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Effect of Coconut Water and GA3 Concentrations on *in vitro* Clonal Propagation of Potato Cultivars from Nepal

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Abstract

Nodal propagation plays a crucial role in mass multiplication of potato plants. Growth regulators and media selection have an impact on the efficacy and quality of propagation. Both coconut water, a naturally occurring organic source of growth-promoting compounds, and the synthetic growth regulator GA3 (gibberellic acid-3), have the ability to accelerate plant growth. The purpose of this study was to evaluate the effects of growth regulators and media, specifically GA3 and coconut water (CW) in agar-based media (ABM) and Clarigel-based media (CBM) which is a Gellan gum-based media, on potato nodal propagation. In contrast to the control group, GA3 in ABM did not produce definitive results, however GA3 in CBM showed a considerable level of efficacy. For the Janak Dev variety, CBM surpassed ABM in terms of root length, root hairs, leaf size, and dry biomass, whereas ABM demonstrated superior root length for the cardinal variety. In comparison to the GA3 alone at concentration ranges from 0 to 2 mg/L, adding CW at 200 mL/L to CBM or combining GA3 (0.25 mg/L) and CW (10 mL/L) substantially enhanced features including shoot length, leaf size, and root growth for both kinds. Acclimatized plantlets had a survival efficiency of 85% to 95%, with CBM supplemented with GA3 showing the highest survival rate and CBM supplemented with CW coming in second. These results highlight the significance of growth regulator and media choice in enhancing potato nodal propagation for improved plant quality and multiplication.

Keywords: Potato, PBS, GA3, Clarigel, Agar, Coconut water, Micropropagation

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Introduction

Micropropagation has revolutionized plant propagation and is a key factor in the mass production of disease-free plants. This method entails growing plant cells, tissues, or organs in an aseptic environment in a controlled laboratory setting [1]. The potato (*Solanum tuberosum* L.), among the plant species extensively researched utilizing tissue culture, stands out for its nutritional value and tolerance to varied agro-climatic situations [2]. The major goal of potato tissue culture techniques is the disease-free propagation and maintenance of plants through meristem culture [3]. This method requires isolating and cultivating the apical meristem, a tiny area near the plant's growing tip that contains cells that are actively dividing. Since meristems frequently have no viral infections, meristem culture is especially useful for getting rid of viruses. But the likelihood of getting virus-free seedlings increases when chemo and thermotherapy are applied to the explants before meristem culture [4].

In Nepal, potatoes have emerged as one of the most important cash crops and vegetables, covering a total of 193,997 hectares of land and producing 3,112,947 metric tons annually as of 2019 [5]. Potatoes like Janakdev, Khumal seto, Cardinal, Kufri Jyoti, and Khumal rato-2, Desiree, are among the most popular kinds in Nepal. In addition to these, there are several domestic and foreign variations. Janak Dev and Cardinal are the most popular in the Nepalese hills, where there are many others. This work focuses on improving the tissue culture conditions of these two types in that environment based on growth regulators and solidifying agents.

The success of commercial potato tissue culture depends on the selection of the right culture media and technology. Plant performance in terms of growth can vary depending on the composition of the medium and the container system. Since the beginning of tissue culture, the most widely used method for micropropagating plants, including the potato itself, has been solid media-based tissue culture utilizing glass jars. The choice of solidifying agent for an example has also been a factor in driving the results [6]. Agar is utilized to build a strong support system in the medium that aids in both the support of the plants and the media's diffusibility. Interest has been generated by gellan gum (Clarigel), an efficient replacement for the commonly employed agar-based media. Clarigel has shown

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encouraging results in terms of plants' shoot multiplication and root growth [7].

In plant tissue culture, the basal media compositions almost remained unchanged since the development of Murashige and Skoog (MS) media in 1962 that supplies with the macro-nutrients, micro-nutrients, iron, and vitamins [8]. The basic elements like nitrogen, phosphate, potassium, iron, and manganese are supplied by the basal media. The basal media has to be enriched with carbon sources (dextrose, sucrose etc.), inositol and hormones depending upon the plants' requirements [9]. Apart from that, plant hormones including auxins, cytokinins and Gibberellic acid (GA3) are commonly employed as plant growth regulators, that play a vital role in regulating physiological processes during tissue culture. In potato tissue culture, GA3 is the most frequently used hormone that promotes shoot elongation and multiplication of the *in vitro* plantlets [10]. However, the high cost and limited availability of such hormones have prompted researchers to explore alternative growth-promoting substances, such as coconut water (CW), which has shown potential as a natural substitute [11,12].

Figure 1: Selected potato varieties (a); dissection of sprouts to expose meristem (b); meristem at the tip of surgical blade (c); meristem with leaf primordia (d); meristem regenerated plantlets in jam jar (e); nodal sub culture to obtain enough plants for the experiment (f); clonal varieties from nodal sub-culture if the meristem cultured potato plants (g); acclimatized plants in a screenhouse (h); Well survived plantlet in the screenhouse transplanted from the seedling tray (i); Janak dev variety (left) with agar without growth regulators (A), with agar+GA3 (G+A) and with Clarigel and CW (C+C); Cardinal variety (right) with agar without growth regulators (A), with agar+GA3 (G+A) and with Clarigel and CW (C+C); comparison of plant morphology of Janak dev and cardinal (L and M respectively); comparison of leaf area using graph paper (n and o).

In light of these key components, this study was conducted with an aim to explore the advantages of the CW over commercially available plant growth regulators, virus elimination, and growth performance in different solidifying agents (agar vs. Clarigel) were examined in two varieties of potato; Janak Dev and Cardinal as these varieties are the most common ones for the hilly regions of Nepal. Furthermore, the study was set to evaluate the effectiveness of GA3 versus CW as growth-promoting substances. Additionally, the use of the MS basal medium will be investigated for its effectiveness in supporting potato tissue culture. It was discovered that GA3 (gibberellic acid) in CBM (coconut broth medium) had notable efficacy while GA3 in ABM failed to produce definitive results. When compared to ABM, the Janak Dev variety outperformed CBM in terms of root length, root hairs, leaf size, and dry biomass. The cardinal variant, however, showed longer roots in ABM. Additionally, both types' shoot length, leaf size, and root growth were greatly enhanced by the combination of GA3 and CW at particular concentrations. The CBM supplemented with GA3 showed the best survival rate for acclimatized plantlets, followed by the CBM supplemented with CW. These results highlight the significance of using the proper growth regulator and medium to improve potato nodal propagation for improved plant quality and multiplication. Through this study, we aim to contribute to the optimization and advancement of potato tissue culture techniques, ultimately enhancing potato production and global food security.

Materials and methods

Selection of potato tubers and sprouting

Janak Dev and Cardinal varieties of *S. tuberosum* that were suspected to be Virus infected were chosen for the study as shown in **Figure 1**. Average sized tubers were labelled separately and stored in warm dark chamber for 8 to 10 days until they sprouted. Healthy sprouts measuring 1.0 to 2.0 cm in length were excised and surface sterilized as suggested by [13]. To begin, potato sprouts were carefully excised from the tubers. These sprouts underwent a thorough cleansing process using distilled water, followed by a brief immersion in 70% alcohol for 30 seconds. Subsequently, they were rinsed again with distilled water and subjected to sterilization within a laminar air flow cabinet using a 0.1% aqueous solution of HgCl2 for a duration of 4 to 7 minutes. After sterilization, the sprouts were further washed with sterilized distilled water, repeating this procedure 4 to 5bntimes to ensure their surface cleanliness and complete removal of sterilant.

Apical Meristem culture

Meristem culture procedure was followed as done by [14]. Primarily, surface sterilized sprouts' tip was

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carefully removed, and the outer leaves and leaf primordia were dissected under a dissecting microscope in a controlled laminar airflow cabinet. The resulting meristem tip was then put onto test-tube with hormone free potato culture media that was composed of Murashige and Skoog [8] media (MS media) supplemented by 2 mg/L calcium d-pantothenate (HiMedia, India), 30 g/l plant tissue culture grade sucrose (Thermo Fisher, India) and 0.8% agar (HiMedia). This meristem tip was made up of the apical dome and the first pair of leaf primordia, which were roughly 0.1 to 0.15 mm in length depending on the particular cultivar. In order for the meristem tips to multiply and generate adventitious shoots measuring roughly 2 cm in length, these culture tubes were put in a growth environment under precise circumstances (22 ± 1°C, 16/8 hours of light/dark frequency per day with an intensity of 18 W/m²). The plants developed from meristem were again explanted for another cycle of meristem culture and this process was repeated for two more times to make sure of viral sterility.

Plant micropropagation

After the development of plants from cultured meristem, they were micropropagated by single nodal sub-culture technique as suggested by [10,15] in jam jars using previously used potato tissue culture agar media supplemented by 0.25 Mg/L of GA3 provided by HiMedia, India and the media pH was maintained at 5.8 using pH Thermo orion-420 pH meter. The process of sub-culture was repeated until the enough number of plants for the further experiment were achieved.

Media composition for optimization of plant's growth

Media composition that remained unchanged: MS basal media [8], Sucrose (30g/l), 2 mg/L calcium d-pantothenate and myo-inositol provided by HiMedia (0.1g/l).

Two experimental sets were prepared as described below:

i) Unchanged media composition + varying concentrations of GA3 + Agar (HiMedia) as gelling agent

The media composition mentioned earlier that remained unchanged was prepared using an already prepared concentrated stock. In order to generate GA3 concentrations of 0, 0.1, 0.25, 0.5, 1, and 2 mg/L, each present in triplicate media jam jars with 50 ml media for each potato variety, a stock solution of GA3 at a concentration of 50 mg/L was first made and added to the media. Agar, at a concentration of 8 g/l, was utilized as the solidifying agent.

Single node with identical sized were cultured in the prepared media under Laminar Air Flow (LAF) condition and the cultured jam jars were incubated at 25 ± 2°C, 16/8 hours of light/dark frequency per day with an intensity of 18 W/m² [16] for 30 days. Results for the plants' shoot length, root length, number of roots, number of leaves, number of nodes, internode length, leaf size and dry biomass were observed at the 30th day of incubation.

ii) Unchanged media composition + varying concentrations of coconut water (CW), 0.25 mg/L GA3 and combination of 0.25 mg/L GA3 and 10 mg/L coconut water 2.5 g/l Gellan gum as gelling agent

The unchanged media composition was supplemented in triplicates for each variety by 0, 10, 20, 50, 100 and 200 mL/L filtered CW in jam jar with net 50 ml media. For comparison, two other experimental sets of media, one with 0.25 mg/L GA3 and the other with the combination of 0.25 mg/L GA3 and 10 mg/L CW were prepared in similar manner. Gellan gum (Clarigel™, Plant tissue culture tested from HiMedia) at a concentration of 2.5 g/L, was utilized as the solidifying agent. Nodal culture and result observation was done same as in previous section.

Analysis of plants growth performance

As plants growth indicators, following parameters were analyzed as suggested by [17]: Shoot length, root length, number of root hairs, number of nodes, dry biomass, leaf size and internode length.

Plant hardening:

In vitro plantlets that were 30 days old in jam jars were brought out of the incubation chamber and placed in normal screen house temperature for 5 to 7 days. Then the plants were transplanted into a plastic seedling tray with a sterile sand and soil substrate in a 2:1:1 ratio. The transplant procedure was carried out as suggested by [18] in an enclosed space (an aphid-proof screen house) with specified soil composition and fertilizer combination. After properly cleaning and removing all media residue from the plant roots, Bavistin 200 mg/ml was applied for 30 seconds to the roots. Prior to transplantation, soil was improved with 2 g/kg of urea, 2 g/kg of DAP, and 1.2 g/kg of potassium fertilizer, taking into account our soil for 0.2 m² area. The plants were irrigated with 5 ml sterile water on daily basis. The growth was observed for 15 days and survival rate of the plants was evaluated.

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Data analysis

Data framing, table construction and bar graph design was done using Microsoft Excel version 2013 and the analysis of variance (ANOVA) followed by post-hoc analysis by Tukey HSD for the mean comparison at 95% confidence interval was done using R Studio V.2022.10.1.

Results and Discussion

The variation in growth regulators used for propagating potato nodal propagation resulted in different responses in plant growth. However, the application of GA3 at various concentrations in agar media did not yield conclusive results compared to the control group without any growth regulators. None of the observed attributes showed any improvement compared to the negative control when GA3 was applied in concentrations ranging from 0 to 2 mg/ml in agar-based media (ABM), with a 95% confidence interval. Nonetheless, the application of GA3 was found to be significantly effective when using Clarigel-based media (CBM).

For the Janak Dev variety, the CBM was found to exhibit greater root length (8.67 ± 1.15 cm), quantity of root hairs (4.33 ± 0.58), leaf size (33.67 ± 5.86 mm^2), and dry biomass content of the plants (12.48 ± 0.88 mg) than the negative control of ABM. However, there were only minor differences in the two varieties' node counts and internode lengths. With a value of 10.03 ± 0.31 cm, root length for the cardinal variety was determined to be superior in ABM to CBM. The number of nodes and the number of nodes did not differ noticeably between the two circumstances. All other characteristics, including shoot length, number of root hairs, leaf size, and dry biomass, respectively being 6 ± 1 cm, 5.33 ± 2.31, 23.33 ± 8.02 mm^2, and 11.84 ± 2.69 mg, were significantly better expressed in CBM than in ABM, both of which lacked growth regulator additions.

When supplemented with 0.25 mg/ml GA3 to both of these two basal media, CBM was better than ABM for both cardinal and Janak Dev varieties in terms of all the attributes under investigation. The results are displayed in **Table 1** and **Table 2**.

Further, comparing the performance of different concentrations of CW (varying from 10 to 200 mg/L) with that of 0.25 mg/L GA3 in CBM, addition of CW up to 200 mL/L expressed significantly higher values for root length, number of root hairs, plant dry biomass and number of nodes for both varieties. Combination of 0.25 mg/ml GA3 and 10 mL/L CW was statistically found to be superior for the enhancement of shoot length and leaf sizes for both varieties. GA3 resulted in longer internode length than CW treatment in CBM for both varieties. The survival efficiency of the screen house acclimatized plantlets ranged in between 85 to 95% for both cultivars from all media systems used. Plants from Clarigel media supplemented with GA3 had survival rate of 95% (19 survivors out of 20 transplanted plants) followed by the plants from CW supplied Clarigel media with the survival rate of 90%. Plants grown in AGM with GA3 had the least survival rate of all (85%).

The study investigated the effects of growth regulators and media on potato nodal propagation. GA3 in ABM showed no conclusive results, but was effective in CBM. CBM outperformed ABM in root length, root hairs, leaf size, and dry biomass for Janak Dev variety. ABM had better root length for cardinal variety. CBM with GA3 showed superior performance for both varieties. The ability of Clarigel, a bacterial polysaccharide to gel more consistently than agar, a polysaccharide from seaweeds and its physical smoothness and softness when compared to agar aid in the uniform growth of *in vitro* plants [19]. Study of Clarigel's advantages over agar in the tissue culture of potato remains limited, it's better water retention qualities than agar, can provide a more constant and suitable moisture level for the tissue culture. Water availability has a significant impact on plant metabolism and nutrient absorption. However, in some, gelrite have been reported to show hyperhydration which in case of some plants like those with hard woods was proven to be a drawback of this very nature of gelrite [20]. The inertness and purity of Clarigel provide more accurate results, especially considering that the MS basal media is already defined based on salts. In contrast, agar contains various impurities, including salts of metals and sulfur. [21].

The results presented here represent the mean values ± standard deviation of three separate observations. The letters superscripted with the numerical values denote the groupings obtained from the Tukey HSD test with an alpha of 0.05 conducted after ANOVA. Values sharing the same letter are not significantly different at the 95% confidence level.

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Table 1: Variation of GA3 concentration using Agar based media (ABM)

Variety	GA3 concent-ration (mg/ml)	Shoot length (cm)	Root length (cm)	Number of root hairs	Number of nodes	Internode length (cm)	Dry biomass (mg)	Biomass (%)
Janak Dev	0	8.07^{a} ± 0.42	4.47^{a} ± 0.93	3.67^{a} ± 1.15	8.33^{ab} ± 1.53	0.99^{b} ± 0.19	9.06^{a} ± 1.76	8.58^{ab} ± 0.85
	0.1	8.57^{a} ± 1.38	5.33^{a} ± 0.15	2^{ab} ± 0	10^{a} ± 1	0.86^{b} ± 0.16	10.05^{a} ± 0.82	9.77^{ab} ± 1.92
	0.25	5.8^{a} ± 0.87	4.37^{a} ± 1.89	1^{b} ± 0	6.67^{ab} ± 2.08	0.92^{b} ± 0.28	10.02^{a} ± 0.61	11.31^{a} ± 0.78
	0.5	7.73^{a} ± 1.42	6.73^{a} ± 0.06	1.67^{ab} ± 1.15	7.67^{ab} ± 0.58	1.01^{b} ± 0.12	9.34^{a} ± 1.22	8.96^{ab} ± 0.42
	1	6.37^{a} ± 1.59	4.4^{a} ± 1.39	1.67^{ab} ± 0.58	6^{ab} ± 1	1.06^{ab} ± 0.15	8.46^{a} ± 2.44	8.35^{b} ± 1.10
	2	8.53^{a} ± 3.06	3.8^{a} ± 0.98	2^{ab} ± 1	5.33^{b} ± 2.08	1.67^{a} ± 0.41	9.37^{a} ± 1.58	9.42^{b} ± 0.33
Cardinal	0	5.5^{b} ± 0.46	10.03^{a} ± 0.31	2.67^{a} ± 0.58	7.67^{a} ±1.53	0.73^{b} ± 0.10	10.46^{a} ± 1.57	9.84^{a} ± 0.68
	0.1	7.93^{a} ± 0.86	9.57^{a} ± 0.50	2.33^{a} ± 0.58	6.67^{ab} ± 1.15	1.31^{ab} ± 0.38	11.56^{a} ± 3.08	10.48^{a} ± 2.01
	0.25	7.47^{a} ± 0.67	8.47^{a} ± 1.01	2.33^{a} ± 0.58	4.33^{bc} ± 1.15	1.18^{ab} ± 0.03	12.33^{a} ± 3.06	10.39^{a} ± 2.03
	0.5	8.27^{a} ±1.17	8.83^{a} ± 0.75	2^{a} ± 0	7.33^{a} ± 1.15	1.14^{ab} ± 0.14	14.99^{a} ± 3.25	12.44^{a} ± 2.87
	1	6.57^{ab} ± .32	4.6^{b} ± 1.11	2.33^{a} ± 0.58	3.67^{c} ± 0.58	1.62^{a} ± 0.57	9.69^{a} ± 1.43	9.04^{a} ± 0.70
	2	6.8^{ab} ± 0.1	8.07a ± 1.34	2.66^{a} ± 0.58	6.33^{abc} ± 1.58	1.89^{a} ± 0.33	13.59^{a} ± 2.10	11.20^{a} ± 0.77

Table 2: Coconut water (CW) versus GA3 in Clarigel-based media (CBM)

Variety	Treatments	Shoot length (cm)	Root length (cm)	Number of root hairs	Number of nodes	Leaf size (mm2)	Internode length (cm)	Dry biomass (mg)
Janak Dev	0	7^{e} ± 1	8.67^{a} ± 1.15	4.33c ± 0.58	7.67^{b} ± 0.58	33.67^{e} ± 5.86	0.92^{c} ± 0.2	12.48^{e} ± 0.88
	CW 10 mL/L	7.83^{e} ± 0.29	8.33^{a} ± 2.31	5.67c ±1.15	8.67^{b} ± 0.53	52.33^{d} ± 4.51	0.93^{c} ± 0.19	15.25de ± 1.27
	CW 20 mL/L	9.5^{de} ± 0.5	5.67^{a} ± 1.15	7c ± 2	9.33^{ab} ± 0.58	54.33^{d} ± 2.52	1.02^{bc} ± 0.11	26.38^{c} ± 0.67
	CW 50 mL/L	12^{cd} ± 1	6.83^{a} ± 1.26	7.33bc ± 2.08	9.67^{ab} ± 0.58	55.67^{cd} ± 5.03	1.25^{bc} ± 0.18	29.52^{bc} ± 2.82
	CW 100 mL/L	13.33^{abc} ± 1.15	5.5^{a} ± 0.5	11.67ab ± 2.52	9.67^{ab} ± 0.58	76.67^{b} ± 3.79	1.39^{bc} ± 0.18	32.63^{ab} ± 1.72
	CW 200 mL/L	15^{ab} ± 1	8.33^{a} ± 2.31	13.67a ± 1.53	11^{a} ± 1	82.67^{ab} ± 7.64	1.37^{bc} ± 0.18	36.42^{a} ±1.83
	GA3 0.25 mg/L	15.33^{a} ± 1.15	5.67^{a} ± 2.08	4.33c ± 0.58	7.67^{b} ± 0.58	71.33^{bc} ± 2.52	2.01^{a} ±0.27	14.13^{de} ± 0.83
	CW 10 mL/L + GA3 0.25 mg/L	12.33^{bcd} ± 1.53	6.67^{a} ± 0.58	7.67bc ± 1.53	8^{b} ± 0	97^{a} ± 10.54	1.54^{ab} ± 0.19	18.44^{d} ± 2.40
Cardinal	0	6^{e} ± 1	7.67^{a} ± 1.15	5.33^{cd} ± 2.31	7.67 ^{b} ± 0.58	23.33^{d} ± 8.02	0.78^{b} ± 0.08	11.84^{e} ± 2.69
	CW 10 mL/L	7.83^{de} ± 0.29	7.67^{a} ± 2.08	6.33^{bcd} ± 1.53	8.67^{ab} ± 1.53	50 ^{c}± 7.21	1.08^{b}±0.45	14.69^{de} ± 1.16
	CW 20 mL/L	9.5^{cd} ± 0.5	5.67^{a} ± 1.15	7^{bc} ± 1	9.33^{ab} ± 0.58	55^{c} ± 6.24	1.02^{b} ± 0.11	22.78^{bc} ± 1.16
	CW 50 mL/L	12^{bc} ± 1	6.83^{a} ± 1.26	8.67^{abc} ± 2.52	9.67^{ab} ± 0.58	65.33^{bc} ± 12.1	1.25^{b} ± 0.18	27.77^{b} ± 2.25
	CW 100 mL/L	13.33^{ab} ± 1.15	5.5^{a} ± 0.5	10 ^{ab} ± 1	9.33^{ab} ± 0.58	86 ^{ab} ± 4	1.43^{ab} ± 0.21	33.46^{a} ± 1.26
	CW 200 mL/L	15^{a} ± 1	8^{a} ± 1.73	11.67^{a} ± 0.58	10.67^{a}± 1.53	97.67 ^{a} ± 6.51	1.42 ^{ab} ± 0.25	38.46^{a} ± 1.57
	GA3 0.25 mg/L	15^{a} ± 1.73	5.67^{a} ± 2.08	2.33^{d} ± 0.58	7.33^{b} ± 0.58	56.33^{c} ± 10.21	1.97^{a}±0.27	15.04^{de} ± 1.69
	CW 10 mL/L + GA3 0.25 mg/L	11^{bc} ± 1	6.67^{a} ± 0.58	9^{abc} ± 1	8^{ab} ± 0	107.67^{a} ± 5.86	1.37^{ab} ± 0.13	19.7^{cd} ± 3.19

CW in CBM enhanced root length, root hairs, biomass, and nodes compared to the most widely used concentration of GA3 in potato tissue culture (0.25 mg/L). GA3 and CW combination improved shoot length and leaf size as shown in **Figure 2**. These findings indicate that the choice of growth regulator and media type significantly influenced the growth and development of potato plants.

CW is a natural, organic source of growth-promoting elements such vitamins, minerals, amino acids, and phytohormone [22].

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Shoot length (cm)
18 16 14 12 10 8 6 4 2 0
a a a a b ab c c
GG 200C GG G GG (G+C) Ag G
Conditions
a
Cardinal Janak Dev

Dry biomass (mg)
45 40 35 30 25 20 15 10 5 0
a a c bc bc c c d
GG 200C GG G GG (G+C) Ag G
Conditions
d
Cardinal Janak Dev

Root length (cm)
14 12 10 8 6 4 2 0
a a ab abc ab ab a c
GG 200C GG G GG (G+C) Ag G
Conditions
b
Cardinal Janak Dev

Number of nodes
14 12 10 8 6 4 2 0
a a c b bc b c c
GG 200C GG G GG (G+C) Ag G
Conditions
e
Cardinal Janak Dev

Number of root hairs
16 14 12 10 8 6 4 2 0
a a c cd bc bc c d
GG 200C GG G GG (G+C) Ag G
Conditions
c
Cardinal Janak Dev

Leaf size (mm^2)
120 100 80 60 40 20 0
a ab b b a a c c
GG 200C GG G GG (G+C) Ag G
Conditions
f
Cardinal Janak Dev

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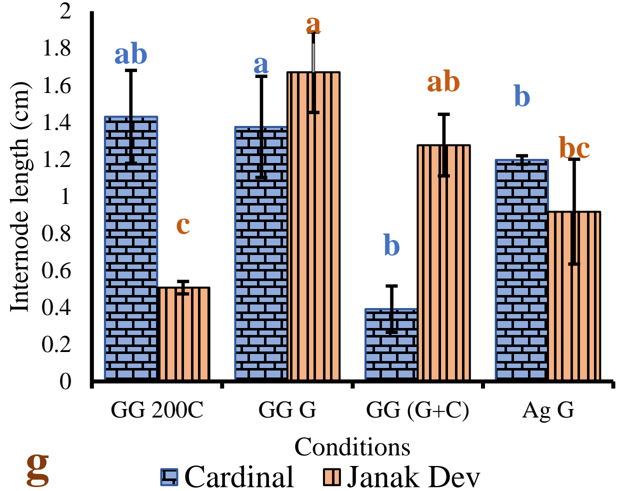

Figure 2: Comparison of shoot length (a), root length (b), number of root hairs (c), dry biomass (d), number of nodes per plant (e), leaf size (f) and average internode length (g) of cardinal and Janak dev varieties of potato plantlets in tissue culture condition grown in: gellan gum/Clarigel based media with 200 mL/L coconut water [GG 200C], gellan gum/Clarigel based media with 0.25 mg/L GA3 [GG G], gellan gum/Clarigel based media with combination of 0.25 mg/L GA3 and 10 mL/L coconut water [GG(G+C)] and agarbased media with 0.25 mg/L GA3 (Ag G).

Compared to synthetic phytohormones like GA3, it comprises a complex blend of substances that can offer a more complete and balanced nutrient profile for plant growth. Auxins, cytokinins, and gibberellins, among other growth-promoting compounds found in CW, can jointly affect different aspects of plant growth and development [23]. This encompassing effect boost a variety of tissue culture performance factors, including root length, root hairs, biomass, and node development [24]. On root initiation and development, CW has reportedly been shown to have remarkable effects. It includes auxins, especially indole-3-acetic acid (IAA), which is essential for promoting root growth and expanding the quantity of root hairs [24].

In this very study, the addition of CW at certain concentrations showed positive effects on various attributes in CBM. These results provide valuable insights for optimizing potato nodal propagation techniques and selecting appropriate growth regulators and media combinations to enhance plant growth and biomass production. Based on the findings of this study, it is recommended that adding CW supplements to Clarigel or gelrite-based media in the range of 50 to 200 mg/ml can be helpful for improving a variety of traits in plant tissue culture. Additionally, 10 mL/L of CW and 0.25 mg/L of GA3 in Clarigel or gelrite-based media have demonstrated encouraging effects when combined.

Conclusion

In comparison to GA3 in agar-based media, the use of coconut water (CW) in Clarigel based medium greatly improves the health and development of *in vitro* potato plants. CW has the potential to be a safe and efficient growth-enhancing agent for potato nodal propagation due to its superior efficacy in promoting root length, root hairs, leaf size, dry biomass, shoot length, and root growth. These results underline how crucial it is to choose growth regulators and media carefully in order to achieve the best plant quality and multiplication in potato plantlet in *in vitro* conditions. Exploring the underlying principles and possible uses of CW in enhancing potato propagation methods call for more investigation.

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