ISSN 1996-3351

# Asian Journal of Biological Sciences

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OPEN ACCESS Asian Journal of Biological Sciences

ISSN 1996-3351
DOI: 10.3923/ajbs.2019.724.732

# Research Article
# Growth, Biochemical and Physiological Responses of Water-stressed African Eggplant Seedlings to Exogenous salicylic Acid

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## Abstract

**Background and Objective:** Water stress is a constraint to the production of African Eggplant. Growth, biochemical, photosynthetic pigments, anti-oxidative defense system responses of pot-grown eggplant to water stress and pre-stress salicylic acid (SA) treatments were investigated. **Materials and Methods:** Treatments consisted of pre-stress foliar application of 0, 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 mM of SA on plants exposed to 10 or 20 days of water-deficit stress and water sufficient control. Randomized complete design with three replicates was used. **Results:** Underwater stress, growth was reduced but anti-oxidant defensive systems enhanced. However, pre-stress foliar application of SA increased growth and further enhanced proline, phenolics, endogenous SA, leakage of electrolytes and activities of superoxide dismutase (SOD), catalase (CAT), peroxidase (POD) and ascorbate peroxidase (APX) to the same level as water sufficient control plants. **Conclusion:** The study concluded that pre-stress foliar application of SA protects water-stressed *S. macrocarpon* against oxidative damage at 2.0 mM concentration.

Key words: African eggplant, external application, moisture deficit, physiological change, phytohormone

Received: December 11, 2018 Accepted: February 01, 2019 Published: August 01, 2019

Citation: Jelili Titilola Opabode, Marufat Omowumi Okewale, Omolara Rukkayat Ibrahim and Adekemi Francisca Dosumu, 2019. Growth, biochemical and physiological responses of water-stressed African eggplant seedlings to exogenous salicylic acid. Asian J. Biol. Sci., 12: 724-732.

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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

More than other crops, water stress is an important constraint to production and post-harvest quality of vegetable crops in Africa[1]. This is attributed to the fact that vegetable crops are herbaceous, whose fresh weight contains more than 70% water. Climate change is expected to increase water scarcity that could aggravate water stress impacts on vegetable crops. Water deficit stress is caused by drought, erratic rainfall and dry season that characterized tropical and subtropical climates[2].

Studies have revealed that water stress elicits morphological, physiological and molecular responses in crops. The most apparent impact of water deficit on crops is cessation of cell and tissue growth arising from reduction in turgor pressure, inhibition of cell elongation occasioned by interruptions of water movement from the xylem tissues to meristematiccells[3]. In addition, Hussain *et al.*[4] presented empirical evidence which confirmed that water stress impaired mitosis, enlargement of cell, growth and yield traits. Chlorophylls, important pigments for photosynthetic process, are object of attack by oxidative stress caused by drought. Quantities of chlorophylls synthesise by a plant has a positive correlation with photosynthetic rate and yield. Under drought stress, chlorophyll content decreases as a result of photo-oxidation and breaking down of chlorophyll molecules[5]. Metabolic activity of a plant correlates with its water status and it is measured as relative water content. Reduction in relative water content and water potential of plant under water stress has been reported. High relative water content under drought stress indicates dehydration tolerance of a plant[6]. Osmotic adjustment is another means of retaining leaf turgor by plants experiencing water deficit through building-up in cytoplasm of soluble carbohydrates, proline, glycine betaine, sucrose and other solutes which enhances absorption of water[7]. Investigations have revealed vital roles of proline in water stress compared with other solutes. To reduce injury to cells and tissues, proline accumulation in plants has been established to be the first physiological response of water-stressed plants. At high concentrations, proline mitigates water-deficit stress through contributions to stabilization of enzymes and proteins, removal of reactive oxygen species, protection of membrane stability and osmotic adjustment of cells[7].

Drought stress trigger production of reactive oxygen species (ROS) in excess of amount produced under normal conditions[8]. Some of the ROS produced in excessive amount under drought are hydrogen peroxides, superoxides and hydroxyl radicals. The ROS breaks down DNA, proteins, carbohydrates and lipids, leading to oxidative stress[8]. Plants have a reliable machinery that collects and removes ROS, shield cells and tissues from oxidative destruction and stabilize metabolism during drought. Superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) are anti-oxidative enzymes that play vital roles in mitigation of oxidative stress. For instance, superoxide radicals are converted to hydrogen peroxide by SOD, the hydrogen peroxide are collected and removed by POD using mainly CAT in the peroxisomes and various substrates as electron donors in the extracellular space and cytosol[9]. Consequently, it has been established that high proline content and high activity of anti-oxidant enzymes are associated with water stress tolerance in most plants[10].

Salicylic acid (SA) is a phenolic phytohormone produced by plants in low quantity and plays vital roles in vegetative and reproductive life of plants[11]. Experimental evidence has established that SA is involved in the processes of thermogenesis, nutrient absorption, flower formation and ethene biosynthesis[12], photosynthesis[13], protection against abiotic and biotic stresses. Data from Hayat *et al.*[14] and Yusuf *et al.*[15] confirmed that exogenous SA mitigates water and salinity stresses. Foliar SA (1.5 ppm) induced defense system in chickpea by increasing activities of peroxidase and polyphenol oxidase and boosting phenols, proteins and hydrogenperoxides[16].

African eggplant (*Solanum macrocarpon* L.), which belongs to Solanaceae, is an important indigenous leaf African vegetable. In west, east and central Africa, fresh and succulent shoots of the vegetable attracted interest of consumers[1]. Nutritional value of *S. macrocarpon* is high. Unprocessed leaves of *S. macrocarpon* contained 89.7% moisture, 4.3% protein[17], 1.3% ash, 0.6% fat, 1.4% crude fibre, 32.6 mg $kg^{-1}$ Ca and 8.2 mg $kg^{-1}$ Zn. Research revealed that the leaves of the vegetable contains 14.0% glutamic acid, 13.3% aspartic acid, 7.5% leucine and 6.6% arginine[1]. Medicinal properties of the vegetable are being exploited to cure many human and animal diseases in Africa and Asia. For example, in Sierra Leone, mature leaves of the vegetable are boiled and chewed to remove throat pain. Decoctions made from the roots is used to treat hookworms in Kenya[1]. Further more, the root of *S. macrocarpon* is part of the herbal mix for curing body aches, bronchitis, asthma and wound healing. Screening of leaf cuticular waxes of two cultivars of *S. macrocarpon* detected an unusual high profile of sterols and low hydrocarbon content, suggesting that the plant is producing phytosterols[18].

In spite of its importance for medicine and nutrition, production of the vegetable does not keep pace with market dem and sat every season of the year[19]. Water-deficit stress

arising from drought, erratic rainfall and dry spell has been identified as a factor limiting cultivation and adequate availability of the vegetable all year round. A recent study has concluded that water stress at 20-45 days after transplanting as the most critical to the cultivation of the leaf vegetable, reducing leaf yield[19] by 78%. Thus, there is the need to protect the vegetable from the impacts of water stress. Ameliorating negative impacts of water-deficit stress on *S. macrocarpon* by exogenous SA is a viable option for a sustainable production of the vegetable. However, impacts of water-deficit on growth, physiology and biochemistry of *S. macrocarpon* has not been investigated. It tested the hypothesis that pre-treatment of *S. macrocarpon* plants with salicylic acid could mitigate negative effects of water deficit. In this report, growth, physiological and anti-oxidant defense system responses of *S. macrocarpon* exposed to 10 or 20 days of water-deficit stress to pre-stress SA foliar application were examined.

## MATERIALS AND METHODS

**Seedling preparations and conditions of growth:** The study was conducted between July, 2017 and October, 2018 at Central Greenhouse, Faculty of Agriculture, Obafemi Awolowo University, Ile-Ife, Nigeria. Plants of *Solanum macrocarpon* cv. Igbagba were raised from sterilized seeds in pot at an average temperature of 26±2°C under 65±5% relative humidity and 7-9 h of daylight. To check reproducibility of the results the study was repeated.

**Treatments and experimental design:** Stress treatment started at 25 days after transplanting of seedlings. The seedlings were arranged into three groups: the first group was subjected to water stress at 15% water holding capacity (by irrigating daily with 150 mL of water) for 10 days and at end of the stress treatment, external application of salicylic acid (SA; 0, 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 mM) were applied on the two leaf surfaces using an atomizer at sunset.

The second group of plants were subjected to water stress at 15% water holding capacity for 20 days and external application of salicylic acid (SA; 0, 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 mM) were applied and third group of plants were watered to full water-holding capacity without SA treatment to serve as control. As a result, the study had 15 treatments as follows:

- T1 = Water sufficient control (100% water holding capacity)
- T2 = Ten days of water stress (10 DWS)
- T3 = 10 DWS+0.5 mM SA
- T4 = 10 DWS+1.0 mM SA
- T5 = 10 DWS+1.5 mM SA
- T6 = 10 DWS+2.0 mM SA
- T7 = 10 DWS+2.5 mM SA
- T8 = 10 DWS+3.0 mM SA
- T9 = Twenty days of water stress (20 DWS)
- T10 = 20 DWS+0.5 mM SA
- T11 = 20 DWS+1.0 mM SA
- T12 = 20 DWS+1.5 mM SA
- T13 = 20 DWS+2.0 mM SA
- T14 = 20 DWS+2.5 mM SA
- T15 = 20 DWS+3.0 mM SA

To ensure an adequate wetting of leaf surfaces, two drops of 0.5% Tween-20 was added to SA solution. Arrangement of the treatments followed completely randomized design with three replicates. A treatment had 15 plants. Data were obtained 14 days after SA treatment.

**Growth parameters:** Dry weight, fresh shoot weight, number of leaf and shoot height were determined at 14 days after SA treatment. Gravimetric method was used to determine leaf area as outlined by Okewale[20].

**Relative water content of leaf:** Relative water content of leaf (RWC) was determined from five fully expanded and mature leaves as outlined by Okewale[20].

**Electrolyte leakage:** Electrolyte leakage was measured as described by Sullivan and Ross[21] from twenty leaf discs after boiling in a test tube containing 10 mL of distilled water.

**Photo synthetic pigments:** Chlorophyll a, chlorophyll b and carotenoids were extracted from 1 g of fresh tissues as described by Lichtentaler and Wellburn[22].

**Leaf proline content:** To examine the osmotic adjustment of plants, proline content of the third fully expanded leaf (3 g) from the top was determined according to Bates *et al.*[23] and absorbance by toluene was measured at 528 nm.

**Phenolics content:** The method of Julkunen-Titto[24] was used to determine leaf total phenolics content from fresh tissues (0.5 g) of 3rd fully expanded leaf from shoot tip and absorbance was read at 750 nm.

**Soluble proteins:** Total soluble proteins were determined as outlined by Bradford[25] of fresh tissues (1.0 g) of 3rd fully expanded leaf and absorbance read at 590 nm wavelength using bovine serum albumin.

**Determination of root and leaf SA contents:** The procedure of Guzman-Tellez *et al.*[26] was adopted for determination of SA from fresh root and leaf tissues (Chromatography (HPLC)). An agilent 1100 system was used for HPLC which was equipped with a fluorescent detector and column preceded by guard column. Salicylic acid was quantified using programmed fluorescent detector.

**Antioxidant enzyme assays:** Enzyme activities were assayed from the fourth fully expanded leaf from the shoot tip. After washing with distilled water, leaf sample (0.5 g) was ground in cold 0.1 mol $L^{-1}$ phosphate buffer (pH 7.5) containing 0.5 mmol $L^{-1}$ EDTA. The homogenized mixture was centrifuged at 4°C for 15 min at 15,000×g. The supernatant served as enzyme assay in this study.

**Ascorbate peroxidase:** Determination of activity of ascorbate peroxidase (APX) as outlined by Nakano and Asada[27] was followed. One unit of APX activity was defined as 1 mmol ascorbate oxidized $mL^{-1}$ $min^{-1}$ at 25°C.

**Superoxide dismutase:** The method of Dhindsa *et al.*[28] was followed for determination of activity of superoxide dismutase (SOD) and expressed as unit $mg^{-1}$ protein.

**Catalase:** Activity of catalase (CAT) was measured as described by Aebi[29] which was determined by recording absorbance of hydrogen peroxide at 240 nm.

**Peroxidase:** The method of Hemeda and Klein[30] was used to determine activity of peroxidase (POD) which was determined by absorbance at 470 nm [$\varepsilon$ = 26.6/mmol $L^{-1}$ cm).

**Statistical analysis:** A two-way analysis of variance were performed on data to determine significance of the treatment effect using statistical analysis systems 9.1.3. At 5% level of probability, treatment means were separated by Ducan multiple range test.

## RESULTS

**Growth parameters as affected by water stress and SA application:** Compare with unstressed (T1) control plants, water stress decreased number of leaves, shoot height, leaf area, fresh shoot weight and dry weight of plants subjected to 10 days of water stress (T2) and 20 days of water stress (T9) (Table 1). T9 exhibited greater reduction in growth than T2. Number of leaves, shoot height, leaf area, fresh shoot weight and dry weight of plants improved as concentration of SA increased in both plants subjected 10 days of water stress (10 DWS) and 20 days of water stress (20 DWS). However, SA-treated plants had peak of growth at 2.0 mM SA in both 10 and 20 DWS plants.

**Effect of SA application on relative water content, photo synthetic pigments and endogenous SA:** Compare with unstressed (T1) control plants, water stress decreased RWC, photo synthetic pigments and endogenous root SA of both 10 DWS plants (T2) and 20 DWS plant (T9) (Table 2). Expectedly, the effect of 20 DWS was more pronounced than that of 10 DWS on relative water content, photosynthetic pigments and endogenous SA. External application of SA improved all the parameters in both 10 and 20 DWS plants. While medium doses (2.0-2.5 mM) of exogenous SA enhanced RWC and photosynthetic pigments, medium and high doses

Table 1: Number of leaves, shoot height, leaf area, fresh shoot weight and dry weight of *S. macrocarpon* seedlings as affected by water stress and pre-stress salicylic acid application

| Treatments | Number of leaves (No plant$^{-1}$) | Shoot height (cm) | Leaf area (cm$^2$ plant$^{-1}$) | FSW (g plant$^{-1}$) | DW (g plant$^{-1}$) |
|---|---|---|---|---|---|
| T1 | 15.9±3.4$^{a}$ | 18.5±4.2$^{a}$ | 35.0±4.6$^{a}$ | 51.8±6.7$^{a}$ | 12.8±2.1$^{a}$ |
| T2 | 8.7±2.8$^{e}$ | 12.2±2.6$^{d}$ | 23.4±4.2$^{e}$ | 35.3±4.3$^{d}$ | 4.6±1.8$^{e}$ |
| T3 | 8.8±2.1$^{e}$ | 13.7±3.2$^{d}$ | 23.9±4.5$^{e}$ | 38.6±4.3$^{c}$ | 4.6±1.1$^{e}$ |
| T4 | 10.5±3.1$^{d}$ | 15.5±2.4$^{c}$ | 27.8±5.1$^{c}$ | 41.9±4.2$^{b}$ | 6.3±1.6$^{d}$ |
| T5 | 14.6±2.7$^{b}$ | 15.2±2.7$^{c}$ | 30.6±5.2$^{b}$ | 45.6±4.6$^{b}$ | 8.9±1.3$^{c}$ |
| T6 | 15.8±3.5$^{a}$ | 18.4±2.9$^{a}$ | 34.6±4.8$^{a}$ | 51.8±4.8$^{a}$ | 12.6±2.1$^{a}$ |
| T7 | 12.8±2.3$^{c}$ | 18.9±2.8$^{a}$ | 34.8±3.8$^{a}$ | 51.7±4.5$^{a}$ | 12.8±2.0$^{a}$ |
| T8 | 12.6±3.2$^{c}$ | 18.6±3.2$^{a}$ | 29.5±3.7$^{c}$ | 51.2±4.7$^{a}$ | 12.6±2.3$^{a}$ |
| T9 | 6.9±1.7$^{g}$ | 8.9±1.8$^{f}$ | 18.5±3.2$^{f}$ | 27.9±4.5$^{e}$ | 3.1±1.1$^{f}$ |
| T10 | 7.8±1.8$^{f}$ | 10.8±1.9$^{e}$ | 18.8±2.4$^{f}$ | 28.5±4.5$^{e}$ | 3.3±0.4$^{f}$ |
| T11 | 10.9±2.1$^{d}$ | 13.8±1.5$^{d}$ | 21.3±3.7$^{e}$ | 34.5±4.8$^{d}$ | 5.7±1.5$^{d}$ |
| T12 | 13.8±3.4$^{b}$ | 13.9±1.7$^{d}$ | 25.8±3.2$^{d}$ | 34.8±4.7$^{c}$ | 5.6±1.4$^{d}$ |
| T13 | 14.6±1.4$^{b}$ | 16.8±3.5$^{b}$ | 28.7±4.3$^{c}$ | 38.8±4.3$^{c}$ | 9.4±2.3$^{b}$ |
| T14 | 14.8±2.1$^{b}$ | 15.9±3.2$^{c}$ | 25.8±5.3$^{d}$ | 38.5±5.5$^{c}$ | 9.0±2.5$^{b}$ |
| T15 | 13.8±2.0$^{b}$ | 15.6±3.3$^{c}$ | 24.8±4.6$^{d}$ | 35.4±5.2$^{d}$ | 9.1±2.6$^{b}$ |

Means followed by different letters in the same column are significantly different at 5% level of probability using Duncan multiple range test, DW: Dry weight and FSW: Fresh shoot weight

Table 2: Leaf relative, chlorophylls, carotenoids and endogenous salicylic acids of *S. macrocarpon* seedlings as affected by water stress and pre-stress salicylic acid application

| SA (mM) | RWC (%) | Chl-a (mg $g^{-1}$) | Chl-b (mg $g^{-1}$) | Carotenoid (mg $g^{-1}$) | Root SA (ng $g^{-1}$) | Leaf SA (ng $g^{-1}$) |
|---|---|---|---|---|---|---|
| T1 | 78.5±7.3$^{c}$ | 38.7±2.4$^{a}$ | 20.2±3.5$^{a}$ | 14.8±2.1$^{a}$ | 25.3±4.5$^{d}$ | 18.5±3.5$^{d}$ |
| T2 | 65.3±5.4$^{d}$ | 18.8±2.2$^{c}$ | 8.7±2.1$^{d}$ | 6.4±1.5$^{c}$ | 34.8±3.7$^{c}$ | 19.3±3.4$^{d}$ |
| T3 | 67.4±5.7$^{d}$ | 16.3±3.2$^{c}$ | 8.9±2.3$^{d}$ | 6.3±1.5$^{c}$ | 43.6±4.2$^{b}$ | 24.6±3.2$^{c}$ |
| T4 | 78.4±6.2$^{c}$ | 22.5±4.4$^{b}$ | 10.6±2.0$^{d}$ | 6.2±1.7$^{c}$ | 48.8±4.7$^{b}$ | 28.9±4.6$^{c}$ |
| T5 | 81.3±7.8$^{b}$ | 29.7±4.3$^{b}$ | 15.6±2.0$^{b}$ | 8.4±1.3$^{c}$ | 53.4±3.8$^{a}$ | 27.6±5.6$^{c}$ |
| T6 | 86.9±7.1$^{a}$ | 34.8±4.7$^{a}$ | 16.8±1.5$^{b}$ | 10.6±2.3$^{b}$ | 54.2±4.8$^{a}$ | 28.8±5.7$^{c}$ |
| T7 | 63.8±6.3$^{d}$ | 34.6±4.8$^{a}$ | 16.2±2.1$^{b}$ | 10.7±2.1$^{b}$ | 53.9±4.9$^{a}$ | 27.5±6.7$^{c}$ |
| T8 | 62.7±5.8$^{d}$ | 31.8±3.2$^{a}$ | 15.4±2.4$^{b}$ | 10.7±2.3$^{b}$ | 52.7±4.6$^{a}$ | 28.7±6.8$^{c}$ |
| T9 | 52.3±4.8$^{e}$ | 13.7±2.4$^{c}$ | 7.2±2.2$^{d}$ | 4.6±1.4$^{e}$ | 38.5±4.8$^{c}$ | 20.4±4.5$^{d}$ |
| T10 | 61.8±6.1$^{d}$ | 15.4±2.3$^{c}$ | 8.4±2.1$^{d}$ | 5.7±1.6$^{d}$ | 42.5±3.2$^{b}$ | 25.9±4.8$^{c}$ |
| T11 | 64.4±5.9$^{d}$ | 18.6±2.3$^{c}$ | 8.4±2.2$^{d}$ | 6.8±1.8$^{c}$ | 48.7±3.7$^{b}$ | 30.8±5.5$^{c}$ |
| T12 | 77.8±6.4$^{c}$ | 23.6±2.7$^{b}$ | 11.7±2.5$^{d}$ | 5.6±1.7$^{d}$ | 54.3±3.7$^{a}$ | 34.8±5.8$^{b}$ |
| T13 | 82.6±5.7$^{b}$ | 28.8±3.2$^{b}$ | 14.4±2.3$^{c}$ | 5.4±1.6$^{d}$ | 56.9±3.8$^{a}$ | 38.7±5.7$^{a}$ |
| T14 | 63.6±6.7$^{c}$ | 28.4±3.1$^{b}$ | 14.1±2.2$^{c}$ | 5.2±1.7$^{d}$ | 55.8±3.8$^{a}$ | 38.9±5.8$^{a}$ |
| T15 | 58.6±5.8$^{e}$ | 26.8±2.4$^{b}$ | 13.6±1.9$^{c}$ | 5.0±1.9$^{d}$ | 54.8±4.7$^{a}$ | 38.5±5.8$^{a}$ |

Means followed by different letters in the same column are significantly different at 5% level of probability using Duncan multiple range test

of exogenous SA promoted synthesis of endogenous leaf and root SA in both 10 and 20 DWS plants.

**Effect of SA application on electrolyte leakage, phenolic, proline and soluble protein:** Compare with unstressed (T1) control plants, water stress decreased soluble protein but increased electrolyte leakage, proline and phenolic contents of T2 and T9 plants (Fig. 1). Foliar SA application increased soluble protein, proline and phenolic but stopped electrolyte leakages in both 10 and 20 DWS plants with medium doses (2.0-2.5 mM) of SA gave the best performance (Fig. 1).

**Effect of SA application on anti-oxidant enzymes:** Water stress increased activity of ascorbate peroxidase (APX), superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD) of T2 and T9 plants when compare with unstressed (TI) control plants (Fig. 2). Foliar application of SA further enhanced APX, SOD, CAT and POD activities in 10 and 20 DWS plants. Medium and high doses of SA enhanced activity of APX in both 10 and 20 DWS plants. While medium dose enhanced activity of SOD, CAT and POD in 10 DWS plants, high doses of SA increased activity of SOD, CAT and POD in 20 DWS plants.

## DISCUSSION

In the current study, water stress decreased shoot height, leaf number, leaf area and fresh and dry weights according to the length of the water deficit. Furthermore, foliar application of SA neutralized the negative impact of the water stress as indicated by the enhanced growth parameters. Previously, exogenous SA mitigated water deficit impacts in wheat, barley and tomato by enhancing growth, photosynthetic attributes, leaf water potentials, activities of antioxidant enzymes, phenolics content and decreasing lipid peroxidation of water-stressed plants[14,31,32]. The enhanced growth of SA-treated water-stressed plants observed in this study was due to protection by exogenous SA against oxidative stress as reflected in high values of chlorophylls, carotenoids, soluble protein, phenolics endogenous SA and increased activities of CAT, SOD, POD and APX. Previously, SA has been reported to improve performance of tomato, barley, chickpea, garlic and red amaranth under water stress[14,16,31-33]. In this study, it observed that water stress decreased leaf RWC of plants. However, exogenous SA improved leaf RWC of both 10 and 20 DWS plants suggesting SA enhanced their dehydration tolerance. Enhanced leaf RWC could be due to osmotic adjustment in the water stressed plants by increased proline and phenolics contents. Water stress reduced the quantities of chl-a, chl-b and carotenoids due to degradation by oxidative stress. But chl-a and b of SA-treated plants were higher than untreated plants, suggesting exogenous SA protected chlorophylls of water stressed plants against degradation or photo-oxidation triggered by oxidative burst. Higher chlorophyll content and photo synthetic rate coupled with high Rubisco activity compare with control have been reported for SA treated wheat seedlings subjected to water stress[31].

High content of endogenous SA synthesized in the roots of water-stressed plants could be triggered by the stress to prevent oxidative damage by detoxifying reactive oxygen species and increased activity of anti-oxidant enzymes. However, high accumulation of SA was not detected in the leaves of water-stressed which was consistent with the reports of Bandurska and Stroinski[32] which was attributed to impairment of water transportation of SA in vascular bundle

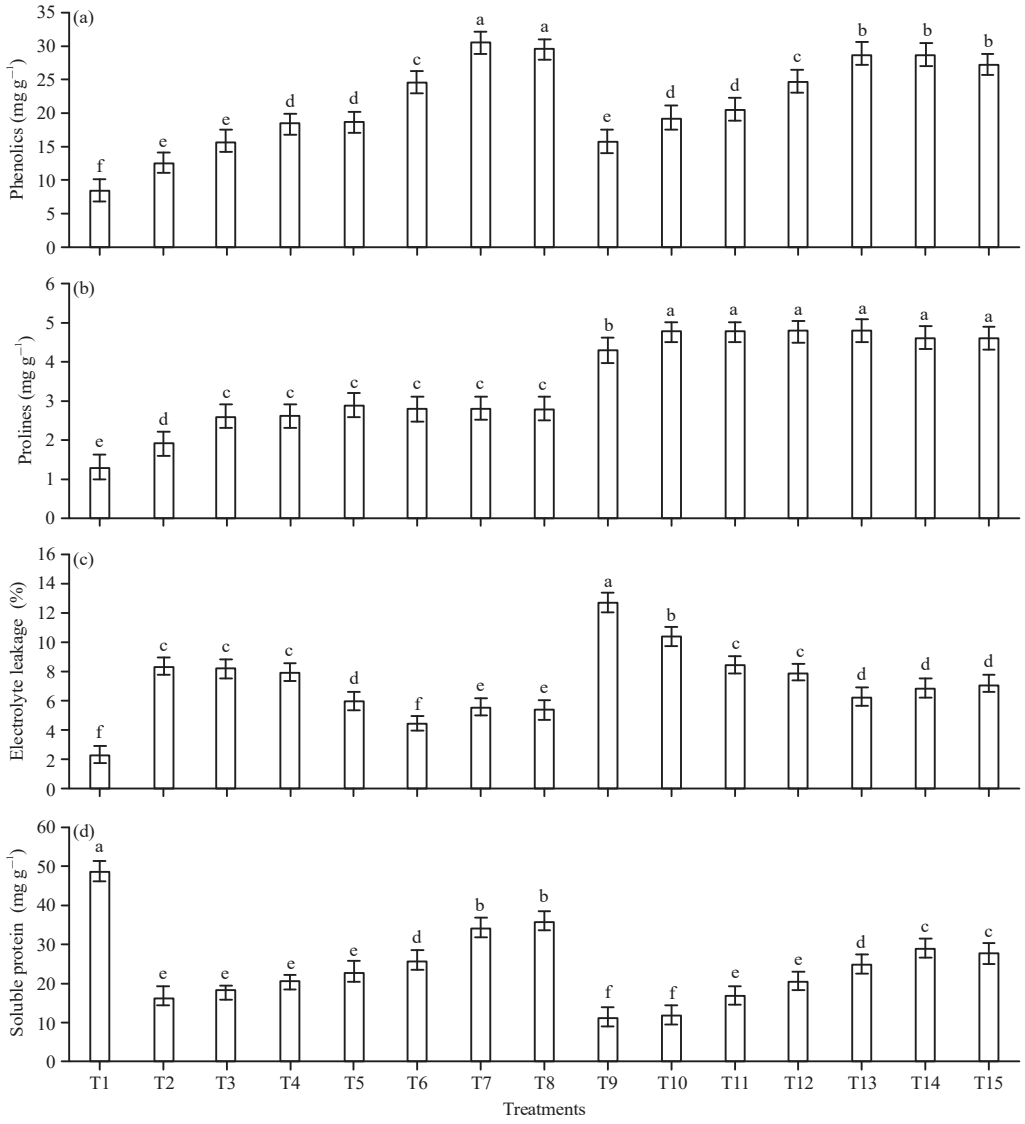


Fig. 1(a-d): Influence of exogenous SA on (a) Phenolics, (b) Proline, (c) Electrolyte leakage and (d) Soluble protein of *Solanium macrocarpa* under water stress

T1: Water sufficient, T2: Ten days of water stress (10 DWS), T3: 10 DWS+0.5 mM SA, T4: 10 DWS+1.0 mM SA, T5: 10 DWS+1.5 mM SA, T6: 10 DWS+2.0 mM SA, T7: 10 DWS+2.5 mM SA, T8: 10 DWS+3.0 mM SA, T9: 20 DWS, T10: 20 DWS+0.5 mM SA, T11: 20 DWS+1.0 mM SA, T12: 20 DWS+1.5 mM SA, T13: 20 DWS+2.0 mM SA, T14: 20 DWS+2.5 mM SA and T15: 20 DWS+3.0 mM SA

from point of production to other parts of water-stressed plants. Furthermore, enhanced SA accumulation in both leaves and roots of SA-treated plants suggested exogenous SA stimulates endogenous SA synthesis. Similarly, empirical evidence that endogenous SA synthesis is stimulated by external stimuli in Arabidopsis was presented by Ogawa *et al.*[34].

Plants under water stress experience in organic ions leakage as a result of membrane and DNA damage. In this study, higher values of electrolyte leakage observed in 20 DWS plants compared with 10 DWS could be due to higher magnitude of disruption to membrane stability and DNA damage in 20 DWS plants. Interestingly, SA-treatment reduced electrolyte leakage to water sufficient control level. Previously, pre-stress SA treatment of two genotypes of barley reduced membrane damage as measured by electrical conductivity[32]. Synthesis of proline is governed by three enzymes: '-pyrroline-5-carboxylate synthetase, '-pyrroline-5-carboxylate reductase and proline dehydrogenase[35]. While activity of '-pyrroline-5-carboxylate reductase increases in cowpea under water stress,

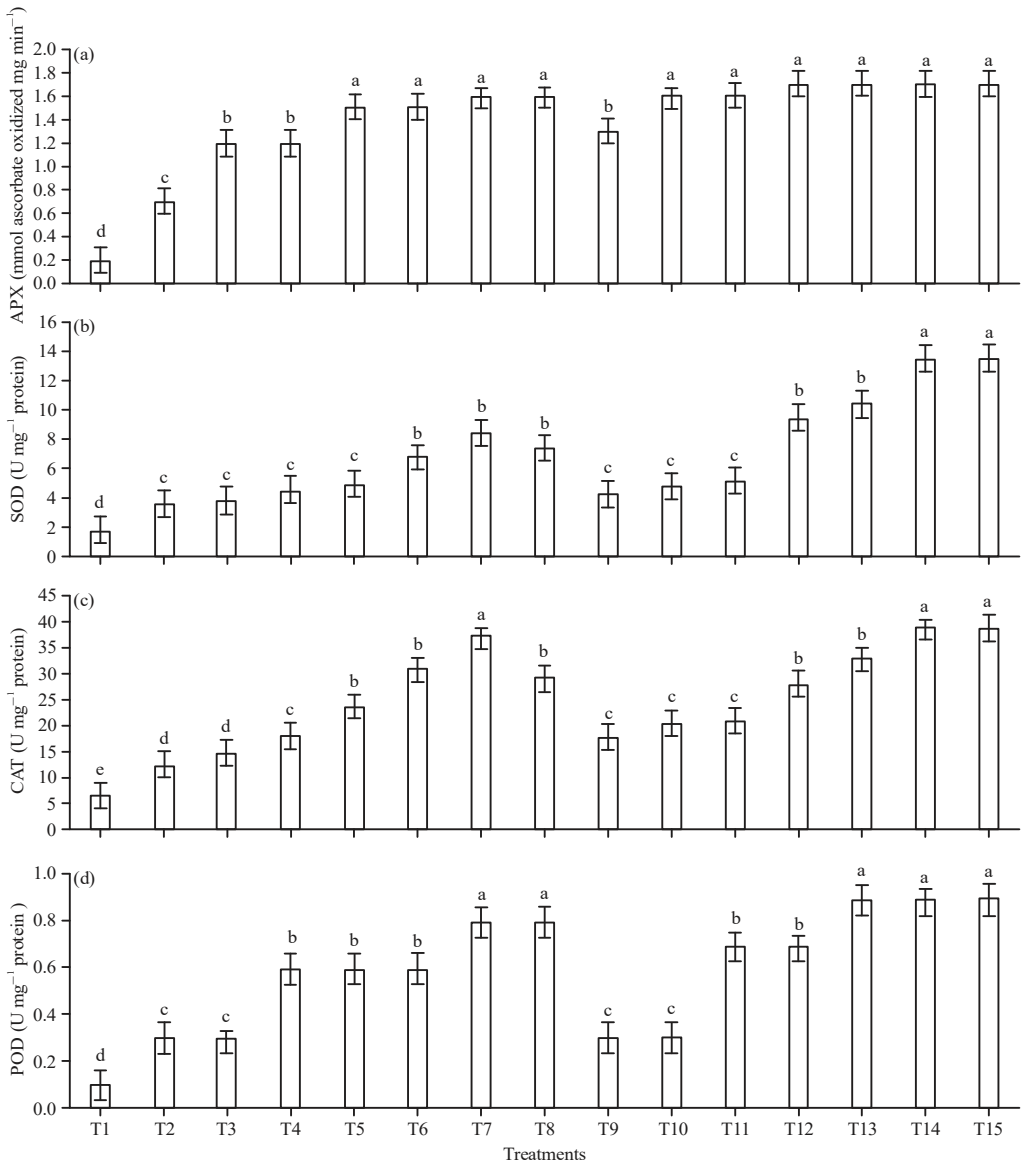


Fig. 2(a-d): Influence of exogenous SA activity of (a) Ascorbate peroxidase, (b) Superoxide dismutase, (c) Catalase and (d) Peroxidase of *Solanum macrocarpon* plants under water stress

T1: Water sufficient, T2: Ten days of water stress (10 DWS), T3: 10 DWS+0.5 mM SA, T4: 10 DWS+1.0 mM SA, T5: 10 DWS+1.5 mM SA, T6: 10 DWS+2.0 mM SA, T7: 10 DWS+2.5 mM SA, T8: 10 DWS+3.0 mM SA, T9: 20 DWS, T10: 20 DWS+0.5 mM SA, T11: 20 DWS+1.0 mM SA, T12: 20 DWS+1.5 mM SA, T13: 20 DWS+2.0 mM SA, T14: 20 DWS+2.5 mM SA and T15: 20 DWS+3.0 mM SA

proline dehydrogenase activity decreases[36]. In this study, proline content of untreated water-stressed plants and SA-treated plants was higher than proline content of water sufficient plants in both 10 and 20 DWS plants. This result suggested that water-stressed and exogenous SA alone and in combination enhanced proline accumulation. It may be possible that water stress and foliar application stimulated metabolism of proline synthesis. Furthermore, higher oxidative stress generated by water stress in 20 DWS plants compared with 10 DWS plants could have responsible for higher quantity of proline accumulated in 20 DWS plants compared with 10 DWS plants. Previous report by Hayat *et al.*[14] found an upsurge in proline content in water-stress and SA-treated tomato plants. However, Bandurska and Stroinski[32] reported that external application of plants with higher concentrations (60 and 120 mM) of SA did not alter proline content of *Hordeum vulgare* but lead to an increase in proline content of *H. spontaneum*.

In this study, water stress low and medium doses of exogenous SA boosted phenolics content of plants, suggesting that SA and water stress and their interaction promotes phenolics synthesis. Results of this work also suggested that the quantity of phenolics produced by water stressed plants depends on magnitude of stress as phenolics content of 20 DWS plants was higher than phenolics content of 10 DWS plants. Plants are protected against destructive impacts of a number of biotic and abiotic stresses by phenolic compounds[36-38].

Disruption of protein synthesis or denaturation by oxidative stress could cause low quantity of soluble proteins in untreated water-stressed plants. High contents of chlorophylls, carotenoids, endogenous SA, proline, phenolic, membrane stability and synthesis of defensive enzymes and other protein based compounds could have caused high soluble protein observed in SA-treated plants. Proteins and enzymes associated with plant defense system have been observed to increase in biosynthesis in plants in response to stress duration[39]. In this report, elevation of POD, CAT and SOD activities of SA-treated plants compared with untreated and water sufficient plants in both 10 and 20 DWS plants suggested that the purpose of increased activities of the enzymes could be for mitigation of oxidative harm caused by water deficit. Previously, SA application enhanced SOD, POD and CAT activities in tomato and wheat seedlings under water stress[14,26].

## CONCLUSION

Water stress for 10 and 20 days at 25% water holding capacity reduced leaf number, height of shoot, area of leaves, dry weight, photosynthetic pigments, leaf RWC and soluble protein. However, water stress for the same duration and intensity increased proline, phenolics, endogenous SA, leakage of electrolyte and SOD, CAT, POD and APX activities. Pre-stress foliar application of SA increased number of leaves, shoot height, leaf area, dry weight, photosynthetic pigments, leaf RWC and soluble protein comparable with water sufficient plants at 2.0 mM concentration. Furthermore, SA application increased proline, phenolics, endogenous SA, leakage of electrolyte and SOD, CAT, POD and APX activities better than untreated water-stressed plants. The study concluded that pre-stress foliar application protects water-stressed *S. macrocarpon* against oxidative damage.

## SIGNIFICANCE STATEMENT

The study discovered that external application of 2.0 mM of salicylic acid restored growth and increased activity of anti-oxidant enzymes such as SOD, CAT, POD and APX that can be beneficial for alleviating drought stress of the African eggplant. This study will help researchers to uncover the critical areas of drought mitigation in vegetable production that many researchers were not able to explore. Thus a new theory on drought mitigation in vegetable production may be arrived at.

## ACKNOWLEDGMENT

The authors are grateful to Professor Oyedele for technical assistance.

## REFERENCES

1. Schippers, R.R., 2000. African Indigenous Vegetables: An Overview of the Cultivated Species. Natural Resources Institute (Great Britain), Chatham, UK., ISBN-13: 9780859545150, Pages: 214.
2. IPCC., 2007. Climate Change 2007-The Physical Science Basis: Working Group I Contribution to the Fourth Assessment Report of the IPCC. Cambridge University Press, Cambridge, MA., USA., ISBN-13: 9780521880091, Pages: 996.
3. Anjum, S.A., X.Y. Xie, L.C. Wang, M.F. Saleem, C. Man and W. Lei, 2011. Morphological, physiological and biochemical responses of plants to drought stress. Afr. J. Agric. Res., 6: 2026-2032.
4. Hussain, M., M.A. Malik, M. Farooq, M.Y. Ashraf and M.A. Cheema, 2008. Improving drought tolerance by exogenous application of glycinebetaine and salicylic acid in sunflower. J. Agron. Crop Sci., 194: 193-199.
5. Farooq, M., A. Wahid, N. Kobayashi, D. Fujita and S.M.A. Basra, 2009. Plant drought stress: Effects, mechanisms and management. Agron. Sustain. Dev., 29: 185-212.
6. Nayyar, H. and D. Gupta, 2006. Differential sensitivity of $C_3$ and $C_4$ plants to water deficit stress: Association with oxidative stress and antioxidants. Environ. Exp. Bot., 58: 106-113.
7. Ashraf, M. and M.R. Foolad, 2007. Roles of glycine betaine and proline in improving plant abiotic stress resistance. Environ. Exp. Bot., 59: 206-216.
8. Gill, S.S. and N. Tuteja, 2010. Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. Plant Physiol. Biochem., 48: 909-930.
9. Kranner, I. and S. Birtic, 2005. A modulating role for antioxidants in desiccation tolerance. Integr. Comp. Biol., 45: 734-740.
10. Fang, Y. and L. Xiong, 2015. General mechanisms of drought response and their application in drought resistance improvement in plants. Cell. Mol. Life Sci., 72: 673-689.
11. Khan, W., B. Prithivirag and D.L. Smith, 2003. Photosynthetic responses of corn and soybean to foliar application of salicylates. J. Plant Physiol., 160: 485-492.

12. Hayat, S. and A. Ahmad, 2007. Salicylic Acid: A Plant Hormone. Springer, Netherlands, ISBN-13: 9781402051838, Pages: 401.
13. Fariduddin, Q., S. Hayat and A. Ahmad, 2003. Salicylic acid influences net photosynthetic rate, carboxylation efficiency, nitrate reductase activity and seed yield in *Brassica juncea*. Photosynthetica, 41: 281-284.
14. Hayat, S., S.A. Hasan, Q. Farriduddun and A. Ahmad, 2008. Growth of tomato (*Lycopersicon esculentum*) in response to salicylic acid under water stress. J. Plant Interact., 3: 297-304.
15. Yusuf, M., S.A. Hasan, B. Ali, S. Hayat, Q. Fariduddin and A. Ahmad, 2008. Effect of salicylic acid on salinity-induced changes in *Brassica juncea*. J. Integr. Plant Biol., 50: 1-4.
16. War, A.R., M.G. Paulraj, M.Y. War and S. Ignacimuthu, 2011. Role of salicylic acid in induction of plant defense system in chickpea (*Cicer arietinum* L.). Plant Signal. Behav., 6: 1787-1792.
17. Oboh, G., M.M. Ekperigin and M.I. Kazeem, 2005. Nutritional and haemolytic properties of eggplants (*Solanum macrocarpon*) leaves. J. Food Compos. Anal., 18: 153-160.
18. Halinski, L.P., M. Paszkiewicz, M. Golebiowski and P. Stepnowski, 2012. The chemical composition of cuticular waxes from leaves of the gboma eggplant (*Solanum macrocarpon* L.). J. Food Comp. Anal., 25: 74-78.
19. Adebooye, O.C. and J.T. Opabode, 2004. Status of conservation of the indigenous leaf vegetables and fruits of Africa. Afr. J. Biotechnol., 3: 700-705.
20. Okewale, M.O., 2018. Response of *Solanum macrocarpon* to water stress. B.Sc. Thesis, ObafemiAwolowo University, Ile-Ife, Nigeria.
21. Sullivan, C.Y. and W.M. Ross, 1979. Selecting the Drought and Heat Resistance in Grain Sorghum. In: Stress Physiology in Crop Plants, Mussel, H. and R.C. Staples (Eds.). John Wiley & Sons, New York, pp: 263-281.
22. Lichtenthaler, H.K. and A.R. Wellburn, 1985. Determinations of total carotenoids and chlorophylls a and b of leaf extracts in different solvents. Biol. Soc. Trans., 11: 591-592.
23. Bates, L.S., R.P. Waldren and I.D. Teare, 1973. Rapid determination of free proline for water-stress studies. Plant Soil, 39: 205-207.
24. Julkunen-Tiitto, R., 1985. Phenolic constituents in the leaves of Northern Willows: Methods for the analysis of certain phenolics. J. Agric. Food Chem., 33: 213-217.
25. Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem., 72: 248-254.
26. Guzman-Tellez, E., D.D. Montenegro and A. Benavides-Mendoza, 2014. Concentration of salicylic acid in tomato leaves after foliar aspersions of this compound. Am. J. Plant Sci., 5: 2048-2056.
27. Nakano, Y. and K. Asada, 1981. Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in Spinach chloroplasts. Plant Cell Physiol., 22: 867-880.
28. Dhindsa, R.S., P. Plumb-Dhindsa and T.A. Thorpe, 1981. Leaf senescence: Correlated with increased levels of membrane permeability and lipid peroxidation and decreased levels of superoxide dismutase and catalase. J. Exp. Bot., 32: 93-101.
29. Aebi, H., 1984. Catalase *in vitro*. Meth. Enzymol., 105: 121-126.
30. Hemeda, H.M. and B.P. Klein, 1990. Effects of naturally occurring antioxidants on peroxidase activity of vegetable extracts. J. Food Sci., 55: 184-185.
31. Singh, B. and K. Usha, 2003. Salicylic acid induced physiological and biochemical changes in wheat seedlings under water stress. Plant Growth Regul., 39: 137-141.
32. Bandurska, H. and A. Stroinski, 2005. The effect of salicylic acid on barley response to water deficit. Acta Physiol. Planta., 27: 379-386.
33. Khandaker, L., A.S.M. Akond and S. Oba, 2011. Foliar application of salicylic acid improved the growth, yield and leaf's bioactive compounds in red amaranth (*Amaranthus tricolor* L.). Veg. Crops Res. Bull., 74: 77-86.
34. Ogawa, T., T. Ara, K. Aoki, H. Suzuki and D. Shibata, 2010. Transient Increase in salicylic acid and its glucose conjugates after wounding in *Arabidopsis* leaves. Plant Biotechnol., 27: 205-209.
35. Hong, Z., K. Kakkenent, Z. Zhang and D.P. Verma, 2000. Removal of feedback inhibition of $\Delta^1$-pyrroline-5-carboxylate synthetase results in increased proline accumulation and protection of plants from osmotic stress. Plant Physiol., 122: 1129-1136.
36. Sumithra, K. and A.R. Reddy, 2004. Changes in proline metabolism of cowpea seedlings under water deficit. J. Plant Biol., 31: 201-204.
37. Rani, P.U. and Y. Jyothsna, 2010. Biochemical and enzymatic changes in rice plants as a mechanism of defense. Acta Physiol. Planta., 32: 695-701.
38. Kawano, T., 2003. Roles of the reactive oxygen species-generating peroxidase reactions in plant defense and growth induction. Plant Cell Rep., 21: 829-837.
39. Rivas-San Vicente, M. and J. Plasencia, 2011. Salicylic acid beyond defence: Its role in plant growth and development. J. Exp. Bot., 62: 3321-3338.