Research Article

Vegetative Growth Characteristics and Flower Sex Expression of *Cucumis sativus* Affected by Exogenous Application of Plant Growth Regulators

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Abstract | The effects of plant growth regulators gibberellic acid (GA_3), paclobutrazol (PBZ), naphthaleneacetic acid (NAA), benzyl amino purine (BAP); and ethrel at 100 ppm concentration on the vegetative and reproductive expression of cucumber were studied at the cotyledonary and two-leaf stages. The study was laid out in a split-plot randomized complete block design with 3 replications. Results showed significant differences in plant responses wherein the application of GA_3 resulted in the longest vine length at the two-leaf stage (3.24 m) and enhanced the staminate flower production. Ethrel enhanced pistillate flower tendency and improves yield when applied at cotyledon stage. Paclobutrazol (PBZ) showed the shortest vine length (1.49 m) at the two-leaf stage and improved pistillate flower production. BAP application enhanced yield and fruit development. NAA attained the least number of pistillate flowers and the lowest yield. The results suggest that the interactions of plant growth regulators applied on the cotyledon and two-leaf stages were significantly different. In conclusion, the application of GA_3 enhanced the maleness of flowers for pollination purposes, and ethrel or paclobutrazol improved pistillate flowers for enhanced fruit production.

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Introduction

Plant hormones are organic substances that influence processes physiologically at minute concentrations that occur naturally. The influenced processes consist primarily of differentiation, growth, and development (Davies, 2010). Plant growth regulators (PGR) are substances applied to regulate plant growth (Ughareja *et al*., 2022), and chemicals used to change and or alter the growth of a plant or part of a crop (Fishel, 2009). The utilization of plant growth hormones is an instant or short-term strategy for manipulating or influencing crop growth and development. Application of these substances together with the combination of plant breeding technology has the upper hand that minute tuning of crop improvement is achievable and the substances used are not costly and often more stable compared to those approaches that have direct hormonal activity as described by Mottley (1983). In studying the effects of these different plant growth regulators, cucumber plants were used as a model crop which is characterized as very tender, warm-season crops that grow best in temperatures ranging from 15.6 (minimum) to 32.2 (maximum) degrees Celsius (Schrader *et al*., 2002). Cucumbers may have indeterminate, determinate, or compact growth patterns with several flowering habits but most cultivars exhibit separate male and female flowers on the same plant or monoecious (Valenzuela *et al*., 1994). Several studies on plant hormones also showed growth regulation effects. A study conducted by Ozgur (2011) showed a significant effect in terms of seedling growth through paclobutrazol application. A decrease in staminate flowers and an increase in pistillate flowers were observed in the study of Putri *et al*. (2022) utilizing 0.375 mL. L-1 concentration of paclobutrazol applied on cucumber plants at various watering frequencies. A combined application of hydrazide and ethephon in 100 ppm showed an early development, maximized sex ratio, morphological, floral, and yield traits, and produced the greatest economic results for the production of cucumber (Thappa *et al*., 2011). Benzyl adenine and gibberellic acid in different concentrations at 25, 50 or 75 mgL-1 significantly affect cucumber mean fruit weight, leaf size, flower and fruit number (Batlang *et al*., 2006). Exogenous application of auxin increases the cucumber female flowers by inducing a sex-controlling gene and promotes the synthesis of ethylene (Niu *et al*., 2022). These previous studies indicate the sensitive responses of cucumber plants towards plant growth regulators; thus, this study was conducted to determine the effects of ethrel, GA_3, NAA, BAP, and PBZ on both vegetative and reproductive attributes of cucumbers grown in tropical lowlands. Moreover, it also aims to evaluate the responses of cucumber plants toward different PGRs in terms of morphological and yield traits for crop production purposes.

Materials and Methods

Seed sowing and experimental field characteristics

Cucumber seeds (variety MEGA C F1) which were bought from the agricultural supplies store located in the local market of San Jorge Samar, Philippines were soaked overnight (6 hours) with tap water and sown in seedling trays filled with medium with a mixture of 1:1 ratio of garden soil and vermicompost. The garden soil used in the growing of seedlings and field plots was characterized as silty clay with 0.20% nitrogen, 2.1 mg/kg phosphorus, 284.33 mg/kg potassium, 3.5 % organic matter, and a soil pH of 6.5 while the vermicast contains 1.20% nitrogen, 1.45% phosphorus, and 1.25% potassium. Experimental field plots were cleaned and plastic mulches were provided in controlling weeds and for soil moisture conservation.

Treatment preparation and application

Plant growth regulators (Ethrel, GA3, NAA, BAP, and PBZ) at 100 ppm concentration were sprayed on sample cucumber plants at the cotyledonary (cotyledon still attached or visible to the growing seedlings) and two-leaf (two fully opened leaves were visible) stage and applied two times at three days intervals.

Transplanting of cucumber seedlings

Cucumber seedlings were planted at a distance of 50 x 50 cm and arranged in a split-plot randomized complete block design with plant stages (cotyledon and two leaf stages) as the main plot and plant growth regulators as sub-plot replicated three times. Conventional management in terms of nutritional recommendations, watering, and pest management was applied. Trellises were made and provided for support as the vines grow.

Data gathered (Plant parameters)

Vine length was measured by the use of a tape measure and the quantification starts from the level of the ground up to the shoot tip of the main vines. The number of leaves was measured by counting the fully opened leaves after treatment application until the termination of the study. The number of nodes formed by the vines was counted at the termination of the study. Days to flower (staminate) were counted by counting the days to bear the first male flowers after treatment application. Days to flower (pistillate) were counted by counting the days to bear the first female flowers after treatment application. Node bearing the first staminate and pistillate flowers were identified by counting the nodes starting from below up to the node to produce the first male and female flowers. The number of staminate and pistillate flowers was measured by counting the male and female flowers formed from transplanting until the termination of the study. The number of fruits was measured by counting the produced fruits 2 weeks after transplanting the cucumber seedlings.

Table 1: *Vine length, number of leaves, and number of nodes in different plant stage as affected by plant growth regulators at termination.*

Plant stage	Treatments	Vine length (m)		Number of leaves		Number of nodes	
Cotyledon stage	T0-Control	2.67	d	38.57	cd	34.50	cd
	T1-Gibberellic acid	3.20	b	46.12	a	43.00	a
	T2-Paclobutrazol	1.76	g	36.00	def	33.00	cd
	T3-BAP	2.93	c	39.44	c	36.50	bc
	T4-NAA	2.41	e	34.71	ef	32.33	de
	T5-Ethrel	2.17	f	33.79	fg	32.00	de
Two-leaf stage	T0-Control	2.68	d	39.02	c	34.50	cd
	T1-Gibberellic acid	3.24	a	46.60	a	43.50	a
	T2-Paclobutrazol	1.49	h	25.04	h	21.67	f
	T3-BAP	2.92	c	42.73	b	39.67	ab
	T4-NAA	2.43	e	37.37	cde	34.33	cd
	T5-Ethrel	2.15	f	31.78	g	28.67	e
CV(%)		1.73		6.67		8.65	

Means within the same column followed by a common letter are not significantly different from each other at 5% level Tukey's HSD.

Data analysis

Data from vegetative and reproductive stage characteristics of cucumbers were analyzed using Analysis of Variance (ANOVA) in a split plot Randomized Complete Block Design (RCBD). Comparison between treatment means was determined using Tukey's HSD at a 5% level of significance.

Results and Discussion

The vegetative growth response of cucumber treated with plant growth regulators varies in terms of vine length, number of leaves and nodes (Table 1). Cucumber plants applied with gibberellic acid at two leaf stage and cotyledon stage exhibited the longest vines (3.20 and 3.24 meters, respectively) compared to other treatments, and paclobutrazol application caused the shortest growth in vine length (1.49 m) at two leaf-stage. The cucumber plants at two leaf stage were more sensitive to paclobutrazol in terms of vine length response since it was shorter compared to cucumbers at the cotyledonary stage. The number of leaves and nodes was also affected by the application of PGR's wherein each PGR had different effects in which the application of GA_3 instigated the greatest number of leaves and nodes. The application of paclobutrazol at two leaf stage attained the least number of leaves and nodes. NAA application was comparable to control treatment in the production of leaves and the number of nodes both in the cotyledon and two-leaf stages. The effect of ethrel was comparable to NAA in the length of vines, and production of leaves and nodes at the cotyledonary stage. The application of paclobutrazol was comparable to control in the number of leaves and nodes sprayed at the cotyledon stage. There were no significant differences in terms of the number of leaves and nodes on plants applied with NAA if compared to the control at the two-leaf stage.

Table 2: *Days to flower a staminate and pistillate in different plant stages as affected by plant growth regulators at termination.*

Plant stage	Treatments	Days to flower (Staminate)		Days to flower (Pistillate)	
Cotyledon Stage	T0-Control	29.00	e	34.11	d
	T1-Gibberellic acid	26.51	g	29.51	h
	T2-Paclobutrazol	27.12	f	34.11	d
	T3-BAP	31.10	c	34.15	d
	T4-NAA	33.43	b	35.59	a
	T5-Ethrel	31.11	c	34.12	d
Two-leaf stage	T0-Control	28.98	e	34.26	d
	T1-Gibberellic acid	27.14	f	33.37	e
	T2-Paclobutrazol	26.88	f	32.20	f
	T3-BAP	29.59	d	35.52	b
	T4-NAA	29.54	d	31.68	g
	T5-Ethrel	36.28	a	34.27	c
cv(%)		0.5080		0.0265	

Means within the same column followed by a common letter are not significantly different from each other at 5% level Tukey's HSD.

Cucumber plants without the application of plant growth regulators produced staminate flowers within 28-29 days and 34 days to initiate pistillate flowers (Table 2). Earliness to produce staminate flowers was attained by the application of GA3 (26.51 days) at the cotyledonary stage compared to other treatments. The application of ethrel caused a delayed staminate flowering response which occurs at 36 days at the two-leaf stage. There were comparable effects of BAP and ethrel application of days to produce staminate flowers at the cotyledonary stage while GA3 and paclobutrazol in two leaf-stage. BAP and NAA applications were also comparable in days to produce staminate flowers at two-leaf stage. In pistillate flower emergence, the application of NAA at the cotyledon stage attained the longest period (35.59 days) to produce pistillate flowers followed by BAP (35.52 days) at the two-leaf stage. The application of BAP, ethrel, and paclobutrazol was comparable to control in days of pistillate flower emergence at the cotyledon stage. The application of GA3 at the cotyledonary stage of cucumber showed earliness to pistillate flower emergence (29.51 days) followed by its application at the two-leaf stage (31.68 days). The cucumber plants' response to GA3 application both in the cotyledon and two-leaf stage showed varied interactions in days of emergence wherein the production of the staminate flower was earlier at the cotyledonary stage about 26.51 days than the two-leaf stage of 27.14 days. NAA application at the cotyledon and two-leaf stage showed a different response in which staminate flower production was delayed applied at cotyledon stage. Ethrel application sprayed at the cotyledonary stage produced staminate flowers in a shorter period (31.11 days) compared to two-leaf stage (36.28 days). BAP also showed a significant difference which resulted in delayed staminate flowering if applied at the cotyledon stage. In the cotyledon and two-leaf stage application of plant growth regulators showed varied responses in days to pistillate flower emergence wherein it was delayed if GA3 is applied on the two-leaf stage compared to cotyledon stage, earlier emergence occurs with the application of paclobutrazol applied on two-leaf stage compared at the cotyledon stage, delayed emergence with BAP application at two-leaf stage than in cotyledon stage, and earlier emergence if NAA applied at two-leaf stage compared to cotyledon stage.

Nodes of first staminate and pistillate flower emergence in cucumber plants showed a varied response applied with plant growth regulators (Table 3). Cucumber plants produced staminate and pistillate flowers mostly in the 3rd to 4th node without the application of PGRs. The application of ethrel stimulates the first staminate flowers in the 5th node both in the cotyledon and two-leaf stage wherein pistillate flowers were different since application in cotyledon stage was mostly visible at the 3rd node and at the two-leaf stage in the 10th node. BAP application at cotyledonary stage together with GA3, and NAA at two–leaf stage was comparable to control on the node of the first staminate flower emergence. There was no significant difference in GA3, ethrel at cotyledon stage, and BAP at two-leaf stage compared to control in the node of first pistillate flower emergence. The application of NAA and ethrel at cotyledon stage, GA3 and BAP at two-leaf stage were comparable to control in terms of node to produce pistillate flowers.

Table 3: *Node bearing the first staminate and pistillate flower in different plant stages as affected by plant growth regulators.*

Plant stage	Treatments	Node bearing first (Staminate flower)		Node bearing first (Pistillate flower)	
Cotyledon stage	T0-Control	3.90	de	4.00	ef
	T1-Gibberellic acid	4.36	cd	3.00	f
	T2-Paclobutrazol	3.45	ef	6.55	bc
	T3-BAP	3.90	de	5.58	bc
	T4-NAA	3.59	ef	4.3	de
	T5-Ethrel	5.34	ab	3.52	ef
Two-leaf stage	T0-Control	3.48	ef	3.33	ef
	T1-Gibberellic acid	4.97	bc	4.27	de
	T2-Paclobutrazol	3.00	f	6.75	b
	T3-BAP	4.66	bc	4.05	ef
	T4-NAA	3.86	de	5.45	cd
	T5-Ethrel	5.94	a	10.42	a
cv(%)		7.48		20.26	

Means within the same column followed by a common letter are not significantly different from each other at 5% level Tukey's HSD.

Staminate flower productions were enhanced by the application of GA3 (48.34) at two leaf stage compared to other regulators (Table 4) followed by BAP (42.48) at two-leaf stage. The application of NAA did not enhance the staminate flower production compared to plants without treatments. BAP at the cotyledon stage and paclobutrazol at two-leaf stage were comparable in effect on the number of staminate flowers. Paclobutrazol both in cotyledon and two-leaf stage enhanced the pistillate flower production (12.66, 17.43), respectively followed by ethrel at cotyledon stage of 15.54 and two-leaf stage of 15.49 as compared to the control with 9.53 to10.45 in quantity. The application of NAA both in the cotyledon and two-leaf stages did not improve the production of pistillate flowers as compared to the control. Numerous fruits were harvested per plant in 5 sessions of harvesting applied with GA3 and ethrel (5.53 and 5.57) respectively at the cotyledon stage while paclobutrazol at two-leaf stage followed by GA3. There were few fruits harvested on cucumber plants applied with NAA (2.25).

Figure 1: *(A) Resembles the staminate flower of a cucumber sprayed with ethrel at the cotyledonary stage with a slight modification on its basal part highlighted with a red circle compared to (B) a normal staminate flower of a cucumber without the application of ethrel.*

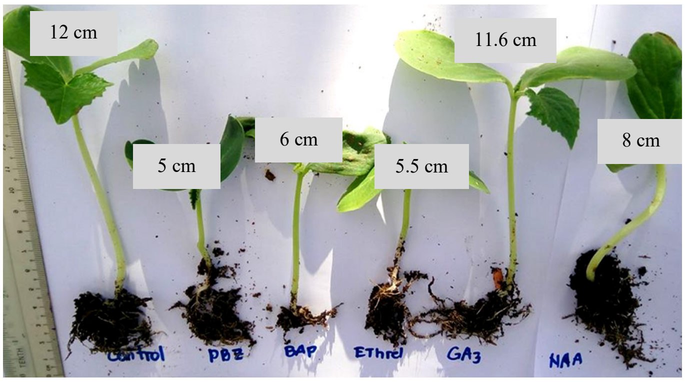

Figure 2: *Cucumber seedlings in 4 days after application of different plant growth regulators at the cotyledonary stage. (The cucumber seedlings exhibited varied responses at cotyledonary stage applied with plant growth regulators in which paclobutrazol (PBZ), 6-Benzylaminopurine (BAP), ethrel, application resulted in shorter seedling's height in average (5,6,5.5 cm long respectively). Seedlings treated with 1-Naphthaleneacetic acid (NAA) showed expanded and bent stems. Gibberellins (GA_3) application and control were most likely similar in appearance).*

The effects of gibberellins on plant growth in terms of cell elongation were previously studied in tissue-cultured plants and were utilized for shoot elongation (George *et al*., 2007). The gibberellic acid (GA) enhanced apical dominance, increased inter-node extension and expand leaf growth (Brian, 1959). There was an increase in sizable elongation of stem tissues due to gibberellic acid (Davies, 2012). Gibberellic acids play a main role in varied processes of growth including organ elongation, seed development, and regulation of flowering time (Santner *et al*., 2009) and daily treatments of GA at 100 ppm increased the node number and postponed the continuous female phase (Perl-Treves, 1999). Thus, cucumber plants sprayed with gibberellins attained the longest vine length and numerous nodes (Table 1). The effects of paclobutrazol on dwarfism in plants were studied by Chen and Zhang (2003); they reported that paclobutrazol (PP333) significantly reduced main stem growth rate of peanuts. Soil drenching of paclobutrazol at 1, 5 or 10 mg 1–1 enhanced chlorophyll content of cucumber leaf discs but inhibited the growth (Wang, 1985); similar results were attained on cucumber plants applied with paclobutrazol at 100 ppm that resulted in shortest in vine length. The action of paclobutrazol in stem elongation reduction is due to its effects on the pathway of isoprenoid and regulates or modify the levels of other plant hormones like inhibiting the gibberellin synthesis and increasing the levels of cytokinins as explained by Desta and Amare (2021).

In terms of flower traits exhibited by cucumber plants, it showed that GA3 enhanced maleness, similar results were reported from the previous study by Sibtehassan and Ali (1986) that GA3 promoted male tendency. Earlier reports showed that the application of GA3 increased male flowers in cucumber (Hidayatullah *et al*., 2009), whereas paclobutrazol and ethrel application promoted pistillate flowers in cucumber (Table 4 and Figure 1), Ullah *et al*. (2011) reported that ethrel application on cucumber significantly reduced the male to female flower sex ratio. On the other hand, paclobutrazol minimized vegetative growth by reducing both the height of plants (Figure 2) and dry weights while simultaneously fruit (diameter, length, and weight) and yield were increased (Kazemi, 2013). The effect of paclobutrazol in cucumber was not yet well studied but its application on other plants like increased flowering and fruiting in apples (Greene, 1986), in strawberry analyses indicated a slight increase in flower number (Lolaei *et al*., 2013). In yield traits, BAP, GA, and ethrel promotes the production of fruit sets since plants treated had numerous fruits harvested. Ethrel promotes femaleness on floral initiation in result to increased fruit yield, GA-enhanced maleness could be a source of pollen grains

Table 4: *Number of staminate and pistillate flower in different plant stages as affected by plant growth regulators in 5 weeks per plant samples.*

Plant stage	Treatments	Number of staminate flower		Number of pistillate flower		Number of fruits harvested in 5 times	
Cotyledon stage	T0-Control	39.95	c	10.45	def	4.40	d
	T1-Gibberellic acid	37.57	d	10.1	f	5.53	a
	T2-Paclobutrazol	32.32	e	12.66	c	4.62	c
	T3-BAP	21.27	g	10.2	ef	4.37	d
	T4-NAA	10.59	j	4.41	h	2.25	g
	T5-Ethrel	18.19	h	15.54	b	5.57	a
Two-leaf stage	T0-Control	40.21	c	10.43	e	4.35	d
	T1-Gibberellic acid	48.34	a	10.79	d	5.26	b
	T2-Paclobutrazol	21.45	g	17.43	a	4.17	e
	T3-BAP	42.48	b	10.53	de	5.58	a
	T4-NAA	23.97	f	7.73	g	3.57	f
	T5-Ethrel	12.75	i	15.49	b	4.34	d
cv(%)		2.29		0.806		1.22	

Means within the same column followed by a common letter are not significantly different from each other at 5% level Tukey's HSD.

for pollination of pistillate flowers so it its chances of fruit set and development, fruits sprayed with the mixtures of high GA3 concentration and middle 6-BA concentration increased by 52.89% with the increase of fruit number (Yang *et al*., 1992).

Conclusions and Recommendations

Significant differences were observed in cucumber plants in response to plant growth regulators applied either at the cotyledonary and two-leaf stages. Each plant growth regulator has their own different functions and effects in the vegetative and reproductive growth of cucumber plants. The roles were specific to a certain hormone or with correlated functions interacting with other types or towards the plant itself. GA3 promoted vine elongation and maleness of flower production. Paclobutrazol stimulated short vine length and enhanced fruit yield. Ethrel promoted pistillate ratio to staminate flowers. Cucumber responses to NAA were delayed pistillate flower emergence and minimal harvested fruits. BAP application improves fruiting. The application of paclobutrazol and ethrel is recommended to enhance pistillate flower production and GA3 is recommended for staminate flower production for favorable pollination to ensure maximum fruit yield.

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Novelty Statement

The study provides insights to students and local farmers on the regulation effects of plant growth regulators. Findings and recommendations will be significant in crop manipulation to ensure and secure food availability within the localities.

Authors' Contribution

Getulio A. Barcenas Jr.: Principal author, performed the experiment and did data analysis.
Luisa Marie I. Barcenas: Helped in treatment formulation, preparation, and application. Reviewed literature and assists in data interpretation.

Conflict of interest
The authors have declared no conflict of interest.

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