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Salicylic Acid Protects Nitrate Reductase Activity, Growth and Proline in Amaranth and Tomato Plants during Water Deficit

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Abstract: Problem statement: Seedlings of *Amaranthus hybridus* cv. NHAC-3 (large green, amaranth) and *Lycopersicum esculentum* cv. Roma (tomato) were subjected to 7 days water stress at Early Vegetative (EV), Late Vegetative (LV), Early Flowering (EF) and Late Flowering (LF) stages of growth to study the impact on leaf water potential (ψ_w), Nitrate Reductase Activity (NRA), growth (plant height, shoot and root biomass) and proline content of both plants. **Approach:** Two concentrations of salicylic acid (1 and 3 mM SA) were applied to stressed plants to study the level of protection given by SA to the plants. Leaf ψ_w was significantly reduced ($p = 0.05$) during stress treatment at nearly all stages of growth in both plants. Leaf ψ_w was in the range -0.25 to -1.42 (unstressed) and -1.45 to -2.02 (stressed) in tomato plants while in amaranth it was -0.7 to -1.62 (unstressed) and -1.62 to -2.68 (stressed). As 3 mM SA increased leaf ψ_w to values close to the control (unstressed plants). NRA was significantly ($p = 0.05$) reduced by stress treatment at the LV stage of amaranth, EF stage of tomato and LF stage of both plants. **Results:** Thus, the reduction of NRA was more pronounced at the reproductive stage of both plants. As 3 mM SA was effective in maintaining NRA at levels similar to the control in both plants. Stress treatment reduced plant height significantly ($p = 0.05$) at the vegetative stages of both plants and 3 mM was also effective in keeping plant height similar to the control. Though shoot biomass was affected by water stress, SA treatment was not very effective in preserving the biomass during stress. Root biomass of plants was reduced by stress treatment at the reproductive stage and only tomato plants responded positively to 3 mM SA. Proline content was only slightly increased at all stages of growth in stressed plants but 3 mM SA induced a two-fold increase in proline content at the vegetative stage of tomato (EV and LV) and significant increases ($p = 0.05$) at almost all stages of growth of amaranth. **Conclusion/Recommendations:** The build up of proline, an osmolyte, by SA in stressed plants increased the capacity of plants to absorb water from the soil as shown by the increase in leaf ψ_w of both plants from -1.45 to -0.25 . SA was more effective in protecting the plants against the adverse effects of water stress when the stress was given at the vegetative stages (EV and LV) than at the flowering stages (EF and LF).

Key words: Amaranth, tomato, water potential, growth, salicylic acid, proline

INTRODUCTION

Water deficit is one of the most limiting factors for plant survival since it regulates growth and development and limits plant productivity. The effect of water deficit varies with the variety, degree and duration of stress and the growth stage of the plant[1-3]. Water deficits cause much lower water potential in soybean during the reproductive stage accompanied by increase in leaf stomatal resistance than the vegetative stage[3]. The resulting effect is a reduction in carbon assimilation and subsequent biomass production. In several plants, growth and yield are slightly affected at the vegetative stage but drastically reduced at the reproductive stage[1,4].

Plants adapt to water deficits by changes in morphology, altered patterns of development and cellular metabolism. A number of these adaptive responses are associated with the accumulation of osmolytes like sugars and proline[5,6]. Salicylic Acid (SA) induces abscisic acid mediated protective reactions of plants to water deficit mainly by increasing proline accumulation[7]. SA protects nitrate reductase activity and maintains protein and nitrogen content and increases the chlorophyll, photosynthetic rate and rubisco activity of wheat plants subjected to water deficit[8].

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Salicylic Acid (SA) is a growth regulator which participates in the regulation of physiological processes in plants. It stimulates flowering in a range of plants, increases flower life, controls ion uptake by roots and stomatal conductivity[8,9]. It acts as an endogenous signal molecule responsible for inducing abiotic stress tolerance in plants[10]. It decreases the inhibitory effect of water stress in wheat seedlings, low and high temperatures in tomato and bean, chilling injury in maize plants and alleviates heavy metal toxicity in Cassia[8,11,12].

This study compares the protective impact of SA on nitrate reductase activity and growth of amaranth and tomato plants subjected to water deficit at different stages of growth. Furthermore, it investigates osmotic adjustments by proline production in amaranth and tomato plants during the stress periods.

MATERIALS AND METHODS

Collection of plant material: Seeds of *Amaranthus hybridus* cv. SPPVH2 and *Lycopersicum esculentus* cv. Roma were collected from the Institute of Agricultural Research and Training (IAR and T) of Obafemi Awolowo University, Ibadan, Oyo State.

Planting procedure: Seeds of amaranth and tomato were planted in nursery beds at the Botanic Garden, University of Lagos and then transplanted into 64 planting pots each, filled with 2 kg of garden topsoil mixed with 2 g of NPK fertilizer.

For each plant, 4 pots per treatment were used with a total of 16 treatments: Unstressed plants (control), water deficit only, water deficit + 1 mM Salicylic Acid (SA), water deficit + 3 mM SA at early vegetative, late vegetative, early flowering and late flowering stages of development of both plants.

Plants were subjected to water stress for seven days at each stage of development. 5 mL SA was given as foliar spray on the first day of each period of stress to each plant at different growth stages (63, 84, 105 and 126 days after planting).

Measurement of leaf water potential: At the last day of each stress period the leaf water potential (ψ_w) was determined using the tissue weight change method[13]. 0.5 g leaves were placed in sucrose solutions of 0.1-1 molal concentration, for an hour. Thereafter, the tissues were removed and blotted to remove excess solution and reweighed. The percentage weight gain or loss was plotted against the solute potential of the sucrose solution (1 molal sucrose had a solute potential of -2.69 MPa at 25°C). Leaf ψ_w is estimated as equivalent to the smotic potential of the solution in which there is no gain or loss in weight. Osmotic potential (ψ_s) of each sucrose solution was calculated using the van't Hoff equation:

$$\psi_s = - C\gamma RT$$

C = The molal concentration
γ = The activity coefficient (a value of 1 for neutral solutes such as sucrose in dilute solution)
R = The gas constant (0.00831 kg MPa moL^{-1} $°K^{-1}$)
T = The absolute temperature (°K = °C + 273)

The change in weight was calculated as a percentage of the original weight and plotted against ψ_s of sucrose solutions. The leaf water potential (ψ_w) is estimated as equivalent to the ψ_s of the solution in which there is no change in weight (the intercept on the abscissa).

Determination of nitrate reductase activity: Nitrate reductase activity (NRA) of leaves was determined[14]. Five ml incubation medium comprising 100 mL 0.1 M phosphate buffer (PH 7.5), 1.5 g potassium nitrate and 1 mL 4% propan-1-ol, was used to incubate 0.4 g finely cut leaves from each replicate, for an hr at a room temperature of 30°C. Then the reaction was stopped by adding 1 mL 1% sulphanilic acid in 2 N HCl, followed by 1 mL 0.02% Naphthylethylenediamine dichloride for 20 min for color development. The absorbance was measured at 540 nm wavelength using a spectrophotometer (Corning 258 model). The concentration of nitrite in the reaction medium was determined by reference to a standard curve prepared using 0-0.8 moles sodium nitrite. NRA is proportional to the concentration of nitrite in the reaction medium[15].

Determination of proline content of leaves: 0.5 g of dried powdered leaves was homogenized in 10 mL 3% aqueous sulfosalicylic acid and the homogenate filtered. 2 mL acid ninhydrin (prepared by warming 1.2 g of ninhydrin in 30 mL glacial acetic acid) was added to 2 mL filtrate in a digestion tube and placed in a boiling water bath for 90 min. The reaction was terminated in an ice bath. 4 mL toluene was added to the reaction mixture and agitated vigorously for 30 min.

The chromophore containing toluene was aspirated from the aqueous phase and the absorbance read at 520 nm using Corning 258 spectrophotometer. Toluene was used as a blank. The concentration was determined by reference to a standard curve prepared using 0-1 mg L^{-1} proline (sigma) and calculated on a dry weight basis[16].

Growth analysis: Plants were harvested on the last day of stress (70, 91, 112, 133 days after sowing) at different growth stages for measurements of height, shoot and root biomass. Biomass was weighed after drying plants in an oven at 80°C for 3 days.

Statistical analysis: Tests of significance between treatments were done using analysis of variance (ANOVA) and Duncan's multiple range tests.

RESULTS

Tomato and amaranth plants subjected to 7 days water stress at different growth stages: early vegetative (EV), Late Vegetative (LV), Early Flowering (EF) and Late Flowering (LF), showed significant decreases ($p = 0.05$) in leaf water potential (ψ_w) during almost all the stress treatments (Table 1).

Salicylic Acid (SA) treatment increased ψ_w in almost all stressed plants to values close to the control and the higher concentration (3 M SA) was more effective.

There was continuous increase in Nitrate Reductase Activity (NRA) of both stressed and unstressed tomato plants from the EV-LF stages (Fig. 1). This was almost the same with amaranth but for the marked drop in activity at the EF stage. Water deficit reduced NRA significantly ($p = 0.05$) at the LV stage of amaranth, EF stage of tomato and LF stage of both plants. Thus, the reduction of NRA was more pronounced at the reproductive stage of both plants. As 3 mM SA was more effective than 1 M SA in enhancing NRA to values close to those of unstressed plants (control).

Stress treatment reduced plant height significantly ($p = 0.05$) at the vegetative stages of both plants and 3 mM was effective in keeping plant height similar to the control (Fig. 2). Shoot biomass (Fig. 3) was significantly reduced ($p = 0.05$) by water stress especially at the reproductive stages of both plants.

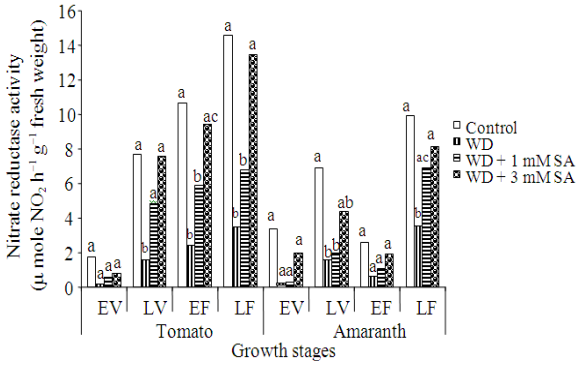

Fig. 1: Effect of Salicylic Acid (SA) on the on Nitrate Reductase Activity (NRA) of tomato and amaranth plants subjected to Water Deficit (WD) at different stages of growth: Early Vegetative (EV), Late Vegetative (LV), Early Flowering (EF) and late flowering.(Bars with same letters at each stage of growth are not significantly different at $p = 0.05$ using the Duncan's multiple range tests)

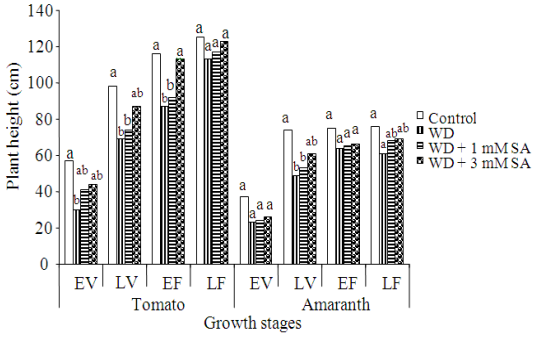

Fig. 2: Effect of SA on height of tomato and amaranth plants subjected to water deficit at different stages of growth. Bars with same letters at each stage of growth are not significantly different at $p = 0.05$ using the Duncan's multiple range tests

Table 1: Effect of Salicylic Acid (SA) on the leaf water potential (ψw) of tomato and amaranth plants subjected to Water Deficit (WD) at Early Vegetative (EV), Late Vegetative (LV), Early Flowering (EF) and Late Flowering (LF) stages

	Ψw (M Pa) at different growth stages							
	Tomato				Amaranth			
Treatments	EV	LV	EF	LF	EV	LV	EF	LF
Control	-0.25^{a}	-0.35^{a}	-0.95^{a}	-1.42^{a}	-0.70^{a}	-0.90^{a}	-1-80^{a}	-1.62^{a}
WD	-1.45^{b}	-1.70^{b}	-2.04^{b}	-2.02^{a}	-1.62^{a}	-2.30^{b}	-2.24^{b}	-2.68^{b}
WD +1 M SA	-0.95^{ab}	-1.25^{ab}	-1.87^{b}	-2.02^{a}	-1.33^{a}	-1.62^{ab}	-2.00^{b}	-2.48^{b}
WD +3 M SA	-0.25^{a}	-0.81^{ab}	-1.57^{ab}	-1.78^{a}	-1.11^{a}	-1.35^{a}	-1.75^{ab}	-2.18^{ab}

Superscripts with similar letters on the same column for each plant are not significantly different at $p = 0.05$, Using the new Duncan's multiple range tests

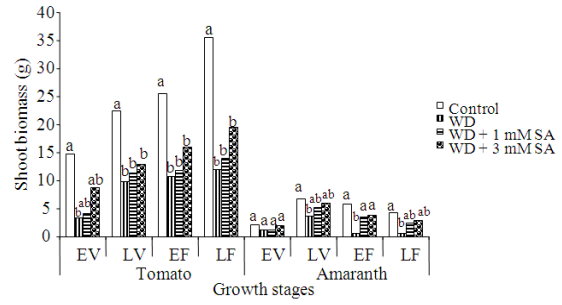

Fig. 3: Effect of SA on shoot biomass of tomato and amaranth plants subjected to water deficit at different stages of growth. Bars with same letters at each stage of growth are not significantly different at p = 0.05 using the Duncan's multiple range tests

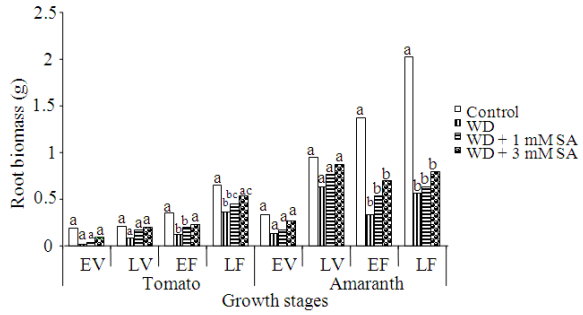

Fig. 4: Effect of SA on root biomass of tomato and amaranth plants subjected to water deficit at different stages of growth. Bars with same letters at each stage of growth are not significantly different at p = 0.05 using the Duncan's multiple range tests

Both concentrations of SA did not improve plant biomass significantly during stress. Root biomass of plants was reduced by stress treatment at the reproductive stage of both plants and the effect was more pronounced in amaranth than in tomato plants. However, only tomato plants responded positively to 3 mM SA (Fig. 4).Generally growth at the flowering stages (EF and LF) was more sensitive to water deficit than the vegetative stages (EV and LV). However, SA was more effective in protecting the plants against the adverse effects of water stress when the stress was given at the vegetative stages (EV and LV) than at the flowering stages (EF and LF).

Proline production was slightly enhanced by water stress in all plants (Fig. 5). At all stages of growth, proline content increased in this order: Deficit with 3 M SA> deficit with 1 M SA> deficit only > control.

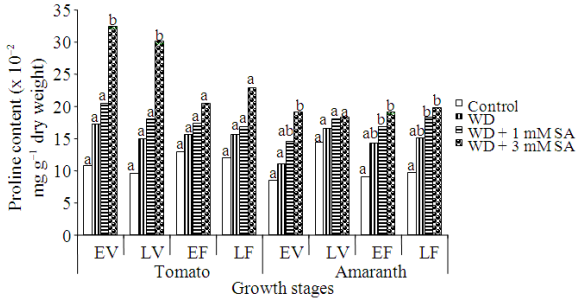

Fig. 5: Effect of SA on the proline content of tomato and amaranth plants subjected to water deficit at different stages of growth. Bars with same letters at each stage of growth are not significantly different at p = 0.05 using the Duncan's multiple range tests

The increase in proline content was significant (p = 0.05) with the application of SA at the EV and LV stages of tomato plants and the EV, EF and LF stages of amaranth plants and 3 mM SA induced a two-fold increase in proline content at the vegetative stage of tomato.

DISCUSSION

Severe water deficits cause significant decreases in ψ_w at all stages of growth of *Glycine max* and there is a strong positive correlation with the decrease in relative water content[3]. Similarly, tomato and amaranth plants subjected to water stress at different stages of growth show similar reductions in water potential which is improved when stressed plants are given salicylic acid treatment. This corroborates the report[17] that SA treated plants conserve more water than plants without SA under drought conditions.

Water stress inhibited Nitrate Reductase Activity (NRA) in both stressed tomato and amaranth plants since NRA is a very labile enzyme[18]. Since water content of plants is higher at the vegetative stage than the flowering stage, a further decrease in water content as a result of deficit treatment reduces NRA markedly at the flowering stage of plants, as observed in tomato and amaranth. Furthermore, reduced stomatal aperture as a result of water stress lowers the carbon gain of the plant and reduces CO_2 assimilation[3]. An efficient N assimilation is said to be favored by a high rate of CO_2 assimilation[19]. SA induced conservation of water in stressed plants also resulted in the protection of NRA in SA treated stressed plants.

The reduction in growth of many plants subjected to water stress at different growth stages has been shown to be more pronounced at the reproductive stage[2,3,6]. Since SA increased water potential of stressed plants, it is

expected that SA will reduce the damaging action of water deficit on growth. SA has been shown to reduce the damaging effect of water stress on seedling growth and accelerate a restoration of growth processes in wheat, bean and tomato[8,12,20]. SA was found to be more effective in protecting amaranth and tomato plants against the adverse effects of water stress when the stress is given at the vegetative stage than at the flowering stage.

Reactive Oxygen Species (ROS) which include oxygen ions, free radicals and peroxides, form as a natural by product of the normal metabolism of oxygen and have important roles in cell signaling. However, during environmental stress such as drought, ROS levels increase dramatically resulting in oxidative damage to proteins, DNA and lipids[21]. The SA induced increase in plant height, shoot biomass and root biomass observed in amaranth and tomato seedlings, is related to the ability of SA to induce antioxidant responses that protect them from damage[12].

Accumulation of proline in plants is a mechanism by which plants resist water stress and develop antistress ability. Plants that produce higher levels of proline are capable of surviving drought[22]. The protective action of SA during water deficit was demonstrated by the enhanced proline production in stressed tomato and amaranth plants. The accumulation of osmolytes allows additional water to be taken up from the environment, thus reducing the immediate effect of water shortage within the plant and they help to stabilize protein tertiary structure and cells[23].

Water potential was significantly reduced by 7 days water stress at the vegetative and flowering stages of amaranth and tomato plants. Generally, water deficit caused a decrease in nitrate reductase activity, plant height, shoot and root biomass of both plants. Salicylic acid induced high proline content, an osmolyte that helps the plant to adapt to drought. The protective effect of SA was shown by the increase in water potential under water stress and enhanced nitrate reductase activity and growth of stressed amaranth and tomato plants. 3 M SA was more effective than 1 M SA in protecting both plants against the damaging effects of drought and the vegetative stage (EV and LV) was more receptive than the flowering stage. Both plants showed almost similar response to water deficit and SA treatments. Further studies will include the effect of higher concentrations of SA on plant growth during drought.

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