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Pre-treatment of *Abelmoschus esculentus* Seeds with Salicylic Acid and Indole Acetic Acid Enhances Nutritional Values of the Fruits

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Authors' contributions

This work was carried out in collaboration between all authors. Author AME designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors COO and KAA managed the analyses of the study. Author OF managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims: This study investigated the effects of phytohormones using indole acetic acid (IAA) and salicylic acid (SA) on some biochemical parameters on LD 88 and 47-4 genotypes of okra.
Study Design: The study was carried out in a screen house condition with 104 pots for each genotype (4 treatments x 5 levels x 3 replicates) in a complete randomized design using phytohormones to evaluate their effect on the nutritional values of okra fruits.
Place and Duration of Study: The study was carried out at the National Horticultural Research Institute (NIHORT), Ibadan, Oyo state, Nigeria for a period of 60 days.

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Methodology: Okra seeds were pretreated with IAA (0.4, 0.5 and 0.6 mM) and SA (10^{-2} , 10^{-4} and 10^{-6} Mm), and distilled water. The treated (experimental) and untreated (control) seeds were germinated and grown to maturity in polythene bags containing 10 kg of clay soil.
Results: Treatment with IAA and SA did not affect proximate composition of okra fruit. Calcium levels were higher in 0.6 mM IAA 47-4 (1.03±0.01) and 10^{-4} mM SA (1.83±0.04), but in LD 8810^{-4} mM SA (1.07 ± 0.03) when compared to control group respectively. Whereas, higher zinc level was observed in 47-4 10^{-6} mM SA (3.93±0.84) and higher phosphorous level in 47-4 10^{-2} mM SA (1.78± 0.06). Sodium and potassium levels were higher in 0.6 mM IAA (47-4) as 3.15±0.01 and 1.83±0.02 when compared to the control group. Higher level of magnesium was observed in 10^{-2} mM SA (47-4) as 1.78 ±0.06 and in 10^{-4} mM SA (LD 88) as 1.62±0.04 as compared to control group. Higher levels of total phenol phytate, oxalate, saponin and tannin were recorded in both phytohormone-treated genotypes than those in the control. The results indicated that IAA and SA phytohormones improved mineral, anti-nutritional, and phytochemical constituents, of okra fruits in contrast with proximate composition.

Keywords: Abelmoschus esculentus; anti-nutrients; indole acetic acid; phytonutrients; proximate content; salicylic acid.

1. INTRODUCTION

Abelmoschus esculentus L. (Moench), commonly known as okra, is an economically important vegetable crop grown in tropical and sub-tropical parts of the world. This crop is suitable for cultivation as a garden crop as well as on large commercial farms. It is grown commercially in India, Turkey, Iran, Western Africa, Yugoslavia, Bangladesh, Afghanistan, Pakistan, Burma, Japan, Malaysia, Brazil, Ghana, Ethiopian, Cyprus and the Southern United States. India ranks first in the world with 3.5 million tonnes (70% of the total world production) of okra produced from over 0.35 million acre of land [1]. It is quite popular in India because of easy cultivation, dependable yield and adaptability to varying moisture conditions [2]. Okra is cultivated for its fibrous fruits or pods containing round, white seeds. The fruits are harvested when immature and eaten as a vegetable. The roots and stems of okra are used for cleaning the cane juice from which gur or brown sugar is prepared [2]. Its ripened seeds are roasted, ground and used as a substitute for coffee in some countries. Mature fruits and stems containing crude fiber are used in the paper industry. Extracts from the seeds of okra is viewed as alternative source for edible oil. The greenish yellow edible oil has a pleasant taste and odor, and is high in unsaturated fats such as oleic acid and linoleic acid. The oil content of the seed is quite high at about 40%. Okra provides an important source of vitamins, calcium, potassium and other minerals which are often lacking in the diet of developing countries [3]. In Asia, okra is typically prepared as traditional medicine for the treatment of gastric irritations [4]. The plant has a wide range of medicinal properties and has been used in the management of several disorders. Its high contents of fiber may help in regulating the absorption of sugar and cholesterol at the intestinal level [5]. Evidence for its benefits against depression, gastric ulcers, lung inflammation, asthma, sore throat as well irritable bowel has been shown [6]. Previous studies reported that okra fiber possesses hypoglycemic activity in normal mice [7]. Also, okra may lower cholesterol levels most likely through its ability to bind bile acids [8]. In the African context, okra has been called as a perfect villager's vegetable because of its medicinal nature, dietary fibers and distinct seed protein balanced in both lysine and tryptophan amino acids [9].

Given the extended functionality for okra products, it would be very beneficial if health-promoting properties of okra can be enhanced using advanced biotechnology techniques. Bioregulators are chemicals that affect the expression of biological responses in plant tissues. Their use is a unique facet of biotechnology and a new approach of manipulating plant biochemistry for enhancing productivity and quality. They act in low concentrations without any negative impact on nutritional values. Salicylic acid and Indole acetic acid have been known as phytohormones that play a stimulatory role on the root and shoot growth of plants [10], thereby controlling plant growth and development, seed germination, fruit yield (guality and quantity), glycolysis, flowering, and heat production in plants [11], ions uptake and transport [12], values of photosynthesis, stomata conductance, and transpiration [13]. Applications of bioregulators are reported to

reduce biotic and abiotic stress in plants. Salicylic acid is known to play an important role in modulating the redox balance across membranes, thereby counteracting the negative effects of reactive oxygen species (ROS) generated by oxidative stress [14] by increasing the activity of antioxidant enzymes such as superoxide dismutase [15]. Salicylic acid is known as an endogenous growth regulator of phenolic type distributing in a wide range of plant species, which induces biotic and abiotic stress tolerance in crops [16-18]. Review of the literatures also demonstrated that SA affects other physiological processes in plants such as growth, photosynthesis, uptake of ions, heat production, flowering and ethylene production [19]. SA was reported to induce an increase of *in vitro* regeneration frequency in *Hibiscus* plants [16]. Exogenous IAA significantly reduced Na^+ concentrations and increased those of Ca^{2+} and K^+. Therefore, the main objective of this study is to investigate the effects of SA and IAA on some biochemical parameters of two genotypes of okra.

2. MATERIALS AND METHODS

2.1 Plant Materials

A. esculentus (L.) Moench seeds of two genotypes (LD 88 and 47-4) were collected from genetic resource laboratory (Product Development Programme), National Horticultural Research Institute, Ibadan, Nigeria. Seeds were sterilized with 1% sodium hypochlorite for 15 min and washed thoroughly with distilled water. The seeds were then pretreated by soaking in 0.4 mM, 0.5 mM and 0.6 mM of indole acetic acid or 10^{-2} mM, 10^{-4} mM and 10^{-6} mM of salicylic acid. The treated (experimental) and untreated (control) seeds were germinated in polythene bags containing 10 kg of clay soil with pH 7.10, Exch. acidity 0.34, clay (%) 12.30, silt (%) 13.90, sand (%) 65.40, organic carbon (g/Kg) 47.32, nitrogen (g/Kg) 2.53, phosphorous (mg/Kg) 20.00, potassium (cmol/Kg) 1.33, sodium (cmol/Kg) 0.89, calcium (cmol/Kg) 45.65, magnesium (cmol/Kg) 13.34 in a screen house at National Horticultural Research Institute, Ibadan, Nigeria (NIHORT). The plants were irrigated with tap water on weekly basis to keep the soil moist. At 60 days after planting , fruits from the three groups (treated with indole acetic acid or salicylic acid, and untreated (control) were harvested, air dried to constant mass and ground to fine powder using Vitamix S30 grinder. The ground samples were stored in an airtight container until analyses for the estimation of proximate composition, minerals content, phytochemicals and anti-nutrient content.

2.2 Proximate Analysis

Proximate analysis was carried out on each of the samples using the AOAC methods [20] in the genetic resource laboratory (Product Development Programme), National Horticultural Research Institute, Ibadan, Oyo state, Nigeria.

2.2.1 Moisture content

Moisture content was estimated by gravimetric measurement of weight loss after drying 5 g of okra sample in a thermostatically controlled oven at 105℃ until constant weight was obtained. The moisture content of each genotype was calculated as loss in weight of the original sample and expressed as percentage moisture content [21].

$$\text{Percentage moisture} = \frac{\text{loss in weight due to drying}}{\text{Weight of original sample}} \times 100 \quad (1)$$

$$= \frac{W1 - W2}{W1} \times 100$$

Where W1 = fresh weight before oven-drying
W2 = oven-dry weight

2.2.2 Determination of crude fibre content

Total crude fibre content was estimated according to Asp et al. [22]. Briefly, about 5 gm dried powders of the sample were taken in a conical flask connected by a condenser. 100 to 150 ml of 1.25% H_2SO_4 solution was added, boiled and refluxed for 30 min. The sample was filtered with boiled distilled water for several times to get rid of sulphate. The residue was transferred into a conical flask and 100 to 150 ml of 1.25% NaOH solution was added and boiled for 30 min. Again it was filtered, washed with boiled distilled water to get rid of alkali. The residue was washed for three times with rectified spirit to wash down the remaining fat. Then, the residue was again washed 3 times with acetone. After which the residue was transferred into silica crucible and dried in a hot air oven at 100℃ and cooled in a desiccators and weighed. After that the residue was ash in a muffle furnace at 500℃ for 3 hrs, cooled in a desiccator and weighed.

% crude fiber = Dry weight of residue- weight of Ash/weight of sample x 100 (2)

2.2.3 Protein determination

This was determined by Kjeldahl method [23]. Briefly, 0.5gm of the sample was taken in a digestion tube containing mixture (copper sulphate and potassium sulphate in the ratio 1:9) and 5 ml of H_2SO_4 (conc). After digestion distilled water was added and shaken. Volume was made up to 100 ml in the digestion flask. An aliquot of 5ml was transferred into micro kjeldahl distillation unit, and 5ml of 40% NaOH (v/v) was added. This was followed by the addition of 5ml methyl orange indicator, and place in the receptor site, the red colour turns green. After that it was titrated against standard H_2SO_4. The percentage nitrogen was calculated and multiplied by 6.25 the conversion factor to obtain the value of the crude protein [20].

2.2.4 Estimation of crude lipid content

This estimation was performed using the Soxhlet extraction method. Briefly, 2 to 3 gm of the dried powder was taken in an extraction thimble. It was placed inside soxhlet extraction unit. A dry and properly cleaned flask beneath the extraction unit was connected with 50 ml of petroleum ether connected with the condenser. After the completion of extraction, the thimble and ether are removed. The flask was dried at 105℃ for 30 min. in a hot air oven and cooled in the desiccator and weighed [16].

Weight of crude lipid = Final weight of the oil flask- Initial weight of the oil flask.

Crude fat % = weight of fat/weight of dry sample x 100 (3)

2.2.5 Analysis of carbohydrate content

The carbohydrate content was determined by subtracting the sum up percentage compositions of moisture, protein, lipid, fibre, and ash contents from 100 [24].

2.3 Minerals Content Determination

About 5 gm of dried fruits powder was digested in a mixture of concentrated nitric acid, sulphuric acid and perchloric acid at the ratios 10: 1: 4, respectively. The ash of plant sample was moistened with an amount of distilled water and 5ml of hydrochloric acid was added to it. The mixture was evaporated to dryness in a water bath. Another 5ml of hydrochloric acid was added and the solution was evaporated to dryness as above. Aliquot 0f 4 ml hydrochloric acid and a few ml of distilled water were then added and the solution warmed over a boiling water bath and filtered into a 100 ml volumetric flask using whatman No. 40 filter paper. After cooling, the volume was made up to 100 ml and suitable aliquots were used for the estimation of calcium, sodium, potassium, zinc, magnesium, and phosphorous. The mineral elements of the plant like Na^+, K^+ and Ca^{++} were estimated by flame photometry, whereas Zn^{++} and Mg^{++} contents were measured by atomic absorption spectrophotometer according to the method of Chapman and Pratt [25] with certain modification.

2.3.1 Phosphorus

The total phosphorous content was determined as described by Murphy and Riley [26]. Briefly, 0.5 ml of the digest, 4 ml of the deionised water, 3 ml of 0.73 M H_2SO_4, 0.4 ml of 10%(w/v) (NH4) 6 $Mo_7O_2.4H_20$ and 0.4 ml of 2% (w/v) ascorbic acid were added and mixed. The solution was allowed to stand for 20 min, and the absorbance reading was recorded at 660 nm. KH_2P0_4 was used as the standard. Results were expressed as mg/g dry weight of the fruit.

2.4 Phytochemicals and Antinutrients Determination

2.4.1 Determination of phytate

The phytate content was determined by the method of Young and Grieve [27] with slight modifications. Briefly, the phytic acid content of the sample was determined spectrophotometrically using UV-Vis spectrophotometer. 0.5 gm of samples were stirred using magnetic stirrer in 10 ml of 3.5% HCl for an hour. The contents were centrifuged at 300gm for 10 min to obtain supernatants. A suitable aliquot of the supernatant was diluted with 3.5% HCl to make up to the 3 ml mark. I ml of wade reagent (0.03% of FeCl3.6H2O containing 0.3% sulphosalicylic acid) was added and it was centrifuged again. The absorbance was measured at 500 nm. Phytic acid was used as standard.

2.4.2 Determination of oxalate

The titrimetric method of Day &Underwood [28] was used in the determination of oxalate in the samples. 150 ml of 15 N H_2SO_4 was added to 5 g of the pulverized samples and the solution was carefully stirred intermittently with a magnetic stirrer for 30 minutes and filtered using Whatman

No 1 filter paper, after which 25 ml of the filtrate was collected and titrated against 0.1 N KMnO4 solution until a faint pink color appeared that persisted for 30 seconds.

2.4.3 Determination of saponin

Saponin composition was determined using the gravimetric method of Hudson & El-Difrawi [29]. Two hundred and twenty millilitres of 20% ethanol was added to 10 g of the pulverized samples and stirred using a magnetic stirrer for 12 hours at 55℃. The solution was filtered using Whatman No 1 filter paper and the extract was reduced to 40 ml under vacuum and 20 ml Diethyl ether was added in a separating funnel and shaken vigorously. The ether layer was discarded while the pH of the aqueous solution was adjusted to 4.5 by adding NaOH. 60 ml of n-butanol was finally used for extraction. The butanol extract were washed twice with 10 ml of 5% NaCl and evaporated to dryness in a fume cupboard to give a crude saponin which was weighed.

2.4.4 Determination of tannins

Spectrophotometric method of Trease & Evans [30] was used in the determination of tannin in the samples. Five grams (5 g) of the powdery form of the samples were extracted with 20 ml of warm water and filtered. 0.5 ml of the filtrate was added to 0.5 ml of 0.5 M ferric solution in an alkaline medium and allowed to stand for 30 minutes for color development. The absorbance was read at 760 nm and the amount of tannin was extrapolated from a standard calibration curve for tannic acid. Tannic acid was used as standard.

2.4.5 Ascorbic acid determination

Vitamin C content was determined according to the method by Campos et al. [31] with some modifications. Briefly, one gm of powdered sample was treated with 4.0 ml of 10% TCA and centrifuged for 20 minutes at 3500 g. 0.5 ml of supernatant was then, mixed with 0.1 ml DTC reagent. The tubes were incubated at 37℃ for three hours. 0.75 ml of ice cold 65% H2SO4 was added and the tubes were allowed to stand at room temperature for an additional 30 minutes. A set of standards containing 10-50 µg of Vitamin C was processed similarly along with a blank containing 0.5 ml of 10% TCA. The colour developed was read at 520 nm. Values are expressed as mg/ gm of dried sample.

2.4.6 Determination of total phenol content

Total phenolic content was determined using the Folin-Ciocalteu reagent as described by [32]. Briefly, 2 gm of the sample was defatted with 100.0 ml of petroleum ether using a soxhlet apparatus for 2 hours. For the extraction of the phenolic component, the fat free sample was boiled with 50 ml of ether for 15 minutes. To 5.0 ml of the extract, 10.0 ml of distilled water, 2.0 ml of ammonium hydroxide and 5.0 ml of concentrated amyl alcohol were added. The sample was left to react for 30 minutes for color development. The absorbance of the solution was read using a spectrophotometer at 760 nm wavelength. Using gallic acid as a standard phenolic compound, the results were expressed as mg of phenol/ gm of dried sample.

2.4.7 Determination of total flavonoid content

Total flavonoid contents were measured according to a colorimetric assay [33]. Briefly, the extract prepared for the estimation of total phenolics was used as sample for this assay. 0.25 ml of the sample was diluted to 1.25 ml with distilled water. 75 µl of 5% sodium nitrite was added and after six minutes 0.15 ml of aluminium chloride solution was added. 0.5 ml of 0.1M NaOH was added after 5 minutes and made up to 2.5 ml with distilled water. The solution was mixed well and the absorbance was read at 510 nm in comparison with standard quercetin at 5-25 µg concentration. The results are expressed as mg of flavonoids as quercetin equivalent/ gm of dried sample.

2.5 Statistical Analysis

The factorial experimental design with two varieties, two genotypes and four salinity levels were arranged in a completely randomized design (CRD) with three replications and the data were analyzed using the software package, SAS windows and the mean separation by LSD at 0.05 level.

3. RESULTS AND DISCUSSION

Pre-treatment with bioregulators did not significantly affect carbohydrate, moisture, crude fiber, fat and protein contents of either genotype of okra tested (Table 1). However, levels of minerals including, P, K, Ca, Zn, Na and Mg were generally increased with the pre-treatments as compared to the values in control. In 47-4, higher level of calcium was recorded in 0.6 mM

IAA and 10^{-4} mM SA as 1.03±0.01 and 1.83±0.04 respectively as compared to control group. But in LD 88, higher level of calcium was observed in 10^{-4} mM SA as 1.07 ± 0.03 when compared to control group. In the same vein, higher level (3.93±0.84) of Zinc was observed in 10^{-6} mM SA (47-4) and (1.78± 0.06) of phosphorous was recorded in 10^{-2} mM SA (47-4) when compared with the control group. Sodium and potassium level were higher in 0.6 mM IAA (47-4) as 3.15±0.01 and 1.83±0.02 respectively when compared to the control group, also, higher level of potassium was observed in 10^{-2} mM SA (LD 88) as 1.72±0.07 as compared to control group, while higher level of magnesium recorded in 10^{-2} mM SA (47-4) as 1.78 ±0.06 and in 10^{-4} mM SA (LD 88) as 1.62±0.04 as compared to control group (Table 2). Moreover, mineral content analysis revealed that calcium and phosphorous are required for bone development and component of energy intermediates respectively [34-35], and Zinc that is required for the proper functioning of the reproductive system [36] was found to be present in the same amount in both genotypes of *A. esculentus*, but the highest level of zinc was observed in the genotype 47-4. Sodium level was also observed to be higher than potassium and magnesium in both genotypes of A*. esculentus* treated with IAA and SA, when compared to control groups. But the groups treated with IAA achieved greater concentration as compared to SA and control, respectively. Similarly, enhanced mineral content were reported when spinach was cultivated in fortified soils [37-39]. Saponin, tannins, oxalate and phytate were studied on the two samples of okra fruits treated with the bioregulators (Table 3). As the concentration of IAA increased, it resulted to decrease in the concentration of antinutrients in the two genotypes with exception of phytate in group treated with 0.6 mM that reported to have highest value of 52.0 mg/100g in 47-4 and oxalate as well as tannins in the groups treated with 0.5 mM and 0.6 mM having 24.3 mg/100g and 19.0 mg/100g respectively. On the contrary to SA treated group, in both genotypes, as the concentration of SA increases, so also the level of antinutrients. The result showed the least content of saponin in 47-4 genotype treated with SA at 10^{-6} mM also in the same vein, the least content of Tannin, oxalate and phytate was recorded in genotype 47-4 treated with 10^{-6} mM of SA as compared to the control values. Indole acetic acid affected the content of antinutrients in the okra which bind Ca, Mg, Fe and Zn, making them unavailable. On the contrary, SA increased the content of antinutrients in the okra genotypes. Tannins and saponin have been reported to have medicinal properties. The presence of tannins and saponin showed that the two *A. esculentus* genotypes have medicinal property.

Table 1. Proximate composition of LD 88 and 47-4 genotypes of okra fruits produced from seeds pre-treated with indoleacetic acid (IAA) and salicylic acid (SA)

Genotype/ bio-regulator	Phytohormones concentrations (mM)	% Total proteins	% Total fat	% Moisture content	% Carbohydrate content	% Crude fibre
47-4 IAA	0	1.86±0.21	0.15±0.12	83.44±0.20	6.20±0.60	1.23±0.32
	0.4	1.85±1.00	0.16±0.33	83.45±0.31	6.10±0.11	1.34± 0.22
	0.5	1.88±0.11	0.32±0.01*	88.60±0.22	6.21±0.23	2.00 ±0.06*
	0.6	1.82±0.32	0.15±0.22	82.10±0.50	5.85±0.33	1.13± 0.12
47-4 SA	10^{-2}	1.64±0.11	0.05±0.34	82.67±0.01	6.15±0.40	1.11±0.33
	10^{-4}	1.88±0.02	0.12±0.62	83.15±0.04	6.00±0.05	1.33±0.21
	10^{-6}	1.32±0.40	0.17±0.11	76.95±0.21	5.89±0.02	1.08±0.03
LD88 IAA	0	1.90±0.30	0.21±2.00	85.40±0.33	6.12±0.33	1.23±0. 20
	0.4	1.96±0.22	0.21±0.04	89.00±0.02**	6.37±1.20	2.3 3±0.04
	0.5	1.53±0.11	0.23±0.50	88.30±0.01	6.23±0.21	2.06± 0.33
	0.6	1.33±0.02	0.12±1.00	75.60±0.55	5.61±0.40	1.07± 0.02
LD88 SA	10^{-2}	1.75±0.60	0.17±0.03	82.45±0.50	4.15±0.40	1.01±0.60
	10^{-4}	1.93±0.04	0.24±0.02	83.31±0.21	6.13±0.20	1.33±0.10
	10^{-6}	1.34±1.00	0.17±0.20	80.11±0.04	5.89±0.05	1.00±0.22

All values are given as means ± standard deviation of three replicates.
**Significant different at P = .05 when compared with normal control of genotype 47-4.*
***Significant different at P = .05 when compared with normal control of genotype LD 88*

Table 2. Mineral content of LD 88 and 47-4 genotypes of okra fruits produced from seeds pre-treated with indole acetic acid (IAA) and salicylic acid (SA)

Genotype/ bioregulator	Phytohormones concentrations (mM)	Ca mg/g	Mg mg/g	K mg/g	Na mg/g	Zn mg/g	P mg/g
47-4 IAA	0	0.51±0.04	0.29±0.02	0.92±0.07	2.52±0.10	0.87±0.10	0 .85±0.07
	0.4	0.93±0.09	0.40±0.01	0.59±0.09	3.11±0.08*	1.79±0.16	1.52±0.09
	0.5	0.87±0.05	0.36±0.02	1.35±0.07*	3.08±0.02	1.72±0.28	1.39±0.08
	0.6	1.03±0.01*	0.42±0.01	1.83±0.02*	3.15±0.01*	1.65±0.2 3	1.75±0.03
47-4 SA	10^{-2}	0.90±0.07*	0.12±0.18*	1.80±0.03	2.70±0.11	2.26±0.12 *	1.78±0.06*
	10^{-4}	1.03±0.04*	0.43±0.01	1.73±0.01	2.78±0.05	1.84±0.18	1.74±0.02
	10^{-6}	0.45±0.10	0.41±0.01	1.67±0.06	2.45±0.04	3.93±0.84*	1.66±0.06
LD 88 IAA	0	0.94±0.02	0.38±0.01	1.37±0.05	2.87±0.07	1.32±0.05	1 .35±0.04
	0.4	0.98±0.03	0.40±0.01	1.38±0.08	2.89±0.17	1.52±0.09	1 .36±0.08
	0.5	0.93±0.02	0.34±0.01	1.38±0.09	2.88±0.12	1.09±0.09	1 .31±0.09
	0.6	0.91±0.04	0.38±0.01	1.30±0.09	2.85±0.15	1.06±0.03	1 .27±0.09
LD 88 SA	10^{-2}	0.90±0.02	0.80±0.02**	1.72±0.07**	2.61±0.13	1.86±0. 03**	1.59±0.07**
	10^{-4}	1.07±0.03**	0.37±0.02	1.63±0.05**	2.64±0.10	1.33±0. 04	1.62±0.04**
	10^{-6}	0.50±0.07**	0.35±0.01	1.61±0.07**	2.23±0.19**	2.39± 0.51**	1.59±0.07**

** Significant different at P = .05 when compared with normal control of genotype 47-4; ** Significant different at P = .05 when compared with normal control of genotype LD 88*

Table 3. Anti-nutrient content of LD 88 and 47-4 genotypes of okra fruits produced from seeds pre-treated with indole acetic acid (IAA) and salicylic acid (SA)

Genotypes	Phytohormones concentrations (mM)	Anti-nutrients (mg/100 g)			
		Saponin	Tannin	Oxalate	Phytate
47-4 IAA	0	40.0±0.01	20.1±0.00	18.2±0.02	47±0.03
	0.4	37.5±0.02	25.0±0.01	18.0±0.01	40.0±0.01
	0.5	44.0±0.00	13.3±0.02*	11.3±0.00*	50.4±0.02
	0.6	24.3±0.01*	10.4±0.01*	10.4±0.01	52.0±0.00*
47-4 SA	10^{-2}	17.1±0.05*	20.1±0.00	17.5±0.00	20.5±0.03*
	10^{-4}	40.0±0.00	14.3±0.02*	19.3±0.00	20.1±0.01*
	10^{-6}	12.4±0.01*	09.5±0.00*	08.6±0.00*	18.0±0.02*
LD 88 IAA	0	43.0±0.04	18.3±0.00	23.1±0.01	47.4±0.01
	0.4	48.4±0.03	20.4±0.02	23.4±0.03	42.2±0.00
	0.5	45.3±0.01	12.4±0.01**	24.3±0.05	39.4±0.02
	0.6	40.3±0.00**	19±0.00	17.3±0.01**	25.3±0.05**
	10^{-2}	18.1±0.05**	16.1±0.00	21.5±0.00	30.5±0.03**
LD 88 SA	10^{-4}	30.0±0.00	24.3±0.02**	17.3±0.00**	30.1±0.01**
	10^{-6}	22.4±0.01**	19.5±0.00	18.6±0.00	28.0±0.02**

** Significant different at P = .05 when compared with normal control of genotype 47-4; **Significant different at P = .05 when compared with normal control of genotype LD88*

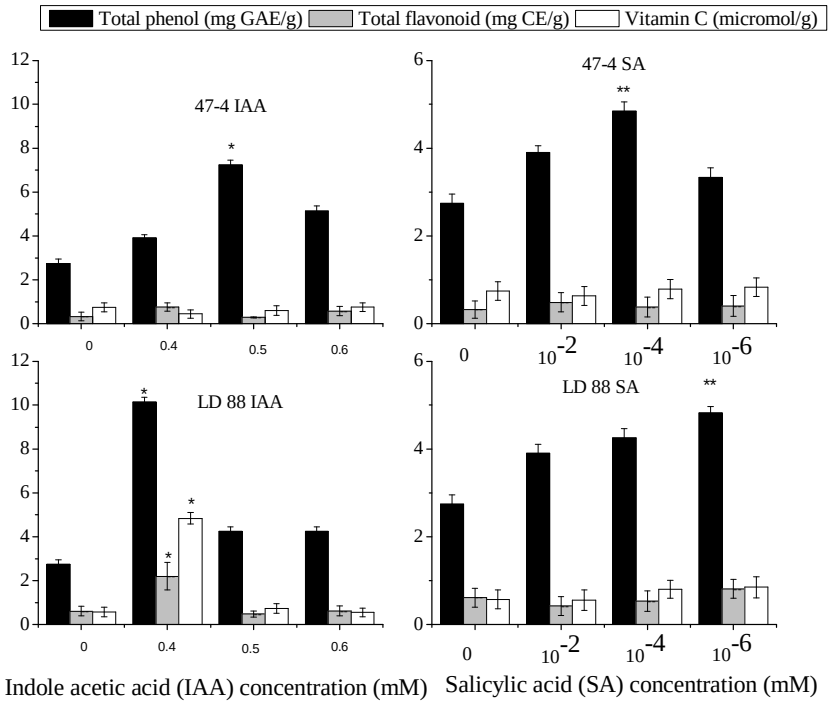

Fig. 1. Total phenolic, flavonoids and vitamin C content of LD 88 and 47-4 genotypes of okra fruits produced from seeds pre-treated with indole acetic acid (IAA) and salicylic acid (SA)

*Vertical bars represent Standard deviation. *Significant different at P = 0.05 for IAA when compared with normal control. **Significant different at P = 0.05 for SA when compared with normal control*

3.1 Phytonutrients Composition

Fig. 1 shows the phytonutrients composition of the okra genotypes pre-treated with bioregulators. In 47-4, treatment with IAA increased phenolic content as compared to the control while only those treated with 10^{-4} mM exhibited highest level of total phenolic content as compared to control. In LD 88 on the other hand, only the those treated with 0.4mM of IAA showed the highest concentration of all the phytonutrients analyzed when compared to the control (Fig. 1). But in SA-treated plants, the phenolic content was increased as the SA concentration decreased when compared to the control. Pre-treatment with both IAA and SA affected the total content of phenolic compounds in okra. Bioregulators affect the concentration of phosphorous, which is important in plant bioenergetics, potassium, that regulates the opening and closing of the stoma by a potassium ion pump, calcium which regulates transport of other nutrients into the plant and also involved in the activation of certain plant enzymes, zinc, that is required in a large number of enzymes and plays an essential role in DNA transcription, sodium, which is involved in the regeneration of phosphoenolpyruvate in CAM and C4 plants as well as magnesium, which is important in photosynthesis. More also, increased in the phenolic, saponin and tannin contents of the plant improved its medicinal properties.

4. CONCLUSION

At the end of this study, we report that pre-treatment of okra seeds with SA and IAA improved the nutritional values of okra fruits. Improvement in the concentration of certain micro-nutirents was observed. Similarly, the levels of tannins and saponins, which are phytochemicals that have been reported to have medicinal properties, were also increased. However, the higher levels of phytate, oxalate, saponin and tannin in the two genotypes of *A. esculentus* treated with salicylic acid may pose problem to the total availability of the minerals and proteins. Hence, the treatment of the seeds with IAA serves as a better choice of bioregulator to reduce the level of antinutrients in the seeds of okra.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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