days after inoculation (DAI). The experimental results were reported as the mean and were compared using Duncan's multiple-range test (DMRT) according to the guidelines provided by IBM SPSS software version 20 (SPSS Inc. USA).

Results and Discussion

The individual nodal explants of sweet potato were discarded and transferred to MS medium containing agar and various concentrations of BA, KIN alone, and in combination with NAA. The objective was to determine the optimal culture media formulation for producing healthy, disease-free microplants. The seven characteristics include days to shoot initiation, the number of node, leaf number, shoots number, shoot height (cm), number of roots, and root length (cm) or any phenotypic abnormalities of the microplants were generated for the justification of the present study. The acquired data were analysed to calculate the mean, standard error, coefficient of variability percentage (CV%), and analysis of variance for comparing mean with DMRT. In the next sub-sections, the results of these numerous statistical analyses are broken down into the following categories.

Table 2: Effect of different plant growth regulators on growth-related parameters of days to shoot initiation, node number, and leaf number at 15 and 30 days after inoculation (DAI).

Treatments	Days to shoot initiation	Node number		Leaf number	
		15 DAI	30 DAI	15 DAI	30 DAI
T-1 (Cont.)	6	2.33±0 a	8.33±0 a	3±0 a	8.333±0 a
T-2	7	0.66± 1.29 cde	2.33±4.16 cd	1±2.154 cd	3.333±8.312 cd
T-3	4	2.00±1.09 b	3.33±4.15 bcd	3.667±1.817 b	6.667±8.295 bcd
T-4	5	1.00±1.28 bcde	0.33±4.17 d	2.33±2.091 bc	2.35±8.329 d
T-5	6	1.00±1.28 bcde	0±4.18 d	1.33±2.144 cd	2.15±8.353 d
T-6	8	0±1.30 e	5.66±3.92 abc	0±2.163 d	11.333±7.71 abc
T-7	7	0.33±1.30 de	7.33±3.49 ab	0±2.168 d	13.667±6.96 ab
T-8	7	0.33±1.30 de	5.66±3.77 abc	0±2.172 d	11±7.84 abc
T-9	7	0.33±1.29 de	4.66±4.09 bc	0±2.178 d	9.333±8.092 bc
T-10	6	1.66±1.14 bc	4.66±4.07 bc	3±1.912 bc	8.667±8.182 bc
T-11	6	3.11± 1.26 bcd	9.11±4.01 bc	2.333±2.11 b	9.333±8.14 bc
T-12	5	1.66±1.18 bc	4.66±4.11 bc	3±1.965 bc	8.667±8.223 bc
T-13	6	1.66±1.20 bc	4.33±4.13 bc	3±2.013 bc	8b±8.259 cd
T-14	5	1.66±1.22 bc	4.66±4.05 bc	3±2.044 bc	9.667±8.019 bc
T-15	5	1.33 ±1.25 bcd	5.66±3.86 abc	2±2.123 bcd	11.333±7.53 abc
T-16	6	1.66±1.24 bc	6.33±3.67 abc	4.25±2.072 bc	12.33±7.332 ab
T-17	6	1.33± 1.27 bcd	5.33±3.98 bc	2±2.134 bcd	10±7.947 b
CV%		9.99	4.00	9.36	7.00

Means with the same letter in the same column are non-significant at 5% significance level.

Variation was observed for days to shoot initiation among the hormonal treatments at 15 days after inoculation. The minimum days required for shoot initiation were noted in treatment T3 (4). Whereas, the maximum days required for shoot initiation at T6 (8), T2 (7), T7 (7), T8 (7) and T9 (7)

respectively followed by T1 (6), T5 (6), T10 (6), T11 (6), T16 (6) and T17 (6) (table 2). Here, needs to mention that the treatment T2, T7, T8, and T9 have no difference, similarly T1, T5, T10, T10, T11, T16, and T17 have no difference at days to shoot initiation. The number of days recorded for shoot initiation in this experiment was near to similar as compared to the previous study reported by Mulugeta and Staden (2004) [16] and delay to the result of Alula *et al*. 2017 [17].

Notable variation was observed for node number among different hormone combinations at 15 DAI. The maximum node was recorded in T11 (3.11) media, and it was followed by T1 (2.33), T3 (2.00), T10 (1.66), T12 (1.66), T13 (1.66), T16 (1.66), T17 (1.33), T4 (1.00), and T5 (1.00). Here, T3, T10, T12, T13, T11, and T12 have no significant difference according to DMRT. The lowest node number was noted in T2 (0.66), T7 (0.33), T8 (0.33), and T9 (0.33). Whereas, there was no node found in the T6 treatment. So, based on the observation of node formation at 15 DAI, hormonal treatment have no effect. On the other hand, only MS media with 30 g/l sugar without any hormone significantly increased node number (table 2).

The observation at 30 DAI for the character of node number among hormonal treatment height was observed at T11 (9.11) and other treatments such as T1 (8.33) T7 (7.33), T6 (5.66), T8 (5.66), T15 (6.33), and T16 (6.33) have no significant difference according to DMRT. Similarly, T3 (3.33), T9 (4.66), T10 (4.66), T11 (5.00), T12 (4.66), T13 (4.33), T14 (4.66) and T17 (5.33) treatment have no significant difference according to DMRT at 30 DAI. The lowest node number was found in T4 (0.33), and no node was found in T5 treatment (table 2).

Notable variation was observed for leaf number among different hormone combinations at 15 DAI. The maximum number of leaves was recorded in T16 (4.25) media, and it was followed by T3 (3.66), T16 (3.00), T14 (3.00), T13 (3.00), T12 (3.00), T10 (3.00), T1 (3.00), T11 (2.33), T4 (2.33), T17 (2.00), T15 (2.00), T5 (1.3) and T2 (1.00). Here, T2, T5, T10, T12, T13 T14, and T16 have no significant difference according to DMRT. There were no leaves found in T9 (0.00), T8 (0.00), T7 (0.00), and T6 (0.00). On the other hand, only MS media with 30 g/l sugar without any hormone significantly increased leaf number (table 2).

The observation at 30 DAI for the character of leaf number among hormonal treatment T16 (12.33) and other treatments such as T6 (11.33), T8 (11.00), T10 (8.66), T12 (8.66), T1 (8.33), T13 (8.00), T14 (9.66) and T17 (10.00) have no significant difference according to DMRT. Similarly, T7 (13.66), T15 (11.33), and T16 (12.33), treatment have no significant difference according to DMRT at 30 DAI. The lowest number of nodes was found in T4 (2.35) and T5 (2.15) respectively (table 2).

Table 3: Effect of plant growth regulators on growth-related parameters of shoot number, shoot height (cm), root number, and root length (cm) at 15 and 30 DAI

Treatments	Shoot number		Shoot height (cm)		Root number		Root length (cm)	
	15 DAI	30 DAI	15 DAI	30 DAI	15 DAI	30 DAI	15 DAI	30 DAI
T-1 (Cont.)	2.00±0 a	11±0 a	1.9±0 a	4.133±0 a	0.12±0 a	0.667±4.56 d	3.667±1.61bcd	2.333±2.983hi
T-2	3.66±3.44 bcde	3.66±5.25 de	0.53±0.295 fg	1.767±3.471ab	0±4.228 d	0±4.592 d	0±1.73 f	0±3.004 i
T-3	5.66±3.27 bc	6.66±5.24 cd	0.567±0.294fg	1.367±3.485ab	0±4.237 d	0±4.569 d	0±1.734 f	0±2.99 i
T-4	3±3.58 cde	3±5.26 e	1.033±0.286cd	1.5±3.478 ab	0±4.245 d	0±4.578 d	0±1.737 f	0±2.996 i
T-5	1.33±3.71 de	2±5.27 e	1.167±0.281c	1.233±3.495ab	0±4.258d	14.333±0a	0±1.742f	6±2.91efg
T-6	3.66±3.50bcde	11.33 ±5.02 bc	1.91±0.246a	4.833±3.153ab	5.667±3.841bc	12.333±4.142ab	2.333±1.698de	8±2.82 cdef
T-7	6.66± 3.11 ab	13±4.40 b	1.533±0.259 b	3.567±3.23 ab	6±3.737 bc	12.333±4.03ab	2.667±1.688cde	4.667±2.943gh
T-8	0±3.72 e	10.66±5.14 bc	1.3±0.266bc	2.9±3.386ab	6.667±3.552b	9.667±4.448bc	2.667±1.657cde	6.333±2.884defg
T-9	0±3.73 e	9±5.21 bc	0.8±0.289def	3.033±3.28ab	3±3.934bcd	13±3.83ab	2±1.706de	8.333±2.776cde
T-10	3±3.55 cde	11.33±5.06 bc	0.9±0.288de	3±3.325ab	3±3.996bcd	9.667±4.408bc	5±1.453b	12±2.506b
T-11	2.33±3.63 cde	12.33±4.76 b	1.2±0.273c	3.9±3.068ab	2.333±4.17cd	8.667±4.497bc	7±0a	16±0a
T-12	4±3.36 bcd	12±4.95 b	1.067±0.284cd	2.967±3.355ab	3±4.05bcd	9±4.475bc	5±1.529b	10.667±2.637bc
T-13	3±3.61 cde	10.33±5.19 b	1.2±0.277c	4.833±2.916ab	3±4.087bcd	7±4.54c	4±1.571bc	9±2.71cd
T-14	2±3.65 de	10.33±5.16 bc	0.7±0.292efg	2.167±3.44ab	2.667±4.149bcd	7±4.52c	2.667±1.672cde	5.333±2.928fg
T-15	9.33±3.67 de	19±5.11 bc	0.6±0.293fg	2.133±3.456ab	3±4.124bcd	10±4.243abc	3±1.635cde	6.667±2.858defg
T-16	2±3.69 de	12.66±4.63 b	0.467±0.295g	2.367±3.423ab	2.333±4.191cd	10±4.31abc	2±1.715de	4.333±2.957gh
T-17	1.66±3.70 de	12.33±4.87 b	0.8±0.291def	2.633±3.406ab	2±4.21cd	10±4.368abc	1.667±1.722ef	4±2.97ghgh
CV%	5.71	7.18	4.88	6.98	6.12	7.44	8.12	7.56

Means with the same letter in the same column are non-significant at 5% significance level.

A significant difference was observed in the character of shoot number at 15 DAI. The highest shoot number was observed in T15 (9.33) and it was followed by T7 (6.66), T3 (5.66), T6 (3.66), and T12 (4) at 15 DAI. Here, T1 and T7 have no significant difference according to DMRT. Similarly, T3, T6, and T12 have no significant difference. The lowest shoot number was found in T4 (3), T10 (3), T11 (2.33), T13 (3), T14 (2), T1 (2), T16 (2) and T 17(1.66). Here, T4, T10, T11, and T13 have no significant difference according to DMRT. Similarly, T14, T15, T16, and T17 have no significant difference according to DMRT (table 3).

A significant difference was observed in the character of shoot number at 30 DAI. The highest shoot number was observed in T15 (19.00) and it was followed by T7 (13.00), T16 (12.66), T17 (12.33), T11 (12.66), T12 (12.00), T10 (11.33), T6 (11.33), T1 (11.00), T14 (10.33) and T13 (10.33) at 30 DAI. Here, T10, T6, and T15 have no significant difference according to DMRT. Similarly, T14 and T13 have no significant difference. The lowest shoot number was found in T4 (3), T5 (2). Here, T4 and T5 have no significant difference according to DMRT (table 3).

A significant difference in the character of shoot height at 15 DAI. T6 (1.91) had the highest shoot height at 15 DAI. This was followed by T1 (1.90), T7 (1.53), T8 (1.30), and T13 (1.2) at 15 DAI. DMRT shows no significant difference between T6 and T1. Similarly, T7, T8, and T13 show no significant differences. The lowest shoot heights were discovered in T11 (1.2), T5 (1.167), T12 (1.06), T4 (1.033),

T10 (0.9), T9 (0.8), T17 (0.8), and T16 (0.4). T11, T5, T12, and T4 show no significant difference according to DMRT. Similarly, T10, T9, T8, and T17 show no significant difference in DMRT (table 3).

A significant difference was observed in the character of shoot height at 30 DAI. The highest shoot height was observed in T6 (4.83) and it was followed by T13 (4.13), T1 (4.13) T11 (3.9), T7 (3.56), T9 (3.03), T10 (3.00), T12 (2.96), T8 (2.9), T17 (2.63) and T16 (2.36) at 30 DAI. Here, T13, T6, T1, and T11 have no significant difference according to DMRT. Similarly, T6 and T7 have no significant difference. The lowest shoot height was found in T3 (1.36), T5 (1.23). Here, T3 and T5 have no significant difference according to DMRT (table 3).

According to the result of this experiment knowing the appropriate concentration of BA and KIN for shoot length is vital for sweet potato. Because further increase in concentration does have negative effect and ends with reduced shoot length. So, far there is no sufficient study report on sweet potato concentration effect of growth regulators on the length of shoots per plantlets. However, Mulugeta and Stedany (2004) [16] reported that the effect of growth regulators on plantlet regeneration from shoot tip explants and reported 3-5 cm shoot length on media with 2.5 mg^{-l} BAP which is less than shoot length reported in this experiment. Among the growth regulators tested T3 (2.00 mg^{-l} BA), T7 (2.00 mg^{-l} KIN) single and T11 (2.00 mg^{-l} BA with 0.5 mg^{-l} NAA), T12 (3.00 mg^{-l} BA with 0.5 mg^{-l} NAA), T13 (4.00 mg^{-l} BA with 0.5 mg^{-l} NAA), T14 (1.00 mg^{-l} with 0.5 mg^{-l} NAA) and T15 (2.00 mg^{-l} KIN with 0.5 mg^{-l} NAA) combination were best for all shoot initiation and growth parameters which was in agreed with the research result of Alula *et al*. 2017 [17].

A significant difference was observed in the character of the root number at 15 DAI. The highest root number was observed in T8 (6.66) and it was followed by T7 (6.00), T6 (5.667), T15 (3.00), and T13 (3.00) at 15 DAI. Here, T8 and T7 have no significant difference according to DMRT. Similarly, T15, and T13, have no significant difference. The lowest root number was found in T13 (3), T12 (3), T10 (3), T9 (3), T14 (2.66), T16 (2.33), T11 (2.33) and T17 (2.00). Here, T13, T12, T10, and T9 have no significant difference according to DMRT. Similarly, T14, T16, T16, and T17 have no significant difference according to DMRT (table 3).

A significant difference was observed in the character of the root number at 30 DAI. The highest root number was observed in T8 (14.33) and it was followed by T9 (13.00), T6 (12.33), T7 (12.33), T17 (10.00), T16 (10.00), T15 (10.00), T8 (9.66), T10 (9.66), T12 (9.00) and T11 (8.66) at 30 DAI. Here, T9, T6, and T7 have no significant difference according to DMRT. Similarly, T17 and T16 have no significant difference. The lowest root number was found in T1 (0.66). There is no root shown in T4 (0.00) and T3 (0.00). Here, T4 and T3 have no significant difference according to DMRT (table 3).

A significant difference was observed in the character of root length at 15 DAI. The highest root length was observed in T11 (7.00) and it was followed by T12 (5.00), T10 (5.00), T13 (4.00), and T1 (3.66) at 15 DAI. Here, T12 and T10 have no significant difference according to DMRT. Similarly, T13 and T1 have no significant difference. The lowest root length was found in T15 (3), T7 (2.66), T14 (2.66), T8 (2.66), T6 (2.33), T16 (2.00), T9 (2.00) and T17 (1.66). Here, T5, T17, T14, and T8 have no significant difference according to DMRT. Similarly, T6, T16, and T9 have no significant difference according to DMRT (table 3).

A significant difference was observed in the character of root length at 30 DAI. The highest root length was observed in T11 (16.00) and it was followed by T10 (12.00), T12 (10.66), T13 (9.00), T9 (8.33), T6 (8.00), T15 (6.66), T8 (6.33), T5 (6.00), T14 (5.33), T7 (4.66), T16 (4.33), T17 (4.00) and T1 (2.33) at 30 DAI. Here, T12, T13, and T9 have no significant difference according to DMRT. Similarly, T15 and T8 have no significant difference. The lowest root length was found in T17 (4.00), T1 (2.33). Here, T17 and T1 have no significant difference according to DMRT (table 3).

Better survival and acclimatization for longer shoots is in accordance with the recommendations of (El Far *et al*. 2009) [18] and Ozturk and Atar, 2004) [19]. Moreover, the significance of appropriate root development *in vitro* for successful establishment of sweet potato shoot during acclimatization agrees with (Zobayed *et al*. 1999) [20]. The minimum root length or root were absent in T2, T3 and T4, were as maximum root length was observed in T9 of single treatment, that were quite similar to the research result of Alula *et al*. 2017 [17].

Sweet potatoes and other vegetative crops will continue to be propagated using tissue culture. Without measures to reduce production costs, many impoverished world smallholder farmers will dismiss the technology as a myth. For years, scientists in numerous labs have attempted to reduce the cost of tissue culture crops such as bananas. Tissue culture technology boosts agricultural output by supplying low-cost disease-free planting materials, hence combating food poverty (Odame *et al.,* 2002) [21]. Significant difference was observed between the media used for number of shoot per microplant, shoot height, number of leaf per microplant, number of node per microplant, number of root per microplant and root growth which was in agree by the research result of (Addae-Frimpomaah *et al*. 2014) [22]. Plant hormones exert profound morphological effects on plant growth and development, the precise action depending on the type and concentration of the hormone and the sensitivity of the organ involved (Addae-Frimpomaah *et al*. 2014) [22]. In this study, the effect of BAP, KIN, and NAA, either in combination or alone, on cultured Sweet potato was investigated. The study aimed to determine the optimal culture media formulation for producing healthy, disease-free microplants by analyzing the characteristics of sweet potato nodal explants. The data was analyzed using mean, standard error, coefficient of variability percentage (CV%), and analysis of variance for comparison with DMRT. Variations were observed in days to shoot initiation, node number, leaf number, shoot number, shoot height, number of roots, and root length among hormonal treatments at 15 days after inoculation. The highest node number was recorded in treatment T3, followed by T2 (2.33), T7 (7.33), T8 (7), T9 (7), T4 (1.00), and T5 (1.00). No significant difference was found in node number, leaf number, shoot number, or shoot height between treatments.

The highest shoot number was observed in treatment T15 (9.33), followed by T7 (6.66), T3 (5.66), T6 (3.66), and T12 (4). No significant difference was found in T4, T10, T11, T16, and T17. The lowest shoot number was found in T4 (3), T10 (3), T11 (2.33), T13 (3), T14 (2), T1 (2), T16 (2), and T 17(1.66). The lowest shoot number was found in T4

(3), T5 (2), and T4 and T5 had no significant difference according to DMRT.

In conclusion, the study found that hormonal treatments did not significantly affect the growth of sweet potato microplants. The results suggest that the optimal culture media formulation for producing healthy, disease-free microplants should be considered. The study analyzed the variability of shoot height, root number, root length, and root length at 15 and 30 days of age (DAI). The highest shoot height was observed in T6 (1.91), followed by T1 (1.90), T7 (1.53), T8 (1.30), and T13 (1.2). The lowest shoot heights were found in T11 (1.2), T5 (1.167), T12 (1.06), T4 (1.033), T10 (0.9), T9 (0.8), T17 (0.8), and T16 (0.4). The highest root number was observed in T1 (11.0), followed by T8 (6.66), T7 (6), T6 (5.66), and T15 (3.0). The lowest root number was found in T13 (3), T12 (3), T10 (3), T9 (3), T14 (2.66), T16 (2.33), T11 (2.00), and T17 (2.00). No significant difference was found in T4 (0.00) and T3 (0.00). The highest root length was observed in T11 (7.00), followed by T12 (5.00), T10 (5.00), T13 (4.00), and T1 (3.66). The lowest root length was found in T15 (3), T7 (2.66), T14 (2.66), T8 (2.66), T6 (2.33), T16 (2.00), T9 (2.00), and T17 (1.66). No significant difference was found in T12, T13, T9, T15, T8, and T17.

Fig 1: Different stages of *in vitro* micropropagation: A-initiation of shoot combination of BA 2.00 mg^{-l} with MS medium; B- Regenerated plantlets with well-developed shoot induced on MS medium containing 2.00 mg^{-l} KIN and NAA 0.5 mg^{-l}; C- Regenerated plantlets with well-developed leaf induced on MS medium containing 3.0 mg^{-l} KIN and NAA 0.5 mg^{-l}; D-Maximum height of plantlet after 30 days combination of KIN 1.0 mg^{-l} with MS medium; E- Regenerated plantlets with well-developed roots induced on MS medium containing 3.0 mg^{-l} KIN.

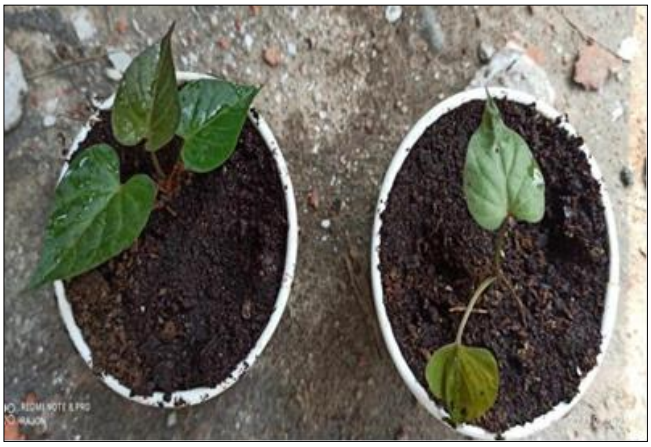

Fig 2: Acclimatization of plantlet on the plastic pot with 2:1:1 (vermicopost: garden soil: sand)

Conclusion

This study evaluates a disease-free *in vitro* microplant production method for sweet potato, a crop rich in vitamins and sugar. Hormonal treatments varied in shoot initiation days, node number, leaf number, shoot number, shoot height, root numbers, and root length. The results showed that hormonal treatment of the microplants were effective. The study suggests that a combination of hormones can be used to produce disease-free microplants, through tissue culture.

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