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Comparative Effect of Indole Acetic Acid and Salicylic Acid on Phytochemical Constituents and Antioxidant Potential of Three Genotypes of Tomato (*Solanum lycopersicum* (L.) Mill)

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Abstract

Indole acetic acid (IAA) and salicylic acid (SA) are plant bioregulators that stimulate the desired growth and quality response in crops. This study examined the impacts of IAA or SA seed pre-treatment on phytochemical constituents and antioxidants potential in three genotypes of tomato plant. The results indicated that IAA and or SA treatments increased height of the three genotypes of tomato significantly ($P \leq 0.05$) when compared with the control group. Lycopene, total flavonoid and phenolic contents of genotypes F2 cobra and panther 17 F1 treated with 40, 80, and 120 mg/L concentrations of IAA, respectively increased significantly as compared to the control group. Furthermore, IAA and or SA significantly increased H_2O_2 scavenging activity of F1 KIARA by 5.0% compared to the control (0.2%). Genotype F1 KIARA treated with IAA and or SA (80 and 120 mg/L) had a significant increase in DPPH scavenging activity by 55.0% and 53.0% respectively, relative to control group (11%). The Fe^{2+}-chelating activity of IAA-treated (80 mg/L) F1 KIARA and F2 COBRA increased by 56.0% and 17.0%, respectively compared to the control. The synergistic effect of IAA and SA showed a significant effect on phytochemical constituents and antioxidant potential than individual treatment. The results showed that IAA application or in combination with SA could be used as an alternative bio-stimulant to improve the quality of tomato.

Keywords: Antioxidant potential; Indole acetic acid; Phytochemicals; Salicylic acid; Tomato.

1. Introduction

Tomato (*Solanum lycopersicum* L.**)** is one of the most cultivated vegetable species worldwide due to its widespread production and dietary value [1]. It reduces the incidence of human diseases like cancer and cardiovascular diseases [2]. Tomato products are very rich in lycopene, ascorbic acid and beta-carotene, which are powerful natural antioxidants against free radicals' generation, and thus maintain the integrity of cells membrane [3]. Tomatoes are an excellent source of antioxidant vitamins [4]. For commercial purposes, consumers desire good quality fruit with attractive size and appearance. A recent trend towards organic farming constitutes to fruit quality by enhancing phytonutrient level and overall quality of fruit crops [5, 6]. However, consumers are still expecting fruits to be free from undesirable chemicals like cyanogenic glucosides, heavy metals, oxalates, and pesticides, and contaminations result from microbes. Plant bioregulators play a significant role in plant metabolism and in the transition of primary to secondary metabolites [7]. Indole acetic acid and salicylic acid are class of plant bioregulators called auxin. Auxins are associated with the regulation of plant growth and development [8]. A study has shown a modern plant biotechnology through the application of plant bioregulators to improve yield and phytochemical constituents of food crop plants[9]. In some instances, plant bioregulators affect levels of RNA, DNA, enzymes, and ultimately the primary and secondary compounds metabolism in plants [10]. So far, there is dearth of information about the possibility of using IAA and SA to improve antioxidant potential and quality value of tomato plant. Here, this study was aimed to investigate the means to increasing phytonutrients and antioxidant capacity in the three genotypes of tomato plant with the use of IAA and SA.

2. Material and Methods

2.1. Chemicals

All the chemicals used are of standard analytical grade and were purchased from Sigma Aldrich (St. Louis, MO).

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2.2. Seed Type and Source

The seeds of the three genotypes of tomato used were F1 Kiara, F2 Cobra and Panther17 F1. They were purchased at the National Horticultural Research Institute, Ibadan, Oyo State, Nigeria (NIHORT).

2.3. Plant Bioregulator

The indole acetic acid and salicylic acid used in this study were obtained from Sigma Aldrich (St. Louis, MO).

2.4. Preparation of Plant Bioregulator

The method of Heydecker and Coolbear [11] was used for the preparation of IAA and SA concentrations, respectively. A 37.5 mg each of indole acetic acid and salicylic acid was dissolved in ethanol (10 mL 60%) containing Tween-20 (0.5%) in 250 mL volumetric flasks to afford a concentration of 150 mg/L. Serial dilution of the concentration was carried out for 120, 80, and 40 mg/L.

2.5. Plant Materials Treatment and Design

Tomato seeds of each genotype were soaked in 120, 80, and 40 mg/L concentrations solution of IAA and SA combined (1:1) and respectively in film containers for 24 h in a dark environment, after which the solution was decanted, and air-dried before sowing. The control seeds were soaked in distilled water differently. Subsequently, the treated and control tomato seeds (n=5) of each genotype were sown in pots containing 10 kg of soil in a screen house with a randomized design, and replicated three times as follows: (a) Control; (b) IAA treatments; (c) SA treatments; (d) IAA and SA treatments. The seedlings were irrigated on a weekly basis for four weeks. After seed germination, the seedlings per pot from each genotype were wet with an equal volume of water thrice in a week. After 45 days of growth, all experimental plant leaves and fruits were harvested, air-dried at room temperature and ground to powder using a mortar and a pestle, and stored before the biochemical assay.

2.6. Growth Determination

After 30 days of planting, growth parameters (shoot height and leaf area) were measured in the three genotypes of tomato respectively.

2.6.1. Height of the Plant

The tomato plant heights were measured using thread and ruler and the unit was expressed in centimeters (cm).

2.6.2. Total Number of the Leaflets

The tomato number of leaflets were determined by counting the number of leaves per stem of each genotype of the tomato plant.

2.7. Phytochemical Activity

2.7.1. Extraction and Determination of Pigments

The extraction of lycopene content was done by using the method of Georgé, *et al.* [12]. Briefly, 1 g of liquidized tomato pulp was mixed with hexane (20 mL), acetone (10 mL) and ethanol (10 mL). The mixture was stored at 3°C until depigmentation occurred. A 20 mL hexane, 10 mL acetone and 10 mL ethanol were added, and filtered by using Whatman No 4 filter paper. To the filtrate, distilled water (50 ml) was added and then transferred into a test tube (150 ml). The absorbance reading was done in the supernatant, and the absorbance for the lycopene content was taken at 503 and 450 nm, respectively using a spectrophotometer (UV/VIS). The result was expressed as mg/100g. The pigment was determined in triplicate by using the equation below:

$$\text{Lycopene (mg/100g)} = 3.956 \times A450 - 0.8061 \times A503 \quad (1)$$

2.8. Antioxidant Potential

2.8.1. Total Phenolic Content Determination

The tomato fruit samples total phenolic content was determined by using the spectrophotometric method of Kim, *et al.* [13]. Briefly, 1 mL of Folin-ciocalteu's phenol reagent was added to 0.5 mL of fruit sample. Five minutes later, a solution of 10 mL of 7% Na_2CO_3 was added to the mixture. Followed by the addition of 13 ml of distilled water using a magnetic stirrer. The mixture was stored in the dark for 1h 30 min at 25 ºC. Gallic acid was applied as the standard. The absorbance for total phenolic content was taken at 750 nm using a spectrophotometer (UV/VIS). The result was expressed as mg GAE/g.

2.8.2. Total Flavonoid Content Determination

The tomato fruit samples total flavonoid content was determined by following the method described by Park, *et al.* [14]. Briefly, 0.15 mL of 0.5 M sodium nitrite ($NaNO_2$) and 0.5 mL of 2% ethanol $AlCl_3$ solutions were mixed with 0.5 mL of the sample extract. Five minutes later, an addition of sodium hydroxide (1 mL of 1 M) solution was done. The mixture was incubated at 25°C for 30 min. The standard used was a quercetin. The reading was taken at 510 nm using a spectrophotometer (UV/VIS). The result was expressed as mg QUE/g.

2.8.3. Determination of Metal Chelating Activity

The metal chelating activity of the extracts was assayed for by using a modified method of Dinis, *et al.* [15]. The extract of about 50 µl was added to 1 mL of 2 mM of ferrous sulphate. The addition of 1mL of 0.25 mM ferrozine initiated the reaction, and the mixture was vortex and stored at room temperature for 10 min. Formation of stable magenta complex occurred by the reaction between ferrozine and divalent iron. A 1 ml distilled water was used to quantify the reaction mixture in control. The absorbance of the mixture with (A1) and without (Ao) the extracts were taken at 517 nm.

The following formula was used to calculate the chelating activity:

$$\text{Chelating rate (\%)} = \frac{Ao - A1}{Ao} \times 100 \qquad 2$$

2.8.4. Determination of Hydrogen Peroxide Scavenging Activity

The ability of the extract to scavenge hydrogen peroxide was determined by using a technique of Ruch, *et al.* [16]. A 2 mL solution of H_2O_2 was dissolved in a test tube containing 50 mM phosphate buffer (pH 7.4) and 0.1 mL of the sample extract. After 10 min of reaction time, the mixture was vortex. The absorbance was taken at 230 nm. The standard used was ascorbic acid. The percentage of hydrogen peroxide scavenging ability was calculated by using the following equation:

$$H_2O_2 \text{ scavenging activity (\%)} = \frac{Ao - A1}{A1} \times 100 \qquad 3$$

where: A_0 = Absorbance of control, A_1 = Absorbance of the sample.

2.8.5. Determination of DPPH Scavenging Activity

The method employed by Gyamfi, *et al.* [17] was followed to determine the 2,2-diphenyl -1- picrylhydrazyl (DPPH) scavenging activity. Briefly, 39.4 mg of DPPH was weighed and dissolved in methanol and made up to 2 mL by the addition of methanol. The baseline control used was methanol. A 0.1 mL extract of tomato was mixed with 3.9 mL of DPPH (0.025 g/L in methanol). The sample was stored for 35 min at 25°C, and the absorbance was read at 517 nm. A 10µl water was used as a control and the scavenging activity percentage was estimated by using the following equation:

$$\text{DPPH radical scavenging activity (\%)} = \frac{Ao - A1}{A1} \times 100 \qquad 4$$

where: A_0 = Absorbance of control, A_1 = Absorbance of the sample.

2.9. Statistical Analysis

All data obtained were subjected to two-way analysis of variance (ANOVA) using the Statistical Package for the Social Sciences (SPSS). Each experiment was repeated in triplicates and the data was expressed as Mean ± SEM, and significant at $P \leq 0.05$.

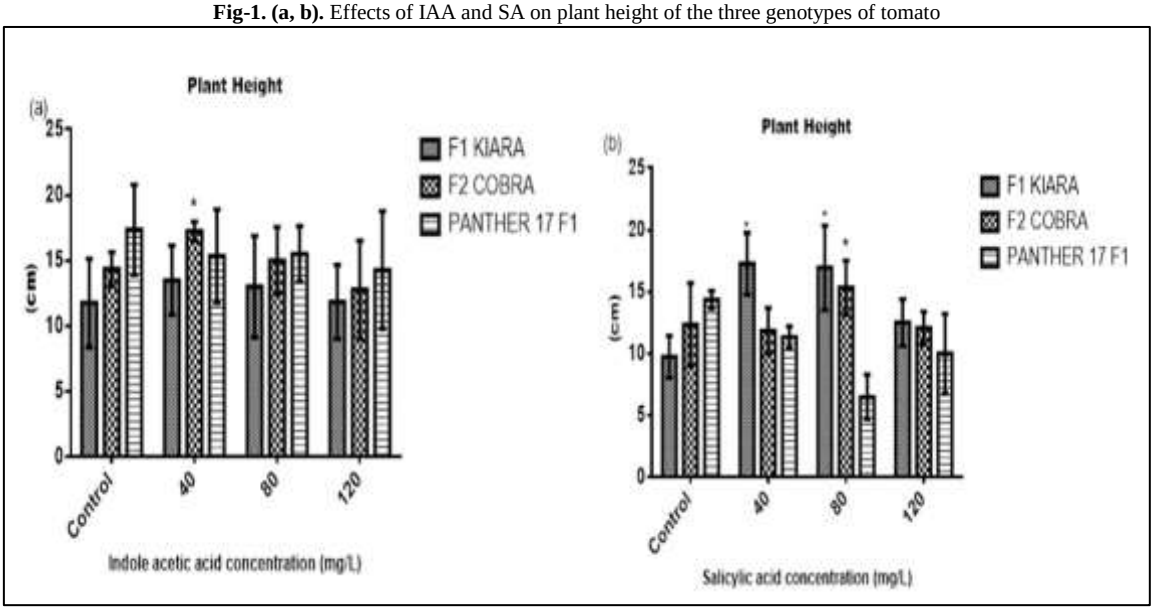
3. Results

3.1. Effects of IAA and SA on Agronomic Traits of the Three Tomato Genotypes

3.1.1. Plant height

In figure (1a, b), IAA and SA application increased height of the three genotypes of tomato significantly ($P \leq 0.05$) when compared with the control group. The 40 and 80 mg/L concentrations of both indole acetic acid and salicylic acid significantly ($P \leq 0.05$) increased the height of genotypes F2 Cobra and F1 Kiara, respectively as compared to the other genotype and control group.

Fig-1. (a, b). Effects of IAA and SA on plant height of the three genotypes of tomato

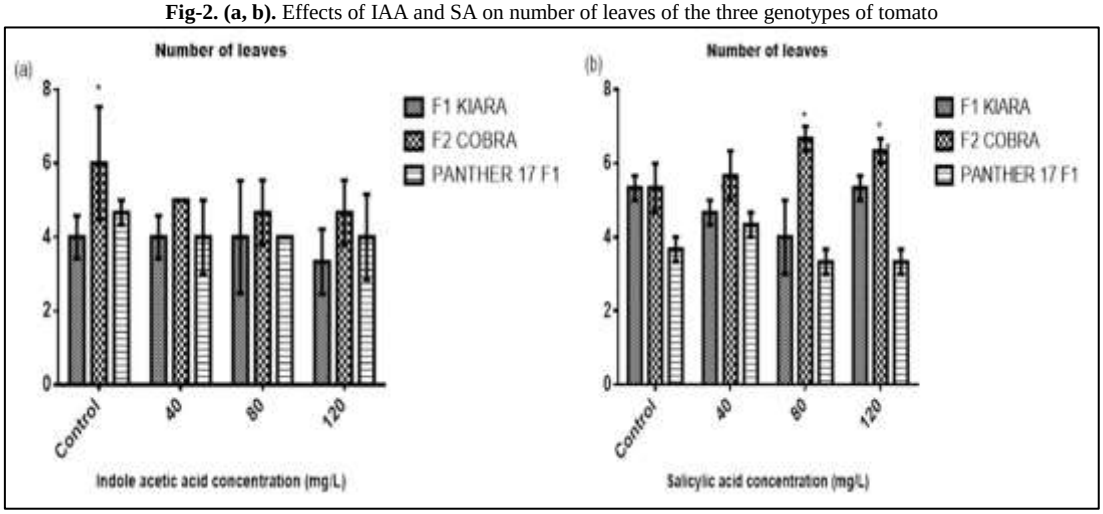


Mean ± SEM, n = 3, was used to express the data. * = Significantly different relative to the control groups.

3.1.2. Number of Leaflets

Figure 2 (a, b) shows the effect of IAA and SA on leaves of the three genotypes. Results showed that the indole acetic acid has little or no effect on the number of leaves in the three genotypes of tomato as compared to the control group (Fig. 2a). However, significant ($P \leq 0.05$) increase in the number of leaves was observed in genotype F2 Cobra treated with 80 and 120 mg/L concentrations of salicylic acid as compared to other genotypes and control group (Fig. 2b).

Fig-2. (a, b). Effects of IAA and SA on number of leaves of the three genotypes of tomato



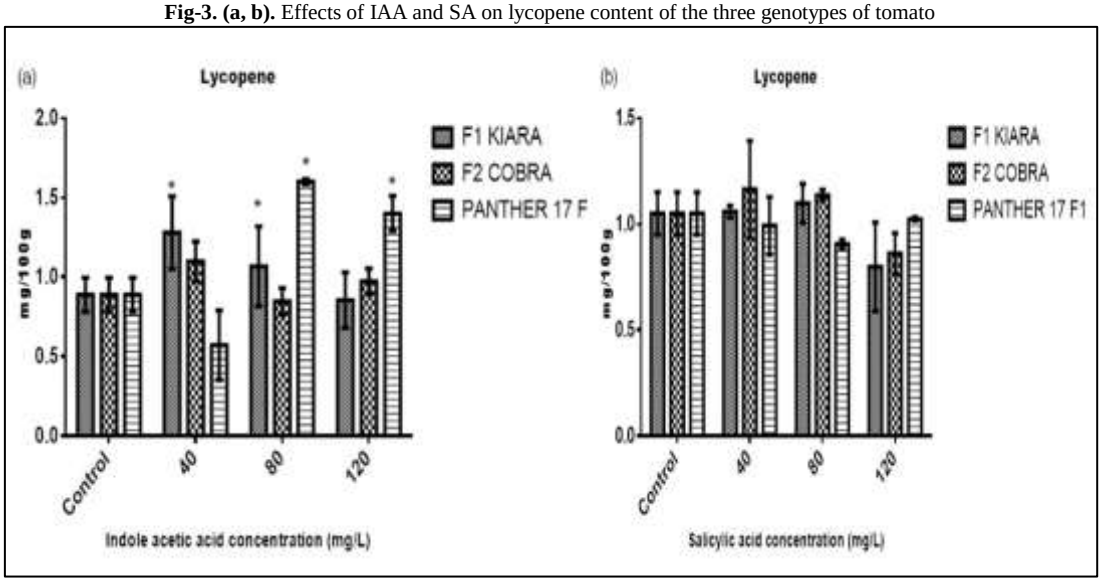
Mean ± SEM, n = 3, was used to express the data. * = Significantly different relative to the control groups.

3.2. Effects of IAA or SA on the Phytochemical Constituents of the Three Tomato Genotypes

3.2.1. Lycopene Content

In figure 3a, the lycopene content of genotypes F2 Cobra and Panther 17 F1 treated with 40, 80, and 120 mg/L concentrations of indole acetic acid, respectively was observed to be significantly ($P \leq 0.05$) increased relativeto the control group. But salicylic acid had little or no effect on the lycopene content, however, the lycopene content of genotype F2 Cobra treated with 120 mg/L concentration of salicylic acid tend to increase when compared with other genotypes and control group (Fig. 3b).

Fig-3. (a, b). Effects of IAA and SA on lycopene content of the three genotypes of tomato



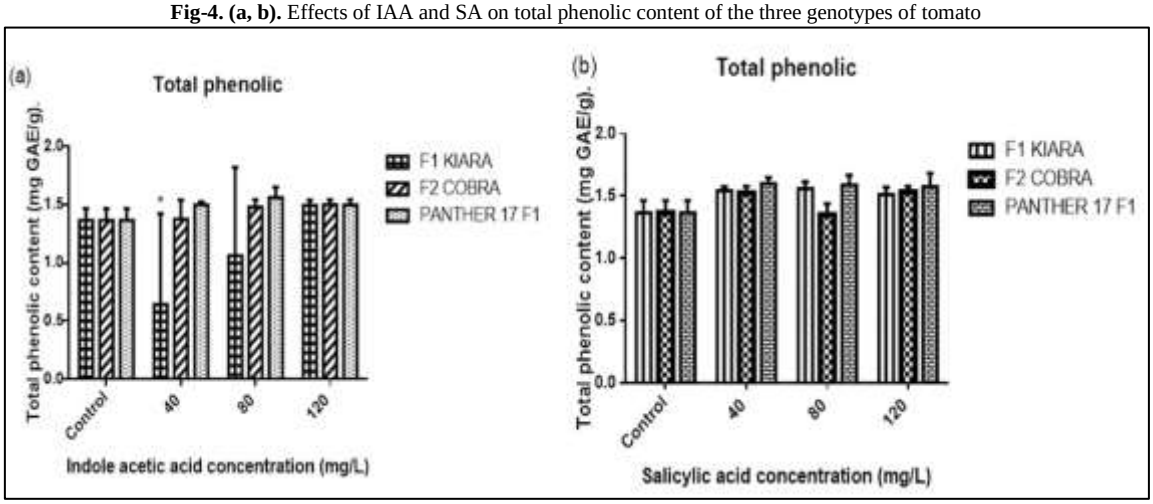
Mean ± SEM, n = 3, was used to express the data. * = Significantly different relative to the control groups.

3.3. Effects of IAA or SA on the Antioxidant Potential of the Three Tomato Genotypes

3.3.1 Total Phenolic Content

Figure 4 (a, b) shows the effect of IAA and or SA on the phenolic content in tomato genotypes. In all the genotypes IAA and SA have no significant effect on phenolic content when compared with the control group. A significant reduction in phenolic content was observed in the genotype F1 Kiara treated with 40 and 80 mg/L concentrations of indole acetic acid compared to the control group.

Fig-4. (a, b). Effects of IAA and SA on total phenolic content of the three genotypes of tomato

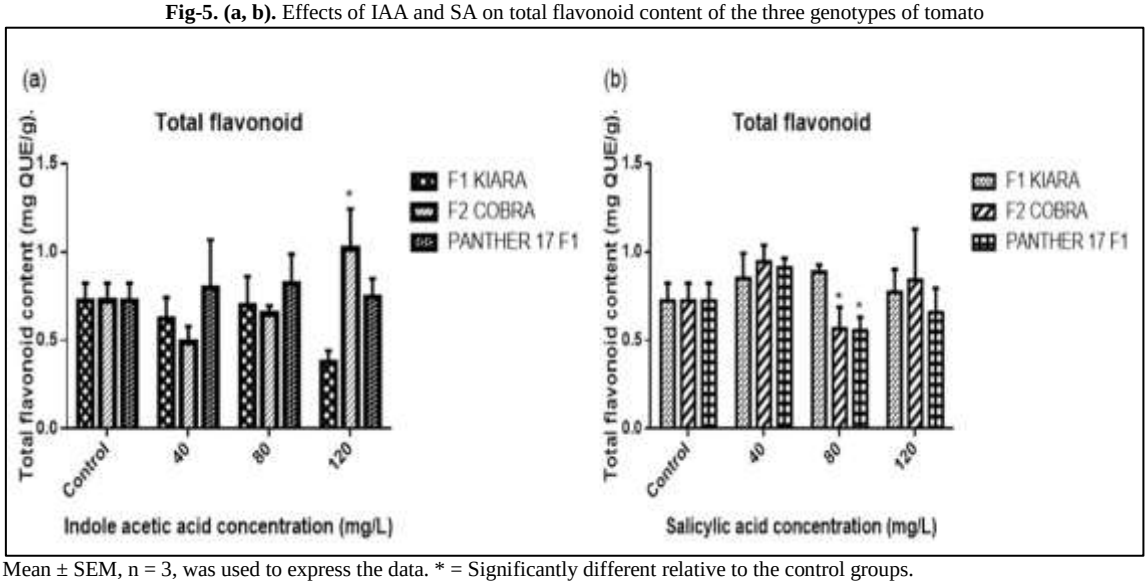


Mean ± SEM, n = 3, was used to express the data. * = Significantly different relative to the control groups.

3.3.2. Total Flavonoid Content

Both IAA and SA have little or no effect on the flavonoid content in the three genotypes of tomato relativeto the control group (Fig. 5 a, b). But, a trend occurred in the group treated with indole acetic acid, and a significant ($P \leq 0.05$) increase flavonoid content of the genotype Panther 17 F1was observed in the group treated with 120 mg/L concentration of indole acetic acid as compared to the control group (Fig. 5a).

Fig-5. (a, b). Effects of IAA and SA on total flavonoid content of the three genotypes of tomato

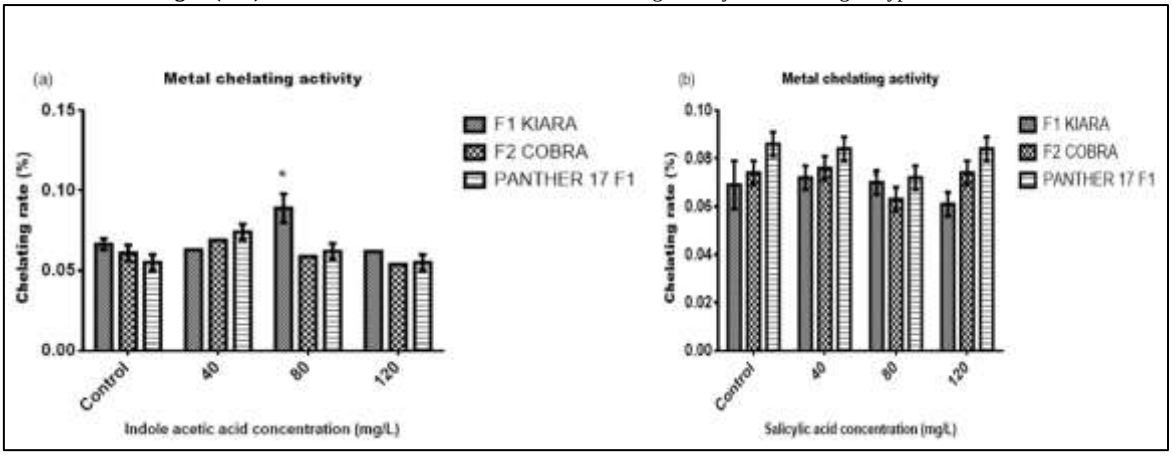


Mean ± SEM, n = 3, was used to express the data. * = Significantly different relative to the control groups.

3.3.3. Metal Ion Chelating Activity

In Figure 6a, metal ion chelating activity of genotype F1 Kiara treated with 80 mg/L concentration of indole acetic acid increased as compared to the control group. However, salicylic acid has no noticeable effect on metal ion chelating activity in the genotypes of tomato in comparison with the control group (Fig. 6b).

Fig-6. (a, b). Effects of IAA and SA on metal ion chelating activity of the three genotypes of tomato

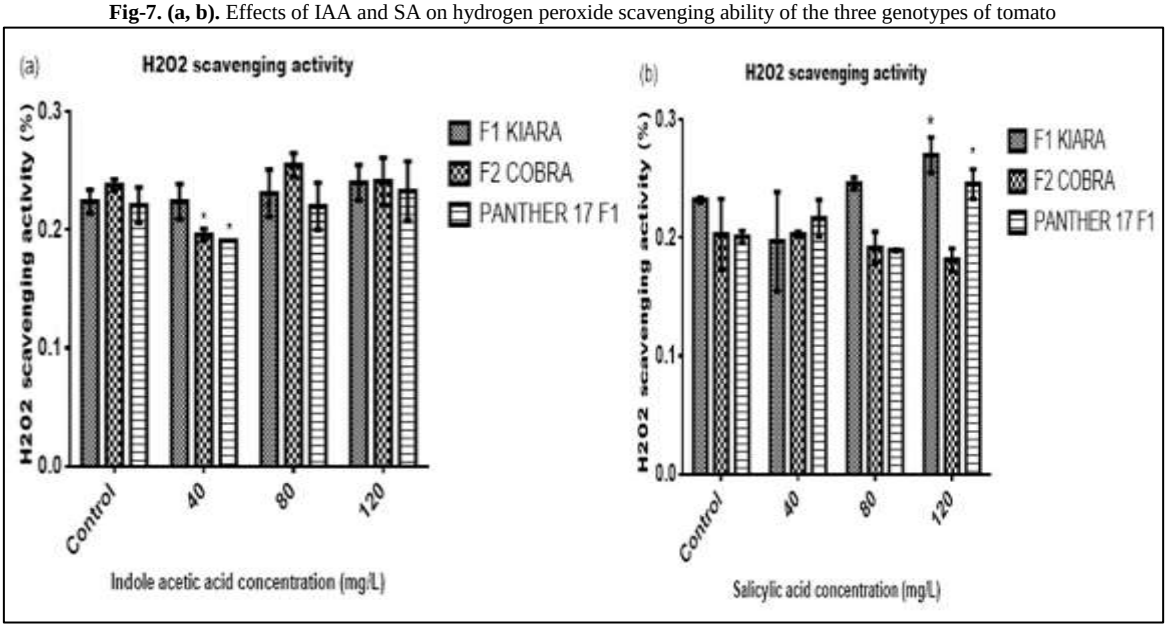


Mean ± SEM, n = 3, was used to express the data. * = Significantly different relative to the control groups.

3.3.4. Hydrogen Peroxide Scavenging Activity

In Figure 7 (a, b), no noticeable effect of IAA and SA on hydrogen peroxide scavenging ability in the three genotypes of tomato. But in groups treated with salicylic acid, a trend occurred and an increase hydrogen peroxide scavenging activity was observed in the genotype F1 Kiara treated with 120 mg/L concentration of salicylic acid as compared to the control (Fig. 7b).

Fig-7. (a, b). Effects of IAA and SA on hydrogen peroxide scavenging ability of the three genotypes of tomato

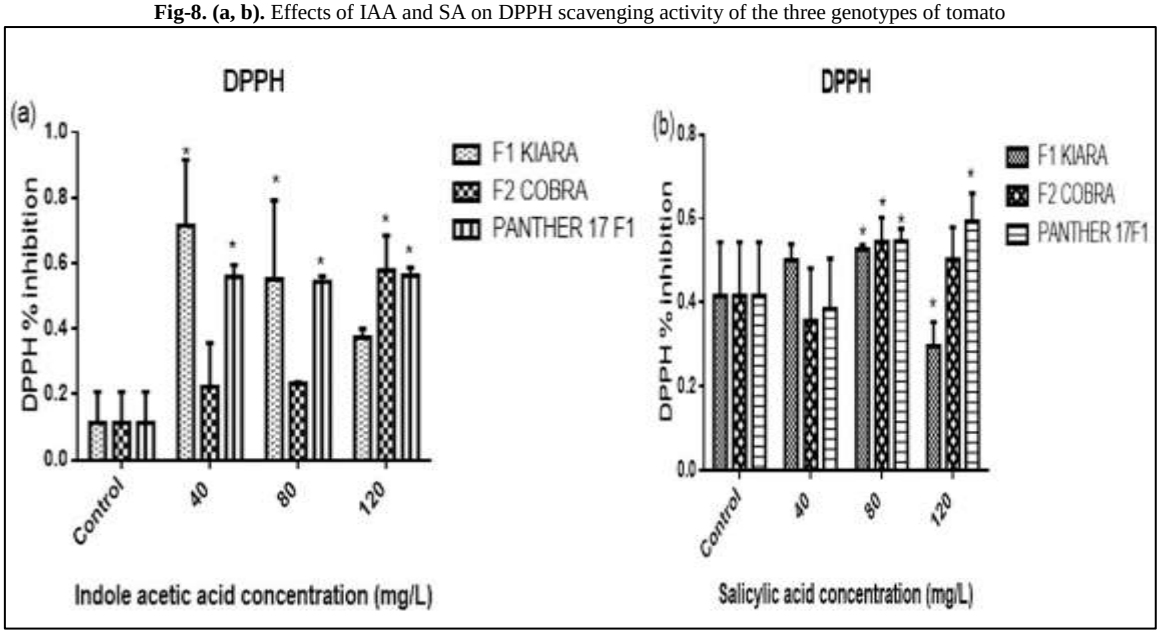


Mean ± SEM, n = 3, was used to express the data. * = Significantly different relative to the control groups.

3.3.5. DPPH Radical Scavenging Activity

Figure 8 (a, b) shows DPPH radical scavenging activity of the three genotypes of tomato. The DPPH radical scavenging activity was increased in all the genotypes treated with varying concentrations of indole acetic acid with exception of the genotype F2 Cobra treated with 40 and 80 mg/L concentrations of IAA when compared with the control group (Fig. 8a). In Figure 7b, little or no effect of SA on scavenging activity of DPPH observed in the three genotypes, but significant increase in DPPH radical scavenging activity was noticed in all the genotypes in a group treated with 80 mg/L concentration of SA when compared with the control group.

Fig-8. (a, b). Effects of IAA and SA on DPPH scavenging activity of the three genotypes of tomato

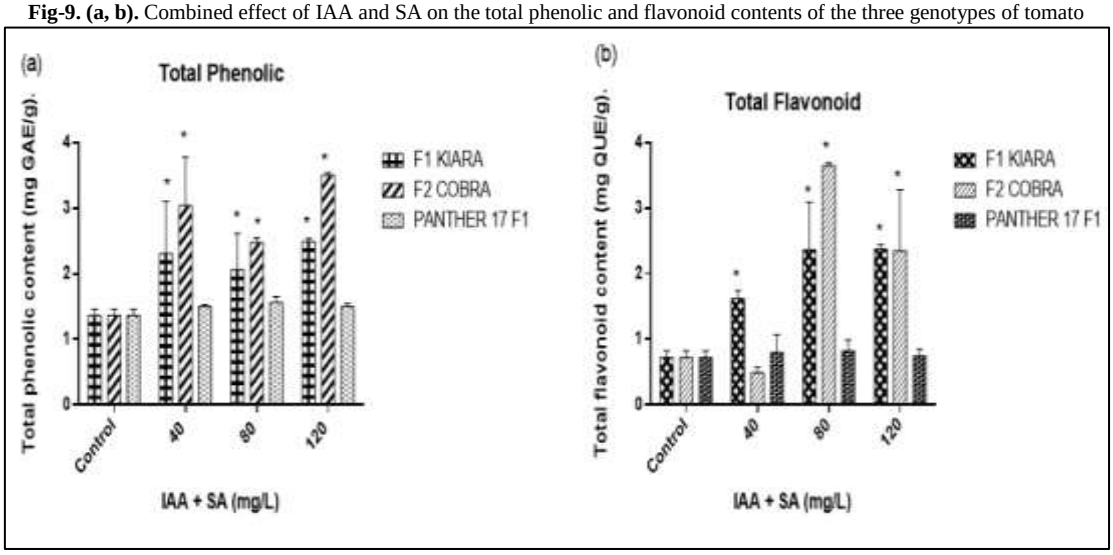


Mean ± SEM, n = 3, was used to express the data. * = Significantly different relative to the control groups.

3.4. Combined Effect of IAA and SA on the Three Genotypes of Tomato

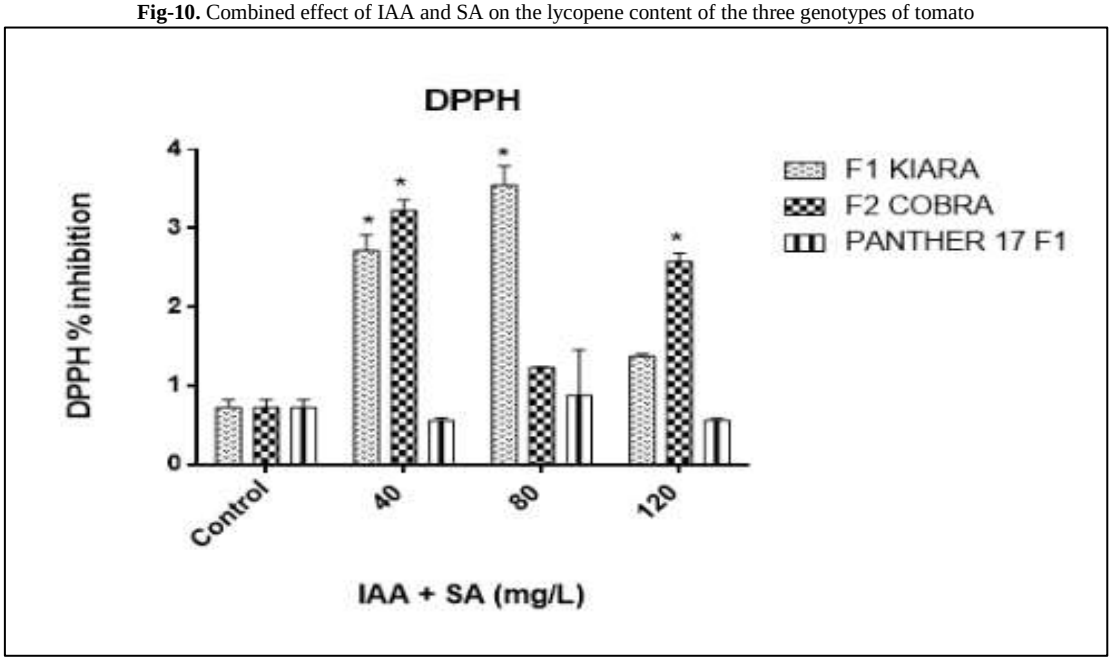
No significant difference observed in the plant height, number of leaflets, metal ion chelating activity, and hydrogen peroxide scavenging activity under the combined effect of IAA and SA when compared with the single treatment. But total phenolic and flavonoid contents in genotypes F1 Kiara and F2 Cobra were significantly ($P \leq 0.05$) increased under the combined effect of IAA and SA concentrations (Figure 9a and b). A similar trend was observed for the DPPH scavenging activities and lycopene content in both genotypes F1 Kiara and F2 Cobra (Figures 10 and 11).

Fig-9. (a, b). Combined effect of IAA and SA on the total phenolic and flavonoid contents of the three genotypes of tomato



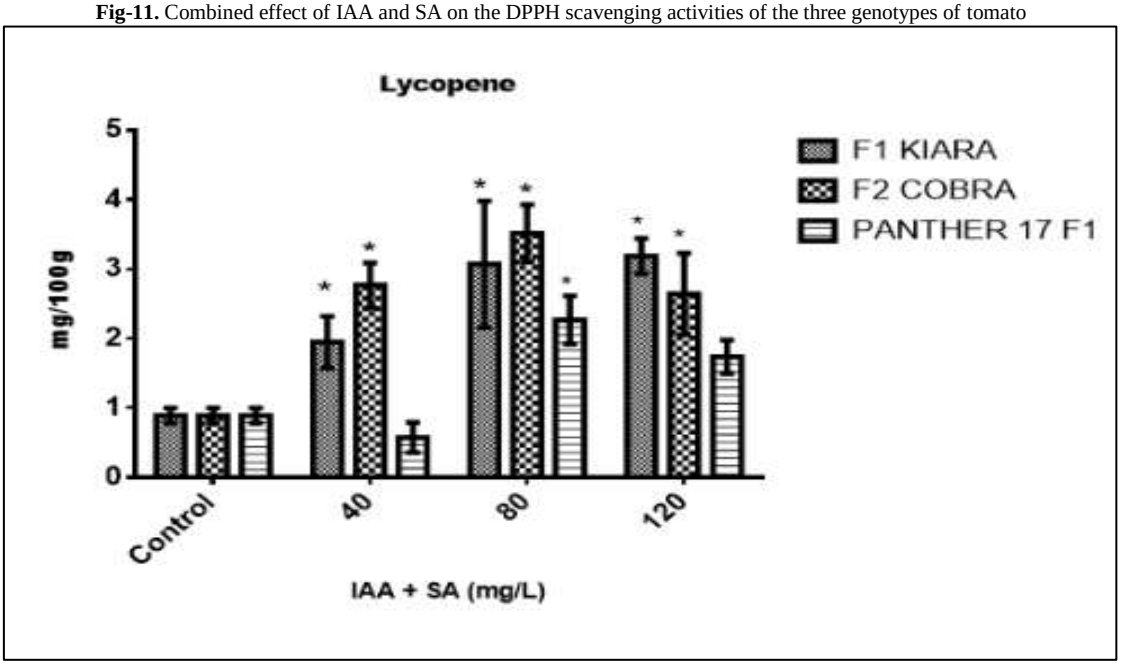
Mean ± SEM, n = 3, was used to express the data. * = Significantly different relative to the control groups.

Fig-10. Combined effect of IAA and SA on the lycopene content of the three genotypes of tomato



Mean ± SEM, n = 3, was used to express the data. * = Significantly different relative to the control groups.

Fig-11. Combined effect of IAA and SA on the DPPH scavenging activities of the three genotypes of tomato



Mean ± SEM, n = 3, was used to express the data. * = Significantly different relative to the control groups.

4. Discussion

Plant bioregulators are natural or synthetic chemicals that manipulate plant chemistry for productivity and quality enhancement with no biocidal or nutritive action [18]. This study is aimed to get a new insight into the effects of IAA and SA on the agronomic traits and phytochemical constituents of the tomato plant. The present study examined the effects of exogenous application of IAA and or SA on phytochemical constituents and antioxidant potential of tomato. Seed pre-treatment with IAA and or SA improved the plant growth and development and the number of vegetative branches (Figs. 1, 2). In tomato plants, increased in the number of vegetative branches might be as a result of auxiliary bud cell elongation and division, which in turn release apical dominance by the application of IAA and or SA. A similar result was observed in the study of Davies [19], who reported an improve quality tomato in respect to cytokinin stimulation.

Indole acetic acid treatments induced the accumulation of total flavonoid content in Panther 17 F1 genotype (Fig. 5a). The significant differences between treatments were observed in total flavonoid content of the genotype Panther 17 F1. IAA-treatment increased total flavonoid content while SA showed no noticeable effect on total flavonoid content compared to levels in control plants. This result agrees with Ali, *et al.* [20], who found an increased amount of total flavonoid content followed by bio-regulator treatment in *Panax ginseng*. An increase concentration of total phenolic content was noticed in genotype F1 Kiara treated with 40 and 80 mg/L concentrations of IAA. But no noticeable effect of salicylic acid on total phenolic content observed in the three genotypes. Lycopene content of genotypes F2 Cobra and Panther 17 F1 treated with 40, 80, and 120 mg/L concentrations of IAA and genotype F2 cobra treated 120 mg/L concentration of SA are generally higher compared to the control plants (Fig. 3a, b). This is evident that IAA and SA facilitate the mobilization of nutrients to the fruit and thus improve fruit lycopene content. A similar observation was reported by Kumari, *et al.* [21], who found increased lycopene content in tomato fruit followed the application of moringa leaf extract. Metal ion chelating is important in reducing the concentration of the transition metal that catalyzes lipid peroxidation. According to the results, indole acetic acid improved metal ion chelating activity of tomato genotype F1 Kiara (Fig. 6a).

DPPH free-radical scavenging and hydrogen peroxide scavenging assays were used to determine the antioxidant capacity of treated and control tomato plants. According to the results, the salicylic acid treatment improved DPPH free-radical scavenging and hydrogen peroxide scavenging activity of tomato genotypes (Fig.8a, b). An increase DPPH and hydrogen peroxide scavenging capacity of tomato genotypes in this study might be due to an increase in the antioxidant enzymes activity in response to plant bioregulator. The combined effect of IAA and SA has a synergistic effect on DPPH scavenging activities, total phenolic and flavonoid contents, as well as on lycopene content (Figures 9, 10, and 11).

5. Conclusion

This study examined whether SA or IAA treatment could improve the phytochemical constituents and antioxidant capacity of tomato. Findings from this study showed that SA or IAA treatment improved bioactive compounds, antioxidant capacity of tomato that is essential for food and pharmaceutical industries. Comparatively, pre-treatment of tomato seeds with IAA have higher total flavonoid, total phenolic content, lycopene content, and metal ion chelating activity than pre-treatment of tomato seeds with SA. The antioxidant potential of tomato improved significantly in IAA-treated tomato plant than SA-treated tomato plant. Furthermore, the phytochemical constituents and anti-oxidative defence system in the three genotypes of tomato improved significantly by the synergistic effect of IAA and SA. Therefore, indole acetic acid application or in combination with SA could be used as a natural source and alternative bio-stimulant to improve the quality of tomato, and to avert food insecurity worldwide.

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Conflict of Interests

Authors declared no competing of interest whatsoever.

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