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RESEARCH ARTICLE

Pre-treatment of two contrasting water-stressed genotypes of cassava (*Manihot esculenta* Crantz) with ascorbic acid. I. Growth, physiological and antioxidant responses

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Abstract Water deficit-stress at early growth stage is a major constraint of cassava production. Ascorbic acid is a non-enzymatic antioxidant that mitigates oxidative damage caused by water stress in plants. Growth, physiological and antioxidant defense system responses of two contrasting water-stressed cassava genotypes to pre-treatment with foliar application of ascorbic acid (AA) were investigated. The objectives of this study were to assess the growth, proline, photosynthesis pigments and antioxidant activities of young water-stressed cassava plants pre-treated with ascorbic acid. The study consisted of IITA-TMS-IBA980581 (drought tolerant) and IITA-TMS-IBA010040 (drought sensitive) cassava genotypes treated with six doses (0.00, 0.25, 0.50, 0.75 and 1.00 mM) of AA before being subjected to water deficit (45.0% field capacity) and a water sufficient AA-untreated control. In both genotypes, water stress reduced shoot height (40.3%), leaf area (42.5%), and number of root (54.5%), biomass (28.6%), relative water content (RWC, 3.2%) and photosynthetic pigments (300.0%). However, water stress increased proline (91.3%), endogenous AA (112.0%), catalase (CAT, 300.0%) and superoxide dismutase (SOD, 15.3%) in both genotypes. Compared with IITA-TMS-IBA010040, leaf area, biomass, number of root and shoot height of IITA-TMS-IBA980581 were higher by 7.3, 24.6, 25.9 and 13.1%, respectively. By less than a quarter, chlorophylls a and b, activity of superoxide dismutase and relative water content of IITA-TMS-IBA980581 were higher compared with IITA-TMS-IBA010040. However, proline content of IITA-TMS-IBA010040 was higher than IITA-TMS-IBA980581 by 14.3%. Pre-treatment with AA improved growth parameters, photosynthetic pigments, RWC, endogenous AA, activity of CAT and SOD, but decreased proline in both genotypes with an optimum concentration at 0.5 mM. Pre-treatment with 0.5 mM AA increased shoot height, area of leaves, leaf number, number of root and dry weight by 46.3, 44.7, 14.4, 88.2 and 37.5%, respectively. Pre-treatment with 0.5 mM AA doubled chlorophylls, tripled carotenoids content, doubled endogenous AA and slightly enhanced RWC (2.1%) and SOD (2.0%) when compared with AA-untreated water stressed plants. But pre-stress application of AA reduced proline content by one-fold, increased CAT activity by one-fold in IITA-TMS-IBA980581 and by one-third in IITA-TMS-IBA010040. The study concluded that pre-treatment of cassava young plants with AA before water deficit could alleviate oxidative stress.

Keywords Antioxidant activities · Drought stress · Exogenous ascorbic acid · Root crop · Photosynthetic pigments

Abbreviations

AA	Ascorbic acid
ABA	Abscisic acid
CAT	Catalase
EDTA	Ethylene diamine tetraacetic acid
ROS	Reactive oxygen species
RWC	Relative water content
SOD	Superoxide dismutase

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Introduction

Next to rice and corn, cassava is the most important source of energy-giving food in the developing countries (Ospina and Ceballos 2012). Currently, 95% of cassava production in developing countries takes place under rain-fed production systems, where precipitation is the only source of moisture for the crop growth and development (Ray et al. 2015; Turyagyenda et al. 2013). However, drought is a limiting factor in cassava production because of climate change (Ray et al. 2015; Turyagyenda et al. 2013). Rainfalls are erratic, coupled with a drastic reduction in amount of rainfall per year. In addition, dry season period in tropical climate is becoming longer than previously experienced (IPCC 2007; Ray et al. 2015). Although, cassava is a root crop that can withstand 4–6 months of drought, the effect of drought during its establishment stage (i.e. first four months of growth) is very critical as drought imposes restriction on a plant's full genetic potential (Santisopasri et al. 2001; Vandegeer et al. 2012; El-Sharkawy 2012; Pereira et al. 2018). For example, water deficit at establishment stage reduces leaf and tuber yield of cassava by 45% and 83% respectively (Vandegeer et al. 2012). Similarly, cassava's starch yield was reduced by 97.8% when young cassava plants are exposed to drought stress (Santisopasri et al. 2001). Furthermore, the cyanide content of cassava plants that experienced water deficit at establishment and post-bulking stages were found to increase by 2.9-fold in immature leaves and four-fold in storage tubers. In addition, cassava has low nitrogen-use efficiency due to an increase in nitrogen concentration in root biomass compared to shoot biomass when exposed to drought stress at young stage (Vandegeer et al. 2012). Exposing cassava young plants to water stress predisposes them to weed competition, pathogen attacks and rapid post-harvest deterioration of tubers. Apart from the first four months of growth, interruption of cassava growth cycle at any stage by more than three months of drought negatively affected physiological processes, resulting in decreased growth, development and economic yield loss (Pardales et al. 2001; Bakayoko et al. 2009). Thus, there is an urgent need to protect cassava plants from drought stress.

Cassava employs stress avoidance mechanisms through alteration of several physiological changes to cope with drought stress at all phenological stages (El-Sharkawy 2012). For example, drought stress promotes nutrient-use efficiency in terms of storage roots development so that there is no difference in the root yields of stressed and unstressed cassava plants (El-Sharkawy 2012). Cassava growth, transpiration and ABA content reduced under water stress (Duque and Setter 2013). However, the crop maintains carbohydrate reserves in leaf blades, petioles and stems to support vital organs and processes during prolonged water deficit at reduced rate (Duque and Setter 2013). In cassava, under water stress, stomata are closed, rooting extended, water use conserved, photosynthetic capacity and proteins reduced but photosynthetic process proceeds at low rates to avoid disruption of metabolism (El-Sharkawy 2012; Zhao et al. 2015; Pereira et al. 2018). In some cultivars, older leaves experience senescence under water stress but growth continues at reduced rate (Zhao et al. 2015). Pereira et al. (2018) observed an increase in total chlorophyll and carotenoids content after 45 days of water deficit but a decrease was observed in chlorophyll a content after 90 days of water deficit at − 70.0 kPa soil tension. At development stage, under a severe water deficit (− 70 kPa), cassava maintains leaf water potential, relative water content and membrane integrity (Pereira et al. 2018).

Reactive oxygen species (ROS), such as hydrogen radicals, superoxide and hydrogen peroxide are produced in excess during water stress (Gill and Tuteja 2010). Excessive ROS in plants are toxic and cause damage to biomolecules, cell organelles and DNA structure (Gill and Tuteja 2010). Plants possess a number of enzymatic and non-enzymatic antioxidants for scavenging ROS to overcome destructive oxidative reactions (Ashraf 2009). An important non-enzymatic antioxidant reported to mitigate damages caused by of ROS in plants is ascorbic acid (Mittler 2002). Under normal growth conditions, AA plays key roles in the growth, metabolisms and physiological processes of plants. For instance, AA is a cofactor for production of many phytohormones such as ethylene, gibberellins and abscisic acid. Also, AA regulates cell division and cell expansion, modulates plant senescence, photosynthesis and synthesis of antioxidants (Naz et al. 2016). In the presence of abiotic stresses, particularly water deficit, AA shields tissues from ROS, which are produced in abundance from oxidative damages (Latif et al. 2016; Naz et al. 2016). Ascorbic acid is synthesized in all plant tissues, however, concentration of AA is high in fruits, meristems and photosynthetic tissues (Mazid et al. 2011).

The concentrations of endogenous AA in most plant species are not sufficient to repair the damaging effects of abiotic stresses efficiently (Shafiq et al. 2014; Latif et al. 2016). Consequently, under abiotic stress conditions particularly water deficit, external application of AA is being used to supplement internal synthesis of AA to neutralize oxidative stress with promising results. For instance, presowing treatment of seeds with 100 and 200 mg/L AA markedly improved germination and seedling growth of sunflower under drought stress (Ahmed et al. 2014). Also, foliar application of 200 mg/L AA on wheat subjected to drought stress enhanced chlorophyll a and b, total soluble proteins, carbohydrates and carotenoids (Hussein and Alva

2014). Both pre-sowing and foliar treatment of water-stressed wheat seedlings at 50 and 150 mg/L enhanced CAT, K, Ca2+, photosynthetic pigments and endogenous AA content (Athar et al. 2008). To protect cassava young plants against water stress, we investigated responses of cassava young plants to pre-water stress application of AA. We hypothesized that exogenous AA could induce morphological and physiological changes for amelioration of oxidative stress in cassava.

The objectives of the study were to (a) determine the effect of foliar application of ascorbic acid on early growth of water-stressed cassava plants; (b) examine the influence of exogenous ascorbic acid on proline and photosynthetic pigment synthesis of the plants; and (c) assess the antioxidant activities of water-stressed cassava plants as influenced by exogenous ascorbic acid.

Materials and methods

Planting materials and growth conditions

Stem-cuttings of cassava genotypes IITA-TMS-IBA010040 (drought sensitive) and IITA-TMS-IBA980581 (drought tolerant) were obtained from the International Institute of Tropical Agriculture (IITA), Ibadan. Cassava plants were raised from the stem-cuttings under greenhouse conditions of the Faculty of Agriculture, Obafemi Awolowo University, Ile-Ife, Nigeria. A stem- cutting (10 cm long), with more than two nodes, was planted per plastic bag containing 8 kg sterilized sandy loam soil with pH of 7.2 and cation exchange capacity of 15.3 cmol kg^{-1}. Daily, each plant was irrigated manually with 600 mL to water holding capacity by tap water, pH 6.8. Plants were grown at an average temperature of 26 ± 2 °C under 65 ± 5% relative humidity and 7–9 h of daylight.

Treatments and experimental design

Forty-five days after planting, young plants of each genotype were divided into two sets. Ascorbic acid (AA) solution was sprayed on the leaves of the first set of plants using an atomizer. The concentration of ascorbic acid applied were 0.00, 0.25, 0.50, 0.75 and 1.00 mM. A few drops of Tween-20 were added to AA solution to ensure adequate wetting of leaf surfaces. Both sides of the leaves were sprayed at sunset. Thereafter, plants were exposed to water stress by withholding water for the next forty-five days at 45% field capacity. The second set of plants were not subjected to water stress but maintained at 100% field capacity, received no AA sprays and served as water sufficient control. Thus, each cassava genotype had six treatments as follows: water sufficient control (WS), water stressed + 0.00 mM AA (ST0), water stressed + 0.25 mM AA (ST1), water stressed + 0.50 mM AA (ST2), water stressed + 0.75 mM AA (ST3), water stressed + 1.00 mM AA (ST4). The experimental design was randomized complete-block in three replications. Each treatment was applied on fifteen plants. The experiment was repeated thrice.

Measurement of growth parameters

At 45 days after water stress treatment (90 days after planting), number of leaf, shoot height, leaf area, number of root and dry weight (biomass) were determined. For dry weight, plants were carefully removed to obtain intact roots. Adhering soil particles on roots were removed by dipping them in water before drying in an oven at 80 °C to a constant weight. Leaf area was measured by a leaf area meter (LI-3000C, LI-COR Biosciences, Nebraska, USA).

Physiological measurements

Relative water content of leaf

Relative water content of leaf (RWC) was determined from five fully expanded and mature leaves. Fresh weight (FW) of the samples were recorded. To determine turgid weight (TW), the samples were put in distilled water for 12 h. The samples were removed from water, dried to a constant dry weight (DW) at 70 °C for 48 h in an oven. The RWC of each leaf was determined as follows: RWC = (FW − DW)/(TW − DW) × 100.

Electrolyte leakage

Electrolyte leakage was measured as described by Sullivan and Ross (1979). Electrical conductivity (A) of twenty leaf discs was determined after boiling in a test tube containing 10 ml of distilled water. Next, the tubes were heated in a water bath at 45 °C and 55 °C for 30 min for their electrical conductivity (B). Finally, the samples were boiled at 100 °C for 15 min and electrical conductivity (C) determined. The electrolyte leakage was deduced from this relationship: Electrolyte leakage (%) = B − A/C × 100.

Photosynthetic pigments

To extract photosynthetic pigments, one gram of fresh tissues from the 4th leaf of the shoot tip was homogenized in 80% acetone. The mixture was separated by centrifugation at 5000× g for 10 min in a Sorvall LYNX 4000 centrifuge (Thermo Fischer Scientific, Wilminton, DE, USA). The absorbance of the supernatant were recorded with a UV/VIS spectrophotometer (Model UV5, Mettler

Toledo, Colombus, OH, USA) at the following wavelengths: 645 and 662 nm for chlorophyll a and b and 470 nm for carotenoids, as described by Lichtenthaler and Wellburn (1985). Measurement were performed in triplicates. The equations used for calculations of the photosynthetic pigments were as follows: Chloropyhll a. = 11.75A662–2.350A645; Chloropyhll b. = 18.61A645–3.960A662; Carotenoids = 100 A470–2.270 chloropyhll a.- 81.4 chloropyhll b/227.

Antioxidant enzyme assays

Enzyme activities were assayed from the fourth fully expanded leaves from the shoot tip. After washing with distilled water, leaf sample (0.5 g) was ground in cold 0.1 mol/l phosphate buffer (pH 7.5) containing 0.5 mmol/l EDTA. The homogenized mixture was centrifuged at 4 °C for 15 min at 15,000× g. The supernatant was used for enzyme assay for measuring superoxide dismutase and catalase activities.

Superoxide dismutase

The method of Dhindsa et al. (1980) was followed for determination of activity of superoxide dismutase (SOD). In this study, a unit of SOD was the enzyme extract that caused photo-reduction of a half of inhibition of nitro-blue tetrazolium and SOD activity was expressed as unit/mg protein.

Catalase

Activity of catalase (CAT) was measured as described by Aebi (1984). A 3 ml-reaction mixture contained 0.1 ml enzyme extract, 50 mmol/l phosphate buffer (pH 7.0) and 30 mmol/l hydrogen peroxide. Activity of CAT was determined by recording absorbance of hydrogen peroxide at 240 nm.

Determination of ascorbic acid content

Determination of ascorbic acid was performed as described by Anwar et al. (1989). One ml of hydrochloric acid (1 N), two gram of 10-phenanthroline monohydrate, 0.16 g of iron (III) ammonium sulphate and 3.2 ml of the homogenized cassava tissue were mixed with a few ml of water. The solution was then diluted to the mark with water. Ascorbic acid standard solution was obtained by dissolving 0.25 g of ascorbic acid in 250 ml of distilled water. A fresh stock solution of the mixture was prepared each time and diluted to get 10 µg/ml solution before use. 0.2–1 ml aliquot of 10 µg/ml solution of ascorbic acid was transferred into five 10 ml calibrated flasks. 2 ml of iron (III)—phenanthroline reagent was added to each flask. After 7 min, the flask contents were diluted to the mark with water. A compensatory blank was prepared by diluting 2 ml of iron (III)-phenanthroline reagents to 10 ml with water. Absorbance of each solution was measured against the blank. A Pye Unicam SP8-400 double beam UV/V spectrometer with 10 mm glass cells was used for absorption measurements. Absorbance was read at 515 nm against the blank after 10 min, and the concentration of ascorbic acid in the sample was determined from the calibration curve.

Leaf proline content

To examine the osmotic adjustment of plants, proline content of the third fully expanded leaf from the top was determined according to Bates et al. (1973). Leaf tissues (3 g) were extracted in 2 ml of sulphosalicylic acid. The same volume of ninhydrin solution and glacial acetic acid was added. The samples were heated at 100 °C for 10 min, cooled in an ice bath and 5 ml of toluene was added. At 528 nm, absorbance by toluene was measured.

Statistical analysis

Data of each independent experiment were subjected to two-way analysis of variance using the General Linear Models procedure of Statistical Analysis Systems (SAS 2003). Means were separated using Tukey's test at 5% level of probability. Results are presented as means (± standard deviations) of the three experiments as there was no significant difference among the values of individual experiments.

Results

Growth parameters

All plants survived pre-stress foliar application of ascorbic acid (AA) and no symptom of injury was observed on plants after water stress treatment. Analysis of variance showed that interaction between stress and genotype were not significant on leaf area, shoot height, biomass, number of leaves and number of root (Table 1). However, significant ($P < 0.01$) influence of stress treatment were detected on leaf area, number of leaf, shoot height, biomass and number of root while genotype significantly ($P < 0.01$) influenced leaf area, shoot height, biomass and number of root (Table 1). The coefficient of determination (R^2) of the growth parameters ranged from 57.3 to 94.9% while coefficient of variation (CV) varied from 5.9 to 10.0. As expected, the leaf area, biomass, number of root and shoot height of drought tolerant genotype (IITA-TMS-IBA980581) were higher than that of drought-sensitive

Table 1 Mean squares, coefficient of determination and variation from the analysis of variance of growth parameters of the two cassava genotypes under water stress, treated with ascorbic acid

SV	DF	LA	B	NL	NR	SH
Rep	2	577.82	104.63	0.13	0.83	56.23
Stress (S)	5	12267.90**	1563.83**	3.45**	202.05**	1022.47**
Genotype (G)	1	3316.39**	4915.20**	1.20^{NS}	396.03**	740.03**
A × G	5	150.77^{NS}	109.20^{NS}	0.12^{NS}	9.28^{NS}	50.53^{NS}
Error	24	187.66	64.00	0.65	3.69	70.60
R^2 (%)		94.1	91.1	57.3	94.9	80.2
CV		4.9	6.8	5.9	7.8	10.0

NS not significant, *LA* leaf area, *B* Biomass, *NL* number of leaves, *NR* number of root, *SH* shoot height

**Highly significant at 0.01 level of probability

genotype (IITA-TMS-IBA010040) by 7.3, 24.6, 25.9 and 13.1%, respectively (Table 2). Compared with water sufficient (WS) plants, water stress reduced shoot height, leaf area, number of root and biomass by an average of 40.3, 42.5, 54.5 and 28.6%, respectively (Table 2). However, pre-water stress application of ascorbic acid (AA) increased all the growth parameters. As the concentration of AA increased, the values of the growth parameters increased up to 0.5 mM (ST2) before declining (Table 2). Consequently, medium (0.5 mM) dose of AA restored the values of most of the growth parameters to the level observed in WS control. Pre-treatment with 0.5 mM AA increased shoot height, leaf area, number of leaves, number of root and biomass by 46.3, 44.7, 14.4, 88.2 and 37.5%, respectively compared with untreated water-stressed plants.

Physiological parameters

Interaction between stress and genotype were not significant on proline, chlorophylls a and b and carotenoid. However, the influence of stress treatment were significant ($P < 0.01$) on proline, chlorophylls a and b. and carotenoids while significant ($P < 0.01$) influence of genotype was detected for proline, chlorophylls a and b. only (Table 3). The coefficient of determination (R^2) of proline and photosynthetic pigments ranged from 96.2 to 99.5% while coefficient of variation (CV) varied from 1.6 to 8.9 (Table 3). Interaction between stress and genotype were significant for catalase and ascorbic acid but not significant for superoxide dismutase and relative water content (Table 4). Significant ($P < 0.01$) influence of stress and genotype were detected for catalase, ascorbic acid, superoxide dismutase and relative water content (Table 4). The coefficient of determination (R^2) of catalase, ascorbic acid, superoxide dismutase and relative water content ranged from 61.8 to 99.9% while coefficient of variation (CV) varied from 0.3 to 0.9.

The chlorophylls a and b of drought tolerant genotype were higher than that of drought sensitive genotype by 12.0 and 16.7%, respectively (Table 5). However, proline content of drought sensitive was higher than drought tolerant

Table 2 Growth parameters of cassava under water stress as influenced by genotype and ascorbic acid treatment

Treatment	NL (no./plant)	LA (cm^2/plant)	B (g/plant)	NR (no./plant)	SH (cm)
Genotype					
TMS 0040	13.4 ± 3.5^a	267.7 ± 6.7^b	104.9 ± 3.8^b	4.2 ± 1.5^b	75.8 ± 3.7^b
TMS 581	13.8 ± 2.7^a	288.7 ± 4.5^a	130.5 ± 3.2^a	5.8 ± 1.4^a	85.7 ± 4.5^a
Stress					
WS	16.4 ± 2.5^a	345.5 ± 7.8^a	140.7 ± 3.3^a	6.2 ± 1.1^a	98.4 ± 3.6^a
ST0	12.5 ± 1.7^c	230.7 ± 5.1^c	100.8 ± 4.1^c	2.2 ± 0.8^c	60.3 ± 3.2^c
ST1	13.8 ± 2.3^b	300.8 ± 6.4^b	120.8 ± 3.2^b	4.0 ± 1.3^b	80.6 ± 3.7^b
ST2	14.0 ± 2.1^b	335.2 ± 4.9^a	138.6 ± 3.5^a	6.0 ± 1.5^a	94.7 ± 3.5^a
ST3	13.5 ± 1.8^b	235.6 ± 5.6^c	115.5 ± 3.4^b	4.0 ± 1.5^b	78.2 ± 3.1^b
ST4	13.5 ± 2.1^b	238.7 ± 5.8^c	100.6 ± 4.7^c	4.2 ± 0.9^b	76.9 ± 3.6^b

LA leaf area, *B* Biomass, *NR* number of root, *SH* shoot height

Values are means (± SD) of three experiments. Each experiment has three replicates. Means followed by different letters within a column under each treatment are significantly different at 5% probability level according to Tukey's Test. TMS 0040- IITA-TMS-IBA010040 (drought sensitive), TMS 581-IITA-TMS-IBA980581 (drought tolerant)

Table 3 Mean squares, coefficients of determination and variation from the analysis of variance for proline and photosynthetic pigments of the two cassava varieties after the application of ascorbic acid

SV	DF	Proline	Chl. a	Chl. b	Carotenoids
Rep	2	2.7×10^{-4}	4.1×10^{-1}	1.1×10^{-1}	1.7×10^{-3}
Stress (S)	5	8.1×10^{-2}**	6.11**	69.60**	2.50**
Genotype (G)	1	4.8×10^{-2}**	3.22**	13.80**	8.8×10^{-2NS}
S × G	5	9.3×10^{-4NS}	8.1×10^{-3NS}	0.20^{NS}	1.8×10^{-2NS}
Error	24	9.0×10^{-5}	2.8×10^{-2}	0.31	2.2×10^{-2}
R^2 (%)		99.5	98.2	98.1	96.2
CV		1.6	3.2	4.2	8.9

AA ascorbic acid, *NS* not significant, *Chl. a* chlorophyll a, *Chl. b* chlorophyll b

**Highly significant at 0.01 of probability

Table 4 Mean squares, coefficients of determination and variation from the analysis of variance for antioxidants and relative water content of the two cassava varieties under water stress treated with ascorbic acid

SV	DF	CAT	AA	SOD	RWC
Rep	2	4.4×10^{-6}	1.0×10^{-6}	9.1×10^{-6}	1.13
Stress (S)	4	0.49**	2.7×10^{-3}**	7.9×10^{-2}**	3.65**
Genotype (G)	1	0.33**	1.8×10^{-3}**	7.2×10^{-2}**	10.45**
S × G	4	1.2×10^{-2}**	4.8×10^{-6}**	2.2×10^{-5NS}	1.53^{NS}
Error	46	8.0×10^{-5NS}	3.7×10^{-7NS}	2.6×10^{-5NS}	1.15^{NS}
R^2 (%)		99.9	99.9	99.8	61.8
CV		0.8	0.5	0.3	0.9

NS not significant, *CAT* catalase, *AA* ascorbic acid, *SOD* superoxide dismutase, *RWC* relative water content

**Highly significant at 0.01 level of probability

Table 5 Proline, photosynthetic pigments, relative water content and activity of superoxide dismutase of cassava under water stress as influenced by genotype and ascorbic acid treatment

Treatment	Proline (mg/g)	Chl. a (mg/g)	Chl. b (mg/g)	Carotenoids (mg/g)	SOD (U/mg)	RWC (%)
Genotype						
TMS 0040	0.7 ± 0.0^{a}	5.0 ± 0.8^{b}	12.7 ± 1.1^{b}	1.8 ± 0.5^{a}	1.7 ± 0.0^{b}	94.8 ± 3.5^{b}
TMS 581	0.6 ± 0.0^{b}	5.6 ± 0.7^{a}	14.1 ± 1.1^{a}	2.0 ± 0.7^{a}	1.8 ± 0.0^{a}	95.9 ± 2.7^{a}
Stress						
WS	0.4 ± 0.0^{d}	17.2 ± 2.8^{a}	7.8 ± 1.7^{a}	2.2 ± 0.5^{a}	1.2 ± 0.0^{c}	98.8 ± 2.3^{a}
ST0	0.8 ± 0.1^{a}	6.2 ± 1.2^{c}	3.2 ± 0.8^{c}	0.5 ± 0.1^{c}	1.5 ± 0.0^{b}	92.0 ± 2.1^{d}
ST1	0.5 ± 0.0^{c}	14.6 ± 2.6^{b}	5.2 ± 0.7^{b}	1.5 ± 0.0^{b}	1.7 ± 0.0^{a}	94.4 ± 1.8^{c}
ST2	0.4 ± 0.0^{d}	15.2 ± 3.2^{b}	6.0 ± 1.1^{b}	1.7 ± 0.0^{b}	1.8 ± 0.1^{a}	96.8 ± 2.7^{b}
ST3	0.5 ± 0.0^{c}	14.5 ± 2.9^{b}	6.1 ± 1.0^{b}	1.5 ± 0.1^{b}	1.5 ± 0.0^{b}	92.1 ± 2.8^{d}
ST4	0.6 ± 0.0^{b}	13.6 ± 3.1^{b}	5.8 ± 1.2^{b}	1.5 ± 0.0^{b}	1.5 ± 0.0^{b}	94.2 ± 2.6^{c}

LA leaf area, *Chl. a* chlorophyll a, *Chl. b* chlorophyll b

Values are means (± SD) of three experiments. Each experiment has three replicates. Means followed by different letters within a column under each treatment are significantly different at 5% probability level according to Tukey's Test. TMS 0040-IITA-TMS-IBA010040 (drought sensitive), TMS 581-IITA-TMS-IBA980581 (drought tolerant)

by 14.3%. The activity of superoxide dismutase and relative water content of drought tolerant genotype were slightly higher than the sensitive genotype by 5.8 and 1.5%, respectively (Table 5). Water stress increased proline by 91.3%. But pre-stress application of AA reduced proline content (by 100.0%) to the same quantity of proline detected in water sufficient (WS) plants at 0.5 mM of AA, beyond this concentration, proline content increased. Water stress reduced chlorophyll a., chlorophyll b. and carotenoids more than three folds (Table 5). However, the three photosynthetic pigments were protected by pre-water stress application of AA. The best pigments protection was

observed at the concentration of 0.5 mM AA. Pre-treatment with 0.5 mM AA doubled chlorophylls and tripled carotenoids content when compared with AA-untreated water stressed (ST0) plants.Water stress reduced relative water content (RWC) by 3.2% and increased the activity of superoxide dismutase (SOD) by 15.3% in both genotypes (Table 5). As the concentration of AA increased, both RWC and SOD increased and reached their peaks at 0.5 mM before decline (Table 5). Compared with AA-untreated water stressed (ST0) plants, pre-treatment with 0.5 mM AA slightly enhanced RWC and SOD by 2.1 and 2.0%, respectively.

Under water sufficient condition, endogenous AA of drought tolerant genotype doubled as compared to drought sensitive genotype but the two genotypes increased synthesis of endogenous AA by the same magnitude with water stress alone and with pre-stress application of AA (Table 6). As the concentration of external AA increased, quantities of endogenous AA also increased in both genotype and reached peaks at 0.5 mM AA and later declined. Pre-treatment of 0.5 mM AA more than doubled endogenous AA when compared with AA-untreated water stressed (ST2) plants in both genotypes. Under water sufficient and water stress alone conditions, activity of catalase in drought tolerant genotype was the same as that of drought sensitive genotype. Water stress increased CAT activity by more than three folds in both genotypes. Pre-water stress application of AA increased CAT activities of both genotypes, drought tolerant one being greater than the drought sensitive genotype. The highest activity of CAT was observed at 0.5 mM AA, where enhancement of 100.3 and 33.4% were obtained in drought tolerant and sensitive genotypes, respectively.

Discussion

Exposure of young (3–4 months-old) cassava plants to drought stress has devastating effects on growth, tuber yield, starch content, cyanogen glucosides, post-harvest quality of tubers and resistance to biotic stresses (Santisopasri et al. 2001; Vandegeer et al. 2012). This is attributed to many factors, including low concentration of endogenous AA in cassava plants under water stress to scavenge reactive oxygen species (Akram et al. 2017). Here, we examined growth and physiological responses of young cassava plants to water deficit after foliar treatment with AA. In our study, water stress reduced shoot height, leaf area, number of leaves and biomass of both drought-tolerant and drought-sensitive genotypes. Our findings were consistent with previous studies that established that water stress at 3–4 months of age significantly reduced growth and tuber yield in cassava (Santisopasri et al. 2001; El-Sharkawy 2012; Duque and Setter 2013; Vandegeer et al. 2012). Our results indicated that pre-water stress exogenous AA was beneficial by restoring shoot height, leaf area, number of leaves and biomass of water-stressed plants to the same level of water sufficient controls in both genotypes. Enhanced growth of AA-treated plants could be due to amelioration of oxidative stress by antioxidant system created by high content of endogenous AA, SOD and CAT observed in this study which protected photosynthetic pigments and supported high RWC. The high photosynthetic pigments may have promoted photosynthesis which made assimilate available for growth process. Naz et al. 2016 observed correlation of photosynthetic rate to high content of chlorophyll a, stomatal conductance and RWC in AA-treated water stressed cucumber. It was

Table 6 Interaction of genotype and stress on activity of catalase and endogenous ascorbic acid content

Genotype	Stress	Catalase (μmol/min/mg FW)	AA content (mg/g)
IITA-TMS-IBA980581	WS	0.2 ± 0.0^{d}	0.08 ± 0.0^{c}
	ST0	0.5 ± 0.0^{c}	0.12 ± 0.0^{b}
	ST1	1.4 ± 0.1^{a}	0.11 ± 0.0^{b}
	ST2	1.4 ± 0.0^{a}	0.15 ± 0.0^{a}
	ST3	1.2 ± 0.0^{b}	0.12 ± 0.0^{b}
	ST4	1.1 ± 0.0^{b}	0.12 ± 0.0^{b}
IITA-TMS-IBA010040	WS	0.2 ± 0.0^{d}	0.04 ± 0.0^{d}
	ST0	0.6 ± 0.1^{c}	0.10 ± 0.0^{b}
	ST1	1.1 ± 0.0^{b}	0.10 ± 0.0^{b}
	ST2	1.2 ± 0.0^{b}	0.16 ± 0.0^{a}
	ST3	1.2 ± 0.0^{b}	0.11 ± 0.0^{b}
	ST4	0.6 ± 0.0^{c}	0.11 ± 0.0^{b}

LA leaf area

Values are means (± SD) of three experiments. Each experiment has three replicates. Means followed by different letters within a column under each treatment are significantly different at 5% probability level according to Tukey's Test

possible that the antioxidant defensive system formed by endogenous AA, SOD and CAT was capable of neutralizing ROS generated by water stress as exogenous AA has been reported to regulate cellular ROS (Akram et al. 2017). This is the first report of protection of young cassava plants subjected to water stress by external foliar application of AA. Previously, exogenous AA has been reported to reduce oxidative stress and stimulate growth of plants under water stress in maize, wheat, canola, sunflower, okra (Dolatabadian et al. 2010; Hussein and Alva 2014; Shafiq et al. 2014; Akram et al. 2017). Genetic difference which allowed superior antioxidant defensive system in drought tolerant genotype could be attributed to higher shoot height, leaf area, number of leaves and biomass of drought tolerant genotype than drought sensitive genotype. In this study, the best concentration of AA for pre-stress treatment of cassava was 0.5 mM, which is consistent with the optimum concentration of AA for mitigation of water stress in other crops. For instance, foliar application of 200 mg/l AA on wheat subjected to drought stress enhanced chlorophyll a and b, total soluble proteins, carbohydrates and carotenoids (Hussein and Alva 2014). Also, 1.0 mM was required to increase dry weights, sugar contents, proline, chlorophyll a and b, carotenoids and leaf area under drought stress in okra (Amin et al. 2009).

Besides growth responses of AA-treated plants to water stress, physiological changes were examined to gain a better understanding of cassava response to AA treatment. Genetic difference could be responsible for higher content of chlorophyll a, b, carotenoids, RWC and SOD in drought tolerant genotypes than drought sensitive one. Proline is an important solute that is involved in osmotic adjustment during water stress to reduce oxidative damages (Szabo and Savoure 2010; Anjum et al. 2011). In this study, proline accumulation of drought sensitive genotype was higher than drought tolerant ones indicating proline accumulation is proportional to degree of stress. This result contradict earlier report that proline accumulation in drought tolerant genotypes of crops was greater than drought sensitive ones (Anjum et al. 2011). Also, in this study, water stress increased proline accumulation but AA treatments decreased proline synthesis by same magnitude in both genotypes, suggesting AA is capable of reducing oxidative stress caused by water stress. Furthermore, reduction of photosynthetic pigments by water stress in this report was consistent with findings of Zhao et al. (2015) but disagreed with Pereira et al. (2018) who reported an increase in total chlorophyll and carotenoids after 45 days of water deficit. The disagreement could be due to differences in drought avoidance mechanism exhibited by various cassava varieties. However, AA-treated plants had enhanced photosynthetic pigments indicating exogenous AA protected photosynthetic pigments in both genotypes against breaking down by oxidative stress. Foliar spray of wheat and basil under drought stress had been reported to have enhanced chlorophyll a and b and carotenoids (Hussein and Alva 2014; Khalil et al. 2010; Amin et al. 2009).

In most crops, leaf relative water content decreases under water stress due to turgor loss and membrane instability (De Faria et al. 2013; Silva et al. 2013; Wedeking et al. 2016). However, studies have shown that cassava water relations is resilient under water deficit (El-Sharkawy 2012; Pereira et al. 2018). For example, at development stage under a severe water deficit (− 70 kPa), cassava cultivar IAC 576-70 maintained leaf water potential, relative water content and membrane integrity (Pereira et al. 2018). In this work, water stress reduced leaf RWC of both genotypes, however, pre-stress application of AA improved leaf RWC in both genotypes. RWC of maize and basil under water stress was enhanced by exogenous AA due to increased water potential and stomatal conductance (Khalil et al. 2010; Ahmad et al. 2014). Under water stress, activities of enzymatic and non-enzymatic antioxidant increase in plants to scavenge ROS and reduce oxidative damages (Anjum et al. 2011). In this work, activities of SOD and CAT and quantity of endogenous AA increased in untreated water stress plants when compared with water sufficient control, confirming physiological mechanism of reducing oxidative stress in cassava. Furthermore, pre-stress foliar of applications AA increased activities of SOD and CAT and quantity of endogenous AA in both genotypes. Pre-sowing and foliar application of 50 and 150 mg/L AA enhanced endogenous AA and activity of CAT of wheat under drought stress (Athar et al. 2008). Higher SOD activity in drought tolerant genotype compared with drought sensitive genotype suggested genotypic influence on SOD activity. In the case of endogenous AA, we observed significant difference between drought tolerant and sensitive genotypes under water sufficient condition. However, the difference in endogenous AA disappeared in AA-treated water-stressed plants, indicating synthesis of AA under water stress in cassava is not affected by genotype. The reverse was observed in case of the activity of CAT as genotypic difference was observed on AA-treated water stressed plants only.

Our work provides alternative approach of protecting cassava plants against oxidative damage from water deficit. Our results provide evidence that pre-water stress foliar application of AA induces physiological changes that mitigate negative effect of water deficit, which manifest in positive growth responses comparable to water-sufficient control plants. The importance of these results lies in the fact that cassava, a root crop with long growth cycle grown in tropical climates prone to soil moisture deficit occasioned by drought and dry season, could be protected by foliar spray of inexpensive compound.

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