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by Susiyanti Susiyanti

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Effect of Benzilaminopurine and Kinetin for shoot multiplication of Indigofera (*Indigofera zollingeriana* Miq.) by in vitro culture

J I Royani[1], S Chotimah[1], R N Utami[1], W S Fatma[2], Susiyanti[2] and A P Fatmawaty[2]

[1]Pusat Teknologi Produksi Tanaman-BPPT, Gd 610-614 Laptiab-Kawasan Puspiptek Serpong Tangerang Banten, Indonesia
[2]Jurusan Agroekoteknologi Fakultas Pertanian-Universitas Sultan Ageng Tirtayasa, Jl. Raya Jakarta KM 4, Panancangan, Cipocok Jaya, Kota Serang, Banten, Indonesia

Corresponding author: juwartina.ida@bppt.go.id

Abstract. Indigofera (*Indigofera zollingeriana* Miq.) is one of legume trees that useful as forage and have advantages in production and quality of forage compared to other types of legumes. This forage contains high protein and mineral sources, good fiber structure and high digestibility value. Nutrient contents are crude protein (31%) with 76% of DMD and 83% of OMD. Indigofera has low germination due to thick seed coat and fungal invasion during germination. Propagation by in vitro culture promise to multiply superior seeds from Indigofera. The aim for this research was to determine the effect of Benzylaminopurine (BAP) and Kinetin for multiply of shoots of Indigofera. The research was designed using Completely Randomized Design (CRD) with 2 factors (BAP and Kinetin concentration) and repeated 3 times. BAP concentration consists of four levels, 0, 1.0, 1.5 and 2 mg/L and Kinetin concentration consists of four levels 0, 1.0, 2 and 3 mg/L. Results showed that BAP gave the best results for number of shoots parameter at any concentration. Compact callus appears in basal of shoots at BAP (1.5 mg/L and 2 mg/L) media. Whereas media with Kinetin showed no significant effect on all parameters and there was no interaction between BAP and Kinetin.

1. Introduction

Indigofera is a tree legume plant that has many species, spread throughout the tropics with high adaptability to a wide range of environments [1]. Indigofera or better known as tarum has about 700 species. This plant has high nutritional content and production and is tolerant of abiotic stress [2]. The existence of Indigofera in Indonesia has long been recognized for the natural dye industry [3].

Currently, the use of Indigofera is not only limited to natural dyes, Indigofera is also known to have many benefits in agriculture and industry. One of the Indigofera species that has the potential as a conservation plant, green manure and plantation protection plant is *Indigofera zollingeriana*. This is because Indigofera can meet nitrogen needs for itself and it is symbiotic with Rhizobium, there are other plant-supporting bacteria that can be isolated from root nodules [4]. Legumes have a unique interaction with Rhizobium because they supply 80-90% of the plant's total nitrogen needs [5].

A part from being a conservation plant, Indigofera is a forage crop which is currently being developed. Indigofera has advantages in production and forage quality compared to other legumes. The average crude protein of Indigofera ranges from 26% -31% with a protein digestibility rate of 83% -86.3%. Meanwhile,

Centrosema mucunoides and *Desmodium intortum* only produced an average of 16% -24% and 14% -23%. The role of Indigofera in feed ingredients as a green concentrate or herbal medicine for livestock because it contains chlorophyll and secondary compounds that are beneficial to livestock [6]. Unfortunately, studies on Indigofera seedlings and propagation have not been done much [7].

Indigofera propagation which is generally carried out is conventionally through seeds. Conventional propagation has constraints that limit large-scale seed supply. Abdullah [3] stated that storing Indigofera seeds for more than 4 weeks reduced seed germination by 24%. The low germination rate is generally due to thick seed coat and fungal invasion during germination. This of course will have an impact on quality and limited distribution [8]. Therefore, it is necessary to do a propagation method that can produce uniform, large and fast seedlings. The aim for this research was to determine the effect of Benzylaminopurine and Kinetin for multiply of shoots of Indigofera.

2. Materials and methods

These researches were done at Laboratorium of Plant Production BPPT at Laptiab Puspiptek Area Serpong Tangerang Indonesia

2.1. Materials

Materials used for these researches was germinated plant from Indigofera 2-month-old after germinated with sterile condition. For shoots induction treatment, Murashige and Skoog media [9] was used as basal media and added with plant growth regulator i.e.: Benzylaminopurine and Kinetin with different concentration.

2.2. Methods

2.2.1. Seed germinated. Seed of Indigofera was sterilized using commercial bleach with 5-6% of sodium hypochlorite content. After rinsed several times until clean, seed was planting on solid MS media. Seed was incubated at Thermostatic room with temperature 25 ± 2 °C for 8 weeks.

2.2.2. Plant planting in media treatments. After 8 weeks germinated, Indigofera plants with 10 cm in height were cutting to get one single nodes with 1 cm in height for explant of treatments. Each single node was planting on media MS with additional of growth regulator i.e.: BAP (B0:0 mg/L, B1:1 mg/L, B2:1.5 mg/L and B3:2 mg/L) and Kinetin (K0: 0 mg/L, K1: 1 mg/L, K2: 2 mg/L and K3: 3 mg/L). Then incubated at Thermostatic room with temperature 25 ± 2 °C for 8 weeks.

2.2.3. Shoots growth observation. Observation of Indigofera shoots growth was done every 2 weeks until 8 weeks. Parameter of observation i.e.: shoot of length (cm), number of shoots, number of leaves, leaf color and callus formation (diameter, texture, and color of callus).

2.2.4. Data analysis. Design of data analysis using a completely randomized design (CRD) with two factors. Concentration of BAP (B0:0 mg/L, B1:1 mg/L, B2:1.5 mg/L and B3:2 mg/L) were the first factor, and second factor was concentration of Kinetin (K0: 0 mg/L, K1: 1 mg/L, K2: 2 mg/L and K3: 3 mg/L). Combination of 2 factor produced 16 treatments and repeated 3 times. The interaction of 2 factor of plant growth regulator was also analyzed.

To know the effect and interaction between 2 factors, data were tested with F test. Further test will be carried out to know the different variance data of each treatments with the DMRT or Duncan Multiple Range Test with significant level at $\alpha = 5\%$.

3. Results and discussion

There were several parameters that observed quantitatively and qualitatively on this research. Quantitative parameters were including shoot appears time, shoot length, number of shoots, number of leaves, callus diameter and callus texture. Meanwhile, the qualitative parameters were including leaf color and callus color.

3.1. The effect of BAP and Kinetin on time of shoots appears, shoots of length, number of shoots and leaves and leaf color

Time of shoot appears was an observational parameter which was an indicator related to the initial response of the expansion to BAP, Kinetin, and the interactions that occur between the two. When these shoots appear, they are marked by the emergence of shoots on the axillary leaves. The buds that appear are observed every week and stop when the shoots are visible in the axillary part.

Table 1. The averages of Indigofera shoots appearance time on BAP and Kinetin treatments

BAP	Kinetin				Averages
	K0	K1	K2	K3	
B0	1.35	1.58	1.34	1.34	1.40
B1	1.31	1.39	1.46	1.54	1.42
B2	1.60	1.42	1.53	1.54	1.52
B3	1.51	1.39	1.50	1.43	1.46
Averages	1.44	1.44	1.46	1.46	

Figure 1. The shoots length of Indigofera in vitro

The adding of BAP or Kinetin did not have a significant effect on the time parameters of Indigofera shoots appearing (Table 1). The result showed that no interaction between BAP and Kinetin added. The mean shoot appears time in all treatments was 1.45 weeks after planting. The shoot appear time were varied from 1.31 week after planting in B1K0 treatment (BAP 1 mg/L) to 1.60 in B2K0 treatment (BAP 1.5 mg/L). However, when the sprouts appeared, they did not have a real effect.

According to Gantait et al [10], the success of in vitro regeneration depends on various aspects such as genetic composition, type of explants, media composition, plant growth regulator and environmental conditions. In this study, it is suspected that the exact composition of plant growth regulator was still unknown to encourage the emergence of Indigofera shoots. Borthakur [11], stated that media with Kinetin caused inhibition of shoot growth of yellow tamarind (*Albizzia lebbeck*). The same result was also reported, in which the adding of BAP and Kinetin did not had a significant effect on the time parameters of yellow Kepok banana shoots (*Musa paradisiaca* L.) [12].

The adding of BAP or Kinetin did not have a significant effect on the length parameters of Indigofera shoots (Table 2, Figure 1). In addition, there was no interaction between BAP and Kinetin. The results showed that shoot length parameter at 2 weeks after planting had an average of 0.85 cm. This value increased, from 1.04 cm at 4 weeks after planting, 1.12 cm at 6 weeks after planting and at the end of the observation at 8 weeks after planting the average shoot length reached 1.20 cm.

Table 2. The averages of Indigofera shoot length (cm) in BAP and Kinetin treatments

Week after planting	BAP	Kinetin				Averages
		K0	K1	K2	K3	
2	B0	0,82	0.84	0.90	0.85	0.85
	B1	0.87	0.91	0.83	0.87	0.87
	B2	0.84	0.84	0.82	0.86	0.84
	B3	0.84	0.91	0.82	0.86	0.86
Averages		0.84	0.88	0.84	0.85	
4	B0	1.01	1.13	1.14	1.09	1.09
	B1	1.09	1.01	0.97	1.06	1.03
	B2	1.02	0.94	0.98	1.05	1.00
	B3	1.01	1.07	0.90	1.09	1.02
Averages		1.03	1.04	1.00	1.07	
6	B0	1.08	1.19	1.25	1.20	1.18
	B1	1.21	1.12	1.04	1.09	1.11
	B2	1.10	1.00	1.10	1.15	1.08
	B3	1.13	1.10	0.94	1.19	1.09
Averages		1.13	1.10	1.08	1.15	
8	B0	1.12	1.24	1.30	1.28	1.23
	B1	1.36	1.15	1.07	1.19	1.19
	B2	1.17	1.08	1.24	1.37	1.21
	B3	1.21	1.18	1.00	1.24	1.16
Averages		1.21	1.16	1.15	1.27	

The increase in shoot length every week indicated that the explants had grown. Growth is defined as a process of increasing size or volume and number of cells, this process occurs irreversibly [13]. The basic media formulation was equipped with vitamins, sucrose, and plant growth regulator gives good results in various plants [14].

BAP gave a significant effect on the parameters of the number of Indigofera shoots at 4, 6, and 8 weeks after planting. Meanwhile, Kinetin did not have a real effect. In addition, there was no interaction between BAP and Kinetin. These data indicated that absence of BAP shows significantly different results. The adding of BAP in B1 (1 mg/L), B2 (1.5 mg/L), and B3 (2 mg/L) did not showed different results.

In 4 weeks, data of explants grown on media without BAP had an average number of shoots of 1.41 shoots/explant on the other hand media with BAP had an average number of shoots 1.68 shoots/explants. This value increased at 6 weeks after planting with an average number of shoots of 1.78 shoots/explant. The development of the number of shoots continued to increase until the end of the observation at 8 weeks after planting with the number of shoots without BAP giving an average shoot of 1.61 shoots/explant and on media added BAP produced an average shoots of 1.92 shoots/explant.

The adding of BAP was more effective to increasing the number of shoots than Kinetin (Table 3). Based on previous research [15], multiplication of shoots of *Amygdalus communis* on MS media added by BA 1 mg/L gave the best results with an average of shoots was 16.1 per explant. According to [16] reported, BAP added 0.75 mg/L on media gave the best results on the number of shoots of *Albizzia odoratissima* with an average of 10 shoots per explant. Meanwhile, Kinetin showed an inability to proliferate shoot multiplication either singly or in combination with BAP. The effectiveness of BAP compared to Kinetin has also been reported in a number of plants such as yellow

trembesi (*Albizzia lebbeck*), *Colutea gifana*, hazelnut, gude bean (*Cajanus cajan* L.), as well as on African Yam Bean (*Sphenostylis stenocarpa*) [16-20].

Table 3. The averages number of Indigofera shoots on BAP and Kinetin concentrations

Week after planting	BAP	Kinetin				Averages
		K0	K1	K2	K3	
2	B0	1.00	1.11	1.00	0.78	0.97
	B1	1.00	1.22	1.11	1.00	1.08
	B2	0.67	1.11	1.00	1.11	0.97
	B3	1.22	1.11	1.11	1.00	1.11
Averages		0.97	1.14	1.06	0.97	
4	B0	1.41	1.31	1.53	1.39	1.41[a]
	B1	1.78	1.72	1.64	1.50	1.66[b]
	B2	1.82	1.64	1.70	1.65	1.71[b]
	B3	1.74	1.75	1.54	1.68	1.68[b]
Averages		1.69	1.60	1.60	1.56	
6	B0	1.41	1.54	1.64	1.39	1.49[a]
	B1	1.81	1.91	1.74	1.50	1.74[b]
	B2	1.96	1.68	1.75	1.85	1.81[b]
	B3	1.83	1.90	1.57	1.84	1.78[b]
Averages		1.75	1.76	1.67	1.64	
8	B0	1.50	1.61	1.86	1.46	1.61[a]
	B1	1.81	1.93	1.78	1.85	1.84[b]
	B2	2.04	1.77	1.95	2.03	1.95[b]
	B3	2.16	1.98	1.75	2.01	1.97[b]
Averages		1.87	1.82	1.84	1.84	

Note: the numbers followed by the same letter in the same row and column showed no difference according to the 5% level of the DMRT test.

However, the result of this average number of shoots with 1.84 shoots/explant, it was still considered less optimal for shoot multiplication. This suboptimal result can be predicted from the inaccurate composition of the addition of exogenous hormones or the poor relationship between BAP and Kinetin. Based on previous research [21], it showed that kinetin had a similar function to BAP which acts as a general signal that regulated protein transcription in plant cell growth and development. BAP activity is known to be stronger than kinetin. So that kinetin can attenuate BAP activity by binding BAP to its receptors.

Regarding Indigofera, a legume plant, previous research [20] states that in other legume plants, NAA combined with BAP provides effective results for shoot multiplication. The administration of BAP together with NAA has been reported on several legume plants and gave the best results such as *Acacia, African Yam Bean*, and *Indigofera viscosa* [20,22,23]. The adding of BAP together with NAA, which is the auxin, is thought to optimize the multiplication of legume shoots.

3.2. The effect of BAP and Kinetin on number of leaves and leaf color

The adding of BAP or Kinetin did not significantly affected of the parameters of the number of Indigofera leaves (Table 4). In addition, there was no interaction between BAP and Kinetin. Observation after 4 weeks showed that the number of leaves reached 1.55 leaves/explant, meanwhile at 6 weeks reached 2.69 leaves /explants and at the last observation reached 3.29 leaves/explants.

The adding of cytokinins did not had a significant effect on leaf number parameters was also reported by [12] which showed that kinetin had no significant effect on the number of in vitro grown of yellow Kepok banana leaves. Meanwhile, [24] stated that the adding of BA also did not had a significant effect on the number of Jatropha leaves.

Table 4. The average number of Indigofera leaves on BAP and Kinetin concentration

Week after planting	BAP	Kinetin				Averages
		K0	K1	K2	K3	
4	B0	1.52	1.69	1.57	1.55	1.58
	B1	1.54	1.58	1.45	1.42	1.50
	B2	1.59	1.45	1.51	1.62	1.54
	B3	1.70	1.64	1.30	1.69	1.58
Averages		1.58	1.59	1.46	1.57	
6	B0	2.44	2.38	2.46	2.48	2.44
	B1	2.90	2.94	3.02	2.26	2.77
	B2	2.72	2.75	2.57	3.33	2.84
	B3	3.06	2.92	1.94	2.96	2.72
Averages		2.77	2.75	2.50	2.76	
8	B0	2.41	2.34	2.85	2.80	2.60
	B1	3.84	3.73	3.27	3.17	3.50
	B2	3.03	2.99	3.93	4.47	3.60
	B3	4.47	3.30	2.80	3.29	3.47
Averages		3.44	3.09	3.21	3.43	

In this study, several explants experienced leaf loss. Leaves that will fall out will initially change color which will become paler and then the segment between the petiole and stem was detached. The fallen leaves will fall on the surface of the media around the explants. Leaf loss certainly affects the leaf number data, making the average number of leaves smaller than the resulting leaves.

Leaf loss on in vitro culture is thought to be due to cytokinins and ethylene gas, both substances have a relationship to the occurrence of leaf loss. According to [25] under certain conditions cytokinins can stimulate ethylene. Lizawati [26] added that leaf loss was related to ethylene production, nutrient deficiency, and toxicity. The effect of ethylene is also thought to cause the small size of the leaves on potato plants in vitro in tightly closed bottles. Harahap [13], reported that ethylene causes several plant responses, such as leaf shedding. Ethylene inhibits growth in an elongated direction (longitudinal) and encourages growth in a transverse direction (transverse) so that the sprouts look swollen. Ethylene also modifies the geotropic response, encouraging the shedding of leaves, flowers, and fruit. To prevent leaf loss, several attempts are needed. The physiological effects of ethylene can be prevented by adding some substances to the culture media such as Silver Thiosulfate (PTS).

Meanwhile, for nutrient deficiency it is necessary to do subcultures at the 4th week after planting. This was thought to be due to an imbalance of endogenous auxin and cytokinin content in plant tissue [26]. Apart from ethylene there is also abscisic acid (ABA) which causes leaf loss. Harahap [13] reported, that leaf shedding occurs because of the abscission process that occurs in the abscission area. The abscission area is a collection of cells found at the base of the petiole. ABA plays an important role in the process of plant growth and development by interacting with other plant growth regulating substances. Usually these interactions are antagonistic in nature. In physical and chemical stress conditions, the ABA content will increase and will be activated after the stress is gone.

Table 5. Color of Indigofera leaves on treatments with BAP and Kinetin concentration

Umur (MST)	BAP	Kinetin			
		K0	K1	K2	K3
4	B0	BYG	SYG	SYG	SYG
	B1	SYG	LYG/BYG/SYG	SYG	SYG
	B2	SYG	SYG	SYG	SYG
	B3	SYG	SYG	BYG	BYG
6	B0	LYG/BYG	SYG	SYG	BYG/SYG
	B1	VYG	LYG	SYG	SYG
	B2	SYG	SYG	BYG	SYG
	B3	SYG	SYG	BYG	LYG/SYG
8	B0	LYG	LYG/BYG/SYG	SYG	SYG
	B1	SYG	SYG	BYG/MYG	SYG
	B2	SYG	SYG	SYG	SYG
	B3	SYG	MYG/SYG	SYG	BYG

Note*:* Determination of leaf color based on Munsell Color Charts guidelines (MCC) For Plant Tissues: LYG: Light Yellow Green, BYG: Brilliant Yellow Green, VYG: Vivid Yellow Green MYG: Moderate Yellow Green, SYG: Strong Yellow Green

Leaf color parameters were observed visually, and statistical data analysis was not performed. Observation of leaf color was carried out based on the guidelines for measuring leaf color suitability with the Munsell Color Charts (MCC) list for Plant Tissues. The result showed that color of leaves from in each treatment varies (Table 5). However, based on these data, media with BAP 1.5 mg/L (B2) produced a darker green color with a dominant color was Strong Yellow Green (SYG). While the lowest green color concentration was seen in the B0K0 treatment (BAP 0 mg/L + Kinetin 0 mg/L) which resulted in the dominant color was Light-Yellow Green (LYG). The resulting color in the B0K0 treatment showed a change in the dominant green color for each week, from Moderate Yellow Green (MYG) to Light Yellow Green (LYG) at 8 weeks after planting. In some treatments, showed the consistency of the dominant color was SYG every week, i.e. B2K0, B3K0, B2K1, B0K2, B1K3, and B2K3 treatments. The dominant leaf color was seen to vary in various treatments. Indriani [27] reported the same case with the research of color variations in Chrysanthemum plant when planted with BA on media. This is due to the chlorophyll content which can affect the green color in the photosynthesis process. The relationship between cytokinins and chlorophyll was also stated by [28], cytokinins can affect the green color in the formation of chlorophyll. For more details, leaf color can be seen in Figure 2.

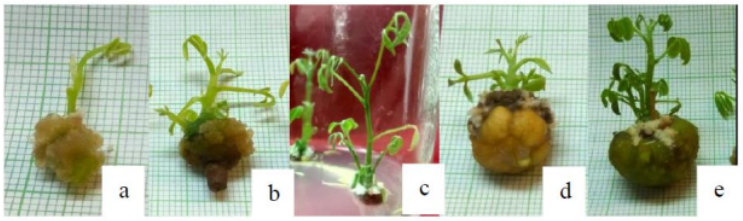

Figure 2. Leaves color: (a) LYG, (b) BYG, (c) VYG, (d) MYG, and (e) SYG

According to [29], the appearance of green on leaves is closely related to chlorophyll content. Changes in chlorophyll pigment can affect leaf color such as changing the color of leaves from greenish yellow to light green to dark green at different levels of leaf development. As leaves age,

chlorophyll content and leaf area increase. However, the older the leaves are, the less their photosynthetic ability will cause damage to chlorophyll even though the leaf area is increasing.

3.3. The effect of BAP and Kinetin on callus diameter, callus textured and callus color

Observation of callus diameter was carried out at the end of the observation at 8 weeks after planting (Figure 3). The measurement of callus diameter was done by placing a soft explant on sterile millimeter block paper. Then the callus formed was photographed with a camera to measure its diameter using Image J, which was Image Analysis software. All callus formed were at the base of the stem, the part of the scar that was in direct contact with the media. This was because callus was formed because of injury to the tissue and response to plant growth regulators. The appearance of callus on the injured part was thought to be due to tissue stimulation on the explants to cover the wound. According to [14], callus consists of an abnormal mass of thin-walled parenchyma cells that are loosely arranged and arise from proliferating stem tissue cells as a result of injury, callus forms at the ends of the cut stems or roots. Based on the data, the adding of BAP or Kinetin on media did not have a significant effect on the Indigofera callus diameter and no interaction between BAP and Kinetin for callus diameter. The callus that was formed had an average diameter of ± 1.34 cm.

Figure 3. Diameter of callus on B3K0 media

Callus texture parameters were observed visually from the callus formed on the explants. Observations were made at 8 weeks after planting. The texture of the callus formed was given a score of 1 for compact callus, 2 for intermediate callus, and 3 for crumb callus.

The adding of BAP was showed significant effect on the texture of Indigofera callus (Table 6, Figure 4). Meanwhile, Kinetin did not show any significant effect and there was no interaction between BAP and Kinetin. These data indicated that BAP with B1 treatment (1 mg/L) resulted an average value of 1.44; with B2 (1.5 mg/L) treatment resulted an average value of 1.36; and with B3 treatment (2 mg/L) resulted in an average value of 1.33. Meanwhile, the treatment without BAP produced an average value of 1.58. This indicated that callus growing on media without BAP was crumbly when compared to media that is nourished by BAP. At B2 and B3 media, callus textures were more compact than those of B1 and B0 media.

Almost all explants were formed callus at the bottom of the stem. The callus formed was compact, intermediate, and crumb callus. Compact callus can be clearly seen from the regular round lumps. The callus feels hard when taken using tweezers. For crumb callus, it was more irregular in shape and feels soft when taken with tweezers. While the intermediate callus was a mixture of the two textures, there were a compact callus and a crumb in one callus lump. According to [14], compact callus is a callus whose growth is lignified and has a coarse texture, while callus that is easily fragmented is termed crumb callus.

Table 6. Average Indigofera Callus Texture on treatment of BAP and Kinetin concentration

BAP	Kinetin				Averages
	K0	K1	K2	K3	
B0	1.22	1.74	1.71	1.63	1.58[a]
B1	1.46	1.54	1.53	1.22	1.44[b]
B2	1.34	1.35	1.39	1.34	1.36[c]
B3	1.31	1.31	1.43	1.27	1.33[c]
Averages	1.34	1.49	1.51	1.37	

Note: the numbers followed by the same letter in the same row and column showed no difference according to the 5% level of the DMRT test.

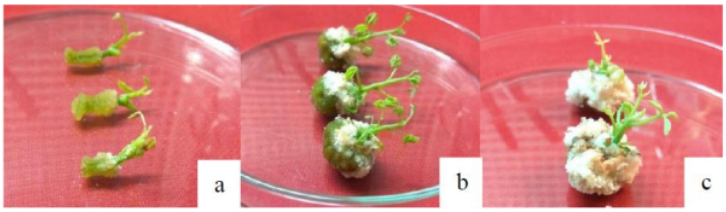

Figure 4. Callus Texture (a) Compact in B0K0 media, (b) Intermediate in B2K3 media and (c) Crumbs in B1K2 media

Callus texture is one of the markers used to assess the growth of a callus and its quality [30], [28]. The criteria for a good callus depend on the purpose. According to [30], callus which has a compact texture is considered good for the purpose of producing secondary metabolites because it can accumulate more secondary metabolites. Meanwhile, Andaryani [28] states that a good callus is one that has a crumb texture. This is because the crumbly texture of the callus facilitates separation into single cells in suspension culture and increases oxygen aeration between cells. Thus, this texture can be attempted to multiply the number of callus through suspension culture more easily. Both compact, intermediate, and crumb calluses have one characteristic in common. From a functional point of view, the most important characteristic of callus is that the abnormal growth has the potential to develop normal roots, shoots and embryoids that can form plants [14].

Callus color were observed visually, and no statistical analysis was performed (Table 7, Figure 5). Observation of leaf color was carried out by measuring criteria based on callus color match with the Munsell Color Charts (MCC) For Plant Tissues color list same with leaves color.

The indicator of explant growth on in vitro culture is callus color. Callus color can describe the visual appearance of the callus. So that later it can be seen whether a callus still has cells that are actively dividing or have died. The callus tissue produced from an explant usually showed different colors [28]. The average callus color produced in the entire study was Moderate Yellow Green (MYG). The adding of BAP was considered to have an average dark green color compared to Kinetin, while callus on kinetin media tends to be more yellowish. In the treatment conditions without giving plant growth regulator (B0K0,) callus was resulted the yellow color (Y). The interaction between BAP and Kinetin were also shown, the green color was more intense when Kinetin was combined with BAP than without BAP. The higher cytokinin content, the color of the leaves will be the darker green. The solid green color was classified as Strong Yellow Green (SYG).

Table 7. Callus color in media with BAP and Kinetin treatments

BAP	Kinetin			
	K0	K1	K2	K3
B0	Y	OY	MYG	Y
B1	MYG	MYG/Y/SYG	MYG	SYG
B2	MYG	MYG/O/BYG	MYG	MYG/SYG
B3	SYG	MYG	SYG	SYG

Note: Determination of callus color based on Munsell Color Charts guidelines (MCC) For Plant Tissues: Y: Yellow, O: Olive, OY: Olive Yellow, BYG: Brilliant Yellow Green, MYG: Moderate Yellow Green, SYG: Strong Yellow Green

The increase in callus color was getting darker (SYG) due to the role of exogenous cytokines added to the media, in this case in with BAP and Kinetin. This study showed that cytokinins can influence chlorophyll formation in callus. Andaryani also [28] reported that the green color of callus was a result of the effect of cytokinins in the formation of chlorophyll. The difference in the color of the callus that appears showed that the level of callus development was different. Meanwhile, a good quality callus had a green color.

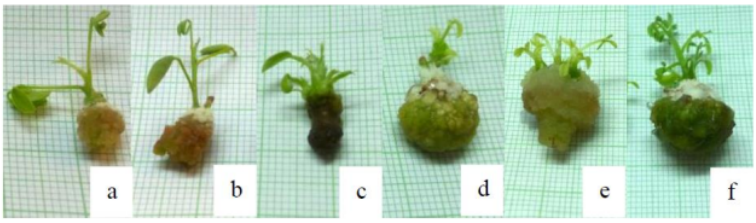

Figure 5. Leaf Color (a) Y, (b) OY, (c) O, (b) BYG, (e) MYG, and (e) SYG

4. Conclusion

Adding BAP at any concentration gave the best results for the parameters of the number of shoots at 4, 6, and 8 weeks after planting. While the lowest number of shoots was produced in the media without BAP. The application of BAP had a significant effect on the callus texture. Crumb callus was produced in B0 media and compact callus was produced in B2 and B3 media. In the parameters of shoot appears time, shoot length, number of leaves, and callus diameter, BAP was not significantly different. There was no interaction between BAP and Kinetin for all parameters observed.

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