EFFECTS OF HORMONES AND COCONUT WATER ON CLONAL MULTIPLICATION OF POTATO (*SOLANUM TUBEROSUM* L.)

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Abstract

Shoots were produced from tissue culture of *Solanum tuberosum* L. Two experiments were carried out at the Plant Tissue Culture Laboratory, Department of Agricultural Research, Htone Bo, Agricultural Research Farm, Taunggyi during March to June, 2022. The shoot tips of potato cultivar Markies were inoculated in Murashige and Skoog (MS) media supplemented with the different concentration of alpha-Naphthalene acetic acid (NAA), 6-benzylaminopurine (BAP) and coconut water. Hormonal effects on plant height, shoots, leaves and roots formation were studied. The maximum plantlet height and the highest number of root formation were observed in potato cultivar Markies on MS + 200 ml coconut water. The highest leaves formation were observed in ½ MS + 200 ml coconut water medium. The highest number of shoot were found in 0.05 mg/L NAA + 1.0 mg/l BAP +200 ml coconut water supplemented with MS medium.

Keywords: shoot tips, MS media, markies.

Introduction

The *Solanum tuberosum* L. (Potato) are members of the Solanaceae family. The Solanaceae, or nightshades, are an economically important family of flowering plants. The family ranges from herbs to trees, and includes a number of important agricultural crops, medicinal plants, spices, weeds, and ornamentals. The Solanaceae, also called nightshades, comprise more than 3000 species many of which evolved in the Amazonian regions of South America. (Jagatheeswari, 2014). Total world area 18.6 million ha^{-1}, 17. 4 t ha^{-1}(FAO 2011). Myanmar, total area 37000 ha^{-1}, yield 15.11 t ha^{-1} (DAP 2021). Potatoes are fat free food containing carbohydrate, protein, vitamins, antioxidants and minerals (FAO 2008)

The *in vitro* and aseptic cultivation of excised plant part on a nutrient medium is termed as plant tissue culture. The plant tissue culture is a branch of biotechnology. This technique is used for plant propagation, plant breeding, preservation and storage, scientific investigation and others. Plant tissue culture or micropropagation is the aseptic culture of cells, pieces of tissue or organs (Kavi Kisher, 1999).

By using micropropagation, the millions of new plants can be derived from a single plant. Micropropagation used for rapid multiplication and getting true-to-type plants on artificial nutrient media under controlled environment. Used in almost all potato seed producing countries in initial stages of seed production (Sein Hla Bo, 1987).

Seed potatoes are tubers that can be planted in a vegetable plot or in containers depending on the variety to produce a crop of harvestable potatoes. A seed potato is a potato that has been grown to be replanted to produce a potato crop (https:// theunconventionalgardener.com).

The purpose of this investigation was to study nutritional effect on clonal propagation of the tested species. To examine suitable culture system for *in vitro* multiplication of selected potato cultivar, production of virus and disease free plantlets. To achieve the aim, shoots of tested species

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were cultured in nutrient media. Various source of nutrition, such as hormones and coconut water were supplied to the media. Response of explants to the media was studied in the present work.

Materials and Methods

Source of Plant Materials and Identification

The explants **of *Solanum tuberosum* L.** (shoots) obtained from initial culture which were well established in Plant Tissue Culture Laboratory of the Htone Bo Agricultural Research Farm, Taunggyi, Department of Agricultural Research. Markies are available from the Department of Agriculture; obtained from Heho Garden.

The morphological and taxonomical studies of the specimen were made at the Department of Botany, Taunggyi University by using available literatures.

Culture Media

The shoot of *Solanum tuberosum* L. were cultured on MS basal medium supplement with 30g sucrose, inositol 0.1g and 6g agar for solid media and various concentrations of hormones: naphathalene acetic acid (NAA), 6-benzylaminopurine (BAP), and coconut water. Finally, the preparation of the medium were inoculated into autoclaved at 121º C for 30 minutes.

Table 1. Different concentrations of NAA, BAP and Coconut water with MS medium

Treatments	Basal Medium	PGR concentration			Remark
		NAA (mg/l)	BAP (mg/l)	Coconut water (ml/l)	
T_1	MS	0	0	0	5 Replication
T_2	MS	0.05	1.0	200	
T_3	MS	0.05	0.5	200	
T_4	MS	0	0	200	

Table 2. Different concentrations of NAA and Coconut water with MS medium

Treatments	Basal Medium	PGR concentration		Remark
		NAA (mg/l)	Coconut water (ml/l)	
T_1	½ MS	0	0	5 Replication
T_2	½ MS	0.01	200	
T_3	½ MS	0.05	200	
T_4	½ MS	0	200	

Media Preparation

According to Murashige and Skoog (1962), method of 1000 ml medium preparation applied in the present study.

Culture of Explants

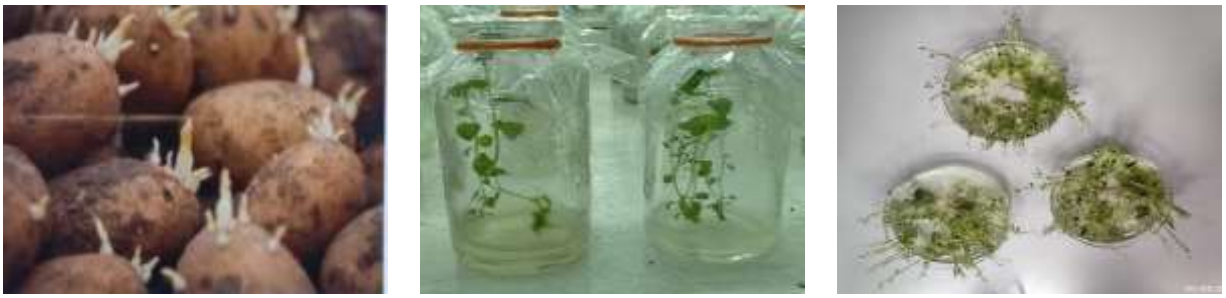
The shoot obtained from initial culture which node cutting of 1 cm in length consisting of one axillary shoot from the middle portion of 6-weeks old plantlets were used as explants source for regeneration. Six weeks old *Solanum tuberosum* L. shoots containing one to two leaves and length of 7.0 – 7.5 cm were selected for each experiment. Two numbers of such shoots were taken and cultured in a prepared medium bottle and each treatment had five replications.

Cultured Conditions

All culture bottles were inoculated in culture room at 20 ± 2˚C with 16/8 hours (light/dark) photoperiod and 30 µmolm^{-1}s^{-1} using white fluorescent lamps.

Measurement and Recording

Growth of shooting stage and rooting stage were measured by recording length of plantlet, number of leaves, the number of shoot and number root. These were recorded at five weeks after culture.



Seed potato | 6-weeks old plantlets in culture | 35- days olded in subculture

Figure 1. *Solanum tuberosum* L. (Potato)

Results

Morphological Characters of *Solanum tuberosum* L.

Scientific Name	:	*Solanum tuberosum* L.
English Name	:	Potato
Myanmar Name	:	A-lu
Family	:	Solanaceae

Annual tufted herbs with tuberous underground stems, cultivated. Stem; ribbed winged, erect or prostrate, about (1-2 ft) in height, pilose. Leaves; alternate, unipinnate, interruptedly imparipinnate compound, petiolate, exstipulate; each pair of large leaflets following pairs of much smaller ones; lowest pair (at base of petiole) small; larger ones ovate-obvate-elliptic; leaflets herbaceous, leaf blade with simple hairs on both surfaces. Inflorescences; terminal or lateral cyme, stout peduncle, pedicels hairy, articulate, 7-20 flowered. Flower; ebracteate, ebracteolate, pedicellate, complete, bisexual, regular, hypogynous. Calyx; sepals 5, synsepalous, lobes long-accuminate, bell-shaped, valvate, pilose, persistent. Corolla; petals 5, synpetalous, campanulate, valvate, white or pale purple, outside hairy; limb slightly lobed. Androecium; stamens 5, apostamenous, antipetalous, epipetalous, the filaments very short, the anthers dithecous, basifixed, introrse, porous dehiscent. Gynoecium; bicarpellary, syncarpous, two locules, axile placentation, many ovules in each locule, the style long, the stigma lobed, ovary superior. Fruits; spherical to ovoid baccate (Figure. 2).

Habit Inflorescence Flower as seem

Figure 2 . Morphological Characters of *Solanum tuberosum* L. (Potato)

Table 3. Survival percentage of potato

Treatments	7 days	14 days	21 days	28 days	35 days
T_1	1	1	1	1	1
T_2	1	1	0.93	1	1
T_3	1	1	1	1	1
T_4	1	1	1	1	1

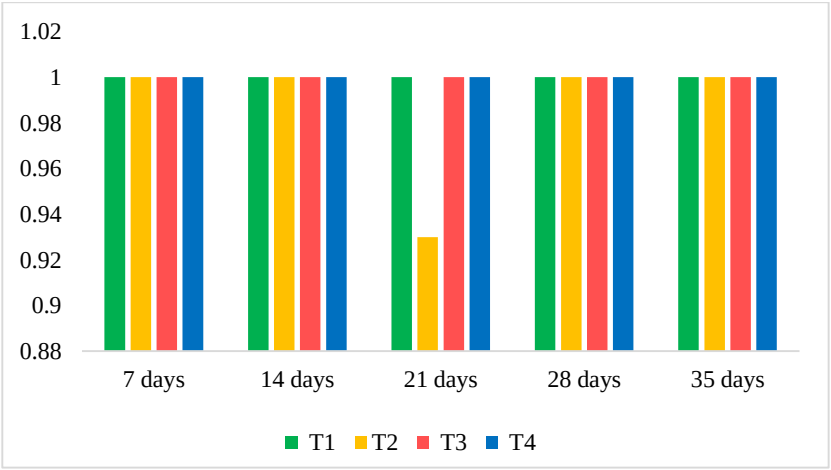


Figure 3 . Survival percentage of potato

Experiment 1: Effects of plant hormone concentration (NAA and BAP) and coconut water in the Shoot Regeneration of potato.

The result obtained in the present investigation using different media, T_1 (control MS only), T_2 (MS + 0.05 mg/l NAA + 1.0 mg/l BAP +200 ml CW), T_3 (MS + 0.05 mg/l NAA + 0.5 mg/l BAP +200 ml CW), T_4 (MS + 200 ml CW) were as follows:

The result of the plant height showed that T_1 (control) had 4.34, T_2 (0.05 mg/l NAA + 1.0 mg/l BAP +200 ml CW) had 4.59, T_3 (0.05 mg/l NAA + 0.5 mg/l BAP +200 ml CW) had 4.2, T_4 (200 ml CW) had 4.74. The maximum plant height were observed in T_4 (200 ml CW).

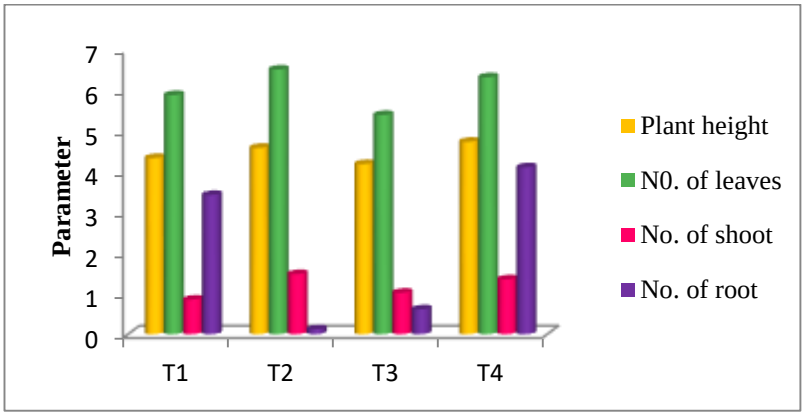
The result of the leaves number T_1 (control) had 5.89, T_2 (0.05 mg/l NAA + 1.0 mg/l BAP +200 ml CW) had 6.52, T_3 (0.05 mg/l NAA + 0.5 mg/l BAP +200 ml CW) had 5.4, T_4 (200 ml CW) had 6.32. The highest number of leaves were observed in T_2 (0.05 mg/l NAA + 1.0 mg/l BAP +200 ml CW).

T_2 (0.05 mg/l NAA + 1.0 mg/l BAP +200 ml CW) produced more shoot number 1.48 in this experiment. The second best result obtained from T_4 (200 ml CW) had 1.35 and T_3 (0.05 mg/l NAA + 0.5 mg/l BAP +200 ml CW) had 1.02 followed by T_1 (control) had 0.85.

The result of the root number T_1 (control) had 3.43, T_2 (0.05 mg/l NAA + 1.0 mg/l BAP +200 ml CW) had 0.12, T_3 (0.05 mg/l NAA + 0.5 mg/l BAP +200 ml CW) had 0.61, T_4 (200 ml CW) had 4.11. The highest number of root were observed T_4 (200 ml CW) (Table 3 and Figure 3 and 4).

Table 4. Effects of plant hormone (NAA + BAP) and coconut water on the Shoot Regeneration of potato

Treatments	Survival %	Plant Height (cm)	No. of Leaves	No. of shoots	No. of Roots
T_1	100	4.336	5.886	**0.852**	3.434
T_2	100	4.587	**6.52**	**1.48**	**0.118**
T_3	100	**4.196**	**5.4**	1.024	0.612
T_4	100	**4.74**	6.328	1.352	**4.114**



T_1 : Control (MS only)
T_2 : 0.05mg/l NAA+1.0 mg/l BAP +200 ml/l coconut water
T_3 : 0.05 mg/l NAA + 0.5 mg/l BAP + 200 ml/l coconut water
T_4 : 200 ml/l coconut water

Figure 4. Effects of plant hormone (NAA + BAP) and coconut water on the Shoot Regeneration of potato

Maximum length of plantlet and root from T_4 Highest number of leaves and shoot from T2

Figure 5. Effects of (NAA + BAP) and coconut water treatments on the 5-week after the start of the experiment

Experiment 2: Effects of plant hormone concentration NAA and coconut water in the Rooting Stage (Pre-hardening stage) of potato

The result obtained in the present investigation using different media, T_1 (control ½MS only), T_2 (½MS + 0.01 mg/l NAA + 200 ml CW), T_3 (½MS + 0.05 mg/l NAA + 200 ml CW), T_4 (½MS + 200 ml CW) were as follows:

The result of the plant height showed that T_1 (control) had 1.83, T_2 (½MS + 0.01 mg/l NAA + 200 ml CW) had 2.14, T_3 (½MS + 0.05 mg/l NAA + 200 ml CW) had 2.81, T_4 (½MS + 200 ml CW) had 3.58. The maximum plant height were observed in T_4 ½ MS + 200 ml CW).

The result of the leaves number T_1 (control) had 3.55, T_2 (½MS + 0.01 mg/l NAA + 200 ml CW) had 4.45, T_3 (½MS + 0.05 mg/l NAA + 200 ml CW) had 5.39, T_4 (½MS + 200 ml CW) had 7.05. The highest number of leaves were observed in T_4 (½MS + 200 ml CW).

T_4 (½MS + 200 ml CW) produced more shoot number 0.65 in this experiment. The second best result obtained from T_3 (½MS + 0.05 mg/l NAA + 200 ml CW) had 0.58 and T_2 (½MS + 0.01 mg/l NAA + 200 ml CW) had 0.51 followed by T_1 (control) had 0.31.

The result of the root number T_1 (control) had 0.69, T_2 (½MS + 0.01 mg/l NAA + 200 ml CW) had 1.85, T_3 (½MS + 0.05 mg/l NAA + + 200 ml CW) had 3.47, T_4 (½MS + 200 ml CW) had 4.05. The highest number of root were observed in T_4 (½MS + 200 ml CW) (Table 4 and Figure 5 and 6).

Table 5. Effects of plant hormone NAA and coconut water on the Rooting Stage (Pre-hardening stage) of potato

Treatments	Survival %	Plant Height (cm)	No. of Leaves	No. of shoots	No. of Roots
T_1	100	1.830	3.548	0.308	0.692
T_2	100	2.136	4.452	0.508	1.846
T_3	100	2.814	5.392	0.578	3.472
T_4	100	3.576	7.052	0.648	4.048

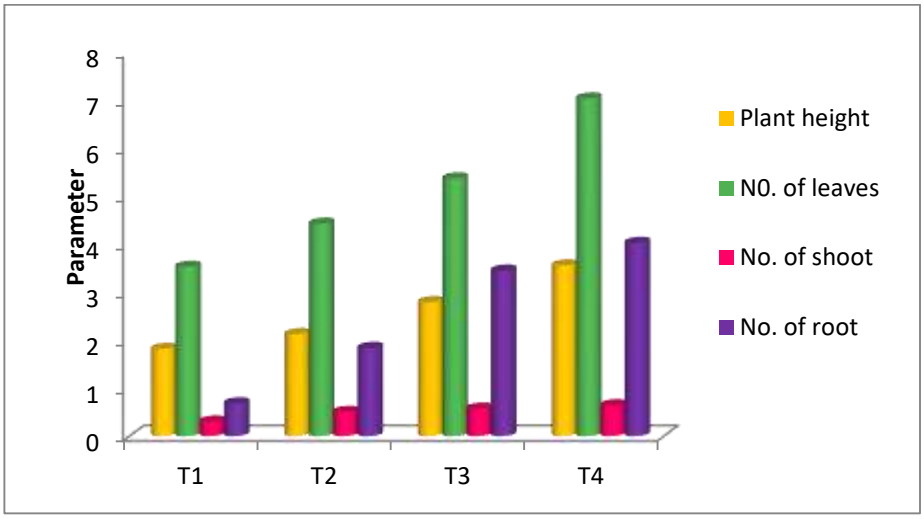


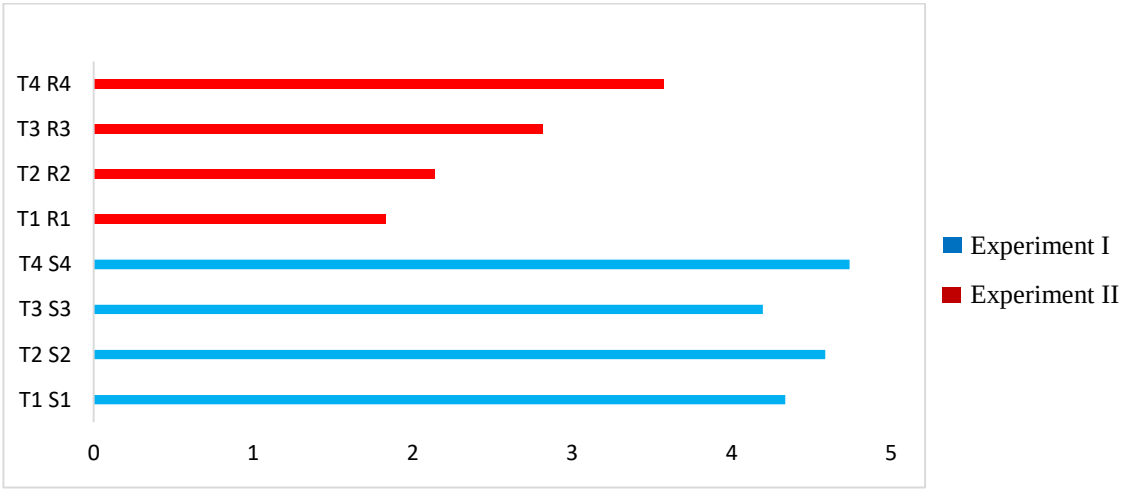
Figure 6. Effects of plant hormone (NAA + BAP) and coconut water on the Shoot Regeneration of potato

Maximum length of plantlet, highest number of leaves, shoot and root from 200 ml/l coconut water

Figure 7. Effects of NAA and coconut water treatments on the 5-week after the start of the experiment

Table 6. Comparison of plant height

$T_1 S_1$	$T_2 S_2$	$T_3 S_3$	$T_4 S_4$	$T_1 R_1$	$T_2 R_2$	$T_3 R_3$	$T_4 R_4$
4.336	4.587	4.196	4.74	1.83	2.136	2.814	3.576



Plant height (cm)

T_1 - Control (MS only)

T_2 - MS + 0.05mg/l NAA+ 1.0mg/l BAP + 200 ml/l coconut water

T_3 - MS + 0.05mg/l NAA+ 0.5mg/l BAP + 200 ml/l coconut water

T_4 - MS + 200 ml/l coconut water

T_1 - Control (½ MS only)

T_2 - ½ strength MS + 0.01 mg/l NAA + 200 ml/l coconut water

T_3 - ½ MS + 0.05 mg/l NAA + 200 ml/l coconut water

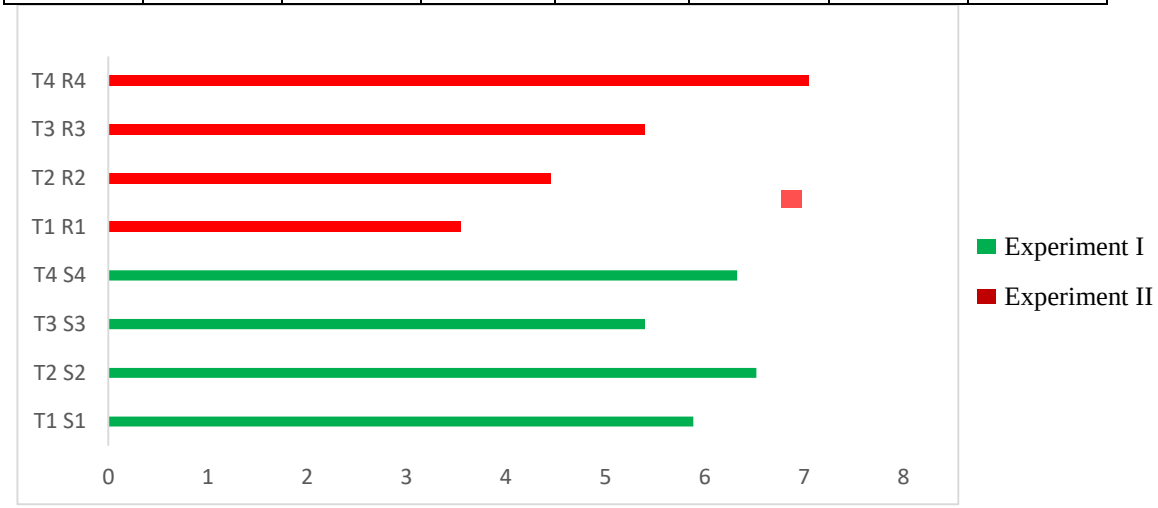
T_4 - ½ MS + 200 ml/l coconut water

S - Experiment I, R- Experiment II

Figure 8. Comparison of plant height (Markies)

Table 7. Comparison on sum of no. of leaves per plant

T_1S_1	T_2S_2	T_3S_3	T_4S_4	T_1R_1	T_2R_2	T_3R_3	T_4R_4
5.886	6.52	5.4	6.328	3.548	4.452	5.392	7.052



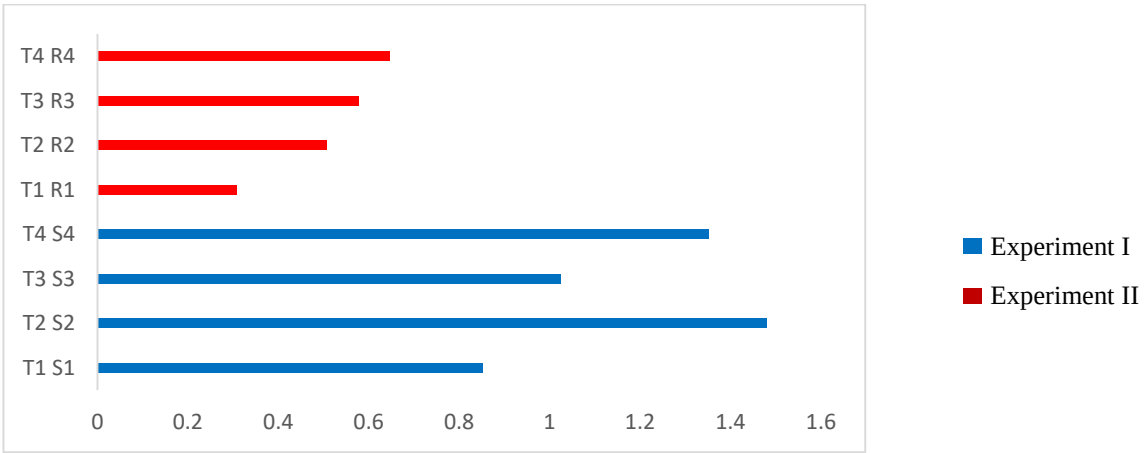
Parameter

T_1 - Control (MS only)
T_2 - MS + 0.05mg/l NAA+ 1.0mg/l BAP + 200 ml/l coconut water
T_3 - MS + 0.05mg/l NAA+ 0.5mg/l BAP + 200 ml/l coconut water
T_4 - MS + 200 ml/l coconut water
T_1 - Control (½ MS only)
T_2 - ½ strength MS + 0.01 mg/l NAA + 200 ml/l coconut water
T_3 - ½ MS + 0.05 mg/l NAA + 200 ml/l coconut water
T_4 - ½ MS + 200 ml/l coconut water
S - Experiment I, R- Experiment II

Figure 9. Comparison of no. of leaves per plant

Table 8. Comparison on sum of no. of shoots per plant

T_1S_1	T_2S_2	T_3S_3	T_4S_4	T_1R_1	T_2R_2	T_3R_3	T_4R_4
0.852	**1.48**	1.024	1.352	0.308	0.508	0.578	0.648



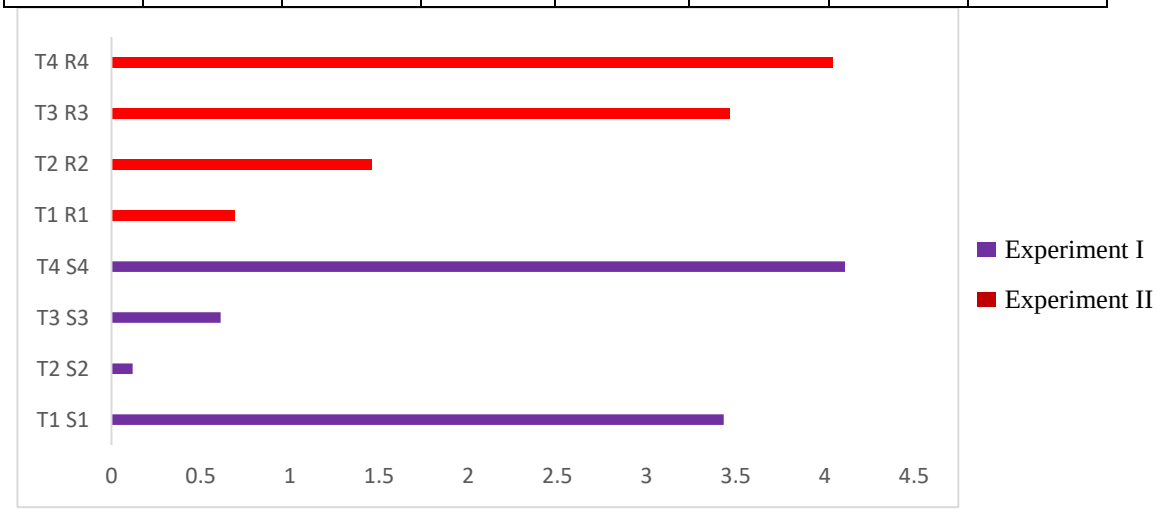
Parameter

T_1 - Control (MS only)
T_2 - MS + 0.05mg/l NAA+ 1.0mg/l BAP + 200 ml/l coconut water
T_3 - MS + 0.05mg/l NAA+ 0.5mg/l BAP + 200 ml/l coconut water
T_4 - MS + 200 ml/l coconut water
T_1 - Control (½ MS only)
T_2 - ½ strength MS + 0.01 mg/l NAA + 200 ml/l coconut water
T_3 - ½ MS + 0.05 mg/l NAA + 200 ml/l coconut water
T_4 - ½ MS + 200 ml/l coconut water
S - Experiment I, R- Experiment II

Figure 10. Comparison of no. of shoots per plant

Table 9. Comparison on sum of no. of roots per plant

$T_1 S_1$	$T_2 S_2$	$T_3 S_3$	$T_4 S_4$	$T_1 R_1$	$T_2 R_2$	$T_3 R_3$	$T_4 R_4$
3.434	0.118	0.612	4.114	0.692	1.46	3.472	4.048



Parameter

T_1 - Control (MS only)
T_2 - MS + 0.05mg/l NAA+ 1.0mg/l BAP + 200 ml/l coconut water
T_3 - MS + 0.05mg/l NAA+ 0.5mg/l BAP + 200 ml/l coconut water
T_4 - MS + 200 ml/l coconut water
T_1 - Control (½ MS only)
T_2 - ½ strength MS + 0.01 mg/l NAA + 200 ml/l coconut water
T_3 - ½ MS + 0.05 mg/l NAA + 200 ml/l coconut water
T_4 - ½ MS + 200 ml/l coconut water
S - Experiment I, R- Experiment II

Figure 11. Comparison of no. of roots per plant

Discussion and Conclusion

The plant of *Solanum tuberosum* L. are annual herbs with tuberous underground stems. The stems are erect or prostrate. Leaves interruptedly imparipinnate compound, each pair of large leaflets following pairs of much smaller ones; lowest pair (at base of petiole) small; leaflets herbaceous, with simple hairs on both surfaces. Inflorescence is a terminal or lateral cyme, erect, on a stout peduncle; pedicels hairy, articulate. Flower white or pale purple, anthers at narrowed apex with 2 lateral pores developing into short slits, ovary superior. These characters were in agreement with Backer and Brink (1965).

In this research, potato cultivar Markies were treated with T_1 (Control -MS only), T_2 (0.05 mg/l NAA + 1.0 mg/l BAP + 200 ml/l coconut water), T_3 (0.05 mg/l NAA + 0.5 mg/l BAP + 200 ml/l coconut water) and T_4 (200 ml/l coconut water) for shoot regeneration stage. The cultivar were treated with T_1 (Control - ½ MS only), T_2 (0.01 mg/l NAA + 200 ml/l coconut water), T_3 (0.05 mg/l NAA + 200 ml/l coconut water) and T_4 (200 ml/l coconut water) for rooting stage.

In the present study, among the combination with NAA, BAP and coconut water, 200 ml coconut water supplemented with MS solid medium gave the maximum length of plantlet 4.74 cm. The minimum length of plantlet were observed in (½ MS medium).

As the number of leaves, 200 ml coconut water supplemented with ½ MS solid medium gave the highest leaves number 7.05 in shoot regeneration stage. Control (½ MS medium) gave the smallest number of leaves 3.5 in rooting stage.

As the number of shoot, the highest number of shoot were found in (0.05 mg/l NAA + 1.0 mg/l BAP + 200 ml/l coconut water) in shoot regeneration stage. The lowest of shoot number 0.3 observed in cultivar Markies on control (½ MS medium) in rooting stage.

The result of the root number, 200 ml coconut water supplemented with MS solid medium gave the highest root number 4.11. The lowest of root number 0.11were observed on 0.05 mg/l NAA + 1.0 mg/l BAP + 200 ml/l coconut water medium.

When compared the results of plant growth regulator and coconut water added basal medium, combination of 200 ml coconut water with MS medium showed longest length of plantlets and 200 ml coconut water with ½ MS medium indicated the highest number of leaves in potato.

The proper medium for potato *(Solanum tuberosum* L.) using 200 ml coconut water with MS medium was suitable for longest length of plantlets and the highest number of roots. 200 ml coconut water with ½ MS medium was suitable for highest number of leaves and 0.05 mg/l NAA + 1.0 mg/l BAP + 200 ml/l coconut water medium was suitable for highest number of shoot.

This finding was in agreement with the finding of (Mauney *et al*., 1952) who reported that the plant growth regulators especially auxin and cytokinin present in coconut water.

The cytokinin (BAP) are also used for shoot proliferation by the release of axillary buds from apical dominance (Bhojwani and Razdan, 1983). The investigation of this study were in agreement with the previous finding.

The finding of this study were agreement with (Tulecke and Nickel, 1960) reported that coconut water contains amino acids, inorganic salts and growth promoting substances and the increase in growth is probably due to the presence of these substances.

Coconut water is rich in various minerals and electrolytes like potassium, calcium, manganese, antioxidants amino acids and cytokinins. Coconut water is the best source of potassium (https://pharmeasy.in>blog>11-in...).

It is concluded that the shoots of potato (*Solanum tuberosum* L.) using different combination and concentration of growth regulators and coconut water can reduce the high cost for commercial plant production.

Potatoes are raw, boiled, peeled, or mashed all have medicinal and healing properties. The potatoes are Carbohydrate plant, a fat free food containing carbohydrate, proteins, vitamins, antioxidants and minerals. Moreover, potato is considered as major food crops after maize, rice and wheat. Therefore, potato should be cultivated by using tissue culture method for the production of many plants.

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