



Seedless okra pod, chlorophyll, vitamin C and mineral content development using plant growth regulator *in vitro* and *in vivo* culture

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ABSTRACT

Vegetables are rich in vitamins, minerals and dietary fiber that keep a significant role in the functioning of the human body to refrain human health benefits. The experiment was carried out to investigate the effect of different concentrations of IAA on the seedless pod, chlorophyll, vitamin and mineral content of okra as human health benefits. The innovative seed soaking method of application using 0, 25, 50, 100 & 200 mg/l of IAA concentrations was used in okra before germination and cultured *in vitro* and *in vivo*. The lower concentrations (25 and 50 mg/l) of IAA significantly increased the pod setting compared to the higher concentration (100 and 200 mg/l). The higher concentration (100 and 200 mg/l) had lower fruit settings than the lower concentration (25 & 50) had higher fruit settings. The higher pod size was obtained in the concentration of 100 & 200 mg/l of IAA (34.18 cm²) as compared to the control and other concentrations. In addition, the highest soluble solid content was obtained by 100 and 200 mg/l of IAA concentration as compared to the other concentrations. The maximum vitamin C was found in the concentration of 100 mg/l of IAA as compared to the control and other concentrations. Moreover, higher mineral contents like K, Ca, Mg, Na and Fe were found in 100 & 200 mg/l of IAA. The higher concentrations (100 and 200 mg/l) of IAA greatly increased the seedless okra percentage as compared to the lower concentration. It seemed that 100 and 200 mg/l concentration IAA was a better concentration for mineral content and seedless okra production as compared to the other concentrations.

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Introduction

Okra is one of the important vegetables grown widely as a commercial crop in many countries such as India, the United States, Western Africa, and other countries in the world. They are considered secure foods as their utilization of avoiding many diseases (1). Okra (*Abelmoschus esculentus*) is one of the superlative and favorable vegetables in tropical and subtropical areas in Africa and Asia (2). It was reported that the total production of okra was 8.9 m tons in the world (3). The okra production was 1.0, 2.4, 8.1 and 8.5 m tons in the USA, Sudan, Nigeria and India respectively (4).

It was observed (5) that okra pods are considered as nutritious, providing some human supplementary vitamins such as vitamin C, A, B- complex, calcium, potassium, iron and other minerals. Okra pod contains many nutrients contents which are foremost for human health. One hundred grams of the fresh pod has moisture (89.6%), K (103 mg), Ca (90 mg), Mg (43 mg), P (56 mg) and vitamin C (18 mg) and some important metals such as iron and aluminum (6,7).

Application of plant growth regulators (PGRs) is one of the most functional tools for horticultural crops to enhance yield, improve crop quality and management (8). Several

reports indicated that the application of the plant growth regulators enhanced germination, growth, fruit set, fresh vegetable weight and seed yield quality (9).

It was stated that growth regulators had a positive effect on fruit quality, carbohydrate, protein, vitamin contents and mineral elements of many horticulture crops (1). It was exhibited (10) that natural or synthetic plant growth regulators controlled the plant activities and their productions by controlling one or more specific physiological processes within a plant.

IAA is an auxin and was produced in cells in the bud and young leaves of a plant. It was exhibited that plant cells mainly produced IAA from tryptophan, but could also produce it independently of tryptophan (11). He also identified that plant growth regulators were used to regulate the vegetative growth of plants. However, research has been done on the use of IAA to improve vegetative growth, pod size, and delay pod maturity in vegetables using the spray method. But little studies have been conducted to evaluate the complete profile of vegetative growth, pod quality and seedless okra production based on IAA application using seed soaking method. Objectives of the experiment were undertaken:

To investigate the effect of different concentrations of IAA on okra plant growth, chlorophyll content, maximum

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quantum yield (Fv/Fm), nutrient and vitamin C content in pod, pod size and yield and also to investigate the effect of the application of seed soaking method for inducing parthenocarpy (aborted seed/seedless) in okra pod.

Materials and Methods

Plant materials and experimental design

The seeds of the local okra (*Abelmoschus esculentus*) variety were sown in the experimental area of Banting, Malaysia. These seeds were soaked in distilled water for 24 hours 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 30°C. Okra seeds germinated *in vitro* (Figure 1) and seedlings were sown directly into the soil by hand in soil fertilized with N-P-K 19 g/hill 14-14-14 as basal fertilizer (1) and plots were irrigated when necessary. The size of the unit plot was 1 x 1 m². The seedling was shown in rows. Hoeing, weeding and other cultural practices were done uniformly.

Preparation of plant growth regulator

The growth regulator employed in the experiment was indole acetic acid (IAA). The concentrations of the growth regulators treatments were 0, 25, 50, 100 and 200 mg/l. The IAA was dissolved in 2ml of 1% ethanol to make desired concentration. Each rate of chemical IAA was added to distilled water to make 100 ml of solutions.

Measurement of parameters

Germination (%): Germination was recorded every day for two weeks and calculated as a total percent.

Plant height and stem girth (cm): Plant height was measured from above ground level up to the uppermost tip of the leaves at the end of harvesting. Both plant height and stem girth were measured using a meter rule with the aid of thread.

Leaf phytochemical as chlorophyll content: The chlorophyll content as phytochemical in the leaves was counted by the SPAD value meter (Minolta Japan).

Leaf chlorophyll fluorescence measurement: Leaf chlorophyll fluorescence was measured on the upper surface of the leaf by using a Plant Efficiency Analyzer (PEA, Hansatech Instruments Ltd., England). A leaf clip was appended to the leaf and kept in the dark for 15 minutes for dark adaptation. After that the shutter plate was opened and the light was applied to the leaf. The initial fluorescence intensity (Fo) when all reaction centers (RCs) are open, the maximal fluorescence intensity when all reactions are closed (Fm), the variable fluorescence, Fv and the time to reach the maximal fluorescence intensity (t-max), were calculated. The quantum yield was determined according to the equation of Fv/Fm.



Figure 1. The partial view of the okra seed-soaked technique *in vitro*.

Pod parameters: Five pods were randomly chosen from each treatment to determine the green pod length (cm), green pod diameter (cm), pod size (cm²), pod size was determined by measuring the length and diameter of the pod per treatment with a Vernier caliper.

Pod weight: Green pod weight (g) was determined with the help of a digital UWE-ESP Digital Electric Balance and the average weight was calculated.

Seed production: The number of healthy seeds and seedless (aborted) percent were counted after the dry stage.

Total soluble solid (TSS): A small fraction of the homogeneous mixture was centrifuged at 4000 ×g for 10 min and the clear supernatant was analyzed for TSS. The total TSS of pods was examined using a hand refractometer at Atago 8469 (Atago Co. LTD., Tokyo, Japan and expressed as ° Brix).

Measurement of potassium (K) content

The K content of the pod was determined using a Cardy Potassium meter instantly after harvesting the pods. 1 gm of the pod was homogenized in 5 ml distilled water in a mixer and centrifuged at 4000 rpm for 10 min. Afterward, 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 was taken from the display pad after it stabilized (30 to 43 sec).

Vitamin C determination

Vitamin C (Ascorbic acid) concentration was determined by applying a redox titration having potassium iodate in the presence of potassium iodide. 1 ml of titrant was utilized for each flask and Calculated. The measurements were taken that were obtained and averaged them.

Average volume = total volume/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 5ml water was mixed with the sample. After that 1ml of the sample was injected into the MOA and readings were calculated.

Statistical analysis

The data were statistically analyzed using SPSS Computer Program, Version 16. The data were analyzed following the Analysis of Variance (ANOVA) technique and mean differences by using the Least significance difference Test (LSDT) at a 5% level of significance.

Results

The highest germination percent was observed in the concentration of 25 mg/l IAA as compared to the 0, 50, 100 & 200mg/ml of concentrations (Figure 2). Plant height was influenced by the application of IAA at different concentrations (Table 1). The lower concentrations (25 and 50 mg/l) of IAA greatly increased the plant height (83.8 and 84.25 cm) compared to the control (75.8 cm). The higher concentrations (100 & 200) had lesser plant height (80.9 and 76.3 cm). A significant variation was

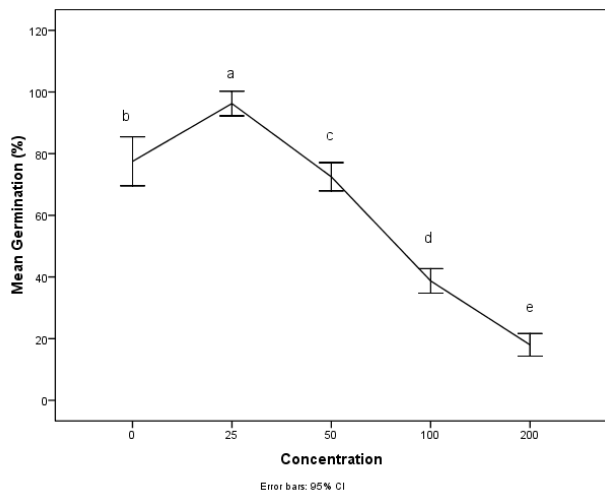


Figure 2. Measurement of germination (%) of okra seed as affected by IAA concentrations. Means followed by the same letter do not differ significantly at the 5% level by the Least Significance test (LSD) test.

found in the number of branches per plant, stem girth and number of leaves due to the application of IAA at different concentrations (Table 1). The treated plants generated a higher number of branches over control. 100 mg/l of IAA induced a maximum number of branches followed by 50 mg/l and 200 mg/l as compared to control. Moreover, 100 mg/l concentrations induced the highest value of stem girth and the number of leaves over the control and other concentrations. It has been shown in Figure 3 the effect of pre-sowing treatment at different concentrations of IAA on chlorophyll fluorescence yield. Chlorophyll fluorescence

yield was higher in 25, 50 and 100 mg/l concentrations than the concentration of 200 mg/l and control. Results in Table 2, indicated that leaves content of chlorophyll was affected significantly by different concentrations of IAA. The results showed that IAA concentrations of 50 and 100 and 200mg/l increased chlorophyll content and number of pods over other concentrations.

It was observed in Table 3, that total pod per plant, pod length, pod diameter, pod size, individual pod weight and healthy seeds percentage per pod were significantly

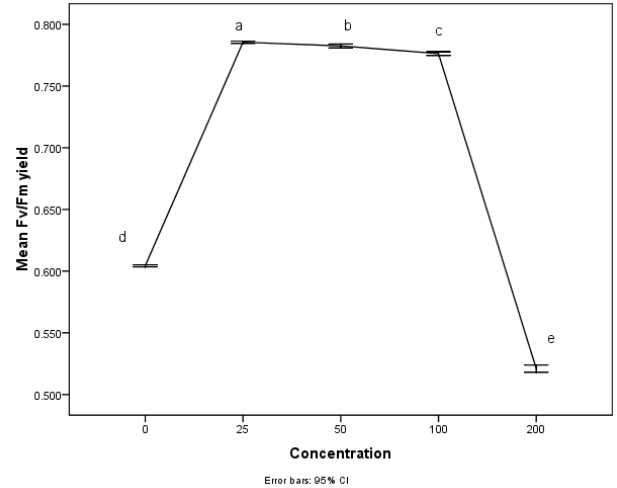


Figure 3. Effect of pre-sowing treatment at different concentrations of IAA on chlorophyll fluorescence yield of okra (Means followed by the same letter do not differ significantly at 5% level by Least Significance test (LSD)).

Table 1. Effect of IAA at different concentrations on plant height and stem girth of okra.

Concentrations (mg/l)	Plant height (cm)	No. of branches/plant	Stem girth (cm)	No. of leaves/plant
0	75.80±0.02e	1.75±0.50c	2.52±02d	19.25±0.95d
25	83.84±0.02b	2.0±0c	2.63±02c	30.00±0.82c
50	84.25±0.01a	2.75±0.50a	2.69±0.02b	33.25±1.26b
100	c ±0.05 80.94	3.0±0a	2.75±0.01a	36.25 ±0.50a
200	76.34±0.01d	1.50±0.57c	2.65±09c	19.75±0.96d
LSD (0.05)	0.021	0.62	0.02	1.40
	*	*	*	*

Values are means ± standard deviation. Means followed by the same letter do not differ significantly at the 5% level by the Least Significance test (LSD).

Table 2. Measurement of chlorophyll content and number of pods/plants at different concentrations of IAA in okra.

Concentrations (mg/l)	Chlorophyll content (Spad value)	No. of pods/plant
0	39.75±0.50d	8.50±0.57d
25	55.13±0.01c	18.00±1.16b
50	60.75±0.01a	23.50 ±1.29a
100	57.21±0.02b	22.00±.82a
200	38.62±0.02e	11.00±1.41c
LSD (0.05)	0.36	1.65
	*	*

Values are means ± standard deviation. Means followed by the same letter do not differ significantly at the 5% level by the Least Significance test (LSD).

affected by different concentrations of IAA. Among the concentrations, 50 mg/l had the maximum number of pods per plant followed by 100 and 50 mg/l in comparison with control. It was revealed that 50 mg/l produced the longest pod followed by 100 mg/l.

Pod diameter was found maximum with 50 mg/l and 100 mg/l and followed by 200 mg/l and 25 mg/l. Significantly highest pod size was obtained at 50 mg/l followed by 100 mg/l. In this respect, pod weight was recorded as significantly highest at 50 mg/l (10.52g). The second heaviest pods were obtained at 100 mg/l (9.4g) followed by 200 mg/l and 25 mg/l. Seedless pod harvested from 200 mg/l treated plants had significantly highest aborted seeds percentage (37.3%) followed by 100 mg/l (19.3%), 50 mg/l (6.75%), 25 mg/l (3.65%) and control (0%). Control, 25 and 50 mg/l treatments had increased the production of healthy seeds compared with 100 and 200 mg/l concentra-

tions (Table 3). Besides, 100 mg/l of IAA treatment exhibited maximum soluble solid content and ascorbic acid (vitamin C) followed by 200, 50 and 25mg/l (Table 4). The higher mineral content (K, Ca, Mg, Fe & Na) was found in the concentration of 200mg/l of IAA as compared to the 100, 50, 25 mg/l and control (Tables 4 & 5).

Discussion

This study compared the bioavailability of different concentrations of IAA for improving growth, yield and fruit quality when applied to okra by seed soaking technique. The use of plant growth regulators (PGRs) is becoming an increasingly important aspect of agricultural and horticulture practices for many cultivated plants (2). Several reports indicated that the application of the plant growth regulators showed the germination, growth, fruit

Table 3. Effect of different concentrations of IAA on okra pod size, weight and seedless production.

Concentrations (mg/l)	Pod length (cm)	Pod diameter (cm)	Pod size (cm ²)	Pod weight (g)	Seeds/pod (%)	Seedless(Aborted seeds)/pod (%)
0	4.92±0.01d	2.16±0.01d	8.46±0.08d	3.62±0.01d	100.0±0.01a	0 e
25	8.67±0.01b	3.83±0.01b	33.23±0.14b	9.81±0.03b	96.33±0.01b	3.65±0.02d
50	8.76±0.02a	3.90 ±0.01a	34.18±0.05a	10.52±0.01a	93.23±0.02c	6.75±0.01c
100	8.52±0.01c	3.53±0.02c	c 10±0. 30.1	9.42±0.02c	80.16±0.01d	19.83±0.02b
200	4.10±0.02e	1.83±0.03e	7.50±0.14e	3.30±0.03e	62.62±0.01e	37.38±0.02a
LSD (0.05)	0.02	0.02	0.16	0.030	0.02	0.02
	*		*	*	*	*

Values are means ± standard deviation. Means followed by the same letter do not differ significantly at 5% level by the Least Significance test (LSD) test.

Table 4. Effect of different concentrations of IAA on total soluble solid, ascorbic acid and K⁺ of okra.

Concentrations (mg/l)	Soluble solid (%Brix)	Ascorbic acid (mg/100g)	K ⁺ (mg/100g)
0	2.35±0.01c	8.98±0.02d	92.16±0.02d
25	2.36±0.01c	10.96±0.02d	92.30±0.02c
50	2.36±0.01c	15.53±0.01c	92.41±0.02b
100	2.44±0.02a	15.76±0.01a	92.44±0.01ab
200	2.37±0.01b	15.56±0.02b	92.46±0.01a
LSD (0.05)	0.015	0.02	0.02
	*	*	*

Values are means ± standard deviation. * : Means followed by the same letter do not differ significantly at 5% level by the Least Significance test (LSD) test.

Table 5. Nutrient element (mg/100g) contents of okra pods as affected by different concentrations of IAA.

Concentrations	Ca	Mg	Fe	Na
0	55.26±0.02e	35.26±0.042e	0.332±0.01e 0.420±0.001d	3.92±0.01e
25	59.19±0.02d	38.52±0.01d		6.03±0.02d
50	59.22±0.02c	38.56±0.01c	0.453±0.01c	6.07±0.01c
100	59.27±0.02b	39.24±0.02b	0.457±0.013b	6.11±0.01b
200	59.31±0.011a	39.28±0.012a	0.463±0.01a	6.16±0.01a
LSD 0.05	0.02	0.016	0.037	0.021
	*	*	*	*

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

set, fresh vegetable weight and seed yield quality (9).

It was reported (10) that natural or synthetic plant growth regulators have controlled plant activities and their productions by controlling one or more of one or more specific physiological processes within a plant. It was reported that IAA was safe for human health and might be used for different aims (12,13). It was reported that plant growth regulators played a central role in the morphology and physiology of the plants (14). They also stated that the effect of the growth regulator was dependent on plant species, variety, growth stage, the concentration of chemicals used, application technique and frequency of application (14). Application of IAA at 25, 50 and 200 mg/l increased the plant height over control. IAA has been considered as enhancing cell division and elongation (15). It was stated (16) that increased stem elongation might be due to the stimulating action of GA₃, which alleviates the cell wall by increasing its plasticity (17). It was found (18,19) that IAA applications increased the plant height of soybean and Red sorrel, respectively. With IAA at 100 and 200 mg/l concentrations, there was a significant difference in the pod in comparison with the control (18). It was observed that GA₃ and IAA at 100 ppm increased leaves number and leaves area and chlorophyll content in *Hibiscus sabdariffa* L. (19). Moreover, It was observed (20) that a significant increase in the leaf length in onion by the application of GA₃. This might be attributed that GA₃ and IAA increased the division and elongation of the cells which led to better vegetative growth of plants. It was stated (21) that the GA₃ application increased branches number by breaking apical dominance. They also reported that increasing yield might be related to the plant height, leaf number and leaf area. Another reason, the physiological role of gibberellin and indole acetic acid might increase cell division, elongation and stimulating the complete growth of plant which is revealed in better pod settings by using IAA. It was stated that IAA allowed water to enter the cells of fruits and dissolved materials which led naturally to an increase in fruit size by increasing the permeability of the fruit cell wall (22).

From the above discussion it can be concluded that lower concentrations (25 and 50 mg/l) of IAA significantly increased the plant height, pod setting, pod growth and pigment development and the higher concentration (100 and 200 mg/l) had lower values. 100 mg/l of IAA was the best for ascorbic acid (vitamin c) and soluble solid content and 200 mg/l of IAA concentrations were the best for minerals and seedless pod production. So it can be recommended that the seed soaking technique can be used commercially in the vegetable industry. The growth regulator application with seed soaking can reduce the chemical and production cost without hazardous any environmental pollution.

Author contributions

Conceptualization, A.B.M.S.H, M.M.A and H.A.R; formal analysis, A.B.M.S.H and A.S.A: funding acquisition, A.B.M.S.H, M.M.A and H.A.R; investigation, A.B.M.S.H; methodology, A.B.M.S.H. F.H. and A.A; project administration, A.B.M.S.H, A.M.A and H.A.R resources, A.B.M.S.H, A.M.A and H.A.R supervision, A.B.M.S.H and H.A.R writing—original draft, A.B.M.S.H and M.M.A; writing review & editing, A.B.M.S.H, F.H, D.K.: and H.A.R; All authors have read and agreed to the published version of the manuscript.

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Conflicts of interest

The authors declare no conflict of interest.

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