Optimizing minimal cytokinin levels to promote sustainable potato micropropagation

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Abstract. Potato micropropagation via tissue culture is essential for producing high-quality, disease-free planting materials and supporting sustainable production in the face of increasing climate change variability. Optimizing cytokinin levels is crucial for improving plantlet growth. This study was conducted from March to May at the Laboratory of Plant Physiology and Biotechnology, Faculty of Agriculture, Sebelas Maret University, to evaluate the effects of two cytokinins, 6-benzylaminopurine and kinetin, on the growth of two potato varieties under tissue culture conditions. A factorial experiment based on RCD with three replications was performed, using MS media with different cytokinin concentrations. Plantlet height, number of leaves, branches, nodes, and roots were measured after two and four weeks of subculture. Results showed that Bonito Agrohorti generally demonstrated better growth than Granola, especially in shoot elongation. 6-benzylaminopurine was more effective than kinetin in promoting plant height and branching, particularly at concentrations between 0.3 and 0.5 ppm. Similarly, BAP at 0.3-0.5 ppm enhanced root development, especially in Bonito Agrohorti. These findings provide valuable insights into optimizing cytokinin concentrations for efficient potato micropropagation.

1 Introduction

The potato (Solanum tuberosum L.) is considered one of the most crucial crops globally, produced in more than 100 countries worldwide, with global production exceeding 300 million tons [1]. Nutritional potatoes provide carbohydrates, dietary fiber, vitamins (notably vitamin C and B6), minerals (such as potassium, iron, and magnesium), and phytonutrients including polyphenols and carotenoids [2,3]. They enhance diet quality and offer health-promoting benefits, including antioxidant, anti-inflammatory, and antidiabetic properties.

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Additionally, potatoes demonstrate greater bioaccessibility of minerals and vitamins in comparison to certain other staple foods[4].

Commonly, it propagates vegetatively by using whole tubers or cuttings, which makes the plants more susceptible to diseases, especially viral diseases [5] . Plant tissue culture is one of the modern propagation methods to obtain disease-free plants in a short time and homogeneous plants [6].

Cytokinins are essential hormones due to their effective role in promoting cell division and shoot formation during tissue culture [7]. Among these, BAP (6-Benzylamine Purine) and kinetin are the most widely used in potato tissue culture, due to their significant and effective roles in enhancing plant growth and development [8]. Although it effectively promotes plant growth, high concentrations can cause an imbalance between vegetative and root development, potentially reducing the adaptability to external conditions [9].

Potato micropropagation and sustainable hormone management are essential componentss for adapting to climate change. Micropropagation facilitates the swift production of disease-free, climate-resilient seed stock, whereas hormone regulation stabilizes yields under heat stress conditions. Together, these practices contribute significantly to food security amid the increasing variability in climate patterns [9] .

Sustainable agriculture emphasizes optimizing hormone application instead of maximizing it, aiming to grow plants that are resilient to environmental stresses [10]. Although the function of cytokinins, particularly BAP and kinetin, in promoting shoot growth during potato micropropagation has been confirmed by numerous studies, most research has centered on optimizing shoot quantity through the application of relatively high cytokinin concentrations. This emphasis has resulted in a knowledge gap concerning the minimal effective cytokinin levels necessary to sustain healthy and balanced plantlet development while reducing hormonal requirements.

Therefore, this study aims to investigate the impact of varying concentrations of BAP and kinetin on the in vitro development of potato plantlets. It seeks to identify the minimum effective levels of cytokinins necessary to induce healthy and balanced shoot growth, examine the response of different cytokinins concentrations on the shoot-to-root ratio, and determine the hormone levels that promote optimal plantlet growth within the existing cytokinins concentrations. This approach aims to facilitate more sustainable micropropagation practices.

2 Materials and Methods

Two in vitro potato plantlets (Granola and Bonito Agrohorti) were obtained from Kebun Benih Tanaman Pangan Hortikultura Kledung and then multiplied to increase the planting material used in the experiment.

The research tools included culture bottles, bottle caps, autoclaves, a LAF (Laminar Air Flow) system, beakers, Erlenmeyer flasks, scalpels, Bunsen burners, analytical scales, pH meters, tweezers, hot plates, magnetic stirrers, petri dishes, matches, label paper, plastic wrap, tissues, and ovens. The media used were MS media, along with macro stock solutions, micro stock solutions, vitamins, Fe-EDTA, agar, sugar, BAP, Kinetin, distilled water, NaOH, HCl, 70% alcohol, and detergent.

A factorial experiment using an RCD design was conducted, with three factors: two potato varieties, two types of cytokinin (BAP and kinetin), and three concentrations (0.3, 0.4, and 0.5 ppm). The research process began with the sterilization of tools and the preparation of MS media, all of which were performed under sterile conditions. The measured variables included plant height, leaf count, branch number, node, and root number at two and four weeks after subculture.

The data were analyzed using GenStat (Computer Program) Version 4, and the means were separated by using Duncan Multiple Range Test (DMRT) at $P \leq 0.05$.

3 Results

3.1 Plant height

Table 1 shows the effects of three factors: varieties, cytokinins, and concentration on plant height. After two weeks, no significant effects were observed for varieties and cytokinins; however, there were highly significant differences among the concentrations, with 0.4 and 0.5 ppm producing the tallest plants. Additionally, a highly significant effect was found among all interactions; the tallest plants were obtained with Granola * Kinetin, Bonito Agrohorti * BAP, and Granola * 0.4 ppm. Most interactions between cytokinin and concentrations resulted in taller plants, except for Kinetin * 0.3 ppm, which produced shorter plants (Table 2). The tallest plants were found in the combinations of variety, cytokinins, and concentrations, recorded by Granola Kinetin at 0.4 ppm, Bonito Agrohorti BAP at 0.5 ppm, and Granola Kinetin at 0.5 ppm, respectively (Table 3).

Four weeks after subculture, a highly significant variation was observed between varieties and concentrations. The variety Bonito Agrohorti had the tallest plants, and among concentrations, 0.5 ppm produced the tallest plants. There was no significant effect of cytokinins (Table 1). A highly significant effect was also observed in the interactions between varieties, specifically in the cytokinins treatment, where Bonito Agrohorti with BAP produced the tallest plants. No significant interaction was found between varieties in terms of plant height. Moreover, the interactions revealed highly significant differences among cytokinin concentrations; Kinetin at 0.5 ppm resulted in the tallest plants (Table 2). Additionally, significant differences were reflected in the interactions among varieties, cytokinins, and concentrations; Bonito Agrohorti * BAP * 0.4 ppm, Bonito Agrohorti * BAP * 0.5 ppm, and Granola * Kinetin * 0.5 ppm were associated with the tallest plants, as shown in Table 3.

3.2 Number of Leaves/plant

As shown in Table 1, a highly significant effect was observed among varieties. Bonito Agrohorti had more leaves than Granola two weeks after subculture. However, no significant effect was seen among cytokinin types and their concentrations two weeks after subculture. Additionally, no significant differences were found among the interactions of Varieties * Cytokinins and Varieties * Concentrations in terms of leaf number after two weeks. Conversely, the interaction between Cytokinin concentration showed significant variation, with BAP 0.3 ppm and kinetin 0.5 ppm producing the highest leaf counts, as shown in Table 2. Furthermore, significant differences were noted among the interactions of varieties * Cytokinins * Concentrations, with Granola * BAP * 0.3 ppm exhibiting the highest leaf number compared to other interactions after two weeks (Table 3).

Additionally, four weeks after subculture, highly significant differences were observed between the varieties; Bonito Agrohorti had the most leaves, while Granola had the fewest. Furthermore, significant differences were noted between cytokinins and their concentrations. Kinetin produced the highest number of leaves, and concentrations of 0.5 ppm and 0.3 ppm resulted in the highest leaf counts (Table 1).

Table 2 shows a highly significant variation in all interactions among treatments after four weeks; the interactions between Varieties * Cytokinin, Bonito Agrohorti * BAP, and Bonito Agrohorti * Kinetin have the highest number of leaves. Varieties * Concentration

interactions, specifically Bonito Agrohorti * 0.3 ppm and Bonito Agrohorti * 0.5 ppm, also resulted in the highest number of leaves. Additionally, Cytokinin * Concentrations interactions varied significantly after four weeks, with Kinetin * 0.5 ppm and BAP * 0.3 recording the highest number of leaves (Table 2). Moreover, the interactions among Varieties, Cytokinins, and Concentrations were highly significant. Bonito Agrohorti * Kinetin * 0.3 ppm, Bonito Agrohorti * BAP * 0.5 ppm, Bonito Agrohorti * BAP * 0.3 ppm, and Granola * Kinetin * 0.5 ppm recorded the highest number of leaves (Table 3).

3.3 Number of Branches/plant

Table 1 showed highly significant differences across all observed treatments, including varieties, cytokinins, and concentrations, two weeks after subculture. Specifically, the cultivar Bonito Agrohorti, BAP, and the 0.5 ppm concentration recorded the highest number of branches. After four weeks, there was no significant difference between varieties and concentrations, but a notable variation between cytokinins was observed in the number of branches; BAP produced the highest number of branches.

The results shown in Table 2 demonstrate the interactions among varieties, cytokinins, and concentrations. No significant differences were observed in the interactions between varieties and cytokinins after two and four weeks from subculture. Additionally, the interaction of Varieties * Concentrations varied significantly; Bonito Agrohorti * 0.3 ppm produced the highest number of branches after two weeks, while Bonito Agrohorti * 0.5 ppm and Granola * 0.4 ppm showed the highest values after four weeks. Furthermore, Cytokinins * Concentrations had a significant effect on the number of branches; BAP * 0.5 ppm yielded the most branches after two weeks, and BAP * 0.3 ppm, BAP * 0.5 ppm, and kinetin from subculture also produced notable results. After four weeks, Bonito Agrohorti * 0.5 ppm, BAP * 0.4 ppm, and kinetin * 0.5 ppm resulted in the highest branch counts. Lastly, there was a highly significant interaction among Varieties * Cytokinins * Concentrations on the number of branches. Specifically, Granola * BAP * 0.5 ppm and Bonito Agrohorti * BAP * 0.3 ppm recorded the highest branch numbers after two weeks. After four weeks from subculture, all interactions showed the highest number of branches, except for Granola * Kinetin * 0.3 ppm and Bonito Agrohorti * Kinetin * 0.4 ppm (Table 3).

3.4 Number of Nodes/Plant

After two weeks, highly significant differences were observed between varieties; Bonito Agrohorti produced the highest number of nodes, while no significant differences were observed between cytokinins or among concentrations. Moreover, after four weeks, significant variations were noted between varieties, cytokinins, and concentrations. Bonito Agrohorti had the highest number of nodes, whereas Kinetin recorded the highest number of nodes, with 0.5 ppm and 0.3 ppm concentrations showing the highest node counts after four weeks (Table 1).

Table 2 indicates that the interaction of the three factors influences the number of nodes. No significant differences were found among Varieties * Cytokinin and Varieties * Concentration after two weeks. Conversely, there were highly significant differences among Cytokinin * Concentration; BAP * 0.3 ppm and Kinetin * 0.4 ppm produced the most nodes after two weeks of subculture.

Four weeks after subculture, a highly significant variation was evident among the interactions of Varieties*Cytokinin and Cytokinin*Concentration regarding the number of nodes. Bonito Agrohorti with BAP and Kinetin at 0.5 ppm, and BAP at 0.3 ppm, respectively, recorded the highest node counts (Table 2). Furthermore, there were highly significant differences in the interactions among Varieties*Cytokinins*Concentrations. Bonito

Agrohorti with BAP at 0.3 and 0.5 ppm and with kinetin at 0.3 ppm recorded the highest node counts after two weeks, while Granola with kinetin at 0.5 ppm and Bonito Agrohorti with BAP at 0.5 ppm had the highest values after four weeks (Table 3).

3.5 Number of roots/plant

As shown in Table 1, there is a significant effect of varieties, cytokinins, and concentrations. The variety Bonito Agrohorti, with a concentration of 0.5 ppm, produced the highest number of roots after two and four weeks from subculture. In contrast, Kinetin after two weeks and BAP after four weeks recorded the highest number of roots. Generally, highly significant differences were observed among the interactions of varieties, cytokinins, and concentrations. The interaction of varieties with cytokinin varied significantly at two and four weeks after subculture, with Granola * Kinetin and Bonito Agrohorti * BAP and Kinetin showing the highest number of roots. Meanwhile, the interaction of varieties and concentration was highly significant after two weeks, with Bonito Agrohorti at 0.5 ppm producing the highest number of roots. However, no significant variation was observed after four weeks. Moreover, there were highly significant differences in the interaction between the Cytokinins * Concentrations after two and four weeks. Kinetin * 0.4 ppm and BAP * 0.3 ppm recorded the highest number of roots, as shown in Table 2. Table 3 revealed highly significant variation among the interactions of Varieties * Cytokinins * Concentrations after two weeks, with Bonito Agrohorti * BAP * 0.5 ppm producing the highest number of roots. However, there were no significant differences among the interactions of Varieties * Cytokinin * Concentration after four weeks.

Fig. 1. Bonito Agrohorti + 0.5ppm BAP

Fig. 2. Granola + 0.5ppm BAP

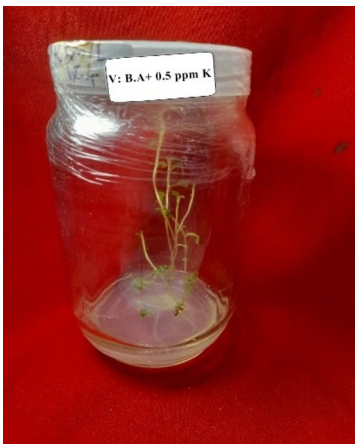

Fig. 3. Bonito Agrohorti + 0.5ppm Kinetin

Fig. 4. Granola + 0.5ppm Kinetin

Table 1. Effect of two cytokinins and their concentration on growth parameters of two potato varieties after two and four weeks from culture.

Varieties	**Plant Height (cm)**		**Number of leaves**		**Number of branches**		**Number of Nodes**		**Number of Roots**	
	After two weeks	**After four weeks**	**After two weeks**	**After four weeks**	**After two weeks**	**After four weeks**	**After two weeks**	**After four weeks**	**After two weeks**	**After four weeks**
Granola	8.32 a	8.99 b	6.28 b	10.11 a	1.67 b	1.83 a	6.06 b	10.0 b	4.22 b	5.45 b
Bonito Agrohorti	8.24 a	11.59 a	7.72 a	13.28 a	2.06 a	1.78a	7.50 a	12.44 a	4.89 a	11.00 a
Sig. L.	NS	***	***	***	***	NS	***	***	**	***
SE±	0.209	0.270	0.190	0.312	0.039	0.072	0.171	0.265	0.157	0.251
LSD	0.611	0.787	0.556	0.910	0.114	0.213	0.500	0.777	0.459	0.737
Cytokinin										
BAP	8.28 a	10.29 a	7.17 a	11.06 a	2.17 a	1.94 a	6.83 a	10.64 b	4.11 b	9.31 a
Kinetin	8.29 a	10.29 a	6.83 a	12.33 a	1.56 b	1.67 b	6.72 a	11.89 a	5.00 a	7.14 b
Sig. L.	NS	NS	NS	**	***	*	NS	**	***	***
SE±	0.209	0.270	0.190	0.312	0.039	0.072	0.171	0.265	0.157	0.251
LSD	0.611	0.787	0.556	0.910	0.114	0.213	0.500	0.777	0.459	0.737
Concentration										
0.3 ppm	7.52 b	10.27 b	7.04 a	12.25 a	1.75 b	1.75 a	7.00 a	11.75 a	4.08 b	8.29 ab
0.4 ppm	8.75 a	9.17 c	6.79 a	10.50 b	1.75 b	1.75 a	6.83 a	9.62 b	4.58 ab	7.50 b
0.5 ppm	8.58 a	11.43 a	7.17 a	12.33 a	2.08 a	1.92 a	6.50 a	12.42 a	5.00 a	8.88 a
Sig. L.	**	***	NS	**	***	NS	NS	***	*	*
SE±	0.256	0.330	0.233	0.382	0.048	0.089	0.210	0.325	0.192	0.308
LSD	0.748	0.963	0.681	1.115	0.140	0.262	0.612	0.951	0.562	0.903
CV%	10.7	11.1	11.5	11.3	9.0	17.1	10.7	10.0	14.6	13.0

Means having the same letter(s) within the same column or row were not significantly different using DMRT at P ≤0.05

Table 2. Effect of the interaction of two Cytokinins, their concentrations, and two potato varieties on growth parameters after two and four weeks

Varieties * Cytokinin	Plant Height (cm)		No. of leaves		No. of branches		No. of Nodes		No. of Roots	
	After two weeks	After four weeks	After two weeks	After four weeks	After two weeks	After four weeks	After two weeks	After four weeks	After two weeks	After four weeks
Granola * BAP	7.26 b	7.19 c	6.22 a	8.56 c	2.00 a	1.89 a	5.89 a	8.17 c	3.33 b	4.00 c
Granola * Kinetin	9.37 a	10.79 b	6.33 a	11.67 b	1.33 a	1.78 a	6.22 a	12.00 b	5.11 a	6.89 b
Bonito Agrohorti * BAP	9.29 a	13.39 a	8.11 a	13.56 a	2.33 a	2.00 a	7.78 a	13.11 a	4.89 a	14.61 a
Bonito Agrohorti * Kinetin	7.20 b	9.79 b	7.33 a	13.00 a	1.78 a	1.56 a	7.22 a	11.78 b	4.89 a	7.39 b
Sig.L.	***	***	NS	***	NS	NS	NS	***	***	***
SE±	0.296	0.381	0.269	0.441	0.055	0.102	0.866	0.375	0.222	0.355
LSD	0.863	1.113	0.786	1.287	0.162	0.302	0.707	1.098	0.649	1.042
Varieties * Concentration										
Granola * 0.3 ppm	6.88 d	8.58 a	6.33 a	10.00 b	1.00 d	1.67 ab	6.00 a	10.17 a	3.33 c	5.83 a
Granola * 0.4 ppm	9.96 a	8.05 a	5.83 a	9.67 b	2.00 b	2.00 a	6.17 a	8.92 a	5.50 ab	5.00 a
Granola * 0.5 ppm	8.11 bc	10.34 a	6.67 a	10.67 b	2.00 b	1.83 ab	6.00 a	11.17 a	3.83 c	5.50 a
Bonito Agrohorti * 0.3 ppm	8.15 bc	11.96 a	7.75 a	14.50 a	2.50 a	1.83 ab	8.00 a	13.33 a	4.83 b	10.75 a
Bonito Agrohorti * 0.4 ppm	7.53 cd	10.30 a	7.75a	11.33 b	1.50 c	1.50 b	7.50 a	10.33 a	3.67 c	10.00 a
Bonito Agrohorti * 0.5 ppm	9.05 ab	12.52 a	7.67	14.00 a	2.17 b	2.00 a	7.00 a	13.67 a	6.17 a	12.25 a
Sig.L.	***	NS	NS	**		*	NS	NS	***	NS
SE±	0.362	0.467	0.330	0.540	0.068	0.126	0.297	0.460	0.272	0.435
LSD	1.058	1.363	0.963	1.576	0.198	0.370	0.866	1.345	0.794	1.276
Cytokinin * Concentration										
BAP * 0.3 ppm	8.32 a	11.5 b	8.33 a	13.33 ab	2.00 b	2.00 a	7.83 a	13.00 a	3.50 c	11.50 a
BAP * 0.4 ppm	8.32 a	9.45 c	6.33 cd	9.00 d	2.00 b	2.00 a	6.33 c	8.08 c	3.50 c	7.17 c
BAP * 0.5 ppm	8.19 a	9.84 c	6.83 bc	10.83 c	2.50 a	1.83 ab	6.33 c	10.83 b	5.33 ab	9.25 b
Kinetin * 0.3 ppm	6.72 b	8.96 c	5.75 d	11.17 c	1.50 c	1.50 b	6.17 c	10.50 b	4.67 b	5.08 d
Kinetin * 0.4 ppm	9.18 a	8.90 c	7.25 bc	12.00 bc	1.50 c	1.50 b	7.33 ab	11.17 b	5.67 a	7.83 c
Kinetin * 0.5 ppm	8.97 a	13.02 a	7.50 ab	13.83 a	1.67 c	2.00 a	6.67 bc	14.00 a	4.67 b	8.50 bc
Sig. L.	**	***	***	***	*	*	***	***	***	***
SE±	0.362	0.467	0.330	0.540	0.068	0.126	0.297	0.460	0.272	0.435
LSD	1.058	1.363	0.963	1.576	0.198	0.370	0.866	1.345	0.794	1.276
CV%	10.7	11.1	11.5	11.3	9.0	17.1	10.7	10.0	14.6	13.0

Means having the same letter(s) within the same column or row were not significantly different using DMRT at P ≤0.05.

Table 3. Effect of the interactions of two cytokinins and their concentration on growth parameters of two potato varieties after two and four weeks from culture

Varieties * Cytokinin * Concentration	Plant Height (cm)		No. of leaves		No. of branches		No. of Nodes		No. of Roots	
	After two weeks	After four weeks	After two weeks	After four weeks	After two weeks	After four weeks	After two weeks	After four weeks	After two weeks	After four weeks
Granola * BAP * 0.3 ppm	8.37 b	10.33 c	8.67 a	12.33 ab	1.00 d	2.00 a	7.67 a	12.33 cd	3.33 d	7.00
Granola * BAP * 0.4 ppm	8.37 b	5.40 d	4.67 c	6.33 c	2.00 c	2.00 a	5.33 cd	5.50 g	3.33 d	2.00
Granola * BAP * 0.5 ppm	5.05 d	5.85 d	5.33 c	7.00 c	3.00 a	1.67 ab	4.67 d	6.67 fg	3.33 d	3.00
Granola * Kinetin * 0.3 ppm	5.40 cd	6.83 d	4.00 c	7.67 c	1.00 d	1.33 bc	4.33 d	8.00 f	3.33 d	4.67
Granola * Kinetin * 0.4 ppm	11.55 a	10.70 c	7.00 b	13.00 ab	2.00 c	2.00 a	7.00 ab	12.33 cd	7.67 a	8.00
Granola * Kinetin * 0.5 ppm	11.17 a	14.83 a	8.00 ab	14.33 a	1.00 d	2.00 a	7.33 a	15.67 a	4.33 cd	8.00
Bonito Agrohorti * BAP * 0.3 ppm	8.27 b	12.83 ab	8.00 ab	14.33 a	3.00 a	2.00 a	8.00 a	13.67 bc	3.67 d	16.00
Bonito Agrohorti * BAP * 0.4 ppm	8.27 b	13.50 a	8.00 ab	11.67 b	2.00 c	2.00 a	7.33 a	10.67 de	3.67 d	12.33
Bonito Agrohorti * BAP * 0.5 ppm	11.33 a	13.83 a	8.33 ab	14.67 a	2.00 c	2.00 a	8.00 a	15.00 ab	7.33 a	15.50
Bonito Agrohorti * Kinetin * 0.3 ppm	8.03 b	11.08 bc	7.50 ab	14.67 a	2.00 c	b1.67 a	8.00 a	13.00 c	6.00 b	5.50
Bonito Agrohorti * Kinetin * 0.4 ppm	6.80 bc	7.10 d	7.50 ab	11.00 b	1.00 d	1.00 c	7.67 a	10.00 e	3.67 d	7.67
Bonito Agrohorti * Kinetin * 0.5 ppm	6.77 bc	11.20 bc	7.00 b	13.33 ab	2.33 b	2.00 a	6.00 bc	12.33 cd	5.00 bc	9.00
Sig.L.	***	***	***	***	***	*	***	***	***	NS
SE±	0.512	0.660	0.466	0.764	0.096	0.178	0.419	0.650	0.385	0.615
LSD	1.496	1.927	1.362	2.229	0.280	0.524	1.224	1.902	1.123	1.805
CV%	10.7	11.1	11.5	11.3	9.0	17.1	6.67	10.0	14.6	13.0

Means having the same letter(s) within the same column or row were not significantly different using DMRT at P ≤0.05.

4 Discussion

There were notable differences in the growth and development of potato plantlets grown under in vitro conditions. The Bonito Agrihorti cultivar outperformed Granola in most measured traits, likely due to the genetic makeup of the Bonito Agrihorti variety, as confirmed by [10], who reported significant variation in the in vitro growth characteristics of potato cultivars, including shoot and root development.

Cytokinins, specifically BAP, were more effective than kinetin, highlighting the highest ability of BAP to promote growth and development in tissue culture. This finding aligns with, who found that BAP outperformed kinetin in stimulating shoot growth and multiplication in vitro cultures. The concentration of 0.5 ppm was most effective in promoting growth and development compared to 0.3 and 0.4 ppm; these findings are consistent with studies by [11], cytokinins promote cell division and overall plant growth and development.

Regarding the interaction between variety and cytokinin, Bonito Agrihorti showed a better response to BAP compared to Granola. This finding is supported by [12], who observed that different genotypes respond differently to cytokinin types, with each genotype having

better growth and development than the others. There was a positive correlation between variety and concentration, with 0.4 and 0.5, having the most significant effect, especially on the Bonito Agrihorti variety. A similar result was mentioned by [13], who found that the response to cytokinins (e.g. 6-benzyladenine, BAP) in tissue culture is specific to the plant variety and the cytokinin concentration.

Although the interaction between cytokinin and concentration is significant, BAP is most effective at all levels, whereas kinetin was less effective except at 0.4 and 0.5 ppm. This could be due to the high biological activity and chemical stability of BAP, which is consistent to the results found by [14], who reported that BAP was most effective in promoting shoot proliferation and yielded stronger plantlets under in vitro culture. Ultimately, the interaction among variety, cytokinin, and concentration showed that the best performance was achieved by the Bonito Agrihorti variety at a BAP concentration of 0.5 ppm, in the parameters.

5 Conclusion

Overall, the Bonito Agrohorti variety showed superior in vitro growth performance compared to Granola across most measured traits, including plant height, number of leaves, branches, nodes, and roots. Among the cytokinins tested, BAP consistently promoted greater vegetative growth than kinetin. Cytokinin concentrations of 0.3–0.5 ppm enhanced growth, with 0.5 ppm producing the strongest responses across parameters. The combination of Bonito Agrohorti with BAP at 0.5 ppm resulted in the best plantlet performance, characterized by taller shoots, more leaves, increased branching, greater node formation, and higher root numbers.

References

1. L. Li, T. Zhu, L. Wen, T. Zhang, and M. Ren, Biofortification of potato nutrition. Journal of Advanced Research. (2024). https://doi.org/10.1016/j.jare.2024.10.033
2. T. Mishra, P. Raigond, N. Thakur, S. Dutt, and B. Singh, Recent Updates on Healthy Phytoconstituents in Potato: a Nutritional Depository. Potato Research. **63**, 3 (2020). https://doi.org/10.1007/s11540-019-09442-z
3. M. Hussain, A. Qayum, Z. Xiuxiu, L. Liu, K. Hussain, P. Yue, S. Yue, M. Y.F Koko, A. Hussain, and X. Li, Potato protein: An emerging source of high quality and allergy free protein, and its possible future based products. Food Research International. **148**, 600 (2021). https://doi.org/10.1016/j.foodres.2021.110583
4. M. S. Rathore, A. Mahakul, K. Tiwari, R. Prajapati, N. Maheshwari, R. Yadav, N. Vasure, N. Vasure, S. Tiwari, and Y. P. Singh, Review on aeroponics based potato seed production for food security and sustainable agriculture. Agriculture Association of Textile Chemical and Critical Reviews. **13**, 1 (2025). https://doi.org/10.21276/aatccreview.2025.13.01.20
5. B. S R and M. R. B, Effect of Varied Levels of Gibberellin, Anti-Gibberellin and Cytokinin on Growth and Tuberization in Potato. Madras Agricultural Journal. **111**, (2024). https://doi.org/10.29321/maj.10.000ma9
6. N. M. Arli, Z. A. Noli, and M. Idris, The Application of Plant Growth Regulators in Propagation of Dendrobium Orchid with Thin Cell Layer (TCL) Technique: A Review. International Journal of Progressive Sciences and Technologies. **39**, 2 (2023). https://doi.org/10.52155/ijpsat.v39.2.5505
7. M. P. Voko, A. O. Aremu, N. P. Makunga, J. Nisler, K. Doležal, and N. A. Masondo, The potential applications of cytokinins and cytokinin oxidase/dehydrogenase

inhibitors for mitigating abiotic stresses in model and non-model plant species. Current Plant Biology. **40**, 100398 (2024). https://doi.org/10.1016/j.cpb.2024.100398

8. S. Thakur, Shruti, S. Hashmi, S. Mishra, S. K. Ekka, A. Kushwaha, and R. Kujur, A Review on Plant Tissue Culture. Asian Journal of Biology. **20**, 2 (2024). https://doi.org/10.9734/ajob/2024/v20i2387
9. N. Li, D. Euring, J. Y. Cha, Z. Lin, M. Lu, L. J. Huang, and W. Y. Kim, Plant Hormone-Mediated Regulation of Heat Tolerance in Response to Global Climate Change. Frontiers in Plant Science. **11**, 627969 (2021). https://doi.org/10.3389/fpls.2020.627969
10. L. Zhang, F. J. Nie, L. Gong, X. Y. Gan, G. H. Zhang, X. Liu, W. J. Yang, L. Shi, Y. C. Chen, R. X. Xie, Z. Q. Guo, and Y. Song, Regenerative plantlets with improved agronomic characteristics caused by anther culture of tetraploid potato (Solanum tuberosum L.). PeerJ. **11**, (2023). https://doi.org/10.7717/PEERJ.14984
11. L. Leto, V. Guarrasi, A. Agosti, M. Nironi, B. Chiancone, and J. Juan Vicedo, Effects of Cytokinins on Morphogenesis, Total (Poly)Phenolic Content and Antioxidant Capacity of In Vitro-Cultured Hop Plantlets, cvs. Cascade and Columbus. Plants. **14**, 3 (2025). https://doi.org/10.3390/plants14030418
12. A. Ariste, M. D. C. Ojeda Zacarías, H. Lozoya Saldaña, E. Olivares Sáenz, E. A. García Zambrano, A. Ibarra López, and A. K. Cham, Optimized in vitro micropropagation and microtuber production in potato (Solanum tuberosum L.) through apical buds using hormone regulation and tissue culture techniques. Journal of Experimental Biology and Agricultural Sciences. **13**, 1 (2025). https://doi.org/10.18006/2025.13(1).86.96
13. L. Van Trong, B. B. Thinh, N. Hung, L. T. Huyen, and H. T. Phuong, Effects of cytokinin on physiological and biochemical indicators of some tomato varieties (Solanum lycopersicum l.) cultivated in Vietnam. Pakistan Journal of Biological Sciences. **23**, 11 (2020). https://doi.org/10.3923/pjbs.2020.1462.1472
14. M. Kamińska, A. Styczynska, A. Szakiel, C. Pączkowski, and A. Kućko, Comprehensive elucidation of the differential physiological kale response to cytokinins under in vitro conditions. BMC Plant Biology. **24**, 1 (2024). https://doi.org/10.1186/s12870-024-05396-8

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