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The Growth of Red Spinach (*Amaranthus tricolor* L.) After Application of Bean *Sprouts* Extract [*Vigna radiata* (L.) R. Wilczek] on *Murashige and Skoog* Medium *In Vitro*

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Abstract *– Red spinach (Amaranthus tricolor L.) is a commercially valuable vegetable known for its high nutritional content and immune-boosting properties, making it popular among the Indonesian population. However, red spinach cultivation in Indonesia is still relatively low and inconsistent, necessitating efforts to enhance its cultivation. One potential approach is through in vitro culture techniques. This study aimed to investigate the impact of applying mung bean sprout [Vigna radiata (L.) R. Wilczek] extract on Murashige and Skoog medium, focusing on identifying the most effective concentration for in vitro growth of red spinach explants. The experimental design employed a Completely Randomized Design (CRD) with five concentration levels: 0%, 5%, 10%, 15%, and 20%. Each concentration was replicated five times, with each replication consisting of five red spinach seeds in individual culture bottles. Data analysis involved Levene's test, one-way ANOVA, and Tukey's Honestly Significant Difference (HSD) test at a significance level of 5%. The findings revealed that the application of mung bean sprout extract significantly influenced the height of plantlets, leaf count, root length, fresh weight, and soluble carbohydrate content of red spinach. The most effective concentration for promoting red spinach explant growth was determined to be 5%.*

Keywords: *cultivation, growth, in vitro, mung bean sprout extract, red spinach*

INTRODUCTION

Red spinach (*Amaranthus tricolor* L.) is a vegetable commodity of high commercial value in Indonesia due to its exceptional nutritional content and potential to enhance human endurance (Puspita et al., 2021). This leafy green vegetable is rich in essential vitamins, fiber, carotenoids, chlorophyll, alkaloids, flavonoids, and saponins in its leaves, as well as polyphenols in its stems. Additionally, it contains vital minerals such as manganese, calcium, phosphorus, and iron, making red spinach a popular choice among health-conscious individuals in Indonesia (Pradana et al., 2021).

Despite its promising potential, red spinach cultivation in Indonesia faces challenges, resulting in low and unstable production levels (Batubara et al., 2022; Khusni et al., 2018). These challenges arise from factors such as genetic variations, environmental influences, and suboptimal management practices, which contribute to the limited success in cultivating this crop (Puspita et al., 2021). To address these issues and improve the efficiency of red spinach cultivation, researchers have turned their attention to in vitro techniques in the field of crop cultivation. In vitro culture techniques offer the advantage of producing high-quality, disease-free seedlings in large quantities, independent of seasonal constraints, thereby fostering increased red spinach production (Budi, 2020).

The successful implementation of in vitro plant culture relies heavily on the selection of an appropriate growth medium. The medium must provide sufficient macro and micro nutrients, such as the well-known Murashige and Skoog medium (Purwanto et al., 2007). Moreover, the effectiveness of in vitro techniques is influenced by the presence of Plant Growth Regulators (PGRs) in the medium. Among them, the auxin hormone plays a crucial role in promoting rapid root, shoot, and leaf formation in plants (Sulasiah, 2015). However, the current use of synthetic auxin as a PGR comes with a considerable cost, posing a burden on farmers. Therefore, there is a pressing need to explore alternative PGRs derived from organic sources, offering comparable benefits to their synthetic counterparts (Tanjung and Darmansyah, 2021).

Bean sprout extract has been identified as one such organic source containing significant concentrations of PGR compounds, including auxin (1.68 mg/L), gibberellin (39.94 mg/L), and cytokinin (96.26 mg/L) (Ulfa, 2014). The extract contains auxins in the form of Indole Acetic Acid (IAA) at 3.74% and Indole-3-Butyric Acid (IBA) at 1.88% (Sunandar et al., 2017). Studies have indicated that bean sprout extract exhibits comparable effects to IBA in stimulating root growth (Rochmah and Rahayu, 2021) and has shown promising results in promoting potato root growth at a concentration of 20 g/L (Fadhillah, 2015). Furthermore, research by Erhani et al. (2020) demonstrated that the addition of bean sprout extract at a concentration of 10% in Murashige and Skoog medium effectively optimized planlet height, leaf count, wet and dry weights of soybean planlets. In light of these findings, this study aims to investigate the impact of bean sprout extract [*Vigna radiata* (L.) R. Wilczek] on Murashige and Skoog medium and determine the most effective concentration for the in vitro growth of red spinach explants.

RESEARCH METHODS

This study employed an experimental design and was conducted using a Completely Randomized Design (CRD) with one factor, which was the concentration of bean sprout extract. The experiment consisted of 5 concentration levels: 0%, 5%, 10%, 15%, and 20%, with 5 replications. The research was conducted between February and April 2023 in the In Vitro Culture room, Botany Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, Lampung University.

The tools and materials used in the study included an ESCO brand Laminar Air Flow (LAF), an autoclave, an analytical balance, a scalpel, Petri dishes, Erlenmeyer flasks, a UV/Vis spectrophotometer, seeds of red spinach (Amaranthus tricolor L.), bean sprout extract, Murashige and Skoog medium, agar, sugar, distilled water, 96% alcohol, 70% alcohol, aluminum foil, Whatman No.1 filter paper, bayclin, phenol, H2SO4, potassium hydroxide (KOH), and hydrochloric acid (HCl).

The research was carried out in several stages. The first stage involved the sterilization of equipment. All the tools used in the study were washed with detergent and rinsed with water, then wrapped in umbrella paper and autoclaved at 121°C and 1 atm pressure for 30 minutes to achieve sterilization. Afterward, the treatment medium was prepared. The Murashige and Skoog medium was prepared with the addition of bean sprout extract at concentrations of 0%, 5%, 10%, 15%, and 20%. The medium was then autoclaved at 121°C and 1 atm pressure for 15 minutes to ensure its sterilization. The sterilized medium was left on a culture rack for 3 - 4 days before use to verify the absence of contaminants.

With the medium ready, the next step was to plant the red spinach explants. Planting of red spinach seed explants was done on the treatment medium according to the specified concentrations. The process took place within the Laminar Air Flow. Firstly, 250 red spinach seeds were soaked in a 10% bayclin solution for 5 minutes, followed by a 3-minute soak in a 70% alcohol solution. After rinsing with distilled water, the seeds were transferred into Petri dishes. The seeds were then planted in the treatment medium containing the appropriate concentration of bean sprout extract. Each culture bottle was planted with 5 red spinach seeds, resulting in a total of 125 seeds distributed among 25 culture bottles. The red spinach seeds were allowed to grow into planlets on Murashige and Skoog medium. The cultures were incubated in a room with approximately 1000 lux of light intensity, 24 hours/day, and at a temperature of approximately 20°C for 3 weeks.

Throughout the study, several variables were observed, including the percentage of live planlets, planlet height, number of leaves, root length, wet weight, and analysis of soluble carbohydrate content. The percentage of live planlets, planlet height, and number of leaves were observed periodically every three days over the three-week planting period. In contrast, root length, wet weight, and soluble carbohydrate content analysis were conducted on the last day after three weeks of planting. The analysis of carbohydrate content was performed using the phenol-sulfur method (Dubois et al., 1956) with a UV spectrophotometer.

The data obtained from the growth of red spinach explants with the addition of bean sprout extract on Murashige and Skoog medium in vitro was in the form of quantitative data. The quantitative data for each variable were homogenized using the Levene test at a significance level of 5%, followed by One Way ANOVA analysis at a significance level of 5%. In the event of significant differences, the Honest Real Difference (BNJ) test was conducted at a significance level of 5%.

RESULTS AND DISCUSSION

The characterisation results of applying bean sprout extract on Murashige and Skoog medium and determining the most effective concentration for the in vitro growth of red spinach explants can be obtained by observing several variables. The primary variable is the percentage of living planlets. The observations on the percentage of living planlets are presented in Table 1 as follows.

Table 1. Percentage of the number of live red spinach planlets

Extract Concentration Bean sprouts (%)	Percentage Number of Live Explants at Week - (%)		
	I	II	III
0	100	100	100
5	100	100	100
10	100	100	100
15	100	100	100
20	100	100	100

Table 1 reveals that the percentage of live planlets, after three weeks of planting and applying the five different treatments with concentrations of bean sprout extract (0% as control, 5%, 10%, 15%, and 20%), generally indicates a positive impact on the growth of red spinach planlets. Notably, all concentrations of bean sprout extract have shown favorable effects. Additionally, Figure 1 illustrates the development of red spinach explants into 3-week-old planlets after being treated with various concentrations of bean sprout extract.

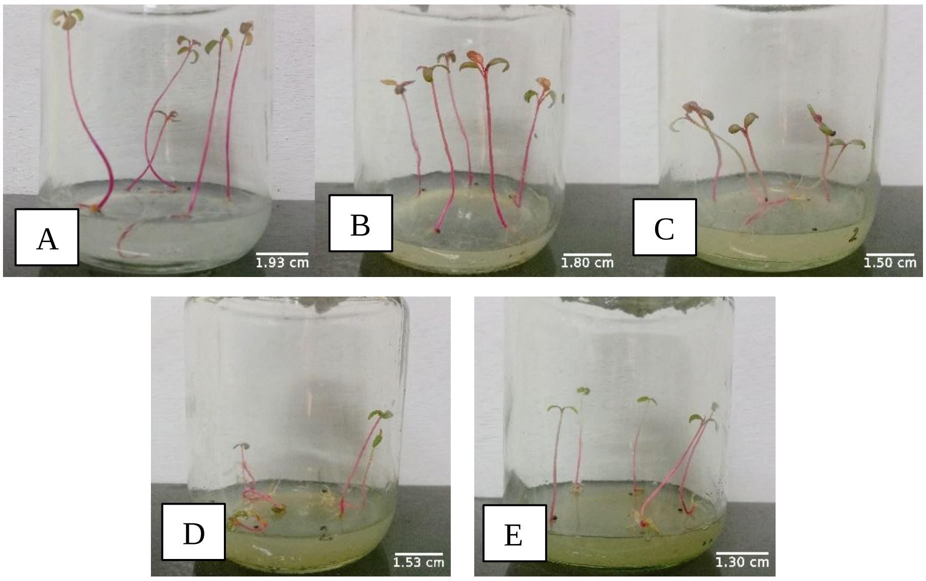

Figure 1. Growth of 3-week-old Red Spinach (*Amaranthus tricolor* L.) Planlets on Murashige and Skoog Medium with the Addition of Various Concentrations of Bean Sprout Extracts

Description:
A = 0% bean sprout extract (control)
B = 5% bean sprout extract
C = 10% bean sprout extract
D = 15% bean sprout extract
E = 20% bean sprout extract

In Figure 1, it is evident that both the red spinach planlets treated with the addition of bean sprout extract and those without the extract exhibit robust growth and healthy development. The planlets in each concentration display a remarkable 100% survival rate, indicating no significant differences among the treatments. This high success rate can be attributed to the medium's uncontaminated nature and precisely controlled environmental conditions. Furthermore, these results demonstrate that the Murashige and Skoog medium, even without the addition of bean sprout extract, is fully capable of meeting the nutritional requirements and providing the necessary nutrients for the explants to thrive. This is attributed to the Murashige and Skoog medium's composition, which includes abundant inorganic salts, macro and micro nutrients, iron, vitamins (such as Thiamine-HCL, Pyridoxine-HCL, Nicotinic acid), myo-inositol, sucrose, and Glycine (Fauzy et al., 2016).

Next, we move on to the planlet height variable. The observation results of planlet height are presented in Table 2 as follows:

Table 2. BNJ test of the average height of red spinach planlets at 3 weeks after treatment

Extract Concentration Bean sprouts (%)	Average Planlet Height at Week 3 (cm)
0	4.04 ± 0.20[a]
5	5.18 ± 0.09[b]
10	3.52 ± 0.14[ac]
15	3.78 ± 0.17[a]
20	2.92 ± 0.29[c]

Planlet height = Ý ± SE
Ý = Average value of planlet height
SE = *Standard Error*
Values followed by the same letter are not significantly different at the 5% BNJ test level. BNJ value (0.05) = 0.80

Based on Table 2, it is evident that the red spinach explants treated with 0% bean sprout extract (control) show significant differences compared to the treatments with 5% and 20% concentrations of bean sprout extract. However, there are no significant differences between the control and the treatments with 10% and 15% concentrations of bean sprout extract. Additionally, the treatment with 5% concentration of bean sprout extract exhibits significant differences when compared to all other treatments. Remarkably, the planlets treated with 5% concentration of bean sprout extract demonstrate the highest growth, and this growth is significantly different from the other treatment groups.

The planlet height serves as a crucial indicator of growth and is an essential variable in assessing the experiment's efficacy. The increase in planlet height is attributed to enhanced cell division resulting from increased assimilation (Laksmita et al., 2018). The notable growth in planlet height observed in the treatment with 5% concentration of bean sprout extract can be attributed to the presence of auxins in the extract, particularly IAA (3.74%) and IBA (1.88%), which significantly support planlet growth (Sunandar et al., 2017). This finding aligns with the research conducted by Pamungkas and Puspitasari (2019), which suggests that optimal auxin levels stimulate plant cell growth. Auxins play a crucial role in cell wall elongation, causing plant cells to lengthen due to water absorption through osmosis.

Furthermore, Alpriyan and Karyawati (2018) have also demonstrated that auxin concentration influences plant height, stem diameter, leaf count, leaf area, root length, and root dry weight in sugarcane plants. The results of this study reveal that higher concentrations of bean sprout extract correspond to lower levels of plant growth. This phenomenon can be explained by Kartiman et al. (2018), who suggest that the application of exogenous auxin hormones in an imbalanced ratio with endogenous hormones can inhibit cell elongation processes in plants. While auxins exhibit optimal effects at low concentrations, excessive concentrations can impede plant growth, as indicated by Dwidjoseputro (2001).

Next is the variable number of leaves on the planlets. The observation results of the number of planlet leaves are as follows.

Table 3. BNJ test of the average number of red spinach leaf planlets at 3 weeks after treatment

Extract Concentration Bean sprouts (%)	Average Number of Leaves in Week 3 (strands)
0	4.00 ± 0.17^{a}
5	4.00 ± 0.33^{a}
10	2.60 ± 0.26^{b}
15	3.20 ± 0.07^{ab}
20	2.40 ± 0.23^{b}

Number of leaves = Ý ± SE
Ý = Average value of the number of leaves that grow
SE = *Standard Error*
Values followed by the same letter are not significantly different at the 5% BNJ test level. BNJ value (0.05) = 0.98

Based on the findings in Table 3, it is evident that red spinach explants treated with 0% bean sprout extract (control) and 5% concentration produce the highest number of leaves, and this number is not significantly different from the treatment with 15% concentration. Conversely, the treatment with 20% concentration of bean sprout extract results in the lowest number of leaves. Consistent with the plant height variable, the observation of leaf count in red spinach planlets reveals that as the concentration of bean sprout extract increases, the number of leaves produced by the plants decreases. This correlation can be explained by Siregar et al. (2015), who propose that taller stems tend to bear more leaves along the plant stem.

The most effective concentrations in this study were found to be 5% and 0%, followed by 15%, 10%, and 20% in descending order. The application of growth regulators with high concentrations can slow down cell division activity, leading to minimal effects on increasing plant growth (Hidayanto et al., 2003). These results highlight the significance of optimizing the concentration of bean sprout extract to achieve the desired outcome in terms of leaf production and overall plant growth. Ensuring an appropriate concentration of growth regulators is essential in balancing plant development and maximizing the benefits of the extract.

Then, for the results of observations on root length variables can be seen in Table 4 as follows.

Table 4. BNJ test of average root length of red spinach at 3 weeks after treatment.

Extract Concentration Bean sprouts (%)	Average Root Length in Week 3 (cm)
0	5.42 ± 0.45[a]
5	4.86 ± 0.45[ab]
10	6.50 ± 0.95[a]
15	2.40 ± 0.70[bc]
20	1.06 ± 0.10[c]

Root length = Ý ± SE
Ý = Average value of root length of red spinach planlets that grow
SE = *Standard Error*
Values followed by the same letter are not significantly different at the 5% BNJ test level. BNJ value (0.05) = 2.54

Based on the results presented in Table 4, the BNJ test with a significance level (α) of 0.05 indicates that the bean sprout extract concentration of 0% shows significant differences when compared to the concentrations of 15% and 20%. However, it does not show significant differences when compared to the concentrations of 5% and 10%. Similarly, the 5% concentration exhibits significant differences when compared to the 20% concentration but not with the concentrations of 0%, 10%, and 15%. The 15% concentration shows significant differences when compared to the concentrations of 0% and 10%, but not with the concentrations of 5% and 20%.

The average results reveal that there is a significant effect of the treatments on the root length of the planlets. Specifically, the treatment with a concentration of 10% bean sprout extract demonstrates the longest root length among all concentrations. However, it is important to note that this significant difference is not observed when compared to the concentrations of 0% and 5%, as the average root length for these treatments is 6.50 cm.

These findings underscore the importance of selecting the appropriate concentration of bean sprout extract to enhance the root development of red spinach planlets. While a concentration of 10% has shown the longest root length, it is essential to consider other factors such as plant health, overall growth, and practical feasibility in determining the most optimal concentration for application. Further investigation and careful consideration are necessary to achieve the best results in promoting robust root development in red spinach planlets.

This result is in accordance with the statement of Himanen *et al.* (2002) which states that auxin triggers cell division so that it is needed for root formation, but in certain situations auxin can also be toxic to plants. As is the case in this study which shows that the higher the concentration of bean sprout extract given causes the lower the growth of red spinach plants. The concentration of auxin that exceeds the optimum range will reduce the growth of a plant (Hopkins and Huner, 2004).

Furthermore, the observation results of explant wet weight are as follows.

Table 5. BNJ test of average wet weight of red spinach at 3 weeks after treatment.

Extract Concentration Bean sprouts (%)	Average Wet Weight at Week 3 (g)
0	0.112 ± 0.001[a]
5	0.092 ± 0.002[b]
10	0.065 ± 0.003[c]
15	0.022 ± 0.002[d]
20	0.020 ± 0.002[d]

Wet weight = Ý ± SE
Ý = Average value of wet weight of red spinach planlets
SE = *Standard Error*
Values followed by the same letter are not significantly different at the 5% BNJ test level. BNJ value (0.05) = 0.010

Based on the data presented in Table 5, the results indicate that the application of various concentrations of bean sprout extract significantly affected the wet weight of red spinach planlets. The control group, with 0% bean sprout extract concentration, showed a significant difference from all other concentrations. However, the 15% concentration of bean sprout extract did not show a significant difference compared to the 20% concentration. Notably, the treatment with 5% concentration of bean sprout extract exhibited the highest average wet weight, amounting to 0.092 g after the control.

Moreover, it is worth noting that the treatment without the addition of bean sprout extract demonstrated the highest wet weight compared to the treatments with bean sprout extract. This phenomenon can be attributed to the fact that the Murashige and Skoog medium already contains a sufficient amount of inorganic salts and nutrients for plant growth (George and Sherrington, 1984). Nevertheless, among the treatments with bean sprout extract, the 5% concentration showed the most promising results with regard to wet weight. These findings suggest that a low concentration of auxin hormone has a positive impact on the growth and wet weight of planlets, whereas excessively high levels of auxin hormone may have detrimental effects on growth and wet weight (Kartiman et al., 2018).

Finally, the observation results of soluble carbohydrate content in red spinach can be seen in Table 6 as follows.

Table 6. BNJ test of average soluble carbohydrates in red spinach at 3 weeks after treatment

Extract Concentration Bean sprouts (%)	Average Dissolved Carbohydrate Content (mg/L)
0	1.89 ± 0.19[a]
5	33.15 ± 0.23[b]
10	80.31 ± 0.19[c]
15	97.99 ± 0.23[d]
20	101.10 ± 0.18[e]

Soluble carbohydrate = Ý ± SE
Ý = Average value of soluble carbohydrate content
SE = *Standard Error*
Values followed by the same letter are not significantly different at the 5% BNJ test level. BNJ value (0.05) = 0.86

Based on the data presented in Table 6, the results indicate that the concentration of bean sprout extract had a significant effect on the total soluble carbohydrate content of red spinach planlets. The control group, with 0% bean sprout extract concentration, showed a significant difference when compared to all other concentrations (5%, 10%, 15%, and 20%). Additionally, the average results suggest that there is a notable effect of the treatments on the total soluble carbohydrate content in the red spinach planlets.

Carbohydrates, a class of organic compounds composed of carbon, hydrogen, and oxygen (Almagfirah and Laenggeng, 2022), serve as the primary energy source for plants, crucially contributing to photosynthesis and the synthesis of storage materials like starch (Novitasari, 2017). In this study, the carbohydrate content of red spinach planlets was assessed to examine the impact of bean sprout extract at various concentrations. The results of the carbohydrate test indicated that higher concentrations of bean sprout extract led to an increase in soluble carbohydrate content in red spinach planlets. Statistical analysis revealed a significant difference between the control group (0% concentration) and bean sprout extract concentrations of 5%, 10%, 15%, and 20%. This suggests that bean sprout extract positively influences carbohydrate accumulation in plants.

The increase in carbohydrate content with increasing concentration of bean sprout extract can be explained by several factors. Firstly, bean sprout extract contains auxins, especially IAA, which can stimulate the rate of photosynthesis. Auxin can also increase the conversion of glucose into carbohydrates through the biosynthesis process (Lee and Huang, 2014). Therefore, increasing the auxin content in bean sprout extract at higher concentrations can stimulate increased carbohydrate production in plants. However, although increasing the concentration of bean sprout extract had a positive impact on carbohydrate content, growth variables such as plant height, leaf number, root length, and wet weight showed different results. This could be due to the toxicity effect at higher auxin concentrations. Although carbohydrate content increased, plant growth was inhibited due to high exposure to bean sprout extract.

Furthermore, the regression curve of the relationship between the total carbohydrate content of red spinach planlets and the application of bean sprout extract at various concentrations is presented in Figure 2.

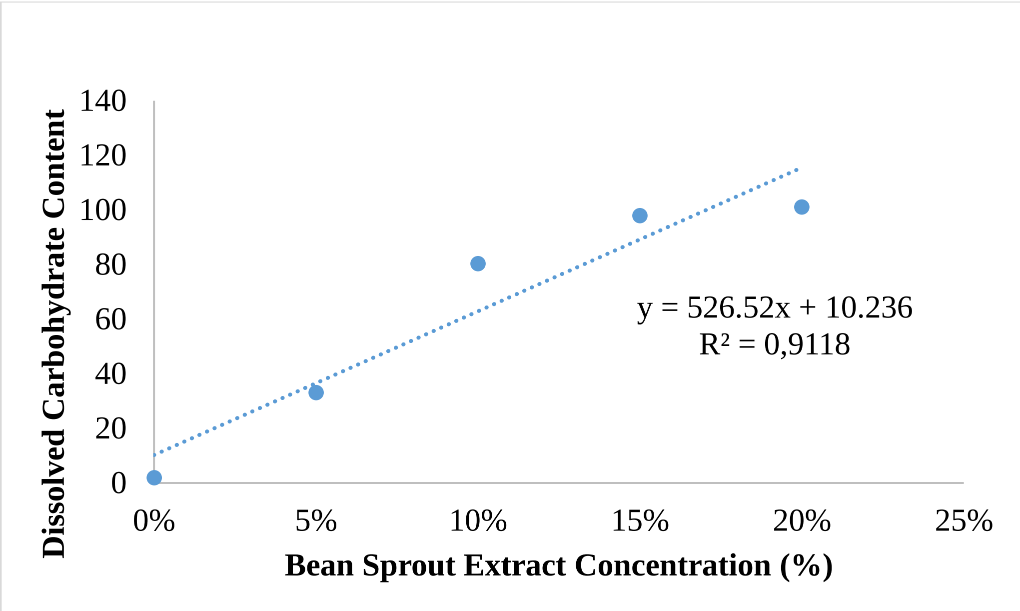

Figure 2. Regression curve of soluble carbohydrate content of red spinach planlets with bean sprout extract at various concentrations

Based on Figure 2, the measurement of the relationship curve of soluble carbohydrate content of red spinach planlets with the administration of bean sprout extract at various concentrations is shown by the equation of a linear regression line ($y = 526.52x + 10.236$) and a very strong correlation value ($R^2 = 0.9118$).

CONCLUSIONS

In conclusion, the use of bean sprout extract (Vigna radiata) at different concentrations had a significant impact on the growth of red spinach (Amaranthus tricolor) planlets. It affected various variables, including planlet height, leaf number, root length, wet weight, and soluble carbohydrate content. Among the concentrations tested, the most effective concentration for the growth of red spinach explants on the Murashige and Skoog medium in vitro was found to be 5% bean sprout extract.

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