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Research Article

Effect of Cadmium Pollution on Nitrogen Assimilation and Biomass Accumulation of *Vigna unguiculata* L.

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Abstract

Background and Objective: With the increased soil pollution caused by heavy metals, identifying accessions that are high nitrogen assimilators has a potential in improving soil fertility and increase crop yield. Therefore, the current study aimed at determining the variation in nitrogen assimilation and biomass accumulation of cowpea accessions to cadmium pollution. **Materials and Methods:** A field experiment was conducted to evaluate the plant nitrogen assimilation and dry weight matter of cowpea accessions in response to cadmium pollution in Benin city located in southern Nigeria. Five cowpea accessions (TVu-91, TVu-92, TVu-93, TVu-95 and TVu-96) were sown in the control (0ESV) and two treatments of cadmium chloride (Cd-2.5ESV and Cd-5ESV), laid out in a randomized block design (RBD) and replicated thrice. The leaves and roots were assessed at 6 and 18 weeks after sowing and (WAS) for total nitrogen, nitrogen assimilated as nitrate-N or ammonia-N form and their percentage. The plants' biomass accumulation was also determined 20 WAS. **Results:** From the result, 6 WAS cadmium pollution significantly reduced foliar TN% irrespective of cowpea accession except in TVu-95. Cowpea accessions distributed nitrogen mainly as nitrate-N with the highest values observed in TVu-91 while TVu-96 recorded the highest N% assimilated as nitrate irrespectively of treatment. In addition to this, cadmium pollution reduced the overall foliar yield and plant dry weight of TVu-accessions with increased metal concentration. TVu-92 and TVu-96 in the Cd-2.5ESV and Cd-5ESV were more closely related in their mode of response to cadmium incidence compared to the other accessions. **Conclusion:** Nitrogen was significantly assimilated more as nitrate-N than ammonium-N and readily distributed to the leaves.

Key words: Cowpea, dry weight matter, high nitrogen assimilators, nitrate, cadmium pollution, dendrogram, ammonia-N form

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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

In soil, heavy metal increases over time and creates a serious environmental problem[1]. One of their main attributes is reducing the available mineral nutrients in soils, inhibiting the mineralization processes[2] and the litter decomposition rate in ecosystems is generally found to be decreased[3-5]. Heavy metals are widely known inhibitors of plant metabolism. Cadmium been a highly toxic element is widely spread in the environment from natural and anthropogenic activities and easily assimilated from agricultural soils through plants root. Once taken up by plants, Cd induce various morphological and phytotoxicity symptoms, e.g. leaf roll, chlorosis, necrosis, growth retardation and finally death[6], even at low concentration, Cd decreases stomatal density and conductance[7] to CO and reduce the number of opened stomata[8,9], which would further affect the rate of photosynthesis. The reduction in photosynthetic rate may be due to the detrimental effects posed on chloroplast biosynthesis and disturbance in chloroplast development[10].

Cadmium has been reported to negatively affect the physiological, biochemical activities of various plant[11] either individually or holistically. Take for instance, Cd ions lowers the activity of ribulose-1,5-bisphosphate carboxylase (RuBPC) and damage its structure by substituting for Mg ions, which are important co-factors for carboxylation reactions. Cadmium can shift RuBPC activity towards oxygenation reactions[12] and can cause an irreversible dissociation of the large and small subunits of RuBPC, thus leading to the total inhibition of the enzyme[13,14].

The survival of plants depends on its ability to perceive the stimulus, transit signals and activate biochemical processes that adjust the metabolism accordingly. Although these biochemical studies have provided a solid groundwork, a complete picture of the N-assimilation process and its regulation in a single plant is still lacking for a number of reasons. Researchers have reported on mechanisms to dissect the process of N-assimilation *in vivo*[15] and nitrogen distribution pattern to Hm exposure[1]. Nitrogen is an essential component of proteins assisting in the buildup of cell materials and plant tissue. Most of the available nitrogen in plant is often gotten from fertilizer application. Although the atmosphere is mostly made up of N, only some leguminous cropscan convert atmospheric N to plant-available forms via a symbiotic biological process involving rhizobium bacteria and the plant roots[16]. Plant available inorganic forms of N include nitrate and nitrite as well as ammonium.

However, there is no information on the rate of nitrogen assimilation of cowpea to cadmium toxicity. Inorganic nitrogen in all higher plants, is first reduced to ammonia prior to its incorporation into organic form. Ammonia is assimilated into organic form as glutamine and glutamate, which serve as the nitrogen donors in the biosynthesis of essentially all amino acids, nucleic acids and other nitrogen-containing compounds such as chlorophyll[17]. Hence, the assimilation of inorganic nitrogen into organic form has a profound effect on the biomass, productivity and yield of crops. In Nigeria the major source of cadmium into the soil is through fertilizers application, fumes from automobiles and compost. Cowpea (*Vigna unguiculata* L.) is mostly cultivated for its edible beans, peas and green pea pods. Originated in Africa, Nigeria is the highest producer and consumer of cowpea worldwide producing 64% in Africa and 58% globally[18]. The ability to fix atmospheric nitrogen makes it an excellent component within the various farming systems because they provide residual nitrogen and reduce the needs for mineral nitrogen fertilizers by associated non-legumes such as cassava and yam. Therefore, the aim of the present study was to determine the variation in nitrogen assimilation and biomass accumulation of cowpea accessions to cadmium pollution.

MATERIALS AND METHODS

Collection of seeds: This study was conducted between late October, 2016 and March, 2017 in the botanic garden of the Department of Plant Biology and Biotechnology, University of Benin, Benin city, Nigeria. The cowpea accessions used in the study were procured from the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria (Table 1). The accessions were selected based on their availability, agricultural input and relevance in legumes production in Nigeria.

Table 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* L.)	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

Table 2: Treatment designations for metal concentrations

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

Metal, soil collection and land preparation: The metal used in the experiment was cadmium in the form of cadmium chloride ($CdCl_2$) obtained from Pyrex Chemical Laboratory, Benin city, Nigeria. The soils were collected from 10 random points at the botanic garden from a depth of 0-30 cm using a soil auger and pooled together to obtain composite sample. The composite soil samples were air-dried and grind to pass through a 2 mm sieve before subjecting to physico-chemical analysis according to standard procedures. The soils were subsequently polluted with cadmium on the basis of its ecological screening value[19].

Treatment designation: The treatment designation for metal concentration was reported (Table 2). There were three treatments separated into 0ESV, Cd-2.5ESV and Cd-5ESV (Ecological screening value), respectively. The 0ESV 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 and Cd-5ESV being soil polluted with Cd at 5 times the ESV.

Experimental design: There were 3 blocks comprising of 15 treatments per block, 2 plant per polythene bag making a total of 90 plants arranged in a randomized block design (RBD). The plants' biomass accumulation was determined 20 weeks after sowing (WAS). Total nitrogen contents of plants leaves and roots were determined at seedling and flowering stage. The percentage of total nitrogen, ammonia-N and nitrate-N were also determined. The proportion of the total N uptake acquired as nitrate and ammonium were subsequently determined as percentages[20]. The formula used for computation of percentage nitrogen, ammonium nitrogen, nitrate nitrogen, nitrogen assimilated as nitrate and ammonia are given below:

$$\text{N-assimilation (\%)} = \frac{\text{Instrument reading} \times \text{Slope reciprocal} \times \text{Color volume} \times \text{Digest volume}}{\text{Weight of sample} \times \text{Aliquot taken}} \times 10000 \quad (1)$$

$$NH_4^+N \text{ (PPM)} = \frac{\text{Instrument reading} \times \text{Slope reciprocal} \times \text{Color volume} \times \text{Digest volume}}{\text{Weight of sample} \times \text{Aliquot taken}} \quad (2)$$

$$NO_3^-N \text{ (ppm)} = \frac{\text{Instrument reading} \times \text{Slope reciprocal} \times \text{Color volume} \times \text{Digest volume}}{\text{Weight of sample} \times \text{Aliquot taken}} \quad (3)$$

$$\text{N assimilated as nitrate (\%)} = \frac{\text{Nitrate nitrogen}}{\text{Total nitrogen (ppm)}} \times 100 \quad (4)$$

$$\text{N assimilated as ammonium (\%)} = \frac{\text{Ammonium nitrogen}}{\text{Total nitrogen (ppm)}} \times 100 \quad (5)$$

Statistical analysis: The SPSS-20® software was used in analyzing the data at $p = 0.05$. A one-way analysis of variance was adopted for data analyses. Results were presented as mean±standard error of mean ($n = 3$). Dendrogen was used to determine any genetic relationship between various legume genotypes.

RESULTS

Total nitrogen composition: The effect of cadmium on percentage total nitrogen (TN %) content in the leaves and root of cowpea accession 6 WAS and 18 WAS had been reported (Fig. 1). The Cd pollution resulted to a significant reduction in the foliar TN% of TVu's with increased metal incidence 6 WAS except in TVu-95. However 18 WAS, Cd incidence significantly increased ($p<0.05$) foliar TN% when compared to control irrespective of accession type. The Cd had no significant effect on root TN%. TVu-96 recorded the lowest values in percentage TN.

Nitrogen composition and distribution as nitrate-N or ammonium-N: Nitrogen distribution in the leaves and root of cowpea as nitrate-N or ammonia-N was shown in Fig. 2. The Cd pollution significantly reduced nitrate-N content of TVu-accessions when compared to the control. TVu-93 was more resistant to the effect ofCd pollution having little to no significant difference ($p<0.05$) in nitrate-N content between the Cd-5ESV and control in both foliar and root respectively. The distribution of Ammonia-N varied from one accession to the other irrespective of leaves or root. Cowpea accessions distributed nitrogen more as nitrate than ammonium both in the leaves and root respectively.

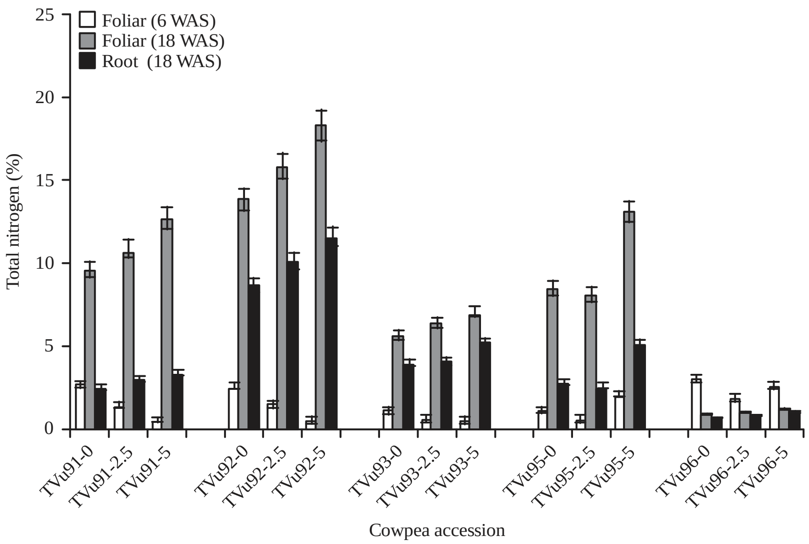

Fig. 1: Effect of cadmium on percentage total nitrogen content of cowpea

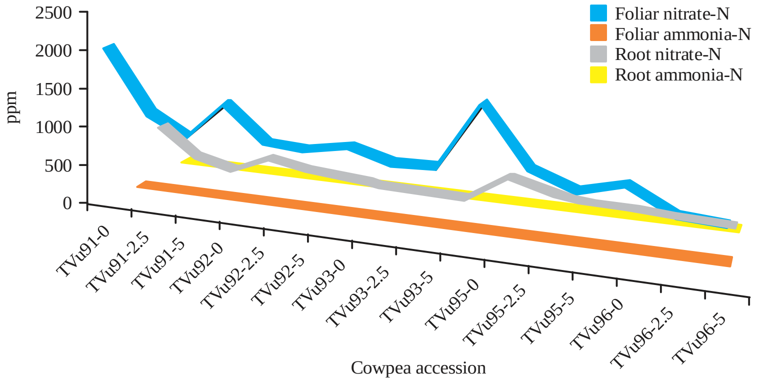

Fig. 2: Effect of cadmium on nitrogen distribution as nitrate-N and ammonium-N forms

Nitrogen assimilated as nitrate (%): The effect of cadmium pollution on percentage N assimilated as nitrate in cowpea accessions was shown in Fig. 3. The Cd pollution resulted to decrease N% assimilated as nitrate with increased metal concentrationwhen compared to the control irrespective of accessions type, however the effect was significantly reduced in TVu-95 and TVu-96. The highest percentage N assimilated as nitrate was recorded in TVu-96 when compared to those recorded in the other accessions..

Nitrogen assimilated as ammonium (%): The N% assimilated as ammonium in the accessions varied to Cd pollution (Fig. 4). The Cd pollution did not result in any significant difference ($p>0.05$) in the ammonium content of TVu-92 and TVu-93 when compared to the control. It was observed that TVu-96 assimilated more ammonium content both in leaves and root than the other accessions.

Effects of cadmium on biomass accumulation of cowpea: The biomass accumulation of cowpea accessions to Cd pollution is shown in Fig. 5. The Cd pollution reduced the overall foliar. The plant dry weight were reduced with increased Cd pollution. TVu-96 was more resistant to Cd toxicity than the other accessions.

Shoot:root ratio: The shoot to root ratio of cowpea accessions to cadmium pollution was shown in Fig. 6. The various accessions varied in their response to Cd pollution. Take for instance, the shoot:root ratio of TVu-91, TVu-92 and TVu-95 were significantly reduced ($p<0.05$) compared to an increase in TVu-93 and TVu-96 with increased metal concentration.

Dendrogram: The phylogenetic relationship of cowpea accessions exposed to cadmium was shown in Fig. 7. TVu-accessions in cluster A1 were more genetically related in

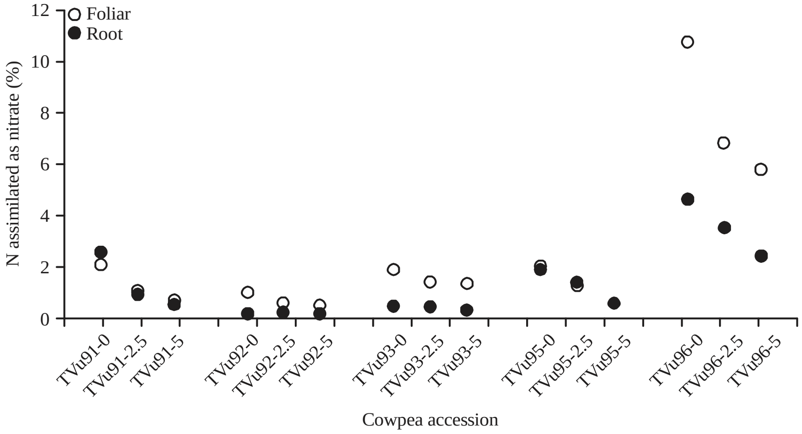

Fig. 3: Effect of cadmium on percentage nitrogen assimilated as nitrate

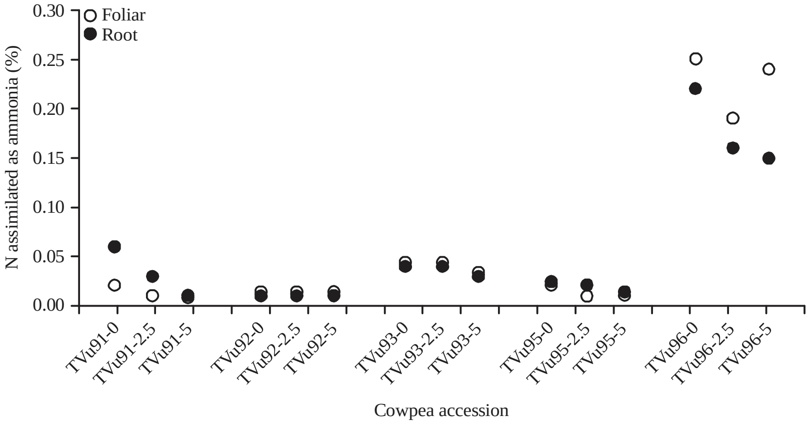

Fig. 4: Effect of cadmium on percentage nitrogen assimilated as ammonium

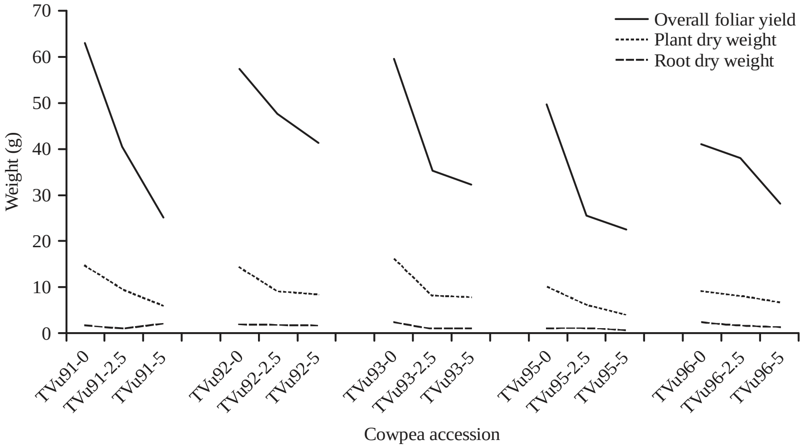

Fig. 5: Effect of cadmium on the biomass accumulation

their behavioral response than those in A2. However, accessions within cluster B1 were more closely related than those accessions in cluster E. The farther the hierarchical distance, the less related the Tvu-accessions.

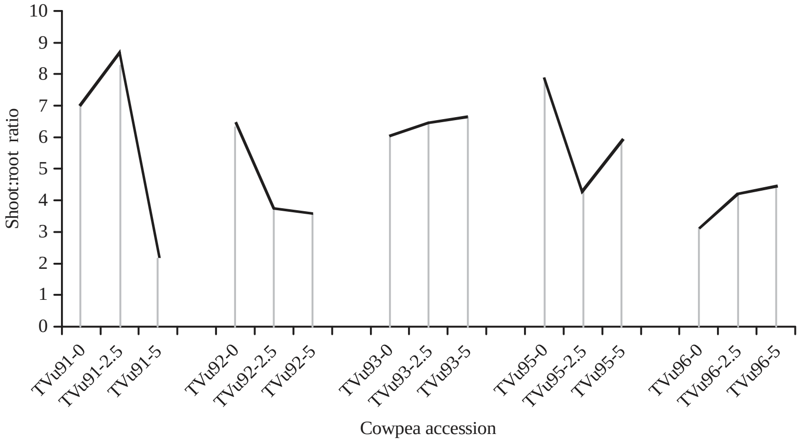

Fig. 6: Shoot:root ratio of cowpea accessions

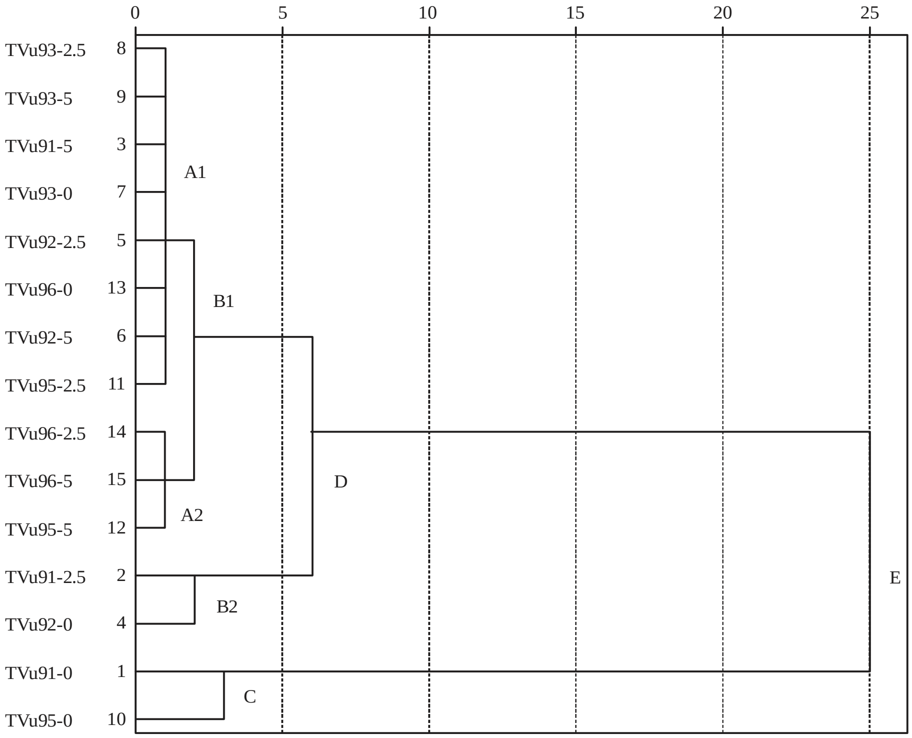

Fig. 7: Phylogenetic relationship of cowpea accessions

DISCUSSION

This experiment distinguished that Cd showed considerable effect on total nitrogen distribution in the foliar and root of cowpea accessions. In the present study, foliar TN% was reduced with increased Cd concentration, except[21] in TVu-95. It was reported that 18 WAS, TN% in the foliar and root increased with increased concentrations of Cd when compared to the control. This is in agreement with previous studies carried out on nitrogen distribution[1]. In this study, the importance of nitrogen was eminent in minimizing the osmotic stress attributed to Cd pollution[22] and its profound effect in elevating photo inhibition and photo damage[23]. The N was assimilated more as nitrate-N than ammonia-N and decreased with increased Cd concentration. The mitigating effects of Cd in increasing osmotic stress on plant were reported[24,25]. This may have resulted to the decreased values observed in nitrate and ammonia distribution in the Cd-2.5ESV and Cd-5ESV, respectively. Nitrate is an important N pool in plant[26]. The result in this study suggested that sufficient N uptake facilitated assimilation and distribution of nitrate to the leaf compared to ammonia, which could partly contribute in limiting the effect of osmotic stress caused by Cd. When N supply is sufficient, a considerable amount of nitrate is stored in vacuoles, which could be reutilized when N supply is limited[23]. This was also reported in this study. During the growth and development of plants, N is moved into and out of plants. Ammonium acts at the center of N flow in plant leaf[27]. The increased ammonia observed in TVu-96 compared to other accessions may be given to the large amount of ammonium produced[23]. It is essential that toxic ammonium is immediately reduced into organic molecules for nitrogen cycling[28]. Nitrate N is used in various processes, including absorption, vacuole storage, xylem transport, reduction and incorporation into organic forms[29]. The cowpea accessions absorbed higher rate of nitrate in leaves than root. According to Aslam *et al.*[30] plants with low rates of nitrate in roots export most of the absorbed nitrate to shoots, where it is reduced and incorporated into amino acids.

Nitrogen is a major macro-element limiting the growth and development of plants in agriculture. The yield of crop strongly depends on the supply of mineral nutrients, particularly nitrogen[31] which is a major macro-element in the growth and development of plants. Reduction in biomass accumulation occur due to physiological disturbances, cytogenetic damage[32] and limited nutrient supply enacted by Cd incidence. This is in agreement with previous studies carried out on heavy metal in reducing crop productivity[33,34]. The decrease in shoot:root ratio observed in TVu-91, TVu-92 and TVu-95 is in correlation with previously reported studies[35-37]. Elevated concentration of Cd in root environment was reported to reduce water and nutrient absorption, disturb water balance and cell metabolism[38]. Van Assche *et al.*[39] and Chaoui *et al.*[40] had earlier reported the sensitivity of legumes to elevated Cd levels however, it was reported in this study that the cowpea accessions varied in their response. The dendrogram illustrated the hierarchical distance that existed among the exposed accessions and was very informative in establishing the genetic relationship that existed in their response.

This study explored extensively the assimilation and distribution of nitrogen in the two most prominent forms available for plant uptake. The observation suggested that cadmium reduced the percentage assimilation of both nitrate and ammonia. In the future, genetic engineering should be used in enhancing plants' capacity to increase nitrogen intake. This will reduce dependence on phosphate fertilization, increase soil fertility and crop productivity. The dendrogram can be employed in the selection process of preferred crop species.

CONCLUSION

The nitrogen was distributed in both ammonia and nitrates form in the foliar and root respectively. TVu's accessions assimilated more nitrogen in the nitrate forms than ammonia in both foliar and root and differed across accessions. However, the nitrate-N were stored mainly in the leaves than the root which increased overall foliar yield. This proved that nitrate played an essential role in the general performance of the plant. It is important to state that the dendrogram highlighted the phylogenetic relationship that existed among the cadmium exposed accessions and their controls.

SIGNIFICANCE STATEMENT

This study discovered the pattern in which nitrogen is assimilated and distributed within the plant system of *Vigna unguiculata* L. Generally, the cowpea accessions assimilated nitrogen more as nitrate-N than ammonia-N. Assimilatory responses were more pronounced in the leaves than the root hence, increased protein for forage animals, fodders and food supplements. This study will help researcher, especially plant breeders and genetics in their selection process in identifying a resistant variety. Thus reducing food insecurity and creating a sustained environment for humanity.

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