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Effect of cytokinin and gibberellic acid applications on seed germination and growth of *Rauvolfia verticillata* plant

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Abstract. *Lestari S, Solichatun, Anggarwulan E. 2018. Effect of cytokinin and gibberellic acid applications on seed germination and growth of* Rauvolfia verticillata *plant. Cell Biol Dev 2: 27-32.* The purposes of this research are to study the effect of cytokinin and gibberellic acid (GA_3) on seed germination and growth of *Rauvolfia verticillata* Lour. or *pule pandak* and to determine the best treatment combination that has an optimal effect on the seed germination and growth of *R. verticillata*. This study was done in a complete randomized design by combination treatment of gibberellic acid and cytokinin that consist of 6 treatment combinations including GA_3 0 ppm/ cytokinin 0 ppm, GA_3 50 ppm/ cytokinin 0 ppm, GA_3 0 ppm/ cytokinin 50 ppm, GA_3 50 ppm/ cytokinin 50 ppm, GA_3 50 ppm/ cytokinin 75 ppm, GA_3 75 ppm/ cytokinin 50 ppm and 8 replicates. The treatments were given by soaking the mature seed in the hormone solution. Some parameters such as germination and growth parameter were measured. The result shows that presoaking treatment with GA_3 50 ppm had a greater influence on germinating time, germination percentage, plant height, leaf number, leaf wide, and dry weight than presoaking use cytokinin 50 ppm. Presoaking treatment using a combination of GA_3 and cytokinin ($G_{50}S_{50}$) has a significant influence on increasing fresh weight and dry weight. The combination of GA_3 and cytokinin in the concentration of $G_{50}S_{75}$ influences fresh weight and dry weight plants. The combination of GA_3 and cytokinin in concentration $G_{75}S_{50}$ influences increased germination percentage, plant height, leaf number, and leaf wide.

Keywords: Cytokinin, GA_3, germination, gibberellic acid, growth, *Rauvolfia verticillata*

INTRODUCTION

Utilizing medicinal plants without considering the sustainability aspect can be viewed as affecting the sustainability and decline of the medicinal plant population, increasing the scarcity of medicinal plant species. Given the Indonesian people's attachment to their cultural traditions of herbal medicine, the use of Indonesian medicinal plants is expected to continue. Some herbal raw materials have proven reliable export commodities, helping the country's foreign exchange reserves. The increased use of medicinal plants as export commodities has not been accompanied by rational cultivation and germplasm preservation (Sulandjari 2008).

One currently widely used plant is the *pule pandak*, a collective name for Genus *Rauvolfia,* such as *Rauvolfia verticillata* Lour and *Rauvolfia serpentina* (L.) Benth. ex Kurz. *R. verticillata*, also known as rat root, is a member of the Apocynaceae family. This plant is found throughout Indonesia, including Sumatra, Java, and Nusa Tenggara islands. It typically grows at elevations ranging from 1000 m to 2100 m above sea level (asl) (Iptekda-LIPI 2001).

Rauvolfia verticillata is effective as a preventative increase in body temperature, sedatives, high blood pressure drugs, and normalized heart rate, among other things. The most important alkaloid found in the root of *R. verticillata* is reserpine, which is commonly used as a hypertension medication (Lilly 1990; Duke 1992; Nigg and Seigler 1992).

Until now, there has been no extensive cultivation of *R. verticillata* in Indonesia (Rosita et al. 1992). *R. verticillata* plants can be propagated by seed, cuttings, or tissue culture. Seeds of *Rauvolfia serpentina* (L.) Benth. ex Kurz species germinated 10-12 days after planting due to the hardness of the seed shell (Iptekda-LIPI 2001). The same occurred to *R. verticillata*. When compared to the germination of peanuts (*Arachis hypogea* L.) in 5 days, corn seeds (*Zea mays* L.) in 2-3 days, and cotton seeds (*Gossypium* sp.) in 5-7 days, this germination time is considered long (Goldsworthy and Fisher 1992).

Pule pandak reproduces naturally by seed, but the germination percentage is low, ranging from 7 to 15%, due to the hard seed coat on pule pandak seeds. The percentage of successful propagation of *R. serpentina* by seed can be increased by removing half of the seed shell before planting or soaking the seeds in a concentrated or semi-concentrated sulfuric acid (H_2SO_4) solution for five minutes before planting (Sulandjari 2008).

Many researchers believe that the growth hormones gibberellins (GA_3) and cytokinins influence enzyme activity in plant metabolic processes (Wringler et al. 1998; Leitei et al. 2003). Previous research on *Brucea javanica* (L.) Merr. seeds by Setyowati and Utami (2008) revealed that soaking seeds in 1000 mg/L GA_3 solution effectively accelerated and increased *B. javanica* germination.

The Forestry Research and Development Agency (2005) researched balsa seeds (*Ochroma* sp.) to improve seedling growth. The findings revealed that soaking hot water for 12 hours effectively increased the seed

germination percentage. Soaking the seeds in hot water for 12 hours, then applying 500 ppm atonic to seedlings, increased height, root length, and seedling dry weight. Combining two types of hormones is more effective than combining three, four, or even more types of hormones (Cavusoglu and Kabar 2007). This is due to the synergistic effect of each hormone, which supports each other because each hormone used has a different effect on growth. Based on this, two types of hormones were used in this study, which were treated separately and then combined to determine their effect on *R. verticillata* seed germination and growth.

The purpose of this study is to determine the effect of the hormones cytokinin and gibberellic acid (GA_3) on the germination and growth of *R. verticillata* and the optimal concentration of cytokinin and gibberellic acid (GA_3) hormones on increasing plant germination and growth of *R. verticillata*.

MATERIALS AND METHODS

Materials

The main materials used in this study were old *R. verticillata* seeds taken from the *R. verticillata* plant that grows in the Boyolali area; cytokinin hormone (kinetin); gibberellic acid (GA_3); and Dhitane M45 2%.

Research design

This study used a completely randomized design (CRD), with each treatment with 5 replications. The types of treatment carried out include:

I = Control (G_0S_0)
II = GA_3 50 ppm: Cytokinin 0 ppm ($G_{50}S_0$)
III = GA_3 0 ppm: Cytokinin 50 ppm (G_0S_{50})
IV = GA_3 50 ppm: Cytokinin 50 ppm ($G_{50}S_{50}$)
V = GA_3 50 ppm: Cytokinin 75 ppm ($G_{50}S_{75}$)
VI = GA_3 75 ppm: Cytokinin 50 ppm ($G_{75}S_{50}$

Research methods

This study begins with collecting seeds from old plants distinguished by their gray color (black). For 2-3 days, the seeds are dried. The seeds were extracted from the seed coat and treated by immersing them in hormones (cytokinins and GA_3). The hormone solution was created by dissolving the powdered hormone in ethanol and aquadest. Before adding aquadest, this ethanol was used to dissolve the hormone. Proceed with the soaking of the seeds once the seeds are ready. The seeds were soaked overnight in the hormone solution at each concentration. These steps were based on Setyowati and Utami's (2008) study on *B. javanica* seeds, which have a seed structure similar to *R. verticillata*. After that, the seeds were planted in polybags. The media was soil collected from the Boyolali area in ¾ polybags (±1 kg/polybag). To avoid mold during germination, the seeds were soaked in a 2 percent Dhitane M45 solution for 5 minutes before planting (Setyowati and Utami 2008). Each polybag contains three seeds spaced about 3-4 cm apart. After the seeds had been planted, the hole was filled with soil and doused with 100 mL of water (Lestari 2008). The polybags containing the planted seeds were placed in a greenhouse with limited sunlight.

Watering was used to treat plants from planting to the end of treatment. Watering was performed regularly to meet the needs of the plant. It was not done if the soil in the polybag was not too dry because it is feared that the seeds will rot if it is too wet.

Data were collected during or at the end of treatment depending on the parameters to be observed. Observations of germination parameters include: (i) the time of sprout emergence. (ii) Germination percentage. The percentage of germination was calculated for each polybag using the formula:

$$\text{Germination percentage} = \frac{\text{Number of sprouted seeds}}{\text{Total of all seeds}}$$

Plant growth observation begins when the plant's first leaves appear. The development of the *R. verticillata* plant was studied. The following parameters were observed: (i) Plant height was measured from the soil surface to the plant's highest tip. (ii) Leaf area and the number of leaves: The number of leaves was calculated after the first leaf appeared, whereas the leaf area was calculated at the end of treatment or at harvest, which was 1 week after the specified time had passed. The gravimetric method was used to calculate leaf area. Leaves were drawn on a piece of paper whose weight and area were known as leaf replicas, which were then cut out, and the leaf area was measured using the following equation:

$$LD = \frac{Wr}{Wt} xLK$$

Where:
LD : leaf area
Wr : leaf replica paperweight
Wt : total paperweight
LK : total paper area (Sitompul dan Guritno 1995)

(iii) Fresh weight: Fresh weight of plants was measured by weighing the plants at the end of the treatment. (iv) Dry Weight: Plants were dried in an oven at a temperature of 60°C until dry, and then the dry weight was weighed. Observations were made daily to determine the germination process and once a week for other parameters. Observations were stopped when the plants were 8 weeks old (± 2 months).

Data analysis

The collected data were then analyzed with analysis of variance (ANOVA) to determine the effect of treatment on the measured parameters. If there is a significant difference between treatments, a second test using the Duncan's Multiple Range Test (DMRT) at a test level of 5% is performed.

RESULTS AND DISCUSSION

Sprouts emergence time

The results of the average germination time of pule seeds pandak with GA_3 and cytokinin treatments are presented in Table 1.

The germination time observations in Table 1 revealed that the seeds began to germinate on the sixth day. Each treatment (control, G_0S_0, $G_{50}S_0$, $G_{50}S_{50}$, $G_{75}S_{50}$, $G_{50}S_{75}$) produced germinated seeds, but the G_0S_{50} concentration treatment showed no signs of seeds beginning to germinate. This suggests that pule seed germination is influenced by internal factors, such as seed age, and external factors, such as hormones, particularly GA_3.

Embryo growth during germination depends on preparing food materials in the endosperm. The embryo's survival depends on the occurrence of enzymatic decomposition, specifically the conversion of starch into sugar, which is then translocated to the embryo as an energy source for its growth, which requires amylase activity (Abidin 1994).

GA_3 is exogenous (from outside) and endogenous (from within the plant). Endogenous GA_3 increases the hydrolysis of starch, fructan, and sucrose into glucose and fructose molecules. The hexose sugar provides energy through respiration, aids cell formation, and causes the cell's water potential to become more negative at times. Water will enter more quickly due to decreased water potential, causing cell enlargement (Salisbury and Ross 1955).

The embryo's production of GA_3 stimulates cells in the aleurone layer to synthesize and produce α-amylase and protease enzymes, which convert endosperm starch into sugar for the growth of young seeds. After the seed absorbs water, the embryo releases GA_3 as a signal to the aleurone, a thin endosperm membrane. Aleurone responds to the response by synthesizing and secreting enzymes that hydrolyze the endosperm's food reserves. α-amylase, for example, hydrolyzes carbohydrates (much like the salivary enzymes in saliva that help break down bread and other carbohydrates). The scutellum (cotyledon) absorbs sugars and nutrients from the endosperm and stores them for use from embryonic development to adulthood (Sponsel 1987).

Cytokinins did not have a significant effect on germination in this study. This could happen if the cytokinin concentration is not optimal. Cytokinins can influence plant germination, growth, and development. Generally, this hormone interacts with the hormone auxin (Abidin 1994). Cytokinins and auxins (IAA) collaborate to promote embryonic growth; cytokinins promote cell division, while auxins (IAA) promote cell elongation (Hess 1970).

Germination percentage

Germination percentage was measured by comparing the number of seeds that germinated with all seeds planted and multiplying by 100%. The results of the average percentage germination of *R. verticillata* seeds with GA_3 and cytokinin treatments are presented in Table 2.

Table 2 shows the germination percentages for 30 days after planting. Table 2 shows that the control had the highest rate of germination. The treatment with the $G_{50}S_{50}$ combination produced the poorest results. Hormone treatment at concentrations greater than cytokinins produced positive results. This suggests that the GA_3 hormone affects seed germination. Gibberellins can remove germination inhibitors, break dormancy, and activate enzymes, resulting in increased metabolic activity (Salisbury and Ross 1995).

Non-germinating seeds mostly rot. This is because too much water ingestion by the seeds creates anaerobic conditions, allowing many rotting seeds to be supported by the state of the seeds, which causes the rot. These findings were reported in a study on the germination of jelutung (*Dyera costulata* (Miq.) Hook.fil.), which found that prolonged soaking of seeds sensitive to anaerobic conditions caused the seeds to rot and become incapable of germinating (Utami et al. 2007).

Germination is an embryonic growth process that includes morphological activity, which is characterized by the appearance of plant organs such as roots, stems, and leaves, as well as chemical activity, which includes several stages such as imbibition, secretion of hormones and enzymes, and hydrolysis of food reserves, particularly carbohydrates and proteins, from simple into complex forms, photosynthesis, translocation of dissolved food and hormones to the growing point area and other parts (Hidayat 1995). Several early germination hormones are activated by water in the cells. Abscisic acid, a phytohormone, decreased while gibberellins increased. Furthermore, the presence of water in the seeds activates active enzymes. The amylase enzyme breaks down flour into maltose, which is then hydrolyzed into glucose by maltase. Proteins are also degraded into amino acids. Glucose compounds enter the metabolic process and are broken down into energy and carbohydrate compounds, which form the body's structure. These amino acids are combined to form proteins, which build cell structures and new enzymes. Fatty acids are primarily used to construct cell membranes (Salisbury dan Ross 1995).

Table 1. Average germination time of *Rauvolfia verticillata* seeds with GA_3 and cytokinin treatment

Cytokinin and GA_3 concentrations	Average germination time (days)
G_0S_0	6
G50S0	6
G0S50	12
G50S50	6
G50S75	6
G75S50	6

Note: G = GA_3 concentration (ppm), G_0= 0, G_{50}= 50, G_{75}= 75; S = Cytokinin concentration (ppm), S_0= 0, S_{50}= 50, S_{75}= 75

Table 2. The average percentage of germination of *Rauvolfia verticillata* seeds with GA_3 and cytokinin treatment 30 days after planting

Cytokinin and GA_3 concentrations	Average (%) of germination
G_0S_0	62.50
G50S0	41.62
G0S50	33.25
G50S50	24.88
G50S75	33.25
G75S50	45.88

Note: G = GA_3 concentration (ppm), G_0= 0, G_{50}= 50, G_{75}= 75; S = Cytokinin concentration (ppm), S_0= 0, S_{50}= 50, S_{75}= 75

Plant height

Plant height is a plant size commonly observed as an indicator of growth and a parameter used to assess the effect of the environment or the treatment used. This is done because plant height is the most visible measure of growth. Plant height is sensitive to environmental factors as a parameter of environmental influence (Sitompul dan Guritno 1995).

The results of the average plant height with GA_3 and cytokinin treatment at the end of the study are presented in Table 3. The table of average plant height shows that for the treatment of hormone administration, $G_{50}S_0$ and $G_{75}S_{50}$ increased plant height compared to other hormone treatments and the control.

The application of GA_3 from the outside on the development of stolons of wild potato plants by Wareing and Philips in Abidin (1994) revealed internodal elongation in shoots growing in leaf axils. GA_3 applied to the plant's tip and kinetin applied to the leaf axils demonstrated that plant height and lateral branch growth were balanced (Abidin 1994).

Internode elongation causes stem elongation, caused by the growth of rib meristems, which form long rows of cells in the cortex and pith meristems. Cell elongation and an increase in the number of cells in the rib meristem occur during stem elongation (Hidayat 1995). Rib meristem is a meristematic tissue composed of a vertical series of cells that divide transversely (Fahn 1995).

Gibberellins stimulate the formation of enzymes that soften cell walls, particularly proteolytic enzymes that release the amino tryptophan (a precursor/former of auxin), increasing auxin levels. Gibberellins also promote the formation of polyhydroxy cinnamic acid, which inhibits the action of the IAA oxidase enzyme (Green Tect 2009).

When used alone, cytokinins (kinetin) stimulate DNA synthesis and are required in the process of mitosis, though IAA is usually more dominant in this phase. Cytokinins work with nucleic acids in cells to increase nuclear RNA synthesis and regulate the amount of RNA in the cytoplasm (Abidin1994).

Number of leaves

Because leaves are the primary photosynthetic organs, they must be observed as a growth parameter to explain the growth process. Once a week, observations on the number of leaves were made. Table 4 shows observation data on the number of leaves.

The best hormone treatment results are shown in the table above for hormone treatment with a concentration of $G_{50}S_0$ and the lowest concentration of G_0S_{50}. This is because gibberellins and cytokinins have opposite effects on leaf formation and meristem development when present in the same concentration or greater concentrations than cytokinins. Some of the effects of cytokinins on epidermal differentiation can also be reversed by GA_3. However, GA_3 and cytokinins can stimulate the formation of epidermal structures known as trichomes (Gan et al. 2007), so the concentration of cytokinins used has no effect.

The increased number of leaves is thought to be due to the increased division of leaf primordia cells and the differentiation of stem tip cells (Hidayat 1995). Leaves, as a means of photosynthesis, will be able to perform optimally if sufficient water, light, and nutrients are available (Loveless 1991; Salisbury and Ross 1995). According to Windarsih (2007), the effect of GA_3 on the process of leaf formation is that administration of the GA_3 hormone does not affect the number of leaves of flax plants (*Boehmeria nivea* (L.) Gaudich.).

Leaf area

Leaf area is a parameter that can be used to determine the rate of photosynthesis per unit plant. The method used to determine leaf area is the gravimetric method, which uses simple tools (Sitompul and Guritno 1995). Observations were made at harvest. The results of the average leaf area can be seen in Table 5.

Table 3. Average plant height of *Rauvolfia verticillata* with GA_3 and cytokinin treatment 60 days after planting

Cytokinin and GA_3 concentrations	Average Plant Height (cm)
G_0S_0	4.14
G50S0	4.64
G0S50	4.06
G50S50	2.2
G50S75	3.38
G75S50	5.06

Note: G = GA_3 concentration (ppm), G_0= 0, G_{50}= 50, G_{75}= 75; S = Cytokinin concentration (ppm), S_0= 0, S_{50}= 50, S_{75}= 75

Table 4. The average number of leaves of *Rauvolfia verticillata* with GA_3 and cytokinin treatment 60 days after planting

Cytokinin and GA_3 concentrations	The average number of leaves
G_0S_0	5.8
G50S0	6.2
G0S50	3.6
G50S50	4.4
G50S75	4.4
G75S50	5.0

Note: G = GA_3 concentration (ppm), G_0= 0, G_{50}= 50, G_{75}= 75; S = Cytokinin concentration (ppm), S_0= 0, S_{50}= 50, S_{75}= 75

Table 5. Average leaf area of *Rauvolfia verticillata* with GA_3 and cytokinin treatment 60 days after planting

Cytokinin and GA_3 concentrations	Average leaf area (cm^2)
G_0S_0	0.519
G50S0	0.663
G0S50	0.362
G50S50	0.362
G50S75	0.504
G75S50	0.612

Note: G = GA_3 concentration (ppm), G_0= 0, G_{50}= 50, G_{75}= 75; S = Cytokinin concentration (ppm), S_0= 0, S_{50}= 50, S_{75}= 75

Table 5 shows that the *R. verticillata* plant with a 50 ppm concentration of GA_3 hormone treatment had the highest leaf area of 0.663 cm2, while the G_0S_{50} and $G_{50}S_{50}$ treatments had the lowest leaf area.

Increased leaf area is one type of plant growth caused by cell division and elongation activity. The hormone GA_3 acting on cells and cytokinins, which are responsible for regulating the degree of leaf development according to soil conditions such as the availability of water and mineralized nitrogen, is one of the effects of cell division and elongation (Goldsworthy dan Fisher 1992).

Plant fresh weight

Plant fresh weight is one of the parameters to describe plant biomass. Fresh weight gain of plants is carried out by harvesting all or part of the plant and weighing it quickly before too much water evaporates from the material (Salisbury and Ross 1995). The results of the average fresh weight of the *R. verticillata* plant are presented in Table 6 below.

The average fresh weight in the control treatment was 0.3308 grams. The results for the $G_{50}S_0$ and $G_{75}S_{50}$ treatments were 0.2662 grams and 0.1244 grams, respectively, which were the lowest results compared to other treatments. The fresh weight of the $G_{50}S_{75}$ treatment was 0.2706 grams. The G_0S_{50} treatment yielded 0.2842 grams, while the $G_{50}S_{50}$ treatment yielded 0.3992 grams, the highest yield of all treatments.

The treatment with the same concentration of GA_3 as cytokinin produced a better fresh weight of the plant based on the average fresh weight obtained from all treatments. These findings suggest that a well-balanced concentration of gibberellins and cytokinins promotes optimal growth in the *R. verticillata* plant. These two hormones mutually benefit plant development, particularly in encouraging cell development (Cavusoglu and Kabar 2007).

Increased endogenous GA_3 content can cause the cell potential to become more negative and water to enter more quickly, resulting in cell enlargement (Lakitan 1996). Increased water uptake by these cells may increase fresh plant weight (Wattimena 1988).

Dry weight

When expressed in terms of dry weight, both the whole plant and its parts, growth as an increase in the material is more accurate. Dry and fresh weight will significantly change water status from time to time, which can vary throughout the day. Water loss causes a significant loss of fresh weight as older tissue dries. Because photosynthesis accounts for 90 percent of plant dry matter, growth analysis is expressed in dry weight, particularly to assess plants' ability to produce photosynthate (Goldsworthy and Fisher 1992). Table 7 shows the results of the average dry weight of the *R. verticillata* plant.

According to Table 7, the highest average dry weight was obtained in the plant treated with $G_{50}S_{50}$, while the lowest was obtained in plants treated with $G_{75}S_{50}$. This indicates that combining the same concentration of GA_3 and cytokinins at 50 ppm produced the best results for plant dry weight. The optimal results for fresh weight are also shown at the concentration of $G_{50}S_{50}$.

A balanced concentration of cytokinins and gibberellic acid has a synergistic effect that supports each other, resulting in optimal cell growth and development, which also affects plant dry weight (Cavusoglu and Kabar 2007).

The addition of cytokinins and exogenous gibberellins will increase the content of cytokinins and gibberellins in the plant (header) as well as the number of cells (via cytokinin hormones) and cell size (via gibberellin hormones), which will accelerate the process along with the increased photosynthate yield at the start of planting. Plant vegetative growth (including the formation of new shoots) and plant stunting are both addressed (Green Tect 2009).

Table 6. Average fresh weight of *Rauvolfia verticillata* in combination treatment with GA_3 and cytokinins 60 days after planting

Cytokinin and GA_3 concentrations	Average of fresh weight (gram)
G_0S_0	0.3308^b
G50S0	0.2662^b
G0S50	0.2842^b
G50S50	0.3992^b
G50S75	0.2706^b
G75S50	0.1244^a

Note: (i) G = GA_3 concentration (ppm), G_0= 0, G_{50}= 50, G_{75}= 75; S = Cytokinin concentration (ppm), S_0= 0, S_{50}= 50, S_{75}= 75. (ii) The numbers followed by the same letter in the column show no significant difference at the 5% DMRT test level

Table 7. The average dry weight of *Rauvolfia verticillata* in combination treatment with GA_3 and cytokinins 60 days after planting

Cytokinin and GA_3 concentrations	Average of dry weight (gram)
G_0S_0	0.0188^{ab}
G50S0	0.0246^b
G0S50	0.0148^{ab}
G50S50	0.0252^b
G50S75	0.0180^{ab}
G75S50	0.0088^a

Note: (i) G = GA_3 concentration (ppm), G_0= 0, G_{50}= 50, G_{75}= 75; S = Cytokinin concentration (ppm), S_0= 0, S_{50}= 50, S_{75}= 75. (ii) The numbers followed by the same letter in the column show no significant difference at the 5% DMRT test level

The increase in dry weight is caused by an increase in the rate of photosynthesis, which produces photosynthate as a byproduct of the metabolic process. Carbohydrates are the end product of the photosynthesis process. Carbohydrates are the basic organic matter building blocks in plant cells, such as structural, metabolic, and important food reserves. These organic materials comprise plant cell components such as cytoplasm, cell nucleus, and cell wall. The accumulation of dry weight resulted from this process (Salisbury and Ross 1992).

According to the research findings, immersion with GA_3 at a concentration of 50 ppm has a greater influence on accelerating germination time, increasing germination percentage, plant height, number of leaves, leaf area, and dry weight than immersion with 50 ppm cytokinins. Immersion treatment with a combination of GA_3 and cytokinin ($G_{50}S_{50}$) increased fresh and dry weight significantly. The combination of GA_3 and cytokinins at a concentration of $G_{50}S_{75}$ increased plants' wet and dry weight. The combination of GA_3 and cytokinins in $G_{75}S_{50}$ increases germination percentage, plant height, number of leaves, and leaf area.

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