Journal of Applied Sciences

ISSN 1812-5654

OPEN ACCESS Journal of Applied Sciences

ISSN 1812-5654
DOI: 10.3923/jas.2022.256.261

Research Article
Biochemical Content, Vitamin and Minerals Development in Okra Using Plant Growth Regulators

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Abstract

Background and Objective: Okra keeps a significant role as a nutritious and medicinal vegetable that causes health benefits. The objective of the study was to evaluate the efficacy of this injection method applied on the flower bud at different concentrations of growth regulators. **Materials and Methods:** The experiment was carried out to investigate the effect of different concentrations of indole acetic acid (IAA), naphthalene acetic acid (NAA) and gibberellic acid (GA3) on okra pod set and size, soluble solids content (SSC), vitamin C and mineral contents using the flower injection method. **Results:** The lower concentrations (25 and 50 mg L^{-1}) of IAA and GA3 significantly increased the fruit set compared to the NAA. The higher concentration of 200 (mg L^{-1}) had a lower fruit setting than the lower concentration (25, 50 and 100) of IAA, GA3 and NAA. The higher pod/fruit size was obtained in IAA (31.01 cm^2) and GA3 (30.8) as compared to the control (0) and NAA 25.6 and 14.6 cm^2) at 100 (mg L^{-1}) of concentration. Pod size (38.4 was found significantly highest with 100 mg L^{-1} of IAA concentration. In addition, the highest SSC was obtained by 100 mg L^{-1} of IAA concentration as compared to the GA3 and NAA. The maximum vitamin C was found in IAA and GA3 as compared to the control (0) and 100 mg L^{-1} NAA concentration. Moreover, higher mineral contents like K, Ca, Mg, Na and Fe were found in 100 mg L^{-1} IAA and GA3 concentrations than 100 mg L^{-1} NAA concentrations. The higher concentrations (100 and 200 mg L^{-1}) of IAA and NAA greatly decreased the healthy seed percentage as compared to the lower concentration. However, GA3 concentration increased the healthy seed percentage at higher concentrations. **Conclusion:** It seemed that IAA was a better growth regulator as compared to the GA3 and NAA for okra growth.

Key words: Fruit size, vitamin C, minerals, SSC, okra, growth regulators

Citation: Hossain, A.B.M.S., M.M. Alenazi and R.M. Taha, 2022. Biochemical content, vitamin and minerals development in okra using plant growth regulators. J. Appl. Sci., 22: 256-261.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Okra is an important nutritious and medicinal vegetable. It is one of the superlative and nutritious vegetables in the tropical and subtropical areas in Asia[1,2] and Africa[2]. It belongs to the family Malvaceae, genus, *Abelmoschus* and species, *esculentus*. It was reported that the total production of okra was 5.9 m tons in the world[3].

It was reported that okra pods were considered nutritious, providing some human supplementary vitamins such as vitamin C, A, B-complex, calcium, potassium, iron and other minerals. Okra pod contains many nutritional contents which are important for human health[4]. One hundred grams of the fresh pod has around moisture (89.6%), K (103 mg), Ca (90 mg), Mg (43 mg), P (56 mg), vitamin C (18 mg) and some important metals such as iron and aluminium[5].

The application of plant growth regulators is known as one of the most important treatments used nowadays in agriculture. Some horticulture crop productions were increased by the application of different growth regulators Growth regulators mainly regulate the plant's physiological and biochemical processes[6]. For example, play a major role in dormancy, organ size, crop improvement, flowering and fruit set, regulation of the chemical composition of plants[2]. The phytohormone auxin affects approximately all developmental processes in plants, including fruit improvement. However, auxin is produced in meristems and young leaves and moved to other parts of the plant in a polar fashion[7].

There are more than 100 distinct gibberellins produced primarily in roots & young leaves but GA3 or gibberellic acid is the most popular available form. GA3 has many effects on plant growth such as enhanced stem and internodes elongation, produce seed germination, enzyme production during germination and fruit setting and growth[8] and breaking of dormancy. It was indicated[6] that plant growth regulators might be used to regulate the vegetative growth of plants. Application of IAA increased the plant height, the number of internodes, leaf area, dry weight of shoot and dry weight of Gram plant, respectively (However, work has been done on the use of GA3 to improve vegetative growth, pod size and delay pod maturity in vegetables using the spray method. But no studies have been conducted to evaluate the complete profile of vegetative growth and pod yield in response to GA3, IAA and NAA application to okra using stem injection. Objectives of the experiment were undertaken to investigate the effect of different concentrations of applied GA3, IAA and NAA at different concentrations on okra pod setting and size, soluble solids content, vitamin C and mineral-like K, Ca, Mg, Na and Fe content. Also to evaluate the efficacy of this injection method (treated flower) of application on the okra pod.

MATERIALS AND METHODS

Study site: The present investigation was carried out on a commercial farm in the summer season (April to September) in Banting, Selangor, Malaysia. The soil in this field was peat with a mean pH of 6.6.

Plant materials: The seeds of the local *Abelmoschus esculentus* variety were sown in the experimental area of Banting. These seeds were soaked in distilled water for 24 hrs after which they were spread on moist filter paper in Petri-dish. The Petri-dish was kept in a dark cupboard at room temperature of 28°C. Okra seeds were sown directly into the soil by hand in soil fertilized with NPK 19 g/plant 14-14-14 as basal fertilization. Thirty days after emergence, side dress with 10 g/hill 46-0-0 and plots was irrigated when necessary. The experiment was laid out in randomized block design having four replications. The whole area was divided into fifteen blocks and each block into 20 unit plots. The size of the unit plot was 1×1 m^2. The seeds were shown in rows made by hand plough. The gaps where seeds failed to germinate were filled up within 2 weeks after germination of seeds. After field preparation, seeds were sown in well-prepared seedbeds in line with a distance of 70 cm when germination was completed thinning was done to maintain the plant to plant distance of 30 cm. The depth of planting was 1cm from the surface of the soil. Hoeing, weeding and other cultural practices were done uniformly.

Preparation of plant growth regulators: The growth regulators employed in the experiment were indole acetic acid (IAA) gibberellic acid (GA3) and naphthalene acetic acid (NAA). The concentrations of the growth regulators treatments were 0, 25, 50, 100 and 200 mg L^{-1}. The GA3, IAA and NAA were dissolved separately in 2 mL of 1% ethanol to make desired concentration. Each concentration of growth hormone was added with distilled water to make 100 mL of solutions. The control plants were injected with 100 mL of water mixed with 2 mL of 1% ethanol.

Application of treatment: One and a half mL of the various concentrations of GA3, IAA and NAA (0, 25, 50, 100 and 200 mg L^{-1}) were applied to the flowers by injecting before anthesis with a needle for a surgical purpose. Four flowers were selected randomly per each replication.

Measurement of parameters

Pod setting and size measurement: Five pods were randomly chosen from each treatment to determine the pod (fruit) size, green pod length (cm) and pod diameter (cm) were measured with a vernier calliper. Then pod size (cm^2) was measured.

Biochemical content represented as soluble solids content (SSC) measurement: Total soluble solids (TSS) content in the fruits/pods were evaluated at 25°C with a Refractometer. TSS was expressed with % Brix. A hand-held refractometer (Atago ATC-1, 32-10 Honcho, Itabashi-ku, Tokyo 173-001, Japan) was used. A few drops of juice were kept on the refractometer prism surface and reading was collected from the screen pad.

Measurement of potassium (K) content: The K content of the pod was determined by using a Cardy Potassium meter instantly after harvesting the pods. One gram of pod was homogenized in 5 mL distilled water in the mixer and centrifuged at 4000 rpm for 10 min. Afterwards, three drops of supernatant were put onto the calibrated sensor pad (Cardy Potassium Meter, Model-2400, USA), having a sampling paper placed on the sensor. The reading in ppm was taken from the display pad after it stabilized (30-43 sec).

Vitamin C determination: Ascorbic acid as an antioxidant was determined by applying redox titration. Vitamin C concentration was determined using a redox titration having potassium iodate in the presence of potassium iodide. Ascorbic acid was oxidized and excess iodine was free to react with starch indicator, following the blue-black starch-iodine complex. The 1 mL of titrant was utilized for each flask and calculated. The measurements[8] were taken that were obtained and averaged:

$$\text{Average volume} = \frac{\text{Total volume}}{\text{Number of trails}}$$

X mL of iodine solution/0.250 g Vit C = X mL iodine solution/
X mL Vit C = g Vit C in that sample

Minerals content measurement: Analysis of mineral contents of okra (Ca, Mg and Na, Fe) was done using a multi-element analyzer (MOA). Samples were grounded properly using a green pod by mortar and pestle. The 5 mL water was mixed with the sample. After that 1 mL of the sample was injected into the MOA and readings were calculated.

Statistical analysis: The obtained data were statistically analysed using SPSS Computer Programme, Version16. The data were analyzed following the Analysis of Variance (ANOVA) technique and mean differences were adjusted by using Duncan's Multiple Test (DMRT) at a 5% level of significance.

RESULTS

Pod/fruit set percent and fruit/pod size: The fruit set was influenced by the application of GA3, NAA and IAA in Fig. 1. IAA and GA3 applied at different concentrations influenced the pod set significantly ($p<0.05$). The lower concentrations (25 and 50 mg L^{-1}) of IAA and GA3 greatly increased the fruit set as compared to the NAA.

A significant variation was evident in the pod set and size due to the application of gibberellic acid (GA3) and IAA at different concentrations in Table 1 compared to the NAA. The analysis of variance showed that IAA exhibited a highly varied influence on fruit size.

Results indicated that pod sizes were significantly affected by the concentration of IAA and GA3. The higher pod/fruit size was obtained in IAA (31.01 cm^2) and GA3 (30.8) as compared to the control (0) and NAA 25.6 and 14.6 cm^2) at 100 (mg L^{-1}) concentration. Pod size (38.4 was found significantly highest with 100 mg L^{-1} of IAA concentration.

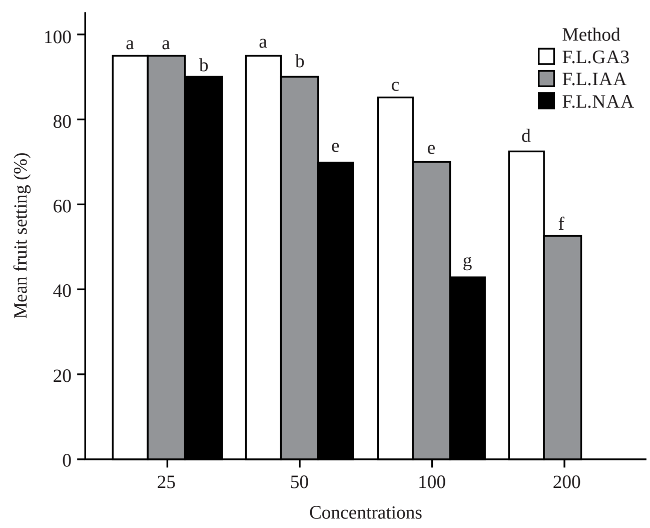

Fig. 1: Effects of different concentrations of IAA, NAA and GA3 on okra fruit setting percentage applied by flower injection method

Means followed by the same letter or no letter do not differ significantly at 5% level by Duncan's Multiple Range Test (DMRT)

Table 1: Fruit size biochemical and mineral contents of *A. esculentus* as affected by different concentrations of IAA, NAA and GA3

Growth regulators	Concentrations (mg L^{-1})	Fruit size (cm^2)	Soluble solids (% brix)	Ascorbic acid (mg/100 g)	K$^+$ (mg/100 g)
	0	25.6±0.30^b	2.74±0.04^b	13.80±0.02^b	92.08±0.02^b
IAA	25	27.83±0.33^a	2.82±0.02^a	14.98±0.02^a	93.15±0.01^a
NAA	25	25.10±0.30^b	2.77±0.02^b	14.08±0.02^a	92.58±0.02^b
GA3	25	26.10±0.26^c	2.81±0.03^a	13.90±0.02^b	93.06±0.03^a
	-	*	*	*	*
IAA	50	30.86±0.40	2.85±0.01^a	12.79±0.03^b	93.23±0.03^a
NAA	50	22.26±0.20^c	2.75±0.02^c	12.06±0.02^b	92.94±0.02^b
GA3	50	30.51±0.31^b	2.80±0.01^b	13.67±0.0^a	93.14±0.05^a
	-	*	*	*	*
IAA	100	31.01±0.37^a	2.89±0.02^a	18.86±0.02^a	93.25±0.02^a
NAA	100	14.65±0.33^b	2.73±0.20^c	11.72±0.70^c	92.40±0.36^b
GA3	100	30.80±0.63^a	2.64±0.02^b	16.33±0.02^b	93.15±0.03^a
		*	*	*	*
IAA	200	14.20±0.25^b	2.22±0.02^b	11.76±0.01^b	92.82±0.03^b
NAA	200	0	0	0	0
GA3	200	24.22±0.27^a	2.82±0.02^a	12.87±0.01^a	93.13±0.02^a
		*	*	*	*

Values are Mean±Standard deviation and means followed by the same letter or no letter do not differ significantly at 5% level by Duncan's Multiple Range Test (DMRT)

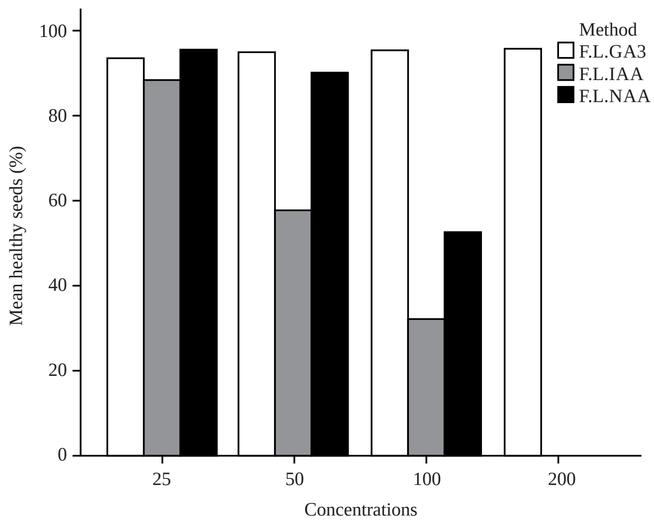

Fig. 2: Effect of plant growth regulators (IAA, NAA and GA3) on healthy seeds percentage in *Abelmoschus esculentus*

Soluble solids (TSS), ascorbic acid (vitamin C), nutrient content and seed percent: One of the most important parameters for pod quality is the soluble solids content of the pod. Total soluble solids (TSS) were markedly increased by IAA application at all different concentrations in Table 1. Under IAA treatment okra pod presented the highest TSS with 100 mg L^{-1} concentration at the same time, the lowest value was in the control treatment. On the other hand, results shown in Table 1 that the vitamin C in okra pods was significantly increased by IAA flower injection treatment. Vitamin C pod content was increased at 25 and 100 mg L^{-1} IAA as compared to the 50 and 200 mg L^{-1} IAA. IAA at 100 mg L^{-1} resulted in the highest pod content of vitamin C while flower injection treatment with 200 mg L^{-1} had the least effect on vitamin C content, which was on par with the control.

There are some important minerals highly concerned with human nutrition, such as potassium (K), calcium (Ca), magnesium (Mg), iron (Fe) and sodium (Na) were analyzed in the samples of okra pods. Table 1 showed that flower injection application of okra plants with IAA and GA3 at 50 and 100 mg L^{-1} concentration led to an obvious increase in the endogenous content of potassium (K). The highest value of K was obtained from the application of 100 mg L^{-1} (93.25 mg/100 g) followed by 25, 50 and 200 mg L^{-1} (93.23 mg/100 g), (93.15 mg/100 g) and (92.82 mg/100 g), respectively. Meanwhile, the control produced the lowest content of potassium (92.08 mg/100 g). In Table 2 the results showed that the calcium content in pods ranged from 59.0-59.31 mg/100 g concerning the effect of IAA application, IAA at 100 mg L^{-1} had a positive effect on calcium pod content and showed the highest Ca contents (59.31 mg/100 g). Also, magnesium (Mg) and sodium (Na) content significantly were increased by IAA application. On the contrary, iron (Fe) content increased significantly with the application of GA3 with 100 mg L^{-1} concentration compared to IAA, NAA and control. The greatest content of Fe content was 0.463 mg/100 g with the 100 mg L^{-1} concentration in this study. The higher concentrations (100 and 200 mg L^{-1}) of IAA and NAA greatly decreased the healthy seed percentage as compared to the lower concentration in Fig. 2. However, GA3 concentration increased the healthy seed percentage at higher concentrations.

Table 2: Nutritional elements (mineral contents) of okra pods as affected by IAA, NAA and GA3 at 100 (mg L^{-1}) applied by pre-sowing treatment

Concentrations	Ca	Mg	Fe	Na
0	59.0±0.012[c]	38.21±0.01[c]	0.410±0.02[c]	5.98±0.012[b]
IAA	59.31±0.011[a]	39.28±0.012[a]	0.420±0.001[c]	6.16±0.01[a]
GA3	59.27±0.02[ab]	39.24±0.02[b]	0.463±0.01[a]	6.11±0.01[a]
NAA	59.19±0.02[b]	38.52±0.01[c]	0.453±0.01[b]	6.03±0.02[a]
LSD 0.05	0.02 *	0.016 *	0.037 *	0.021 *

Values are Mean±Standard deviation and *Means followed by the same letter or no letter do not differ significantly at 5% level by Duncan's Multiple Range Test (DMRT)

DISCUSSION

The use of plant growth regulators (PGRs) has been an increasingly important aspect of the practices of many cultivated plants[2]. Several reports observed that application of the plant growth regulators influenced growth, fruit set, fresh vegetable weight and pod yield quality[9]. Application of IAA and GA3 at 25, 50, 100 and 200 mg L^{-1} increased the fruit setting and size over NAA applied by flower injection method. GA3 is considered as enhancing cell division and elongation[10]. It was stated[12] that increased stem elongation might be due to the stimulating action of GA3, which alleviated the cell wall by increasing its plasticity. The results confirm with other studies[11-13] which observed that GA3 and IAA application increased the plant growth and yield of soybean and red sorrel, respectively. But both investigations found IAA at 100 ppm was more efficient than GA3 and NAA. Also, earlier studies reported that growth regulators increased plant and fruit growth in various crops[12] soybean and sesame.

With GA3 at 100 and 200 mg L^{-1} concentrations, there was a significant difference in the pod in comparison with IAA and NAA. GA3 increased fruit number per plant in Bell pepper[14]. It was found[14] that GA3 and IAA treatment at 100 ppm increased leaves number and leaves area and chlorophyll content in *Hibiscus sabdariffa* L. It was also mentioned[15] a significant increase in the leaf length in onion by application of GA3. This might be attributed to that GA3 and IAA increased the division and elongation of the cells leading to better vegetative growth of plants. GA3 application increased branch number by breaking apical dominance. It was informed that GA3 delays the loss of chlorophyll. Moreover, IAA and GA3 at 50 and 100 mg L^{-1} had a better effect than lower concentrations (25 and 50 mg L^{-1}) and control. GA3 and IAA developed yield and physicochemical characteristics of leafy vegetables[3]. In addition, it was reported[14] that increasing yield might be related to the plant height, leaf number, leaf area. Another reason might be the physiological role of gibberellin and indole acetic acid in increasing cell division and elongation and stimulating the complete growth of a plant which is revealed in a better pod setting by using IAA and GA3. IAA and GA3 allow water to enter the cells of fruits and dissolved materials which lead naturally to an increase fruit size by increasing the permeability of fruit cell wall[12]. IAA and GA3 application at 100 ppm increased the yield of rice and soybean[3].

CONCLUSION

From the above discussion, it can be concluded that 25, 50 and 100 mg L^{-1} of GA3, IAA and NAA concentrations were better than control (0) and 200 mg L^{-1} of concentration for fruit setting, pod size, vitamin C, biochemical content (TSS) and minerals. So it can be recommended that the flower injection technique can be used commercially in the vegetable industry. The internal application as flower injection can reduce the chemical and production cost without hazardous any environmental pollution.

SIGNIFICANCE STATEMENT

The flower injection method was effective and applied on okra using different growth regulators and an important technique instead of the spray method. The internal application reduces the chemical and production cost that can be used for future purposes without hazarding any environmental pollution.

ACKNOWLEDGMENT

We acknowledge the University of Malaya for financial support and grant for this project. Also, the authors are wowed to the King Saud University to support a Scholarship to Mekhled M. Alenazi.

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