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EVALUATION OF BIOCHEMICAL, PHOTOSYNTHETIC AND PHYSIOLOGICAL CHARACTERISTICS OF COWPEA (*VIGNA UNGUICULATA* L. WALP) ACCESSIONS TO CADMIUM STRESS

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Abstract: Cadmium stress are known to reduce crop productivity, increase reactive oxygen species and impair photosynthetic processes. The present study aimed to determine the physiological and biochemical characters of cowpea accessions (TVu-91, TVu-92, TVu-93, TVu-95, and TVu-96) to cadmium stress. Cowpea seeds were exposed to three concentration of CdCl2; the control (0ESV), Cd-2.5ESV and Cd-5ESV and laid out in randomized block design (RBD) for five months. Parameters were evaluated both above and below ground level at the seedling stage. The leaves were partitioned into young leaf (YP), intermediate leaf (IP) and old leaf (OP) for a concise distribution of foliar chlorosis and necrosis 84 days after sowing. The physiological characters, chlorophyll content, enzymatic and non-enzymatic activities were also determined. From the result, the shoot, root and petiole length, number of primary stem and root branches of TVu-91, TVu-92, TVu-93, TVu-95 and TVu-96 were reduced with increased cadmium concentration. Cadmium stress also resulted to a significant difference in the number of root nodule of TVu-96 as compared to TVu-95. Cadmium stress increased foliar chlorosis and necrosis with increased concentration however, the effect were more in older leaves (OP) as compared to the young leaves (YP) and intermediate leaves (IP). There was an insignificant increase in the chlorophyll-a/b content with increased concentration. Superoxide dismutase (SOD) and catalase (CAT) showed the highest activities in TVu-93 while the MDA content of TVu-92 and TVu-93 were significantly increased. Generally, cadmium stress reduced the overall foliar yield, plant dry weight and root dry weight of cowpea accessions. Cadmium significantly reduced the above and below shoot parameters, resulting in increased MDA levels however, the plant responded by employing proline and catalase as a defense mechanism in combating the oxidative stress mitigated by the metal.

Keywords: proline, mda, cowpea, crop productivity, chlorosis, necrosis

INTRODUCTION

In the last few decades, there have been a drastic climatic change probably due to global warming, which has brought about not only a serious environmental threat but also substantial reductions in the yield and quality of crops (Umar and Siddiqui, 2018). Soil pollution by heavy metal (Hm) is a critical global and environmental problem. Hm toxicity in soil is apparent in reducing the growth and development of plants and harming the health of the ecosystem (Ohanmu and Ikhajiagbe, 2018). Researchers have studied the effects of various HMs on soil (Ohanmu et al., 2018), plant (Ohanmu and Ikhajiagbe) and aquatic life form (Shahwar et al., 2018). Cd enters the environment through natural (rock weathering and forest fires); or anthropogenic (industrial effluents, mining, fuel and agricultural chemicals) (Sharma and Dubey, 2005), including the use of phosphate fertilizer.

Of the studied metals, cadmium (Cd) is the second most toxic after mercury, posing a great threat to human health and the environment due to their persistent toxic nature and bioaccumulation in food chain. Cd is known to interfere with chlorophyll synthesis either through the direct inhibition of an enzymatic step or by inducing deficiency of an essential nutrient (Van Assche and Clijsters, 1990)6. This stimulates the formation of reactive oxygen species (ROS) with the potential of damaging the production of biomolecules such as lipids, proteins and nucleic acids (Gill and Tuteja, 2010). The peroxidation of lipid membrane is a major damaging consequence of ROS. If not properly scavenged, results in the inhibition of photosynthesis and respiration processes which then impair plant growth and development. To cope with the effect of metals toxicity, plant has evolved complex mechanisms to control the uptake, accumulation and detoxification of metals (Ohanmu and Ikhajiagbe, 2018). Both enzymatic and non-enzymatic activities are employed as a vital tolerant mechanisms against oxidative stress.

Cowpea (Vigna unguiculata L. Walp) is a globally cultivated and the major staple crop in Nigeria. Cowpea plays a key role in the agricultural and food supply sector of Nigeria which happen to be the largest producer and consumer of the crop in the world. Unfortunately, improvement in legume crop yields have not kept pace with those of cereals. In Nigeria, reduction in agricultural productivity is affected by industrialization, salinization, encroachment of arable lands, famers/herdsmen crisis and environmental stress e.g. heavy metals. Taking into account the role of leguminous plants in the Nigeria economy as well as the resulting benefits for the environment, it is therefore important to understudy the response of cowpea to Cd stress with a view to screening resistant accessions for cultivation. The aim of the present study was to investigate the biochemical, photosynthetic and

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physiological characteristics of selected cowpea accessions to cadmium stress.

MATERIALS AND METHODS

Plant material and experimental setup

The experiment was carried out in the botanic garden of Plant Biology and Biotechnology (PBB), University of Benin, Benin City, Nigeria. Polythene bags (50 x 50 cm) were filled with 15 kg of air dried soil. From the physicochemical analysis, the soil type was sandy loam, pH 7.3. Seeds of V. unguiculata (Cowpea accessions: TVu-91, TVu-92, TVu-93, TVu-95 and TVu-96) (Table 1) were procured from the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria. The physiological performance of these accessions have not been explained under cadmium stressed environment. The experiment spanned from late October, 2016 to March, 2017.

Tab. 1.

Description of accessions of test crop used in the study

Designations	Accession name	ID	Accession number	Country of origin	Cultivar name
Cowpea (Vigna unguiculata)	TVu-91	108154	91	South Africa	51C-421-2
	TVu-92	108155	92	South Africa	WITZENBORG
	TVu-93	108156	93	South Africa	53-C-91-1
	TVu-95	108157	95	South Africa	Renoster
	TVu-96	108158	96	South Africa	51C-428

Experimental design

Soil samples were collected from 10 random point at a depth of 0 to 30 cm in the botanic garden of PBB, University of Benin, Nigeria, pooled together to form a bulk soil. The treatment designation for metal concentration is shown in Table 2. There were three treatments separated into 0 ESV, Cd-2.5ESV and Cd-5ESV (Ecological screening value) respectively. The 0 ESV was free from cadmium pollution and served as the control, Cd-2.5ESV was soil polluted with Cd at the rate of 2.5 times the ecological screening value of cadmium and Cd-5ESV were soil polluted with Cd at 5 times the ecological screening value. The cadmium used was the whitish crystallized form of CdCl2. The experimental setup consisted of three treatments, three replicates, two plants per bag arranged in a randomized block design (RBD) making a total of ninety (90) plants. The various seeds were sowed into each bags. The plants were watered everyday till the end of the experiment. Data were collected at the seedling and flowering stages. The leaves were partitioned into young (YP), intermediate (IP) and old (OP) leaf and crushed together to obtain a composite sample for proper representation of the plant response. Parameters measured were biochemical, photosynthetic and physiological characters.

Tab. 2.

Treatment designations for metal concentrations

Designations	Description	Replications
0 ESV	Control (unpolluted soil)	3
Cd-2.5 ESV	0.15g of cadmium chloride diluted in 2L of water and mixed in 15kg soil	3
Cd-5 ESV	0.30g of cadmium chloride diluted in 2L of water and mixed with 15kg soil	3

Physiological characters

The foliar chlorosis was measured / evaluated by manual counting of chlorotic (yellowing or whitening plant's leaves) leaf for 28 days after sowing (DAS). The percentage chlorosis was calculated as stated in formula (1). Foliar necrosis was by manual counting of necrotic (the death of cells in a tissue) leaves for 28 DAS. Percentage necrosis was achieved using the equation in formula (2). The percentage senesced leaves was calculated using the equation in formula (3). Where, CL = No. of chlorotic leaves, NL = No. of necrotic leaves, N = No. of leaves at 28 DAS, SL = senesced leaves and TN = total number of plant per treatment.

$$\%chlorosis = CL/N \times 100 \quad - \quad (1)$$

$$\%Necrosis = NL/N \times 100 \quad - \quad (2)$$

$$(SL) \div (SL+TN) \times 100- \quad - \quad (3)$$

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Photosynthetic pigments and biochemical assay

Fresh leaf (young, intermediate and older) samples (500 mg) were used for the extraction of pigments in 10 mL of 96% methanol. Chlorophyll a, b and carotenoid contents were determined (Lichtenthaler, 1987) and expressed in µg mg–1 fresh weight (FW). The proline (Marin et al., 2009), SOD (Beyer and Fridovich, 1987), CAT (Patterson et al., 1984) and MDA (Heath and Packer, 1968) activities were also determined.

Statistical analysis

The data collected were subjected to one way analysis of variance using IBM SPSS-20® software. The least significant difference was used to separate differences in their mean at 5% level of significance ($p \leq 0.05$). The resultant values were expressed in bar and line graph.

RESULTS

The observed data for shoot, root and petiole length of the studied cowpea accessions is shown in Fig. 1. Cd stress resulted in a significant decrease ($p<0.05$) in the shoot, root and petiole length of all accessions when compared to the control with increased metal concentration. The highest reduction in shoot length was recorded in TVu-91.

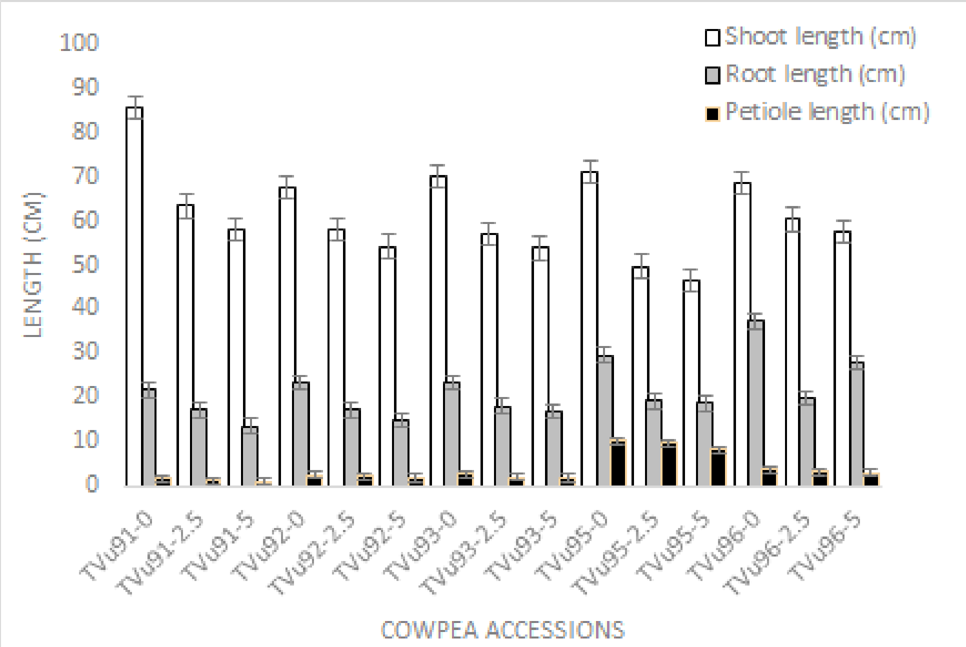


Fig. 1. Effect of cadmium stress on plant parts

The effect of Cd on above and below ground parameters are presented in Table 3. Cd stress resulted to suppression in the number of primary root branches in the Cd-2.5ESV and Cd-5ESV respectively. Increased cadmium stress also resulted to significant decrease ($p<0.05$) in nodules yield irrespective of the plant accessions. TVu-95 and TVu-96 in Cd-5ESV recorded the highest reduction in nodules yield when compared to the control.

Tab. 3.

Effects of cadmium stress on some parameters of cowpea

Accessions	Cd conc. (ESV)	No of primary stem branches	Stem width (mm)	Internodal distance (cm)	No. of primary root branches	No. of root nodules per plant	Nodules yield (g)
TVu-91	0	3.72	6.00	5.92	5.04	14.02	1.75
	2.5	2.56	5.00	3.76	3.02	8.06	1.11
	5	2.11	5.00	3.44	2.06	6.04	0.91
TVu-92	0	5.92	8.00	4.70	6.09	21.07	2.37
	2.5	3.45	8.00	3.81	4.05	14.03	1.58
	5	3.07	7.00	2.87	3.02	11.01	1.10
TVu-93	0	3.68	9.00	5.09	3.54	25.06	3.45

	2.5	3.11	6.00	4.31	3.11	20.04	2.50
	5	3.02	5.00	3.21	2.66	17.05	2.13
TVu-95	0	3.37	7.00	6.47	6.45	22.54	3.19
	2.5	3.11	6.00	4.93	7.10	18.23	2.25
	5	2.07	6.00	4.37	5.48	15.75	1.88
TVu-96	0	4.26	10.00	7.21	8.07	19.95	2.62
	2.5	3.34	7.00	4.90	5.11	12.13	1.65
	5	3.04	5.00	3.62	3.04	9.06	1.25
P-value		0.396	0.420	0.556	0.127	0.031	0.109
Sig*		P>0.05	P>0.05	P>0.05	P>0.05	P<0.05	P>0.05

Morphological character

The variation in partitioned aged leaves of cowpea to cadmium stress is shown in Table 4. The amount of chlorotic leaves were significantly increased in the OP when compared to YP irrespective of accession type with increased Cd concentration. From the result, it was observed that the cowpea accessions had more necrotic leaves in the OP than the YP and IP with increased Cd stress. This could be a form of survival strategy employed by the plant to detoxify excess Cd taken up through the root. TVu-91 recorded the highest necrotic values in the Cd-5ESV.

Tab. 4.

Variation in partitioned aged leaves of cowpea to cadmium stress.

Accessions	Cd conc. (ESV)	Chlorotic leaves			Necrotic leaves		
		YP	IP	OP	YP	IP	OP
TVu-91	0	0	0	3.97	1.99	3.97	5.96
	2.5	2.85	5.70	8.55	5.70	11.39	14.24
	5	3.82	7.67	15.33	7.67	15.33	26.83
TVu-92	0	0	1.89	5.66	0	5.66	9.43
	2.5	2.41	2.41	12.07	4.93	7.72	20.51
	5	3.06	6.11	18.34	6.11	15.28	24.45
TVu-93	0	0	0	2.90	1.45	1.45	4.35
	2.5	4.38	6.57	13.15	4.38	4.382	10.96
	5	5.01	10.03	17.55	5.01	7.52	20.06
TVu-95	0	0	1.85	3.70	1.85	3.70	7.40
	2.5	2.55	5.09	12.73	5.09	7.64	15.28
	5	6.44	9.66	19.32	6.44	12.88	24.76
TVu-96	0	0	3.33	5.00	1.67	1.67	6.67
	2.5	2.48	7.44	9.92	4.96	7.44	17.36
	5	2.98	8.94	14.90	8.94	8.94	26.98
P-value		0.921	0.874	0.979	0.964	0.256	0.609
Sig*		P>0.05	P>0.05	P>0.05	P>0.05	P>0.05	P>0.05

Cd stress had a pronounced effect on % total foliar chlorosis (TFC), necrosis (TFN) and senescence (TNS) of cowpea (Fig. 2). The accessions varied in their foliar chlorotic response to Cd stress, TVu-95 was more susceptible when compared to the other accessions. TVu-91 had the highest percentage necrotic values. Cd stress increased percentage senesced leaf in TVu-93 with increased metal concentration compared with the control. However, the highest and lowest reduction were recorded in the TVu-95 and TVu-92 respectively.

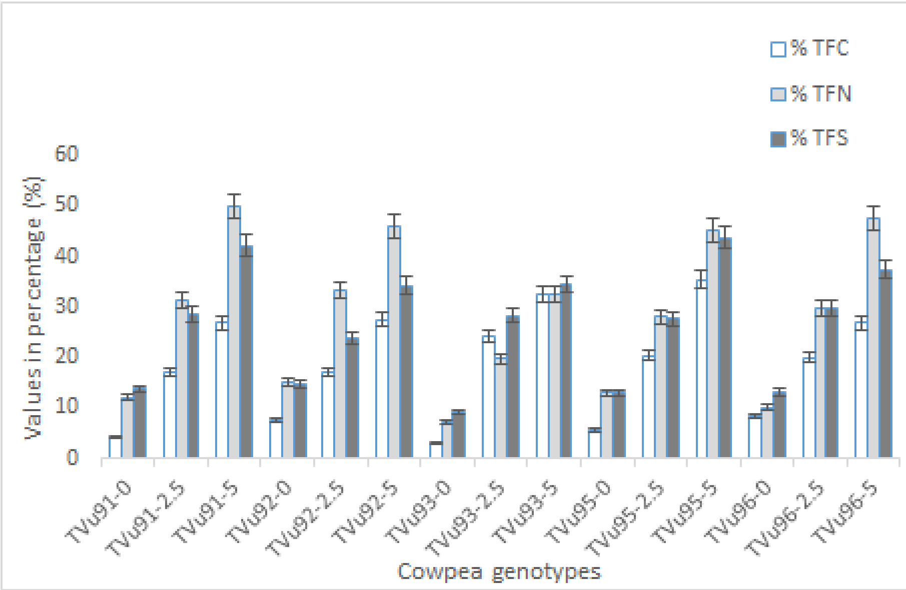


Fig. 2. Effect of cadmium on the total percentage of foliar chlorosis, necrosis and senescence.

Chlorophyll content

The effect of Cd stress on dominant foliar colour and chlorophyll a/b content of TVu-accessions are shown in Fig. 3. There was an insignificant increase in the chlorophyll-a content of TVu-95 between the Cd-5ESV and the control. The changes in chlorophyll-b content of cowpea exposed to Cd stress was also reported. Cd resulted to an insignificant difference in chlorophyll-b contents among TVu-accessions. The modal foliar colour of cowpea to cadmium stress is a way to compare the morphological response of the leaves with the photosynthetic pigment of the plant. Cd stress resulted in variation in the modal foliar colours of TVu-92 treatments. However, TVu-93 maintained a modal foliar colouration of green.

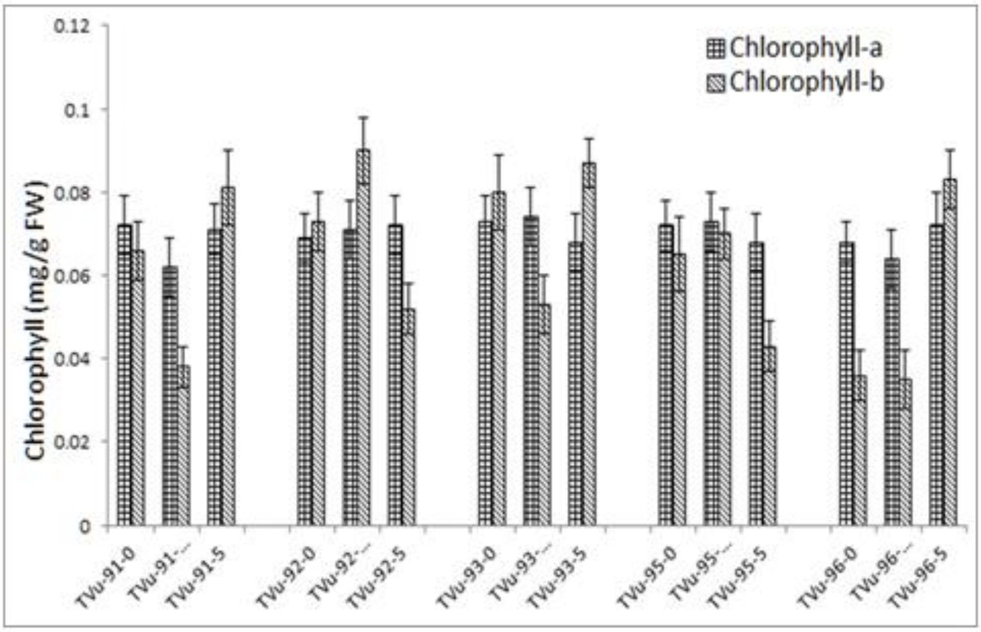


Fig. 3. Effect of cadmium stress on the chlorophyll a/b content and modal foliar colour. TVu-91-0ESV, TVu-92-0ESV, TVu-93-0ESV, TVu-96-0ESV (Green #008000); TVu-91-2.5ESV, TVu-91-5ESV, TVu-95-2.5ESV, TVu-95-5ESV (Olive drab #6B8E23); TVu-92-2.5ESV (Light green #90EE90); TVu-92-5ESV, TVu-93-2.5ESV, TVu-96-5ESV (Dark sea green #8FBC8F); TVu-93-5ESV, TVu-96-2.5ESV (Aquamarine); and TVu-95-0ESV (Sea green) respectively.

Carotenoid

There was variation in the carotenoid levels of cowpea accession to Cd stress (Fig. 4). There was a decrease in the carotenoid content of TVu-95 and TVu-96 with increased metal concentration. However, there was no significant difference (P>0.05) in the carotenoid levels of TVu-92 and TVu-93 between the Cd-5ESV and the control.

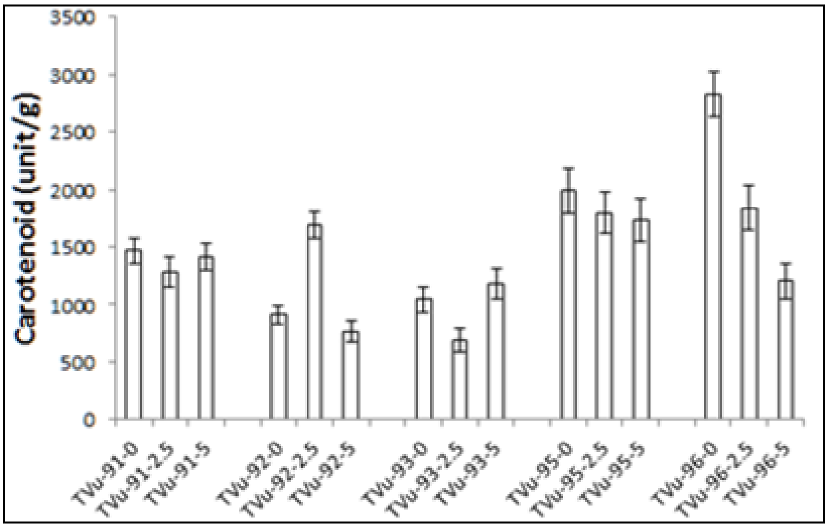
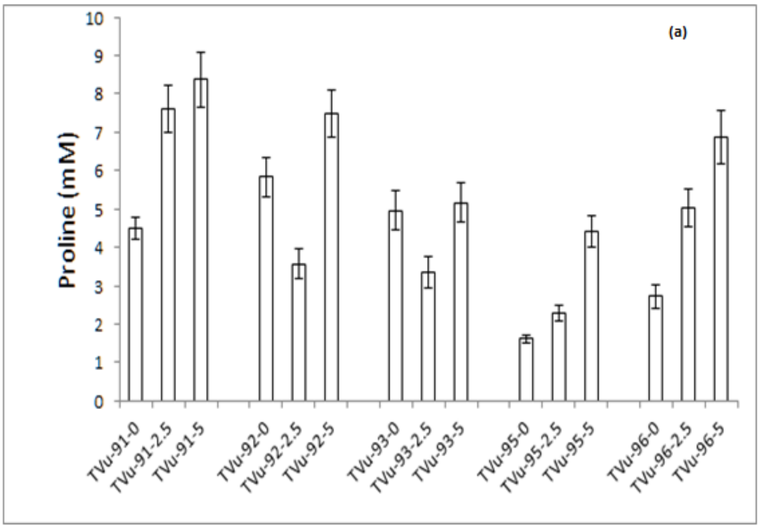


Fig. 4. Effect of cadmium stress on the carotenoid content of cowpea.

The biochemical response of cowpea accession to cadmium stress are shown in Fig. 5 (a-d). The proline content increased with increased metal concentration. TVu-91 and TVu-95 had significantly increased ($p<0.05$) proline contents compared to the other accessions. However, the proline levels of TVu-92 in Cd-2.5ESV was reduced when compared to control and increased in the Cd-5ESV. Enzymatic activities like SOD and CAT were examined. Cadmium stress resulted in increased SOD levels with increased concentration except in TVu-93. There was a decrease in the catalase activities of all TVu's with increased metal concentration except in TVu-95. The CAT activity of TVu-95 in Cd-2.5ESV was significantly heightened. The effect of Cd was more pronounced with a highly significant increase ($P<0.00$) observed in the MDA activities of TVu-92 and TVu-93.



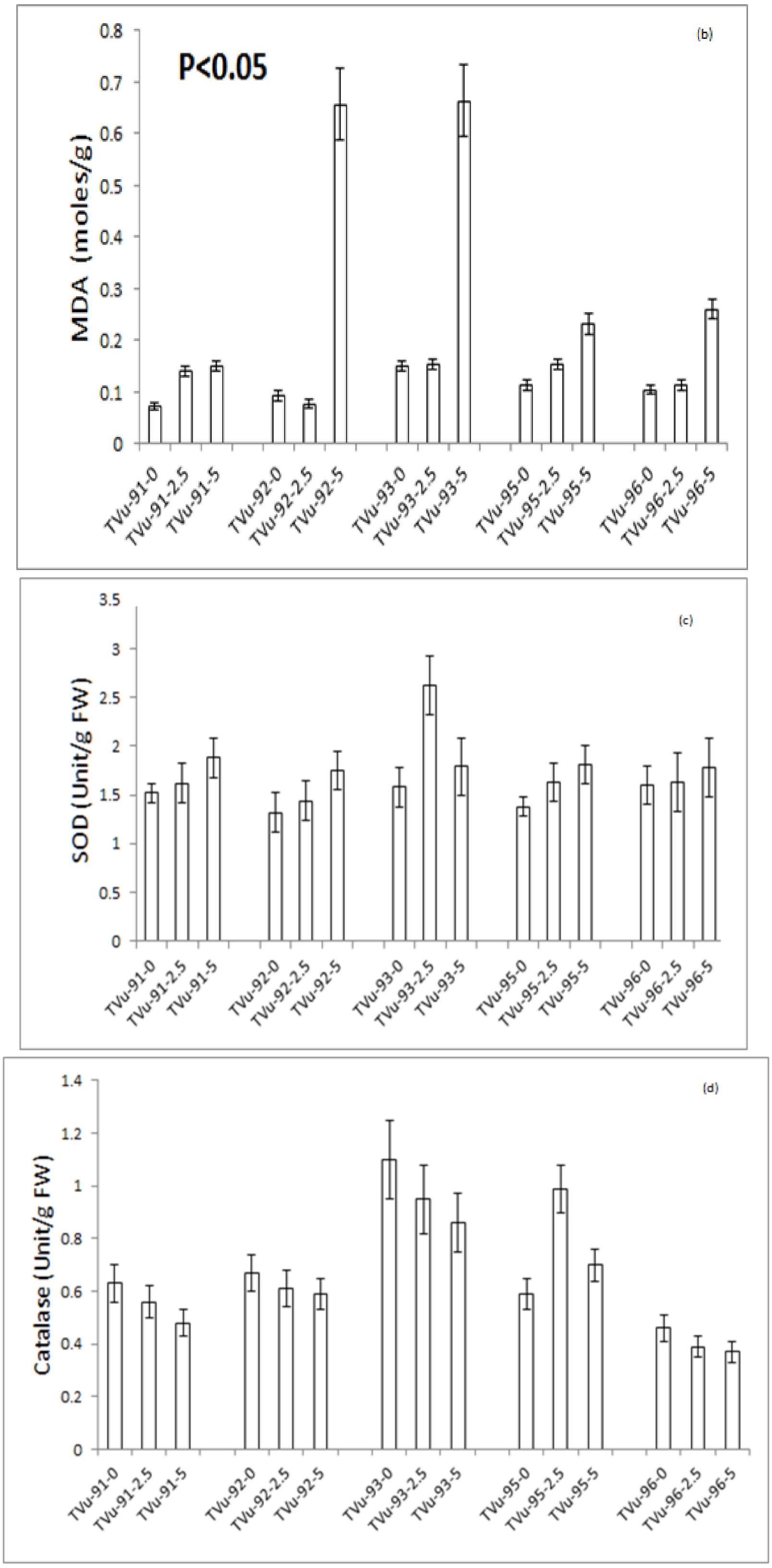


Fig. 5 (a-d). Effect of cadmium stress on proline, malondialdehyde, superoxide dismutase and catalase activities of cowpea.

DISCUSSION

This experiment distinguishes that Cd showed considerable effect on the physiology and biochemical process of cowpea accessions. It was observed that soil exposed to Cd stress resulted to reduction in the morphology and development of the plant with increased metal concentration. Seedling growth were most affected because Hms disturbs plant metabolism by interacting with enzymes and biochemical reactions taking place inside the plant cell (Ashraf et al., 1998). This disturbance was observed in the suppressed number of primary stem and root branches of TVu-92. Of all the non-essential heavy metals, Cd has attracted the most attention in soil science and plant nutrition due to its impending toxicity to humans, and also its relative mobility in the soil-plant system. Cd toxicity resulted to reduction in the number of root nodules by making essential plant nutrients unavailable for root absorption (Ohanmu et al., 2017). Similarly, the reduction in crop yields sown in soils polluted with cadmium has been reported (Bhardwaj et al., 2009; Ghani, 2010). The increased foliar chlorosis, necrosis and senescence to Cd stress was age-dependent on leaves (YP, IP and OP). Plant responds to environmental stresses by a variety of means. It is noted frequently that many crop species tolerance to prevailing stress conditions changes with plants' aging. This was observed as cowpea accessions recorded more necrosis in older leaves than in younger leaves. The increased senesced leaves correlated with reports that Cd causes multiple direct and indirect effects on plant growth by disrupting metabolic processes (Ohanmu et al., 2017). The transportation of more cadmium ions to older leaves may be a survival strategy employed by the plant to overcome the oxidative stress produced; due to Cd absorption (Ghoshroy and Nadakavukaren, 1990). This interference in plant development via attacking photosynthetic pigments in leaves resulted in the high percentage of senesced leaves observed.

The accessions however, responded differently which may be due to the genetic buildup inherent in seed cells. Plants adapt two different tolerant strategies during abiotic stress: either by decreasing their leaf size and/or stomatal conductance (Siddiqui et al., 2014) or by increasing stomatal conductance to increase carbon gain and avoid stress (Umar and Siddiqui, 2018). Although the leaves presented different green color shades, no significant reduction in the multiple roles of light-harvesting chlorophyll a/b contents was reported. This may be attributed to reported differences within accessory pigments (e.g. carotenoids). The increased ROS production resulted in the activation of antioxidant response as the last line of defense. The MDA content were significantly increased in TVu-92 and TVu-93 in the Cd-5ESV. MDA is a widely used marker that estimates the oxidative lipid injury caused by environmental stress. A number of studies have investigated MDA of plants under different stress conditions (Zhou et al., 2015). The proline also increased with increased Cd irrespective of accession. Proline protects the cells from various stresses and assist in cell recovering by maintaining cell turgor thus preventing oxidative burst as observed in TVu-92 and TVu-95. The SOD activities were significantly higher than control, serving as the first line of defense by alternately catalyzing the dismutase of superoxide (O2-) radicals into either ordinary molecular oxygen (O2) or hydroxide peroxide (H2O2) while CAT catalyzes the decomposition of hydrogen peroxide produced to water and oxygen (Sharma and Ahmad, 2014). However, no significant difference in the CAT activities between treatments and control except in TVu-95.

This study gives an extensive knowledge of the physiological and biochemical response of cowpea accessions to the effect of Cd stress. This observation conforms reports that plant response to oxidative stress by activating antioxidant as the last line of defense. Although the accessions activated both enzymatic and non-enzymatic mechanism in combating the oxidative stress produced, proline and catalase activities were the best line of defense employed by the various accessions.

CONCLUSION

The present research work determined that cadmium (Cd) had a significant effect on the physiological, photosynthetic and biochemical characters of Vigna unguiculata L. Walp accessions. The accessions varied in their mode of response to Cd stress and activated both enzymatic and non-enzymatic defense mechanism in combating oxidative stress. It was recorded that the increased oxidative injury estimated by MDA were combated by proline and catalase activity.

This study discover the genotypic response in TVu accessions of cowpea exposed to Cd stress. Variation in accessions may produce different filial generations that are resistant to metal toxicity and improve qualitative and quantitative traits that can be beneficial for breeding and gene modification to improve crop productivity especially in developing worlds. This study will help the researcher to uncover the critical areas of stress response that many researchers were not able to explore.

Thus a new theory on genetic tolerance may be arrived at. Enhance antioxidant and photosynthetic pigments plays a greater role in reducing the mitigating effect of oxidative stress caused mainly by abiotic factors resulting into desirable traits. This will go a long way in reducing health risk taken by consumption of cowpea due to increased chemicals applications in Nigeria. Therefore it may be suggested that the accessions TVu-92 and TVu-93 could be used in field trials in cadmium contaminated farms. However, more detailed molecular work is needed to provide a more convincing arguments and the seed's proximate content determined against possible translocation of Cd.

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