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Effects of Foliar Application of 6-benzylaminopurine on *Zingiber officinal*e Rosc. (Zingerberaceae) Boziab Variety Growth and Rhizome Production in Ethiopia

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Authors' contributions

This work was carried out in collaboration between all authors. Authors MB and GG carried out all experimental work, data analysis and literature search. Author NMC was responsible for study concept, designing and coordinating the research and supervising the work. Authors STH and NMC contributed to writing and preparing the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aim: Various studies have been conducted on the effect of plant growth regulators *in vitro*. However, studies on foliar exogenous spray of 6-benzylaminopurine (BAP) in ginger are lacking. Therefore, the aim of this study is to evaluate the foliar application of BAP on ginger cultivated in pot as well as on plain land.
Materials and Methods: To accomplish these task different concentrations of BAP i.e. 0 ppm, 50 ppm, 100 ppm and 150 ppm were applied exogenously on four months old ginger plants. Plants sprayed with distilled water were served as control. After two consecutive months of foliar spray with BAP, various physio-morphological characters were measured.
Results: It was observed that 100 ppm of BAP was found to be effective to regulate shoot growth

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for ginger plants grown in pot and 50 ppm of BAP was effective for ginger plants cultivated on plain land in comparison to control. BAP was also found to be effective for number of fingers/rhizome and rhizome width at 100 ppm and 50 ppm when cultivated in pot and on plain land respectively. Decrease in the rhizome length and fresh weight was observed at higher concentration of BAP. Thus, this study is the first from Ethiopia that explains the effect of foliar application of BAP on morpho-physiological effect in Boziab variety of ginger.

Conclusion: It may be concluded from the present investigation that plant growth regulator particularly BAP was found to be potent plant growth regulator to enhance the different morphological features of ginger shoots. Future research on the relationship between the effect of different class of growth regulators on shoot growth and rhizome production in ginger is necessary to derive a certain conclusive mechanism.

Keywords: Zingiber officinale; 6-Benzylaminopurine; foliar application; shoot growth; rhizome production; bio-regulator.

1. INTRODUCTION

Ginger (*Zingiber officinale* Rosc.), belonging to the family Zingerberaceae is one of the oldest and familiar commercial spice esteemed for its aroma, flavor and pungency. It is an important source of essential oils as well as the direct use of rhizomes for culinary purposes is increasing worldwide [1]. In 2007, ginger has become second widely cultivated spice next to chilies in Ethiopia [2-3]. Although, there are more than 45 ginger cultivars reported in the country [3], their production and productivity is low. One of the major problems of ginger production in Ethiopia is the non-availability of planting materials [3].

Plant productivity is highly affected by various complex morphological and physiological processes which occur in the plant body, including the plant growth regulator (PGR) hormone balancing systems. The major five class of hormone i.e. auxin, gibberellins, cytokine, ethylene and abscisic acid can work together and independently to influence plant growth. Gibberellins hormone production control in the plant tissues is among the most important aspect during preserving planting material, since this hormone is able to control stem elongation and cell division [4].

Plant growth regulators (PGR's) are organic, natural or synthetic compounds which when exogenously applied in small quantities, exhibit similar actions to those of endogenous plant hormones [5]. Bio-regulator is an organic non-nutrient compound that promotes, inhibits or modifies morphological and physiological processes of the plant when applied at low concentrations [6]. To whatever extent in natural condition, the growth regulators are not functional under balancing condition that causes the plant growing abnormally. Meanwhile, these circumstances creates a condition through manipulating PGR content and its combinations in plant tissues would lead to better plant production and quality [7]. The hormones can be applied directly on plants parts such as leaves, fruits and seeds which may lead to changes in structural and functional processes, in order to increase production as well as to enhance the quality of plant. The PGR's are reported to regulate some processes in plants, such as germination, rooting, flowering, fruiting and senescence [8].

Several studies have shown that plant growth regulators such as cytokine could improve shoot growth [9]. Few studies have been conducted on evaluating the effect of cytokines on root system of ginger. Cytokine is a plant hormone synthesized in root and considering plant type and hormone concentration it has irritating or inhibiting effects on root development. High cytokine concentrations prevent roots growth, but lower concentrations result in improved root development and growth [10].

Therefore, the aim of this study was to evaluate the foliar application of various concentration of BAP (6-Benzylamino-Purine) Boziab variety of ginger.

2. MATERIALS AND METHODS

2.1 Experimental Site and Materials

The experiments were conducted at Areka Agricultural Research Center, Areka, SNNPR, Ethiopia during the period of January to July, 2017 under greenhouse. The place is situated at the distance of 300 km from the capital city of Addis Ababa. The ginger variety, Boziab (37/79)

was obtained from the Areka Agricultural Research Center, Areka, SNNPR, Ethiopia. Boziab (37/79) variety was selected by the Crop Research Process Unit and Biotechnology Research Team of the center. The variety is known to adapt to various stress condition, having high ginger yield and productive in nature (National Spices Research Center, Tepi, Ethiopia). The plant materials were randomly selected for the experiment. All the plants selected were 4 months old and belong to same batch having uniform size of 36-38cm in length, showed the presence of 9-11 number of leaf, their leaf length was 4-13cm and all plants were checked for the absence of tillers. After selection, the plants were safely transferred to the pots and planted with mixture of garden soil, farmyard manure (coffee husk) and sand soil at a ratio of 2:1:1 respectively. The greenhouse condition was maintained at a temperature of 30ºC during day and 22ºC in the night. Greenhouse temperature and humidity were maintained by using sprinkled machine on the outer roof of the greenhouse throughout the experiment.

2.2 PGR Stock Solution Preparation

The stock solution of BAP i.e. 4 mg/ml was diluted to obtain working concentrations of 50, 100 and 150 ppm in water containing 10 ml of 0.1% Tween-20 as surfactant. All the mixtures were formulated and sprayed within one week after plantation. All the media used for the experiments were of analytical grade and purchased from Hi-Media Ltd. Mumbai, India.

2.3 Experimental Design

Both the pot and flat land experiments were laid out in a factorial treatment combination in a Completely Randomized Design (CRD) under greenhouse conditions. The first pot experiment was a two factorial experiment consisting of four concentrations of BAP i.e. 0, 50, 100 and 150 ppm and one ginger Boziab (37/79) variety with four treatments. Each treatment consisted of twelve pots with two plants for every pot per treatment (i.e. for each treatment 24 plants were considered) Plants treated without BAP were served as control. All the treatments were replicated three times. Foliar applications of various concentrations of BAP were applied for a single time once in a month for two consecutive months. For every spray, different concentrations of BAP were applied four times at the same period sprinkled manually with the help of spray machine. Application of BAP was carried out in the morning by watering the plant with a volume of 2000 ml within three days of interval time per plant according to Saeid et al. [11]. The second experiment was conducted on the flat land with two factorial experiments consisting of four concentrations of BAP (0, 50, 100 and 150ppm) and one ginger Boziab (37/79) variety forming four treatments. Accordingly, each experimental plot composed of 2 rows that constitute 12 plants per row. The distance between rows was 30cm and the distance between each plot was 40cm. Each plot area composed of 24 plants with 15cm spacing. Treatment without any PGR served as control experiment. Each treatment was replicated three times. Applications of PGR treatment were applied once every single time in a month for two consecutive months. For every spray various concentration of growth hormones were applied four times at the same period sprinkled manually with the help of spray machine. Applications of PGR treatments were carried out in the morning by watering the plants, based on field water capacity, were uniformly irrigated within three day interval according to the proposed research center which was adopted from National Spices Research Center, Tepi, Ethiopia.

2.4 Measurement of Plant Growth Parameters and Data Collection

Observation of plant in terms of number of leaves per shoot, leaf length (cm), average shoot length (cm) and number of tillers were recorded. To accomplish this task, 10 ginger plants were randomly selected from each treatment after 30 days of first treatment of application for two consecutive months. Plant shoot was measured from the stem base to the tip of highest leaf and leaf length was measured from the leaf base to the tip of the leaf with the help of ruler. The number of tillers was determined by counting the tillers whereas number of leaves was counted from the tip of stem (growing point) towards the stem base. All the observations were carried out monthly. Observations on the rhizome growth such as number of fingers/rhizome, fresh weight (gm), rhizome length (cm) and width of rhizome (cm) were collected once the crop was harvested after 9 months after planting (9MAP). The data was subjected to statistical analysis to test the effective concentration.

2.5 Statistical Analysis

Data were statistically analyzed using one way analysis of variance (SPPS version 20 software).

The significance of differences among treatment means were compared by Fisher's least-significant difference test (LSD) at *P* value of $P \leq 0.05$ was considered statistically significant.

3. RESULTS AND DISCUSSION

3.1 Results

3.1.1 Effect of exogenous foliar application of BAP on physical characteristics of shoots in Boziab ginger variety

Spraying the various concentrations of BAP on some physical characteristics of shoot was investigated in Boziab variety of ginger when cultivated in pot as well as on flat land under green house.

3.1.1.1 Effect of BAP on shoot growth in ginger when cultivated in pot

As shown in Table 1, BAP has significant effect on various physical parameters of shoot such as number of leaf, leaf length, number of tillers except average shoot length. Number of leaf per shoot was increased in concentration dependent manner and higher number of leaf was obtained at 150 ppm of BAP i.e. 19.40. BAP at the concentration of 50 and 100ppm produces 17.40 and 19.0 number of leaves when compared to control. 100 and 150ppm of BAP has significant effect on number of leaf per shoot. BAP at 100ppm resulted in high significant effect on leaf length where 13.64 cm of leaf length was observed. While, 150 ppm of BAP produced 12.86 cm of leaf length in comparison to control. Varying concentrations of BAP on average shoot length failed to produce significant effect in ginger when compare to untreated one. Effect of BAP on number of tiller was found to be effective. High significant effect of BAP on number of tiller was recorded at 50 and 100ppm where, 9.70 and 9.60 tiller number was visible. 150 ppm of BAP has also produced significant number of tillers i.e. 9.30 in comparison to control where 6.60 number of tiller were observed.

3.1.1.2 Effect of BAP on shoot growth in ginger when cultivated on flat land

Results of Table 2 highlighted the effect of BAP on various physical parameters of ginger when cultivated on flat land. BAP has significant effect on number of leaf per shoot in concentration independent manner i.e. higher leaf number was observed at 50 ppm (21.30) and least leaf number was observed at 150 ppm of BAP (20.50). 100 ppm of BAP also produced significant leaf number where 20.70 number of leaf was recorded. Leaf length in presence of different concentrations of BAP was enhanced effectively when compared to that of control. For example, BAP at 100 ppm has maximum leaf length of 14.00 cm on the other hand 50 ppm of BAP recorded 13.43 cm of leaf length. However, 150 ppm of BAP did not have significant effect on leaf length. As seen in ginger plant when cultivated in pot, similar effect of BAP on shoot length was noted where BAP did not alter shoot length when plants are cultivated on flat land except 50ppm had least significant effect on average shoot length. Effect of BAP on number of tillers produces significant effect on ginger and maximum number of tillers was reported at 50 ppm of BAP i.e. 11.10. Whereas, at 100 and 150 ppm of BAP resulted in 10.20 and 9.90 number of tiller. The effect of BAP was recorded better mean on the ginger plant shoot height only at 50 ppm notably. These increasing were in accordance with those results achieved by Saeid et al. [11].

When the concentration of BAP increased to high level the result on shoot length was not significant. The current findings were according to Cato et al. [12] as they observed a negative interaction between gibberellin and cytokine on elongation of the *Phaseolus vulgaris* stem. Since, plants can synthesize gibberellin naturally and when BAP was applied exogenous, the hormone may influence on gibberellin that has been synthesized endogenously through negative interaction as observed by Cato et al. [12]. Exogenous application of plant growth regulators affects the endogenous hormonal pattern of the plant, either by supplementation of sub-optimal levels or by interaction with their synthesis, translocation or inactivation of existing hormone levels [13]. And also in some instances, plants with cytokine applied have appeared smaller and have a less developed root system. Although reduced plant height and root system differences may not correlate to yield differences, it is important to realize that the plant is being impacted by these treatments [14].

Application of BAP on *Zantedeschia aethiopiea* caused to increase number of leaves [15]. In this research finding it is true on BAP as 50ppm caused to increase number of leaves than other concentrations as shown in Table 1 than that of control treatment without hormone application. According to Salehi et al. [16] studies on the application combination of (GA3+ BAP) to

Dizigotheeca elegantissima plant, the results showed that by increasing concentration of growth regulators, number of leaf/plant is increased, too. As the result denoted in Table 1 and 2 shown the same pattern reported by Salehi et al. [16] but in different plant species The results showed that in first and second year and in pooled data, maximum leaf length was recorded when the plants were treated with GA3 150ppm and it is statistically as par with the treatment when the plants treated with GA3 100ppm than the researcher used other plant growth hormone. However, in the current finding the research was not done in the presence of GA3 but BAP was used as bio-regulator that and was effective on leaf length when ginger plants treated with BAP at 100 ppm at both planting site (Tables 1 and 2).

In this experiment BAP increased the proliferation of tillers in both planting site. It is in accordance with Jewiss [17], Langer et al. [18], and Clifford and Langer [19] as they further reported that local applications of kinetin and benzylaminopurine (BAP) promote tiller bud elongation. In both cultivation site the effect of BAP was significant on some ginger physical characteristics of the shoot but there was some difference on the number of leaf and leaf length; when the concentration increase, the effect of this hormone showed high significant in comparison cultivation in the open field. And also in all parameter were observed slightly different mean result that was obtained maximum mean result in the open field. These different mean result found may be due to the planting material as four month old plant were transferred from open field to the pot, plants needs recovery time to survive when planting in the pot and highly sophisticated where grown in the pot when increased their tiller proliferations. Considering the cytokines effects, it was entirely predictable that spraying BAP on plants stimulates cell division and increased cell number [20]. Therefore, application of BAP results in significance increases on the number of leaf per shoot, leaf length and number of tillers and shoots length than control treatment.

3.1.2 Effect of exogenous foliar application of BAP on physical characteristics of rhizome in Boziab ginger variety

3.1.2.1 Effect of BAP on growth of rhizome in ginger when cultivated in pot

Effect of BAP on various physical characteristics of rhizome was investigated and is highlighted in Table 3. Number of fingers/rhizome was recorded higher at 100 ppm of BAP where, 8.40 fingers were noticed. While, 50 and 150 ppm of BAP produced 7.60 and 7.10 fingers/rhizome compared to that of control (6.20). Length of rhizome was not alter in presence of BAP and found to decrease in concentration dependent manner. Untreated plants has 9.80 cm of rhizome length whereas, 50, 100 and 150 ppm concentration of BAP showed 9.68, 9.11 and 7.41 cm of rhizome length respectively. Rhizome weight was also found to be ineffective in presence of BAP. BAP at a concentration of 50, 100 and 150 ppm has 53.46, 49.52 and 49.44 gm rhizome weight respectively when compared to untreated plants which recorded 56.83 gm of rhizome weight. When effect of BAP on width of rhizome was studied, it was observed that BAP at 100 ppm produced higher width i.e. 7.87 cm whereas, 50 and 150 ppm of BAP has 7.27 and 6.80 cm width of rhizome in comparison to control which produced 6.44 cm of rhizome width.

3.1.2.2 Effect of BAP on growth of rhizome in ginger when cultivated on flat land

Table 4 highlighted the effect of BAP on rhizome growth in ginger when cultivated on flat land. Similar results were obtained for rhizome growth in ginger when cultivated on land in comparison to ginger when cultivated in pot. When effect of BAP on number of fingers/rhizome was studied, maximum rhizome finger number was observed at 50 ppm of BAP where 8.50. Whereas, 100 and 150 ppm has 8.30 and 7.80 number of fingers/rhizome in comparison to control which resulted in 6.40 number of fingers/rhizome. Effect of BAP on length of rhizome as well as on fresh weight was found to be non-effective at all the level of concentrations of BAP studied in comparison to control. However, effect of BAP on width of rhizome was found to be effective and maximum width of rhizome was seen at 50 ppm of BAP i.e. 8.10 cm. On the other hand BAP at a concentration of 100 and 150 ppm produced 8.05 and 7.21 cm of rhizome width compare to that of untreated one which produce 7.05 cm of rhizome width.

Results of Tables 3 and 4 showed that application of BAP on ginger rhizome parameters like number of fingers/rhizome and width of rhizome was increased which was due to the biological effect of cytokine compound that has potential to increase bud development and propagation. These increases were in

accordance with the report of Khalighi et al. [21]. In a study by Garner et al. [22], results showed that BAP application on host plants can increase bud development and propagate production ratio. Baque et al. [23] showed that application of BAP, Tidiazuron, and a combination of these two substances decreased roots fresh and dry weight in *Morinda citriofolia*.

Table 1. Influence of foliar application of BAP on some physical parameters of shoot in ginger Boziab variety when cultivated in pot

Treatment	Number of leaf per shoot	Leaf length (cm)	Average shoot length (cm)	Number of tillers
Control	16.80±0.00	10.55±0.00	60.21±0.00	6.60±0.00
BAP 50 ppm	17.40±.60ns	12.61±2.06*	62.26±2.05ns	9.70±3.10***
BAP 100 ppm	19.0±2.20***	13.64±3.09***	62.76±2.55ns	9.60±3.00***
BAP 150 ppm	19.40±2.60***	12.86±2.31**	61.01±.800ns	9.30±2.70**
LSD at (0.05)	.000	.000	.224	.000

*Values are the mean of ten samples. ± indicated the mean difference from control. Where, ***: High significant; **: Moderate significant; *: Less significant; ns: Not significant*

Table 2. Influence of foliar application of BAP on some physical parameters of shoot in ginger Boziab variety when cultivated on plain land

Treatment	Number of leaf per shoot	Leaf length (cm)	Average shoot length (cm)	Number of tillers
Control	18.40±0.00	11.73±0.00	62.71±0.00	8.10±0.00
BAP 50 ppm	21.30±2.90**	13.43±1.70*	67.56±4.85*	11.10±3.00***
BAP 100 ppm	20.70±2.30*	14.00±2.27**	66.44±3.73ns	10.20±2.10**
BAP 150 ppm	20.50±2.10*	12.61±.880ns	62.89±.180ns	9.90±1.80*
LSD at (0.05)	.013	.032	.061	.005

*Values are the mean of ten samples. ± indicated the mean difference from control. Where, ***: High significant; **: Moderate significant; *: Less significant; ns: Not significant*

Table 3. Influence of foliar application of BAP on some physical parameters of rhizome in ginger Boziab variety when cultivated in pot

Treatment	Number of fingers/rhizome	Length of rhizomes (cm)	Fresh weight of rhizomes (gm)	Width of rhizome (cm)
Control	6.20±1.68ns	9.80±1.58*	56.83±8.00	6.44±.774 ns
BAP 50 ppm	7.60±1.57*	9.68±1.87ns	53.46±9.14ns	7.27±.998*
BAP 100 ppm	8.40±1.89*	9.11±2.36ns	49.52±6.68ns	7.87±1.11*
BAP 150 ppm	7.10±1.59ns	7.41±1.58ns	49.44±11.10ns	6.80±1.34ns
LSD at (0.05)	.045	.027	.208	.031

*Values are the mean of ten samples. ± indicated the standard deviation. Where, ***: High significant; **: Moderate significant; *: Less significant; ns: Not significant*

Table 4. Influence of foliar application of BAP on some physical parameters of rhizome in ginger Boziab variety when cultivated on plain land

Treatment	Number of fingers/rhizome	Length of rhizomes (cm)	Fresh weight of rhizomes (gm)	Width of rhizome (cm)
Control	6.40±1.71ns	10.96±1.76*	57.68±11.05	7.05±1.03ns
BAP 50 ppm	8.50±1.58**	10.94±1.65ns	57.18±11.40ns	8.10±.83*
BAP 100 ppm	8.30±2.31**	9.86±2.10ns	55.72±9.21ns	8.05±1.11*
BAP 150 ppm	7.80±2.04*	8.91±1.47ns	51.99±8.80ns	7.21±1.24ns
LSD at (0.05)	.054	.039	.595	.044

*Values are the mean of ten samples. ± indicated the standard deviation. Where, ***: High significant; **: Moderate significant; *: Less significant; ns: Not significant*

4. CONCLUSION

In general, from the current result it can be concluded that exogenous foliar application of BAP is important for shoot growth such as number of leaf, leaf and shoot length and initiation of tiller proliferation which can be stimulated to increase the growth of ginger through the application of this hormone. However, the production of rhizome was decrease when increasing BAP concentration which shows that plants production may not only be affected by lack of soil fertility but also due to the hormonal imbalance condition. In the end, growth and productivity of plants depend on types of plant growth regulators used with suitable method and concentration. The environmental factors also play an important role in optimization of growth in plants. In the present research, it was found that the application of BAP at lower concentration through foliar spray method has significant effect on various parameters like number of leaf, leaf length, proliferation of tiller, number of rhizome and width of rhizome. Since, our study was only restricted to only one plant hormone i.e. BAP which belongs to cytokine family, further research on the relationship between the effect of different class of growth regulators on shoot growth and rhizome production in ginger is necessary to derive a certain conclusive mechanism.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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