

# In Vitro Propagation and Sulforaphane Content Analysis *Brassica oleraceae* var. Botrytis with Treatment Kinetin and Methionine

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**Abstract:** *Brassica oleraceae* var. *Botrytis* is a vegetable plant that is quite popular in Indonesia which has high economic value because it contains sulforaphane which has benefits to prevent cancer. This research aims to obtain the best concentration of kinetin and methionine in in vitro culture *Brassica oleraceae* var. *Botrytis*, and obtain the best concentration of kinetin and methionine for enhancement of sulforaphane from in vitro culture *Brassica oleraceae* var. *Botrytis*. The results showed that the concentration to obtain the best amount of shoot and leaf number was in the treatment of 5 mg/L kinetin without methionine, while the concentration to obtain the best plant height on the treatment of 100 mg/L methionine without kinetin, and the best plant fresh weight was at treatment combination of 10 mg/L kinetin and 50 mg/L methionine. The plant fresh weight of *Brassica oleraceae* var. *Botrytis* is negatively correlated with sulforaphane content in it. The treatment of 50 mg/L of methionine without kinetin was the best treatment in producing a high sulforaphane content of 47.7 ng/g wet weight.

**Keywords:** sulforaphane, kinetin, methionine, *Brassica oleraceae* var. *Botrytis*, culture in vitro

## 1. Introduction

White flower cabbage has an important role for human health, because it contains vitamins and minerals are needed by the body, so the demand for vegetables is increasing. Cabbage is included in cabbage or *Brassicaceae*. *Brassicaceae* vegetables contain many types of compounds sianohidroksibutena and sulforaphane. Sulforaphane serves to prevent cancer. In addition, this class of vegetables contain high vitamin C which can function as an antioxidant [1].

Sulforaphane is formed from methionine and glucoraphanin which is a precursor or precursor to sulforaphane [2]. The initial precursor in the synthesis of sulforaphane from *cruciferaeae* is methionine. The final precursor according to the synthetic line of the sulforaphane substance is glucoraphanin [3]. This can increase the production of sulforaphane.

*In vitro* propagation (*in vitro* culture) has a broad sense that is a method to isolate parts of plants such as protoplasm, cells, a group of cells, tissues, and organs, and grow it in aseptic conditions, so that parts can multiply and generation in to complete plant again [4]. The use of this method has the advantage of being able to produce a culture free of contamination because it is carried out under controlled environmental conditions and is independent of the climate and soil conditions. Another advantage is that cells can be easily propagated to produce specific metabolites [5].

Kinetin is a class of cytokinin hormones. The main function of cytokinin is to stimulate cell division and organ formation. Some of these proteins can be the enzymes needed in mitosis [6]. Cytokines are often added kinetin, zeatin and benzilaminopurin (BAP). Kinetin and BAP are resistant to degradation and are cheaper. In this study used kinetin as growth regulator substances of cytokines [7].

The results of Tilaaret al, (1990) [17]. showed that the growing hypocotyl response and broccoli shoots collected on the best MS medium in shoot induction was at a concentration of 5 mg/L BAP without NAA and a combination of NAA 0.01 ppm and 2.5 ppm BAP produce 2-7 shoots. Further research results from Tilaaret al. (2012), sulforaphane content in broccoli (*Brassica oleraceae* L var. *Italica*) given combination of methionine treatment 0, 50, 100, and 150 mg/L with broccoli seed extract 0, 1, 2, and 3 gram, the highest was in combination treatment of 100 mg/L methionine and 1 gram of broccoli seed extract that is 182.09 ng/g, followed by treatment of 100 mg/L of methionine without broccoli seed extract of 162.89 ng/g. The highest sulforaphane content in the treatment of 100 mg/L methionine.

Based on this research, this research is done by combining it in one stage that is treatment with combination of growth regulator kinetin and amino acid metionin which is sulforafan precursor, using the White Flower Cabbage (*Brassica oleraceae* var. *Botrytis*), aimsto obtain the best concentration of Kinetin and Methionine in the formation of shoots from *in vitro* culture of white flower cabbage (*Brassica oleraceae* var. *Botrytis*), and to get the best concentration from Kinetin and Methionine to increase sulforaphane from in vitro culture Flower Cabbage White (*Brassica oleraceae* var. *Botrytis*).

## 2. Research Methods

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This research used factorial design in completely randomized design, with 9 treatment combinations of kinetin with methionine. Kinetin with concentration of 0 mg/L, 5 mg/L, and 10mg/L, while the concentration of Metionin is 0 mg/L, 50 mg/L, and 100 mg/L. *In vitro* propagation each treatment consisted of 5 replications, whereas in the analysis of sulforaphane content each treatment consisted of 3 replications.

The variables observed were: number of shoots, number of leaves, plant height, plant fresh weight, and sulforaphane content. Data were observed in the fourth week after subculture. The data were analyzed by various analysis and continued with 5% BNT test. Each treatment is done sulforafan analysis.

Data obtained, tabulated and analyzed statistically for conclusions. Data of research result is presented in table, percentage, graph or curve according to the type of data obtained in this research. The statistical test to find out the difference of sulforafan content in shoots and medium is done by analysis of variation (ANOVA) in Completely Randomized Design that is arranged factorially. If different then proceed with 5% BNT test.

### 3. Results and Discussion

Based on the observations, it was shown that the addition of kinetin and methionine to the white flower cabbage *in vitro* culture medium with different concentration level gave different effect to some observation parameters. The parameters consist of number of shoots, number of leaves, plant height, plant fresh weight, and sulforaphane content.

#### 3.1. Number of Shoots

The highest number of shoots was in the combination of K<sub>5</sub> M<sub>0</sub> or Kinetin 5 mg/L treatment without Methionine, which was on average 15 shoots (Tables 1 and Fig. 1A), followed by a combination of K<sub>5</sub> M<sub>100</sub> and K<sub>10</sub> M<sub>100</sub> that was an average of 13 shoots. The lowest number of shoots is in the control treatment or K<sub>0</sub> M<sub>0</sub> which is an average of 6.40 shoots (Table 1 and Figure 1B).

Table 1 shows that for the highest number of shoots in the Kinetin treatment at 5 mg/L kinetin treatment and decreased at the treatment of 10 mg / L. This decrease in the number of shoots may be due to high Kinetin concentration and high endogenous cytokinin content causes no longer optimal in stimulation of shoot induction. In contrast to the combination treatment of Kinetin and Methionine the higher the concentration of the increasing number of shoots. Single treatments of Methionine without Kinetin showed a small number of shoots and was close to the number of untreated shoots (K<sub>0</sub> M<sub>0</sub>). The number of shoots is very low in the treatment without Kinetin, this is in accordance with Winarsihet *al.*, (1998) [8] suggests that cytokines (kinetin) can spur shoot growth. Even if the availability of cytokines in the culture medium is so limited, cell division in cultured tissue is inhibited

#### 3.2 Number of Leaves

The highest number of leaves was in the combination of K<sub>5</sub> M<sub>0</sub> treatment which was 14.20 leaf average, followed by the combination of K<sub>10</sub> M<sub>100</sub> treatment which was 11.60 leaf average, and the combination of K<sub>5</sub> M<sub>50</sub> and K<sub>5</sub> M<sub>100</sub> treatment which was 10.0 leaf average, while the lowest leaf number was the combination of control treatment is an average of 4 leaves. This can be seen in Table 1 and Figure 1.

Table 1 shows that in the control treatment (K<sub>0</sub> and M<sub>0</sub>) did not differ significantly with the combination of 0 mg/L Kinetin and 50 mg/L Methionine (K<sub>0</sub> and M<sub>50</sub>), and a combination of 0 mg/L Kinetin and 100 mg/L Methionine (K<sub>0</sub> and M<sub>100</sub>) in effect on the number of leaves. In the combination treatment of 5 mg/L Kinetin without Methionine (K<sub>5</sub> and M<sub>0</sub>) was significantly different from the number of leaves in the combination treatment of 5 mg/L Kinetin and 50 mg/L Methionine (K<sub>5</sub> and M<sub>50</sub>), and a combination of 5 mg/L Kinetin and 100 mg/L Methionine (K<sub>5</sub> and M<sub>100</sub>). The highest number of leaves is in combination treatment of K<sub>5</sub> and M<sub>0</sub>. Results on the combined treatment of K<sub>5</sub> and M<sub>0</sub> were more leaves than in other K<sub>5</sub> combination treatments. Combination treatment 5 mg/L Kinetin without Methionine is the best to get a lot of leaves. The condition is thought to be due to the added concentration of growth regulating kinetin substances. If the availability of cytokinin in medium culture is too high then cell division in tissue cultured becomes obstructed. However, if the tissue is subcultured on a medium with optimal cytokinin content then cell division can take place faster and plantlet growth can take place optimally [9].

**Table 1.** Mean and Standard Deviation Result of Observation Number of Plants, Number of Leaves, Plant Height (cm), Fresh Weight (g wet weight), Sulforaphane Content (ng/g wet weight) from *in vitro* Culture *Brassica oleraceae* var. Botrytis after treatment of Kinetin (K) 0, 5, 10 mg/L and Methionine (M) 0, 50, 100 mg/L.

No	Treatments	Mean and Standar Deviation Result of Observation				
		Number of Shoots	Number of Leaves	Plant Height (cm)	Fresh Weight (gr)	Sulforaphane (ng/g)
		Mean ±SD	Mean ±SD	Mean ±SD	Mean ±SD	Mean ±SD
1	K0 M0	6.40 ± 0.89 <sup>a</sup>	4.40 ± 1.67 <sup>a</sup>	2.58 ± 0.60 <sup>a</sup>	0.40 ± 0.38 <sup>a</sup>	0.50 ± 0.05 <sup>a</sup>
2	K0 M50	7.00 ± 1.22 <sup>a</sup>	4.80 ± 1.30 <sup>a</sup>	3.52 ± 0.73 <sup>a</sup>	0.12 ± 0.05 <sup>a</sup>	4.77 ± 2.16 <sup>b</sup>
3	K0 M100	7.00 ± 2.45 <sup>a</sup>	6.40 ± 1.95 <sup>a</sup>	8.16 ± 3.44 <sup>c*</sup>	0.67 ± 0.30 <sup>a</sup>	1.52 ± 0.11 <sup>a</sup>
4	K5 M0	15.00 ± 2.24 <sup>d*</sup>	14.20 ± 2.86 <sup>c*</sup>	3.82 ± 1.08 <sup>ab</sup>	3.84 ± 1.00 <sup>b</sup>	0.47 ± 0.06 <sup>a</sup>
5	K5 M50	11.80 ± 4.49 <sup>bc</sup>	10.00 ± 3.32 <sup>b</sup>	4.38 ± 1.17 <sup>ab</sup>	3.72 ± 1.18 <sup>b</sup>	0.31 ± 0.01 <sup>a</sup>
6	K5 M100	13.00 ± 2.45 <sup>cd</sup>	10.00 ± 2.12 <sup>b</sup>	4.34 ± 0.79 <sup>ab</sup>	3.78 ± 1.30 <sup>b</sup>	0.42 ± 0.09 <sup>a</sup>
7	K10 M0	6.40 ± 0.55 <sup>a</sup>	4.40 ± 0.55 <sup>a</sup>	4.46 ± 0.71 <sup>ab</sup>	5.60 ± 2.83 <sup>c</sup>	0.67 ± 0.07 <sup>a</sup>
8	K10 M50	9.20 ± 2.17 <sup>ab</sup>	6.80 ± 2.39 <sup>a</sup>	5.66 ± 2.19 <sup>b</sup>	5.61 ± 4.69 <sup>c</sup>	0.32 ± 0.04 <sup>a</sup>
9	K10 M100	13.00 ± 2.24 <sup>cd</sup>	11.60 ± 2.07 <sup>c</sup>	4.66 ± 0.71 <sup>ab</sup>	5.60 ± 2.83 <sup>c</sup>	0.32 ± 0.04 <sup>a</sup>

Note: The numbers followed by different letters, significantly different from the BNT 5% TEST  
 \* highest results

### 3.3 Plant Height

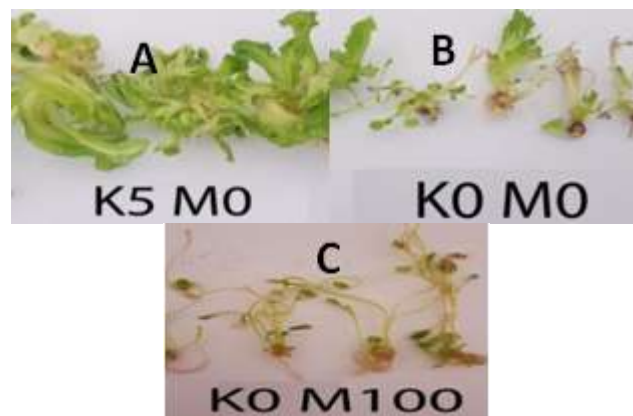
Based on the result of the research, the highest plant height was in combination of treatment K<sub>0</sub> M<sub>100</sub> (Kinetin 0 mg/L and Metionin 100mg/L) that is average 8.16 cm (Figure 1C), then combination of treatment K<sub>10</sub> M<sub>50</sub> that is equal to 5.66 cm and followed by combination the treatment of K<sub>10</sub> M<sub>0</sub> and K<sub>10</sub> M<sub>100</sub> is an average of 4.46 cm, while the lowest is in the control treatment (K<sub>0</sub> M<sub>0</sub>) which is an average of 2.58 cm (Figure 1B). The higher the concentration of Methionine given the higher the plant height.

Table 1 shows that for plant height in control treatment (K<sub>0</sub> and M<sub>0</sub>) did not differ significantly with treatment without Kinetin and 50 mg/L Methionine (K<sub>0</sub> and M<sub>50</sub>), but significantly different from treatment without Kinetin and 100 mg/L Methionine (K<sub>0</sub> and M<sub>100</sub>). In combination with 5 mg/L Kinetin without Methionine (K<sub>5</sub> and M<sub>0</sub>) was not significantly different with plant height in combination treatment of 5 mg/L Kinetin and 50 mg/L Methionine (K<sub>5</sub> and M<sub>50</sub>), and combination treatment 5 mg/L Kinetin and 100 mg/L of Methionine (K<sub>5</sub> and M<sub>100</sub>). Increasing the number of shoots in this combination of treatments is not followed by the increase in plant height, this is in line with the opinion Handayani, (2000)[10], that if the shoots produced more, there will be competition between shoots in fighting for food that will disrupt the process of growth of the shoots, eventually the elongation of the buds is running slowly.

### 3.4 Plant Fresh Weight

The results of the analysis of variance showed no interaction between Kinetin and Methionine even single Methionine was not significantly different to the fresh weight of the plant, but the single Kinetin treatment of 0 mg /L, 5 mg /L and 10 mg /L was significantly different in its effect on fresh weight of white flower cabbage plants *in vitro* cultures (Table 1). The highest fresh weight was on Kinetin 10 mg /L treatment while the lowest was in Kinetin without treatment. The higher the Kinetin concentration the higher the fresh weight of the plant. This may be due to the shoots formed in the treatment without Kinetin and some other treatment combinations having small stems.

Treatment K<sub>0</sub> and M<sub>100</sub> is the highest yielding treatment of plants, but has small shoots and the number of shoots and leaves are small. The combination of Kinetin 10 mg/L and Methionine 50 mg/L (K<sub>10</sub> M<sub>50</sub>) was the combination of treatments that produced the largest fresh weight of plants (Table 1 and Figure 2A), which was an average of 5.61 grams, followed by a combination of K<sub>10</sub> M<sub>0</sub> and K<sub>10</sub> M<sub>100</sub> the average of 5.60 grams, while the lowest is on the combination treatment K<sub>0</sub> M<sub>50</sub> is an average of 0.12 grams (Figure 2B). According to Pierik (1987)[11] and Wattimena (1998)[12] that cytokines are used in culture to stimulate the growth and development of plants. Hess, (1975)[13] states that cytokinin works in stimulating cell division and shoot organ formation. Based on this, it is concluded that Kinetin as a cytokinin group is very encouraging to the addition of fresh weight in terms of cell division and shoot budding. So the more shoots means the division of many cells and affect the fresh weight of the plant.



**Figure 1:** Appearance of *in vitro* culture *Brassica oleraceae* var. Botrytis in the treatment of Kinetin 5 mg /L without methionine (A), treatment (control) (B), and treatment without kinetin and methionine 100 mg /L (C)

### 3.5 Sulforaphane Content

The content of sulforaphane analyzed by LCMS (Liquid Chromatography Mass Spectrometer) is the largest in the treatment of K<sub>0</sub> M<sub>50</sub> (Kinetin 0 mg/L and Metionin 50 mg/L) which is average of 4.77 ng / g wet weight (ww), then 1.52 ng/g ww at the treatment of K<sub>0</sub> M<sub>100</sub>, while the lowest is on the treatment K<sub>10</sub> M<sub>50</sub> and K<sub>10</sub> M<sub>100</sub> that is equal to 0.04 ng/g ww. This shows that the treatment without Kinetin and 50

mg/L Methionine is the best combination to produce a higher sulforaphane content. Table 1 shows that the highest sulforaphane content was treated without Kinetin and 50 mg/L Methionine, and was significantly different from other combinations of treatments. Pandiangan (2010)[14] states that the addition of prazat (prekursor) is one of the ways to increase secondary metabolite content in plant tissue culture, for example anticancer activity of catarantin on Mammary Mouse cells[15], growth and cation content of *Catharanthusroseus* cell aggregate cultures given tryptophan[14]. According to Ding, *et al* (2006)[2], Sulforafan is formed from methionine and glucorafan which is a precursor or precursor to sulforaphan. The earliest precursors in sulforaphane synthesis in the *Cruciferaeae* plant are Methionine[3]. In a single treatment of Methionine, the best concentration for producing high sulforaphane content is 50 mg/L, wherein sulforaphane content at 100 mg/L Methionine is lower when compared with 50 mg/L Methionine but higher than treatment without Methionine. This suggests that the appropriate concentration of Methionine to produce high sulforaphane content is 50 mg/L, if added concentration can suppress sulforaphane synthesis.

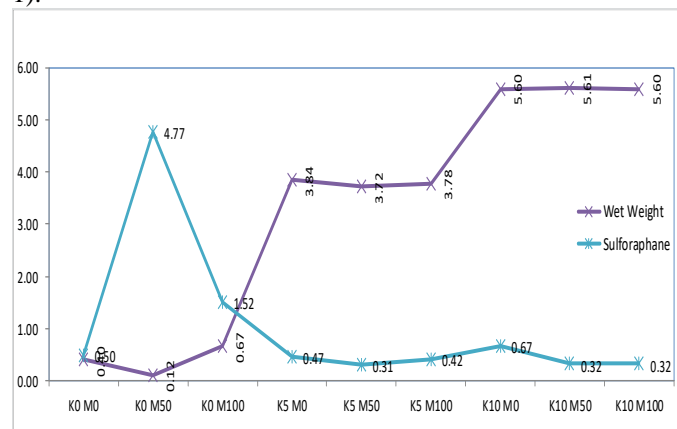


**Figure 2:** *In vitro* culture appearance of *Brassica oleraceae* var. Botrytis on minetin 10 mg/L and methionine 50 mg/L (A) treatment without kinetin and methionine 50 mg/L (B) treatment with kinetin and methionine 50 mg/L

In contrast to the results of Tilaaret *al*, (2012)[16], the sulforaphane content of *Brassica oleraceae* L var. Italica given combination of methionine treatment 0, 50, 100, and 150 mg/L with broccoli seed extract 0, 1, 2, and 3 gram, the highest was in combination treatment of 100 mg/L methionine and 1 gram of broccoli seed extract that is 182.09 ng/g, followed by treatment of 100 mg/L of methionine without broccoli seed extract of 162.89 ng/g. The highest sulforaphane content in the treatment of 100 mg/L methionine, with very high sulforafan content. The best concentration differences of methionine and sulforaphane content may be due to differences in varieties used although still in one species of *Brassica oleraceae*. It can be concluded that the best concentration of methionine to produce sulforaphane in Broccoli (*Brassica oleraceae* L var Italica) is 100 mg/L while in the White Flower Cabbage (*Brassica oleraceae* L var Botrytis) 50 mg/L, and the sulforafan content of Broccoli (*Brassica oleraceae* L var Italica) is higher than the sulforaphane content of the White Flower Cabbage (*Brassica oleraceae* L var. Botrytis).

When it is linked between sulforaphane content and fresh weight of the plant, it is seen that the treatment with the smallest fresh weight of 0.5g is the highest sulforaphane content of 4.77 ng/gww, ie in the combination treatment without Kinetinand 50 mg/L Methionine (K<sub>0</sub> and M<sub>50</sub>). While the combination treatment of 10 mg/L Kinetin and 50

mg/L Methionine (K<sub>10</sub> M<sub>50</sub>) which has the largest fresh weight of 5.61 grams yields the lowest sulforaphane content of 0.32 ng/gww. This suggests that fresh weight of *Brassica oleraceae* is negatively correlated the sulforaphane content in it. The higher the fresh weight the lower the sulforaphane content is produced, and the lower the fresh weight the higher the resulting sulforaphane content (Fig. 3 and Table 1).



**Figure 3:** The Fresh Weight of Plant and Sulforaphane *Brassica oleraceae* var. Botrytis for Kinetin (K) 0, 5, 10 mg/L and Methionine (M) 0, 50, 100 mg/L *in vitro* cultures.

#### 4. Conclusions

Based on the results of research that has been done, it can be concluded several things:

1. *In vitro* propagation of White Flower Cabbage (*Brassica oleraceae* var. Botrytis), to obtain the best number of shoots and leaf counts is in combination treatment of 5 mg/L Kinetin without Methionine (K<sub>5</sub>M<sub>0</sub>), while to obtain the best plant height available in the combination treatment without Kinetin and 100 mg/L Methionine (K<sub>0</sub> M<sub>100</sub>), and Fresh Weight is best in combination treatment of 10 mg/L Kinetin and 50 mg/L Methionine (K<sub>10</sub> M<sub>50</sub>). The interaction of Kinetin and Methionine showed a significant difference to the number of shoots, the number of leaves, the height of plants, and the content of Sulforaphane, but not significantly different from the fresh weight of plants
2. The fresh weight of the plant in negatively correlated with the sulforaphane content of the White Flower Cabbage (*Brassica oleraceae* var. Botrytis). Treatment without Kinetin and 50 mg/L Methionine (K<sub>0</sub> M<sub>50</sub>) was the best treatment in producing high sulforaphane content (4.77 ng/gww) was the combination treatment with the lowest fresh weight (0.12g), The combination of 10 mg/L Kinetin and 50 mg/L Methionine (K<sub>10</sub> M<sub>50</sub>) is the lowest combined treatment in yielding sulforaphane (0.32 ng/gww) is the combined treatment with the largest fresh weight of the plant (5.61g).

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