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Article

The Effect of Benzyl Amino Purine (BAP) On Potato (*Solanum tuberosum* L.) Axillary Buds Micropropagation

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Abstract. Potato (*Solanum tuberosum* L.) production in Indonesia increased by 4.21% in 2022 compared to the previous year, but this production is still low by international standards. This is caused by low quality and quantity of potato seeds which is sourced from tubers. It is necessary to modify the in vitro cultivation of potato seed sources by utilising seeds that are often wasted with the addition of cytokinins, such as Benzyl Amino Purine (BAP). The aim of this study was to determine the effect of BAP on potato axillary shoot explants sourced from seeds. The study was conducted using a Completely Randomized Design with one factor, namely BAP concentrations of 0, 1, 2 and 3 ppm with 3 replications. The results showed that there is an influence of BAP on the parameters of shoot growth percentage, leaf and root emergence time, plant height, number of shoots, roots, and leaves of potato axillary buds planlet. The 2 ppm BAP treatment tends to give the best response in increasing the number of shoots with an average of 4.33 shoots, making it effective for producing potato seedlings from axillary buds in vitro.

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1. Introduction

Potato production in Indonesia increased by 4.21%, reaching 1.42 million tonnes in 2022, compared to 1.36 million tonnes in the previous year [1], but that is still considered low by international standards at 17.5 t/ha [2]. This is caused by low quality and quantity of potato seeds, the relatively high price of quality seeds, and the reduction in the area of potato farms in several regions in Indonesia [3-4]. Potato seedlings usually only use potato tubers that are grown by sprouting, rarely growing directly from the

seeds. In fact, potato tubers are a special seed material because they can carry many disease-causing organisms [4-5] so that potato plants will grow poorly. Therefore, other cultivation alternatives are needed to maximise seed potatoes so that the need for seed potatoes in Indonesia can be met safely, such as tissue culture.

Tissue culture is a plant propagation technique that can produce large numbers of plant seeds in a relatively short time [6-7] Tissue culture techniques can be done directly without going through the callus phase, such as axillary bud induction. The advantages of using axillary buds include the relatively small size of the explants used, namely 1-2 cm so that it is more efficient, the most reliable way to produce clonal plants, which are genetically identical to the starting material [8] so that they can be used as a source of propagation. In this study, axillary buds grown from potato seeds in vitro were used. In inducing axillary buds, the addition of Plant Growth Regulator (PGR) can support shoot growth [9]. PGR that play a role in shoot growth are cytokinins [10-11].

Cytokinin is a PGR that is often used in in vitro techniques, usually used for shoot multiplication. Benzyl Amino Purine (BAP) is one type of cytokinin that is very effective in the induction and proliferation of shoots in vitro. The addition of BAP can increase cell division and growth in a explants [7][12]. BAP has more stable activity [13-14] and is resistant to oxidation, environmentally friendly, and cheaper than other cytokinins [15]. In this research, the culture of potato axillary buds sourced from seeds with the addition of BAP will be carried out.

Research on the maximization of potato seeds for seedlings with the addition of BAP has been conducted previously using stem explants grown from seeds [16].The research showed that optimal stem callus growth was obtained in media treatments with 2 ppm BAP, while optimal root, shoot and leaf formation was obtained in media with the addition of 1 ppm BAP. Also, research about the effect of BAP on the growth axillary buds sourced from the potato plant in nature has been observed [17]. The research shows that 1.5 ppm BAP is the best treatment that can increase the number of buds, number of leaves, and planlet height. Therefore, it is important to improvise this research by using potato axillary buds grown in vitro from seeds to maximise the quality of potato seedlings by utilising seeds that are often wasted.

2. Experimental Section

2.1. Materials

The tools used were culture bottle, petri dish, spatula, sprayer, analytical scale, bunsen, hot plate, magnetic stirrer, pH meter, laminar air flow, autoclave, and digital camera. Materials used were potato seeds, distilled water, methylated spirits, MS base medium (Murashige & skoog with vitamins-Caisson Laboratories.inc,), solidifying agent (gelzan), sucrose, HCl, NaOH, 70% alcohol, liquid detergent, sodium hypochlorite (NaOCl), fungicide, BAP and 2,4 D.

2.2. Methods

The research was conducted at the Plant Structure and Function Laboratory, Department of Biology, Faculty of Science and Mathematics, Diponegoro University, Semarang from February to June 2022. The research flowchart is shown in Figure 1. Potato fruits were obtained from Wonosobo, Central Java.

Seeds were separated from the pulp and then sterilised with 5% fungicide, then 10% Sodium Hypochlorite (NaClO), and 25% alcohol, and lastly rinsed with sterile distilled water three times. The sterilised seeds were planted on ½ MS media. After that, the culture was incubated in culture bottles at 25°C with 1000 lux lighting continuously. Axillary shoots on potato sprouts with a height of about 1 cm (Figure 2) were used as explants. Explants in the form of axillary shoots of potato sprouts were cut and placed on ½ MS (Murashige and Skoog) medium supplemented with (0, 1, 2, 3) ppm BAP, and the culture was maintained for one month at 25°C with 1000 lux light.

The experimental design used a completely randomised design (CRD) with one treatment factor, namely 0, 1, 2, and 3 ppm BAP. Each treatment was given 3 replications. The parameters observed were quantitative and qualitative. Quantitative parameters are shoot growth percentage (%), time of shoot and root emergence, plant height, number of shoots, roots, and leaves. While the qualitative parameters is visual shoot morphology. Data were analysed using Analysis of Variance (ANOVA) and if there were significant differences, it was followed by Duncan's Multiple Range Test (DMRT) at 95% significance level.

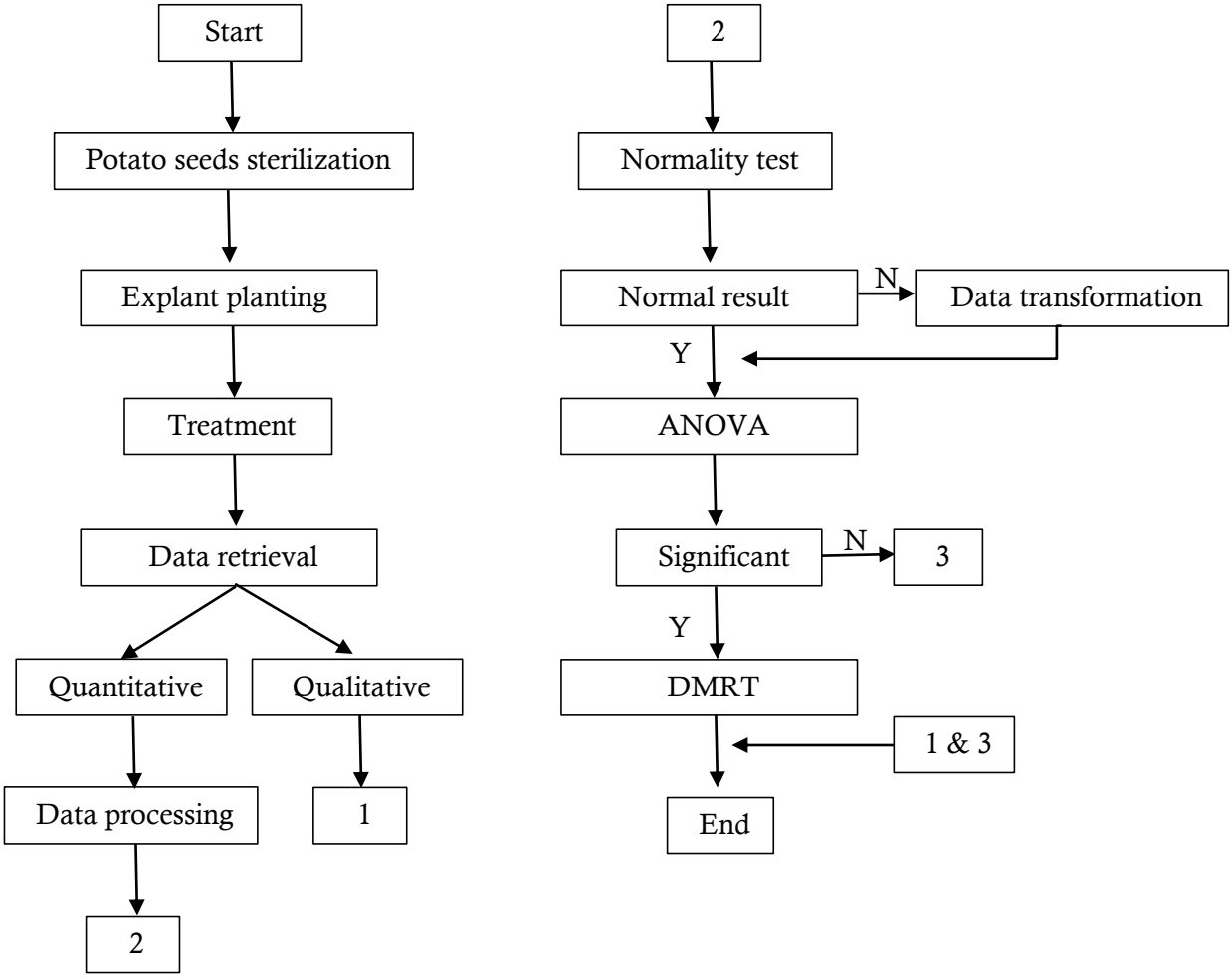

Figure 1. Schematic/flowchart of research. Y is a sign of a normal or significant result and N is a sign of abnormal or non-significant result from the test.

3. Results and Discussion

3.1. Percentage of Shoots Growth, Shoots and Roots Emerge Time

The results showed that the percentage of shoot growth was 100% in all treatments. Meanwhile, there are significant differences in the parameters of leaf and root emergence time to the addition of BAP with different concentrations. Based on Figure 1, the treatment of 0, 1, and 3 ppm BAP is a faster treatment and not significantly different in producing new shoots on average at 3 days after planting (DAP). The treatment of 2 ppm BAP showed the slowest shoots appeared at 3.67 DAP. The treatment of 0 ppm BAP (Figure 2) without the addition of BAP was the fastest treatment to induce new roots at 4 DAP. Treatments of 1, 2, and 3 ppm BAP induced the first roots to appear at 5 DAP, 6 DAP, and 5.67 DAP.

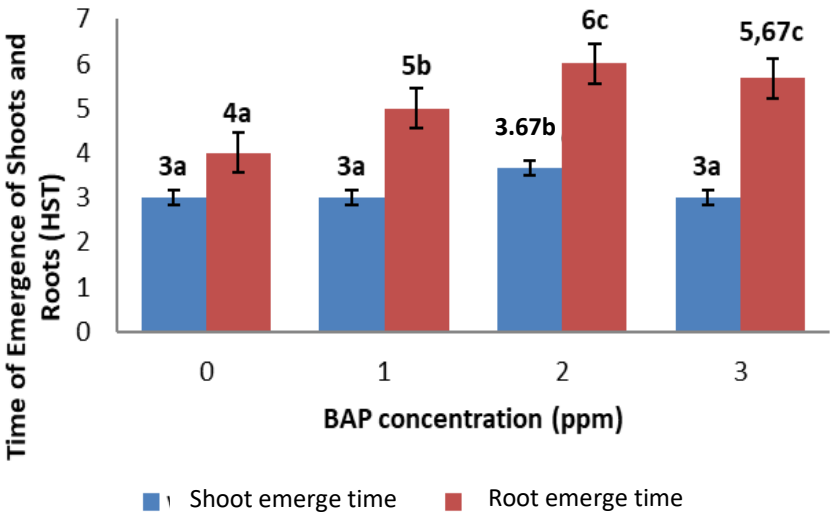

Figure 2. Time of emergence of shoots and roots on potato stem (*Solanum tuberosum* L.) explants after planting

The treatment of 0, 1, and 3 ppm BAP can produce shoots and roots faster than the treatment of 2 ppm BAP, this is due to the influence of endogenous hormones contained in axillary explants. Endogenous cytokinins and auxins are sufficient to induce shoots and roots, so that they can appear without the addition of exogenous PGR [18]. Auxins and cytokinins can interact antagonistically and synergistically [19].

Exogenous cytokinins cause the cytokinin content to be higher than endogenous auxins resulting in slower root growth, while too much exogenous cytokinin also causes slow shoot growth. Although at 2 ppm treatment the shoots and roots grew more slowly, they still grew 100% with a higher number of shoots. This result is similar to previous research [20] on pamelo (*Citrus maxima*) seed explants that the addition of 2 ppm BAP at the beginning caused the seeds to be slower to form shoots and roots, but at the end of the observation, the seeds produced more shoots and leaves.

3.2. Plant height

Based on the analysis of the different concentrations of BAP on potato axillary buds, it shows a significant difference in plant height. The most optimal plant height in the 0 ppm BAP treatment with an average of 7.97 cm, while the lowest plant height in the 3 ppm BAP treatment was 4.70 cm. The 1 ppm BAP treatment had an average plant height of 7.03 cm and the 2 ppm BAP treatment had an average plant height of 6.27 cm (Figure 3).

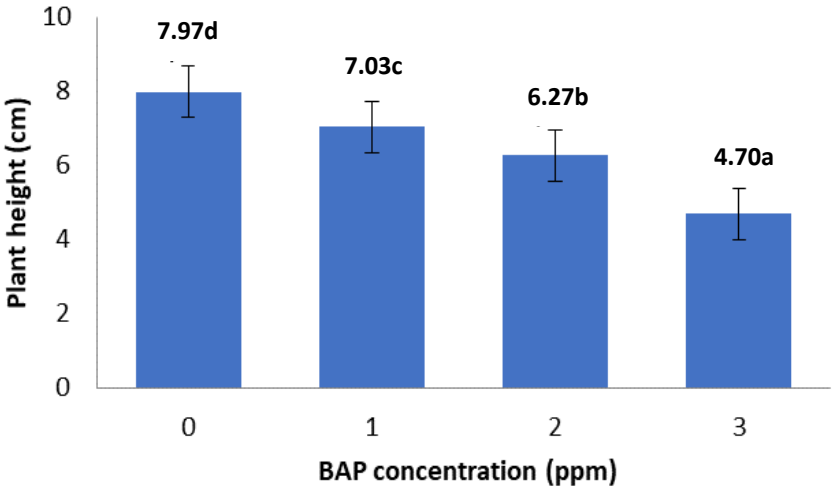

Figure 3. The average height of potato plants (*Solanum tuberosum* L.) with BAP treatment 5th weeks after planting

The 0 ppm treatment can reach the optimal height because the endogenous cytokinin and auxin content is appropriate so that normal growth occurs, not requiring exogenous hormones again. Meanwhile, the addition of exogenous cytokinin causes the ratio between cytokinin and auxin to be higher, causing auxin performance to decrease [21]. In fact, auxin plays a greater role in plant height increases because its related to cell elongation [22] [23]. Auxin activates the proton pump found in the plasma membrane, so that the pH of auxin becomes lower. Activation of the proton pump will break the hydrogen bonds between cellulose microfibrils so that the cell wall will stretch. This makes it easier for the cells to take up water through osmosis and the cells will grow longer [24] [23].

BAP at a relatively low concentration is optimal in the shoots growth [25]. Based on Figure 3, a higher concentration of BAP added, the lower the plant height. This happens because too high cytokinin content inhibits the effect of cytokinin itself [25] and inhibits the performance of endogenous auxin in cell elongation. This result is in line to previous research that higher concentrations of BAP inhibit callus formation in *Zea mays* L. [26] and *Calotropis gigantea* [27] explants. Plant tissues contain the enzyme cytokinin oxidase. This enzyme cleaves the N6 side of the N6 chain of the cytokinin subset [28]. Oxidation of cytokinin enzymes can limit the effects of cytokinin if the concentration applied is too high [29].

3.3. Number of Shoots, Roots, and Leaves

Based on the analysis of the different concentrations of BAP on potato axillary buds, it shows a significant difference on the number of shoots (Figure 4). The number of shoots in this research was optimal in the treatment of 2 ppm BAP. The number of shoots in the 3 ppm BAP produced 1.67, while the treatment of 0 ppm BAP and 1 ppm BAP produced 1 shoot. The treatments of 0, 1, and 3 ppm BAP have different concentrations, but they are not significantly different. This is shows that the addition of 2 ppm BAP is optimal for shoots multiplication.

The 0 and 1 ppm BAP treatment showed the least number of shoots because the cytokinin requirement was insufficient to produce more shoots [30]. Meanwhile, the 3 ppm BAP treatment showed a small number of shoots because the excess cytokinin content inhibited shoot formation. Therefore, the 2 ppm BAP treatment is the most optimal treatment because it produces the highest number of shoots. There is previous studies that also proved that too high BAP concentrations (>3 ppm) resulted in fewer shoots in *Tectona grandis* and a concentration of 2 ppm was the optimal

treatment [31]. Based on these results, it proves that the addition of 2 ppm BAP to potato axillary explants is the best treatment for cultivating potato seedlings with more and faster results.

Cytokinin, in this case BAP, plays a role in protein synthesis in the translational process by increasing the performance of mRNA [32] to translate codons into amino acids that make up proteins in ribosomes [33]. One of the proteins produced is the phosphatase enzyme which plays a role in the cell cycle. Furthermore, cytokinin plays a role in 2 stages of the cell cycle, namely in the G1 to S and G2 to M phases [34]. In the G1 to S phase, cytokinin helps cyclin to activate CDK (cyclin-dependent kinase) so that cell phosphorylation occurs and the cell cycle continues. Whereas in the G2 to M phase, 2 phosphates on CDKs make them inactive, so to reactivate CDKs, the phosphatase enzyme produced from protein synthesis acts to remove the inactive phosphates, and the cell cycle can continue to the M phase [10-11].

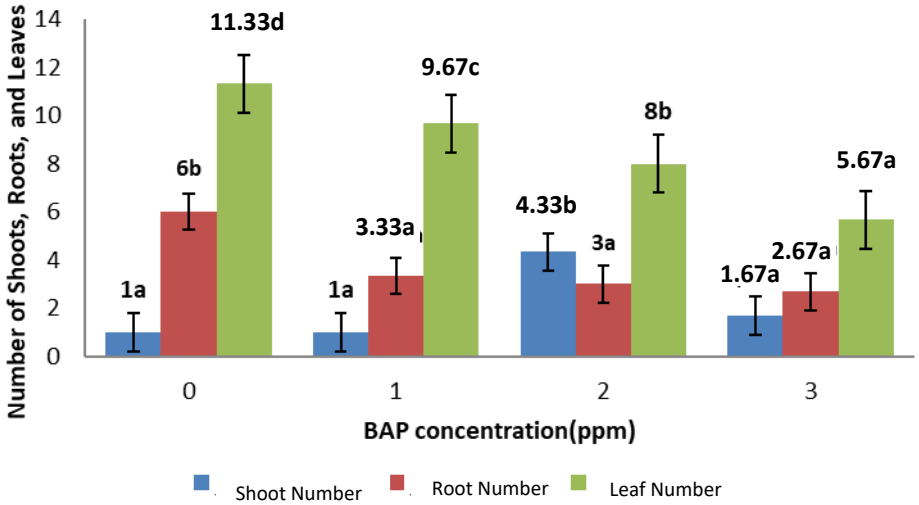

Figure 4. The number of shoots, roots, and leaves formed on the shoots of potato plants (*Solanum tuberosum* L.)

At 0 ppm BAP treatment showed the highest number of roots, namely 6 roots, in the 1 ppm BAP treatment was 3.33 roots, 2 ppm BAP treatment was 3 roots, and 3 ppm BAP treatment with the least number of roots produced 2.67 roots (Figure 4). At 1-3 ppm BAP showed no significant difference, but all three were significantly different from 0 ppm BAP. This indicates that endogenous hormones from explants are sufficient to stimulate root growth. The hormone that responsible for root formation is auxin [35-36], therefore when exogenous cytokinin is added, the root growth slows down. Cytokinin through pericellular cells can inhibit the process of lateral root formation [37-38]. This caused a slow cell division in root formation. The number of roots formed in the propagation of ginger buds (*Zingiber officinale* Roscoe) in vitro decreased as the concentration of BAP increased [39].

Same with the number of roots parameter, the highest number of leaves at 0 ppm BAP, which is 11.33 strands, while the least number of leaves is 3 ppm BAP with a total of 5.67 strands. The treatment of 1 ppm BAP has 9.67 leaves and 2 ppm BAP has 8 leaves (Figure 4). Shoot growth at 0 ppm BAP tends to be faster and taller, so it has a large number of nodes, this can affect the number of leaves formed. The addition of BAP showed a decreasing number of leaves. Increasing the concentration of BAP can inhibit the work of auxin in cell elongation, so that it can affect plant height and fewer nodes are formed [40]. This causes a decrease in the number of leaves formed.

3.4. Shoots Morphology

Shoot morphology is a qualitative parameter that was observed visually from planting to week 5. Figure 5 shows the morphology of shoots grown from axillary buds of potato stems on MS media with different concentrations of BAP. The 0 and 1 ppm BAP treatments have relatively the same shoot colour, which is pale green with a soft shoot texture, but the size of stems and leaves in the 1 ppm BAP treatment is relatively larger. The 2 ppm BAP treatment also visually showed a colour that tended to be greener with a harder texture and larger stem and leaf size. The 3 ppm BAP treatment has a yellowish green colour with a soft texture and relatively small stem and leaf size.

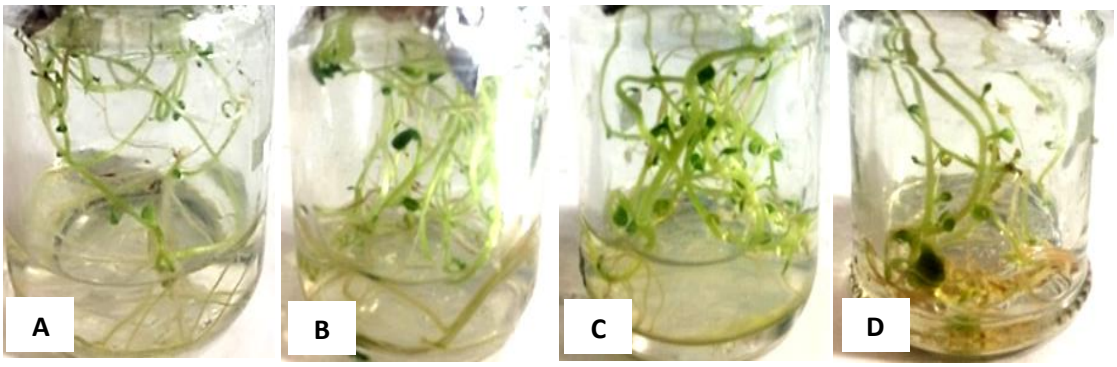

Figure 5. Morphology of potato bud growth 35 DAP a. 0 ppm BAP b. 1 ppm BAP c. 2 ppm BAP d. 3 ppm BAP

The shoot colour variable shows the chlorophyll formed. The addition of BAP affects the colour and size of the shoots. The higher the concentration of BAP, the larger and greener the shoots. Cytokinin plays a role in chloroplast development. Cytokinin application results in chlorophyll accumulation and increases the conversion of etioplasts into chloroplasts through chlorophyll biosynthesis [41]. In chlorophyll synthesis, cytokinins induce the activation of NADH protochlorophyllide reductase, which is a key enzyme in the chlorophyll biosynthesis pathway [42]. Increasing chlorophyll content will make the leaves greener and increase the photosynthesis rate. In addition, plants absorb water and nutrients in the growing medium so that the enlargement of plant organs occurs.

The 2 ppm BAP treatment (Figure 5) visually has a larger stem shape, wider leaves, and a harder texture compared to the other treatments. This is thought to be due to the endogenous auxin content and the addition of exogenous cytokinin in a balanced condition so as to induce the growth and development of shoots. In *Rosa damascena* bud multiplication research, the balance and interaction of PGR in in vitro culture can control the process of plant organogenesis [43]. The balance of auxin and cytokinin stimulates cell elongation and division [38]. Auxin stimulates cell elongation [44], then the cell can absorb more water so that cell plasma increases and causes cells to elongate. Cell elongation is followed by the role of cytokinin in cell division which is very active so that the number of cells increases and stimulates differentiation to form an optimal plant size.

In addition, the larger stem and leaf sizes are thought to be due to the cells formed in the 2 ppm BAP treatment having a larger size. This is caused by high chlorophyll so that the photosynthesis process is more optimal. Cytokinins can increase chlorophyll levels by stimulating chlorophyll synthesis [45-46]. Chlorophyll is the main factor in the photosynthesis process [47]. So, cytokinins assist chlorophyll in producing optimal ATP [48]. This can affect the growth and development of plant organs.

4. Conclusion

The difference in BAP concentrations influence the parameters of shoot growth percentage, leaf and root emergence time, plant height, number of shoots, roots, and leaves in potato axillary buds planlet. The 2 ppm BAP treatment tends to give the best response in increasing the number of shoots with an average of 4.33 shoots, making it effective for producing potato seedlings from axillary buds in vitro.

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