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Research Article

Growth and Yield Assessment of Selected Cowpea (*Vigna unguiculata* L.) Genotypes to Elevated Iron Levels in a Ferruginous Ultisol

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Abstract

Background and Objective: The screening of iron-tolerant cultivar is imperative to sustain and improve overall cowpea production. This study investigated the growth and yield responses of selected cowpea (*Vigna unguiculata*) accession to iron toxicity in a ferruginous ultisol. **Materials and Methods:** Top soil (a ferruginous ultisol) was obtained from an undisturbed garden and sun-dried to constant weight. The soils were divided into 2 groups. One group was the selected ferruginous garden ultisol, whereas the iron level in the other group was elevated by twice the ecological screening benchmark of iron in agricultural soils (400 mg kg⁻¹). One week later, 15 accessions of cowpea, *Vigna unguiculata* (TVu-3742, TVu-3769, TVu-5348, TVu-5760, TVu-5768, TVu-5782, TVu-5883, TVu-6102, TVu-6193, TVu-6219, TVu-6290, TVu-10600, TVu-10881, TVu-11114 and TVu-11214) were sown in both iron-amended and control soils. **Results:** Twenty weeks later, results showed differential responses in carotenoids, lycopenes, NAR and DWP across accessions exposed to elevated iron levels. There was general growth suppression accessions planted in Fe-elevated soils ($p < 0.05$) with TVu-3742, 3769 and 6290 were the worst hit while elevated Fe-enhanced rooting parameters in TVu-3769 and 6219 were observed. Per plant yield in TVu-5760, 5768, 6102, 10600, 11114 and 11214 were significantly reduced by over 35%. However, no significant yield changes were reported for TVu-3742, 5768, 5782, 5883, 6193, 6219, 6290 and 10881 under elevated soil iron condition. **Conclusion:** Given the fact that the control soil was ferruginous, with iron levels higher than 1 g kg⁻¹, the reported capacities for selected accessions to maintain yield levels under further elevated iron conditions suggest possible iron tolerance for those tolerant accessions.

Key words: Antioxidant, ferruginous, growth responses, iron, ultisol, *Vigna unguiculata*

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Humans need proteins for proper development and regulation of all biochemical activities and so therefore, the drive for proteins requires acquisition through animal or meat sources as well as from plant or non-meat sources of protein¹. In many developing countries like Nigeria, where the citizenry are impoverished by the harsh economic situation, it is difficult to access the meat sources. Moreover, many health practitioners propose that over-reliance on meat may have led to cardiovascular and other health disorders; therefore further justifying why plant sources of protein may become a better and cheaper alternative^{2,3}.

Legumes of African origin have been shown to be among the best sources of non-meat proteins during austere times. World-wide, about 70 million people consume leguminous crops among these, cowpea is the most consumed. Cowpea [*Vigna unguiculata* (L.) Walp.] is widely cultivated in Nigeria for the high protein content of the edible grain. It is also an important component of the region's farming systems⁴. In the bid to achieve food security, the emphasis has always been hinged on the need to set up farms, improve auricular practices and storage facilities for harvested crops and tackles the menace of plant diseases and pests^{5,6}.

Crop productivity may be significantly affected by environmental and natural factors such as drought, excessive rainfall as well as edaphic factors. One of the most important factors is that which is attributed to anthropogenic interference on the ecosystem and an example is the incident of soil pollution particularly by industrial activities which lead to aggravated soil metal levels. Soil heavy metal concentrations may not however be entirely due to industrial activities as some soils are naturally ferruginous and hence have increasingly high levels of iron yet some others have increased levels of aluminium which predisposes such soils to increased soil acidity⁷.

Most soils in Edo state, Nigeria have been shown to be Ferruginous and laden with iron from the chemical and geological evidence of soils in Edo Central and South; most are ferric ultisols^{8,9}.

Iron toxicity is a problem associated primarily with crops grown on iron rich, low land, red and laterite soils and under these conditions, Fe^{3+} is reduced to Fe^{2+} , which is absorbed by the plant in larger quantities and causes Fe-toxicity when it exceed 300 mg/kg¹⁰⁻¹². More emphatically, in this study, iron (II) sulphate is selected not only to increase the levels of Fe^{2+} in the soil by which iron toxicity is most conveniently instigated, but also to create a sulphate-acidity situation in the soil, for which the effects of iron on plants are even graver¹². Iron

toxicity is particularly pronounced in acid sulfate soils¹³ or in soils with light texture, high extractable acidity and low fertility which has the tendency of disrupting plant metabolism, leading to overall damage of crop plant¹²⁻¹⁵.

The response of cowpea genotypes to multiple abiotic stresses was investigated by Singh *et al.*¹⁶ using atmospheric carbon dioxide (CO_2), temperature and UVB radiations and established the yield decreasing ability of abiotic stressors. Similarly, Gogile *et al.*¹⁷ screened for salt tolerant genotypes during the seedling growth stage of cowpeas and discovered that the extent of variation of genotypes response to elevated salt conditions varies with their genetic differences although the research centered on the prevailing salted soils in Ethiopia. Though it appears that numerous research works have been done on the impacts of abiotic stress on cowpea genotypes, it is necessary to state here that abiotic stresses are relatives and peculiar to geographical locations. Therefore, genotypes which are tolerant in a region predominantly exposed to certain abiotic stress may not be tolerant when exposed to different stress for which it was not investigated. Though, the idea of investigation of tolerant genotypes is too improve cowpea production in relation to the predominant abiotic stress(es) for which cowpea are exposed, there is a dearth of information on the selection of a tolerant genotype of cowpea to iron toxicity and most of the research studies were carried out *in vitro* and under controlled environment which may not be practicable in a farmland.

There is therefore need to investigate which among selected cowpea cultivars is best suited for cultivation in iron-toxic soils in order to sustain cowpea cultivation. The above information is only possible if a study on the individual plants' response to Fe stress is undertaken in a soil mimicking a farmland where cowpea is cultivated and produced. Hence, it is the aim of this study to investigate iron toxicity in cowpea as well as the identification of iron-tolerant genotypes using comparative growth and physiological responses of selected cowpea accessions.

MATERIALS AND METHODS

Experimental site and soil collection: The study was carried out in the Experimental Garden of the Department of Plant Biology and Biotechnology, University of Benin, Benin city, Nigeria. The duration of this study was between¹⁸ April-September, 2017. Top soil (5-10 cm) was obtained from the Departmental Botanic Garden and sun-dried to constant weight. Samples of this soil (an ultisol) were taken to the Lab for determination of selected physical and chemical characteristics prior to use according to methods described^{19,20}

Table 1: Physical and chemical properties of soil before application of treatments

Parameters	Soil content
pH	5.27
Electrical conductivity ($\mu\text{S cm}^{-1}$)	301.21
Total organic carbon (%)	0.49
Total nitrogen (%)	0.18
Exchangeable acidity (meq/100 g soil)	0.22
Na (meq/100 g soil)	10.90
K (meq/100 g soil)	1.48
Ca (meq/100 g soil)	14.32
Mg (meq/100 g soil)	12.01
NO_2^- (mg kg^{-1})	16.43
NO_3^- (mg kg^{-1})	30.01
Clay (%)	5.13
Silt (%)	7.06
Sand (%)	87.81
Fe (mg kg^{-1})	1011.92

and the result obtained is reported in Table 1. The soils were eventually measured (20 kg) into holding plastic experimental bags.

Experimental design: The soils were divided into 2 groups: the first group being contaminated with iron using iron sulphate (Fe^+GS) and the second group which was not contaminated with iron (GS-only). Addition of iron was to the extent that iron level was raised by a factor of twice the ecological screening benchmark of iron in agricultural soils ($\text{ESV} = 200 \text{ mg kg}^{-1}$)²¹.

After one week following exposure of soil to FeSO_4 , 15 accessions of cowpea (*Vigna unguiculata*): TVu-3742, TVu-3769, TVu-5348, TVu-5760, TVu-5768, TVu-5782, TVu-5883, TVu-6102, TVu-6193, TVu-6219, TVu-6290, TVu-10600, TVu-10881, TVu-11114 and TVu-11214 were sown in both iron-elevated ($\text{Fe}+\text{GS}$) and control (GS-only) soils.

The plants in experimental bags were constantly weeded and carefully irrigated every other day with 500 mL of water (pH 6.6-6.8) especially during dry and hot days. Care was taken to ensure that soil moisture level was adequate for plant development, following laid out procedures.

The experimental design chosen was the completely randomized design (CRD) following assumption of homogeneity of the experimental plot in use. As a result, treatments were randomized over the whole plot in the screen house. Each treatment consisted of 5 replicates. In order to avoid bias and misidentification, treatment bowls were properly labelled.

Data collection: Plant growth and yield responses including plant height, stem branching, leaf number and area, root length and branching as well as seed yield per plant were monitored⁷. Chlorophyll-a, chlorophyll-b, tocopherol, lycopene

and carotenoids contents of plant leaves were determined during flowering with a view to assessing plant productivity capacity on the basis of pigment availability^{22,23}. Most of the laboratory experiments were performed at the Environmental Biotechnology and Sustainability Research (EBSR) laboratory, University of Benin, Benin-city, Nigeria.

Data analysis: Results were therefore analysed using SPSS-20® statistical software for one-way ANOVA, principal components analysis as well as hierarchical cluster analyses where necessary with the levels of significance at 5% ($p = 0.05$).

RESULTS

Physical and chemical properties of soil: Analysis of the garden soil used for the experiment was slightly acidic (pH of 5.27), with an electric conductivity of $301.21 \mu\text{S cm}^{-1}$, a total nitrogen content of 0.18%, high sandy content of 87.81% and a low clayey and silt contents of 5.13 and 7.06%, respectively. It is a ferruginous soil with high iron content ($\text{Fe} = 1011.92 \text{ mg kg}^{-1}$) prior to use (Table 1).

Effects of treatment on growth parameters of cowpea:

Twenty weeks after exposure of plant to elevated Fe concentration, results showed significant negative impact on growth parameters (Table 2). Plant heights in control plants were significantly higher than those in Fe-amended soils ($p < 0.05$) however, no marked differences in plant heights were reported between Fe-exposed and control plants in TVu-5883, 6102 and 10881. Significant reductions in leaf number, leaflet area, length of main root and root dry weight was reported for Fe-exposed accessions. Most of these accessions showed growth repression due to addition iron exposure in the ferruginous soil, but TVu-3742, 3769 and 6290 were the worst hit. Exposure to iron significantly reduced leaf area index to 0.079-0.085 from 0.099-0.127 in the control plants. There were minimal changes in relative growth rates of metal-exposed plants, compared to the control. Net assimilation rate was higher for some metal-exposed accessions than their control counterparts (TVu-3742, 3769, 5348, 5768, 5718, 5883, 6102, 6193, 10881, 11114 and 11214) and lower for others (TVu-5760, 6219 and 10600). Generally, net assimilation rate ranged from $1.7\text{-}4.5 \text{ mg cm}^{-2} \text{ day}^{-1}$ all-together (Table 3).

Effects of treatment on yield capacity of cowpea accessions:

The effects of treatment on yield capacity of cowpea accessions were presented in Table 4. Exposure of accessions

Table 2: Effects of treatment on growth parameters of cowpea at 20 WAS

Plant accession	Plant status	Shoot length (cm)	No. leaves /plant *	Leaflet area (cm ²)	Main root length (cm)	No. of primary root branches*	Root dry wt. (g)	Shoot dry wt. (g)	Per plant nodule yield (g)
TVu-3742	Fe+GS	52.90	26	25.21	16.51	24	10.48	23.34	0.72
	GS-only	62.20	36	35.22	14.11	30	13.65	27.42	1.02
TVu-3769	Fe+GS	48.10	28	21.23	13.85	31	11.65	33.74	1.05
	GS-only	69.70	23	32.33	19.25	27	11.24	30.40	2.52
TVu-5348	Fe+GS	49.76	29	25.32	10.24	18	17.76	24.55	0.43
	GS-only	56.56	37	32.23	12.55	20	13.22	26.82	1.15
TVu-5760	Fe+GS	40.30	32	25.56	12.73	23	11.16	29.5	0.94
	GS-only	57.43	42	30.72	12.21	35	17.2	29.91	1.16
TVu-5768	Fe+GS	55.43	24	23.74	11.52	17	17.32	29.83	0.43
	GS-only	65.44	28	29.43	12.65	20	16.13	24.83	0.95
TVu-5782	Fe+GS	52.32	25	25.23	11.24	18	10.1	25.61	0.67
	GS-only	58.77	37	32.34	18.54	23	10.43	22.48	1.04
TVu-5883	Fe+GS	63.24	21	22.53	17.33	19	6.88	27.84	0.76
	GS-only	65.66	35	39.38	21.53	29	14.47	29.51	2.24
TVu-6102	Fe+GS	59.32	21	25.33	16.61	20	9.41	23.85	0.84
	GS-only	58.65	34	32.34	8.54	20	9.3	29.3	1.25
TVu-6193	Fe+GS	59.54	23	28.32	16.84	28	9.28	25.66	0.92
	GS-only	63.46	39	32.44	17.56	26	13.43	31.34	1.64
TVu-6219	Fe+GS	53.25	26	25.43	18.52	19	9.5	23.84	0.32
	GS-only	59.67	44	29.32	9.53	17	11.54	27.3	0.84
TVu-6290	Fe+GS	52.34	27	24.33	9.18	33	8.95	21.87	0.52
	GS-only	61.01	32	32.54	10.57	28	12.04	25.46	0.79
TVu-10600	Fe+GS	49.56	36	29.32	10.36	26	9.77	21.5	1.12
	GS-only	63.12	32	32.46	12.44	29	10.76	28.65	1.53
TVu-10881	Fe+GS	51.34	27	23.33	12.86	27	10.95	27.94	1.37
	GS-only	53.56	32	29.42	14.74	30	10.67	27.92	1.65
TVu-11114	Fe+GS	49.45	22	21.26	10.24	20	10.94	22.44	0.94
	GS-only	53.53	37	28.32	12.63	22	14.52	20.66	2.02
TVu-11214	Fe+GS	54.36	21	26.43	12.13	22	12.98	23.36	1.22
	GS-only	59.42	34	32.43	8.76	22	12.16	22.54	2.45
p-value		0.007	0.000	0.000	0.856	0.234	0.724	0.290	0.034
LSD (0.05)		5.01	10	10.93	14.43	12	9.64	9.21	0.39

to additional Fe prolonged the time required for 50% maturity from a low as 45 days to as high as 58 days. There were between 2 and 3 pods per peduncle ($p>0.05$). There was no significant on seed number pod however significant reduction in plant yield was adduced to iron exposure ($p<0.05$). Per plant yield in TVu-5768, 6102, 10600 and 11114 were significantly reduced by 35-50% while the reduction in TVu-5760 and 11214 is over 50%. Plant yield was not significantly affected in TVu-3742, 5768, 5782, 5883, 6193, 6219, 6290 and 10881.

Effects of treatment on seed characteristics: Significant changes in physical appearances of seeds after harvest were presented in Table 5. As reported herein, the parameters are not necessarily uniform characteristics of the entire seeds harvested, but the most pre-dominant among other characteristics. Seed shapes in TVu-3742, 5760, 5883, 6290 and 11114 were not affected iron exposure. Testa texture was also affected as changes from smooth texture to rough and vice versa was observed. There were similar changes in testa basal color and the presence of cavity on pods was observed for all plants accessions.

Effects of treatment on pigmentation and antioxidation:

The effects of treatment on some pigmentation parameters of developed emergent of Cowpea at 42 days after sowing were shown in Table 6. No significant changes were observed in plant chlorophyll. The same was reported for tocopherol ($0.1939\text{--}2.3610\text{ mg g}^{-1}$) and lycopene contents ($102.68\text{--}216.84\text{ mg g}^{-1}$) while total carotenoids ($0.828\text{--}1.900\text{ g g}^{-1}$) were significantly reduced.

Effects of treatment on germination: There were significant differences in plant dry weight production (DWP) as shown in Fig. 1. Generally, TVu-5768 showed better DWP (0.33 g day^{-1}) among the iron-exposed plants, whereas TVu-10600 was least (0.15 g day^{-1}). Figure 2 shows the net assimilation rate of accessions following plant's exposure to treatments at 20 WAS. There is a differential response in the Fe-exposed groups across all the accessions although these responses are not statistically significant.

Correlation analysis: Bivariate correlation coefficients were computed for 13 parameters associated with pigmentation,

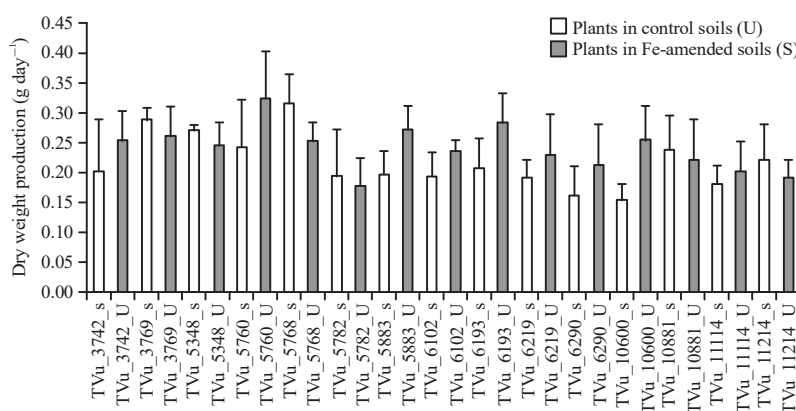


Fig. 1: Dry weight production of cowpea accessions after 20 weeks after exposure to iron contamination
Data are represented as (Mean $\pm$ SD)

Table 3: Presentation of plant growth indices as affected by plant's exposure to treatments at 20 WAS

Plant accessions	Plant status	Leaf area index	Relative growth rate (g day ⁻¹)	Net assimilation rate (mg cm ⁻² day ⁻¹)	Leaf area ratio (cm ² g ⁻¹)	Leaf area duration
TVu-3742	Fe+GS	0.081	0.016	2.6	0.447	1.789
	GS-only	0.114	0.018	2.4	0.388	1.789
TVu-3769	Fe+GS	0.068	0.019	4.5	0.712	1.789
	GS-only	0.104	0.018	2.7	0.429	1.789
TVu-5348	Fe+GS	0.082	0.019	3.5	0.557	1.789
	GS-only	0.104	0.017	2.5	0.413	1.789
TVu-5760	Fe+GS	0.082	0.016	3.1	0.530	1.789
	GS-only	0.099	0.025	3.5	0.511	1.789
TVu-5768	Fe+GS	0.077	0.023	4.4	0.662	1.789
	GS-only	0.095	0.018	2.9	0.463	2.088
TVu-5782	Fe+GS	0.081	0.013	2.5	0.471	1.790
	GS-only	0.104	0.013	1.8	0.339	2.295
TVu-5883	Fe+GS	0.073	0.014	2.9	0.513	1.598
	GS-only	0.127	0.017	2.3	0.372	2.794
TVu-6102	Fe+GS	0.082	0.015	2.5	0.437	1.797
	GS-only	0.104	0.017	2.4	0.397	2.295
TVu-6193	Fe+GS	0.091	0.016	2.4	0.411	2.009
	GS-only	0.105	0.019	2.9	0.460	2.302
TVu-6219	Fe+GS	0.082	0.015	2.5	0.437	1.804
	GS-only	0.095	0.016	2.6	0.441	2.080
TVu-6290	Fe+GS	0.078	0.012	2.2	0.422	1.726
	GS-only	0.105	0.014	2.2	0.384	2.309
TVu-10600	Fe+GS	0.095	0.010	1.7	0.355	2.080
	GS-only	0.105	0.020	2.6	0.404	2.303
TVu-10881	Fe+GS	0.075	0.017	3.4	0.555	1.655
	GS-only	0.095	0.014	2.5	0.437	2.087
TVu-11114	Fe+GS	0.069	0.013	2.8	0.523	1.508
	GS-only	0.091	0.015	2.3	0.414	2.009
TVu-11214	Fe+GS	0.085	0.017	2.8	0.458	1.875
	GS-only	0.105	0.013	1.9	0.356	2.301

productivity and yield for every two selected parameters to ascertain level of correlation at 2-tailed (Table 7). Leaflet area showed a positive significance with shoot length and No. of leaves plant ($p < 0.01$). Total lycopene showed a weak negative statistical significance with No. of leaves plants;

$p < 0.05$, positive significance with chlorophyll a and total carotenoids; $p < 0.01$. Per plant yield shown a positive significance with number of plant leaves, leaflet area, nodule yield and No. of seeds per pod (0.429, 0.412, 0.438; $p < 0.05$ and 0.512; $p < 0.01$, respectively).

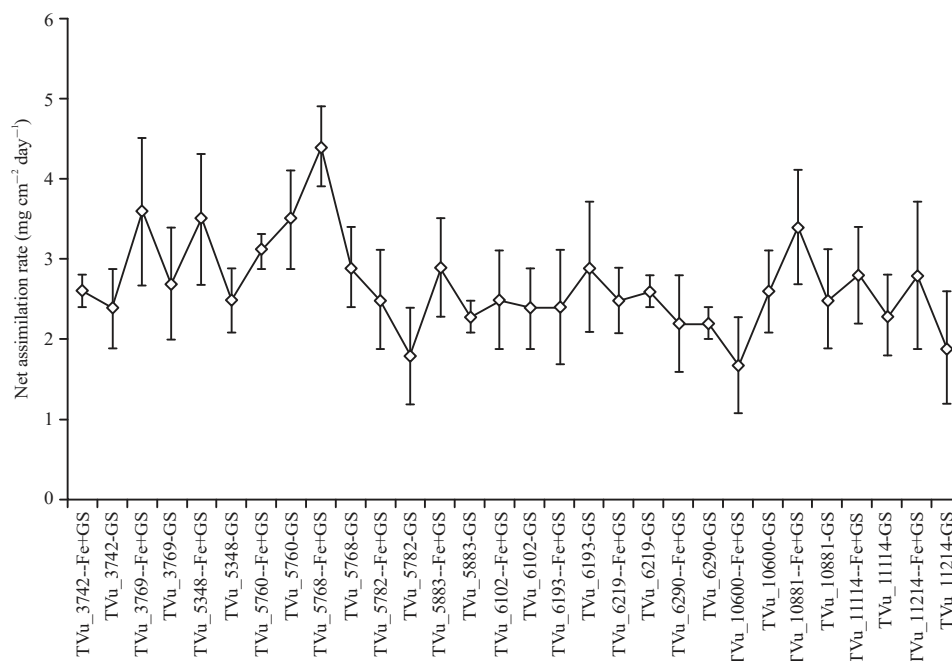


Fig. 2: Net assimilation rates of accessions following plant's exposure to treatments at 20 weeks after seedling

Table 4: Effects of treatment on yield capacity of cowpea (n = 5)

Plant accessions	Plant status	Days to 50% maturity (days)*	No. of peduncle/plant*	Length of peduncle (cm)	Pods/peduncle*^	No. of viable pods/plant**	Seed No./pods*	Mean seed wt. (g)	Per plant yield (g)
TVu-3742	Fe+GS	52	10	8.53	3	12	9	0.15	16.98
	GS-only	55	12	9.21	2	14	11	0.12	18.16
TVu-3769	Fe+GS	59	11	10.33	2	16	7	0.13	14.56
	GS-only	53	14	9.76	3	21	9	0.12	22.68
TVu-5348	Fe+GS	52	14	8.34	2	14	8	0.11	13.96
	GS-only	52	16	7.64	2	18	8	0.14	21.42
TVu-5760	Fe+GS	56	13	9.02	2	13	11	0.10	14.94
	GS-only	49	14	9.25	3	18	13	0.15	33.21
TVu-5768	Fe+GS	56	10	10.22	3	11	7	0.08	7.43
	GS-only	50	12	11.42	3	13	8	0.11	12.83
TVu-5782	Fe+GS	55	15	6.24	2	10	8	0.11	8.42
	GS-only	48	18	7.24	2	11	8	0.13	12.06
TVu-5883	Fe+GS	47	14	10.32	3	15	10	0.09	14.66
	GS-only	45	16	8.73	2	14	9	0.11	14.23
TVu-6102	Fe+GS	58	13	7.32	3	17	10	0.11	17.82
	GS-only	51	15	10.33	2	20	8	0.17	27.52
TVu-6193	Fe+GS	53	10	7.85	2	12	10	0.13	15.12
	GS-only	49	12	7.48	3	16	9	0.12	16.88
TVu-6219	Fe+GS	53	9	11.23	2	13	9	0.08	10.52
	GS-only	53	13	10.32	3	16	8	0.12	14.75
TVu-6290	Fe+GS	56	10	9.24	2	11	11	0.13	15.46
	GS-only	58	15	7.34	3	15	9	0.13	17.95
TVu-10600	Fe+GS	56	12	8.43	2	14	8	0.08	9.18
	GS-only	50	15	10.43	2	21	7	0.13	17.22
TVu-10881	Fe+GS	55	14	8.87	3	12	7	0.11	8.85
	GS-only	52	14	7.53	2	17	9	0.09	13.46
TVu-11114	Fe+GS	57	13	10.02	2	15	8	0.11	12.30
	GS-only	51	12	9.24	2	19	8	0.15	21.85
TVu-11214	Fe+GS	53	10	9.40	3	11	7	0.11	8.32
	GS-only	49	13	8.37	2	15	9	0.13	16.80
p-value		0.003	0.003	0.883	0.996	0.001	0.709	0.013	0.001
LSD(0.05)		5	3	6.45	2	5	5	0.03	5.05

*Mean has been rounded off to the nearest whole number. **Only pods with viable seeds therein were counted, otherwise ignored. ^This took both viable and non-viable pods into account

Table 5: Presentation of the most prominent seed parameters among cowpea seeds harvested from iron-exposed and control plants

Plant accession	Plant status	Shape of seeds	Testa texture	Seed cavity ridges on pods	Testa basal colour
TVu-3742	Fe+GS	Rhomboid	Rough	Present	Light brown (6D8)
	GS-only	Rhomboid	Smooth	Present	Light brown (6D8)
TVu-3769	Fe+GS	Oblong	Rough	Present	Light brown (6D8)
	GS-only	Rhomboid	Rough	Present	Cream (4A3)
TVu-5348	Fe+GS	Oblong	Rough	Present	Light brown (6D8)
	GS-only	Round/globular	Smooth	Present	Light brown (6D8)
TVu-5760	Fe+GS	Rhomboid	Smooth	Present	Grey (E1)
	GS-only	Rhomboid	Smooth	Present	Grey (E1)
TVu-5768	Fe+GS	Rhomboid	Smooth	Present	Grey (E1)
	GS-only	Oblong	Smooth	Present	Grey (E1)
TVu-5782	Fe+GS	Rhomboid	Smooth	Present	Cream (4A3)
	GS-only	Oblong	Smooth	Present	Cream (4A3)
TVu-5883	Fe+GS	Oblong	Rough	Present	Light brown (6D8)
	GS-only	Oblong	Rough	Present	Grey (E1)
TVu-6102	Fe+GS	Round/globular	Rough	Present	Cream (4A3)
	GS-only	Rhomboid	Smooth	Present	Cream (4A3)
TVu-6193	Fe+GS	Round/globular	Rough	Present	Cream (4A3)
	GS-only	Oval	Smooth	Present	Cream (4A3)
TVu-6219	Fe+GS	Oblong	Smooth	Present	Grey (E1)
	GS-only	Oval	Smooth	Present	Grey (E1)
TVu-6290	Fe+GS	Oval	Smooth	Present	Grey (E1)
	GS-only	Oval	Rough	Present	Light brown (6D8)
TVu-10600	Fe+GS	Oval	Rough	Present	Cream (4A3)
	GS-only	Round/globular	Rough	Present	Cream (4A3)
TVu-10881	Fe+GS	Oblong	Smooth	Present	Dark brown (6F8)
	GS-only	Rhomboid	Rough	Present	Dark brown (6F8)
TVu-11114	Fe+GS	Oval	Smooth	Present	Grey (E1)
	GS-only	Oval	Rough	Present	Grey (E1)
TVu-11214	Fe+GS	Rhomboid	Rough	Present	Light brown (6D8)
	GS-only	Oblong	Smooth	Present	Grey (E1)

Table 6: Effects of treatment on chlorophyll and antioxidant composition of developed emergent of cowpea at 42 days after sowing

Plant accession	Plant status	Chlorophyll-a (mg g ⁻¹)	Chlorophyll-b (mg g ⁻¹)	Tocopherol (mg g ⁻¹)	Total carotenoids (g g ⁻¹)	Total lycopene (mg g ⁻¹)
TVu-3742	Fe+GS	0.1478	0.1049	0.1939	0.123	168.48
	GS-only	0.1519	0.0675	1.3050	0.162	157.56
TVu-3769	Fe+GS	0.1458	0.0501	0.0602	0.102	118.56
	GS-only	0.1478	0.0797	0.4009	0.157	152.88
TVu-5348	Fe+GS	0.1503	0.0406	0.1021	0.124	132.6
	GS-only	0.1494	0.0855	1.5853	0.09	102.68
TVu-5760	Fe+GS	0.1485	0.104	1.8186	0.109	107.64
	GS-only	0.1461	0.1401	0.8385	0.155	165.36
TVu-5768	Fe+GS	0.1499	0.0691	1.7137	0.12	140.4
	GS-only	0.1488	0.765	2.3610	0.097	101.12
TVu-5782	Fe+GS	0.1441	0.038	0.8988	0.087	112.04
	GS-only	0.1431	0.0472	1.7662	0.113	138.84
TVu-5883	Fe+GS	0.1489	0.0875	1.6430	0.196	235.56
	GS-only	0.1489	0.0553	0.4061	0.138	165.36
TVu-6102	Fe+GS	0.1495	0.0705	0.5371	0.158	163.8
	GS-only	0.1471	0.1103	0.4978	0.13	137.28
TVu-6193	Fe+GS	0.1466	0.1176	1.7609	0.151	148.2
	GS-only	0.1423	0.1182	1.6132	0.122	142.2
TVu-6219	Fe+GS	0.1455	0.1293	0.9590	0.095	129.48
	GS-only	0.1323	0.0337	0.959	0.083	79.56
TVu-6290	Fe+GS	0.1402	0.0277	0.1912	0.093	119.84
	GS-only	0.1442	0.2682	1.5644	0.104	110.48
TVu-10600	Fe+GS	0.1507	0.0533	0.6656	0.154	165.36
	GS-only	0.1477	0.0456	0.0340	0.14	166.92
TVu-10881	Fe+GS	0.1560	0.0713	0.1310	0.135	156.21
	GS-only	0.1484	0.0943	1.7006	0.175	176.28
TVu-11114	Fe+GS	0.1447	0.1186	0.4190	0.19	201.24
	GS-only	0.1464	0.1194	0.8590	0.125	132.61
TVu-11214	Fe+GS	0.1522	0.0547	0.4669	0.187	216.84
	GS-only	0.1398	0.1799	0.9270	0.146	151.32
p-value		0.251	0.201	0.332	0.631	0.121

NA: Not applicable

Table 7: Bivariate correlation of pigmentation, growth and productivity parameters

Parameters	Shoot length	No leaves plant	Leaflet area	Weight of dry root	Shoot weight	Nodule yield	Seed no pods	Chloro- phyll_a	Chloro- phyll_b	Toco- pherol	Total carotenoids	Total lycopene	Per plant yield
Shoot length cm	1												
No leaves plant	0.145	1											
Leaflet area	0.584**	0.618**	1										
Weight of dry root	0.018	-0.209	-0.135	1									
Shoot weight	0.042	0.138	0.144	0.373*	1								
Nodule yield	0.321	0.163	0.293	0.026	0.302	1							
Seed no pods	0.126	0.156	0.158	-0.300	0.045	0.388*	1						
Chlorophyll-a	-0.199	-0.299	-0.170	0.033	-0.259	-0.289	-0.039	1					
Chlorophyll-b	0.257	-0.068	0.079	-0.042	-0.149	-0.081	-0.001	0.157	1				
Tocopherol	0.174	0.049	0.110	-0.273	-0.074	0.021	0.188	0.250	0.513**	1			
Total carotenoids	0.188	-0.321	-0.025	-0.110	-0.046	0.178	0.163	0.124	-0.156	-0.090	1		
Total lycopene	-0.042	-0.438*	-0.197	-0.125	-0.194	-0.084	0.035	0.608**	-0.158	-0.057	0.796**	1	
Per plant yield	0.205	0.429*	0.412*	-0.006	0.251	0.438*	0.512**	-0.053	0.036	-0.095	0.058	-0.070	1

*Correlation is significant at the 0.05 level (2-tailed). **Correlation is significant at the 0.01 level (2-tailed)

Principal component and cluster analysis: A rotated component matrix of the principal component analyses conducted identified total carotenoids and total lycopene (component-1) as well as chlorophyll-a (component-2). The KMO measure of sampling adequacy amounted to 0.354 and Bartlett's test of sphericity of 0.000 showing the results of the PCA were considered fair²⁴. Also presented was a dendrogram from a hierarchical cluster analysis of parameters (Fig. 4). Results from the dendrogram captured control TVu-3742 and Fe-exposed TVu-6193 as closest (Cluster I) while Fe-exposed TVu-3742 is in cluster with TVu-10881 (Cluster III). The result observably showed most of the closets clusters formed were between plants in similar soil conditions as a clear demarcation is shown between TVu-6193 in Fe-elevated and TVu-5348 in control (Cluster II).

DISCUSSION

Iron is a critical nutrient required by plants. Its importance is associated with its roles in the electron-transport chains of photosynthesis and respiration; its capacity to accept and donate electrons is quite noted²⁵. However, when accumulated at significant levels, they present a number of phytotoxic effects. One of such, as reported by Connolly and Guerinot²⁵, is its ability to act catalytically using the Fenton reaction to generate hydroxyl radicals; these radicals are known for impairing structures and functions of lipids, proteins and DNA.

Analysis of the garden soil used for the experiment showed the sample soil was slightly acidic (pH of 5.27) with a high sandy content of 87.81%. This naturally should favor the cultivation of cowpea but it is ferruginous (Fe = 1011.92 mg kg⁻¹) which may be a limiting factor. Twenty weeks after exposure of plant to elevated Fe concentration, results showed significant negative impact on growth parameters; leaf number, leaflet area, length of main root and root dry weight. The effects of treatment on yield capacity showed that per plant yield in TVu-5768, 6102, 10600 and 11114 were significantly reduced by over 35-50% while the reduction in TVu-5760 and 11214 is over 50%. However, plant yield was not significantly affected in TVu-3742, 5768, 5782, 5883, 6193, 6219, 6290 and 10881. Therefore, these accessions are perhaps best suited for cultivation in highly ferruginous soils. Exposure of plant accessions to iron present significant changes in physical appearances of seeds after harvest. Pigmentation parameters of developed emergent of Cowpea at 42 days after sowing however showed no

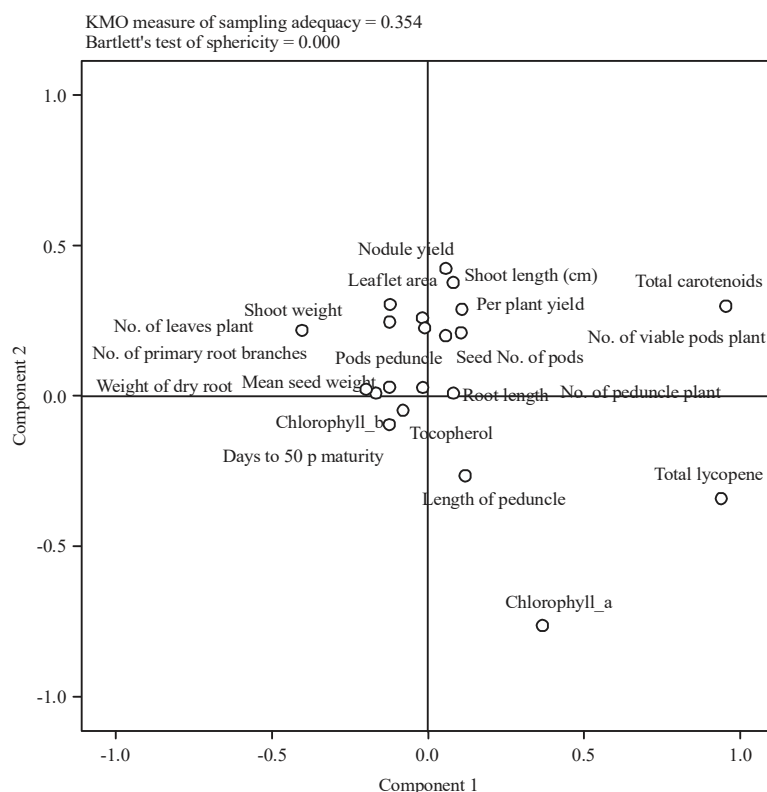


Fig. 3: Rescaled rotated component plot from principal component analysis

significant changes was observed in plant chlorophyll, tocopherol and lycopene contents while total carotenoid were significantly reduced. Differential responses were observed for germination parameters however, it is expected that accession with same genetic make-up should cluster. For example, Tvu-5348(U) is expected to form cluster with Tvu-5348(S) and Tvu-11114(U) with Tvu-11114(S) but the principal component analysis observably showed most of the closets clusters formed were between accessions in similar soil conditions as a clear demarcation in their response to the iron treatments.

Suresh²⁶ reported that toxicity of Iron is a yield decreasing physiological disorder ascribed to the unnecessary uptake of Fe. It is not clear if changes in ability for each of the accessions to access soil nutrients may account for significant differences in growth and yield parameters. However, Mitra *et al.*¹² earlier reported that increased iron in soil impedes N, P, K uptake by plants that generally would affect that plant's development and yield dispositions. The results obtained from this study are in line with the findings of Eun *et al.*²⁷, Jamal *et al.*²⁸, Kopittke *et al.*²⁹ and Egharevba³⁰.

Malkowski *et al.*³¹ showed that the presence of heavy metals in higher concentration in the soil can also induce

lower yield production by stressed plants. The plant yield is a crucial determining factor in the cultivation of plants and some correlation between the severity of iron-toxicity symptom expression and yield were observed. This result is in line with the findings of Kopittke *et al.*²⁹ and Ganesh and Selvaraju³². An attempt to pin-point the exact mechanism(s) by which this reduction in growth and yield parameters in the cowpea accessions occurs may be difficult but with an understanding of the fact that metals induce toxicity by restricting the plants ability to reproduce through cell division and elongation, it is safe to say that iron toxicity also follow same metal toxicity path^{27,31}.

Exposure of plant accessions to iron presented significant changes in physical appearances of seeds after harvest. As reported herein, the parameters are not necessarily uniform characteristics of the entire seeds harvested, but the most predominant among other characteristics. Measurement of the contents of chlorophyll and its accessory pigments such as lycopenes and carotenoids in plants is one of the ways of assessing the presence of heavy metals in plants as well as a potent indicator of plants undergoing abiotic stress. Zengin and Munzuroglu³³ investigated the effects of some heavy metals on the chlorophyll, proline and tocopherol contents in

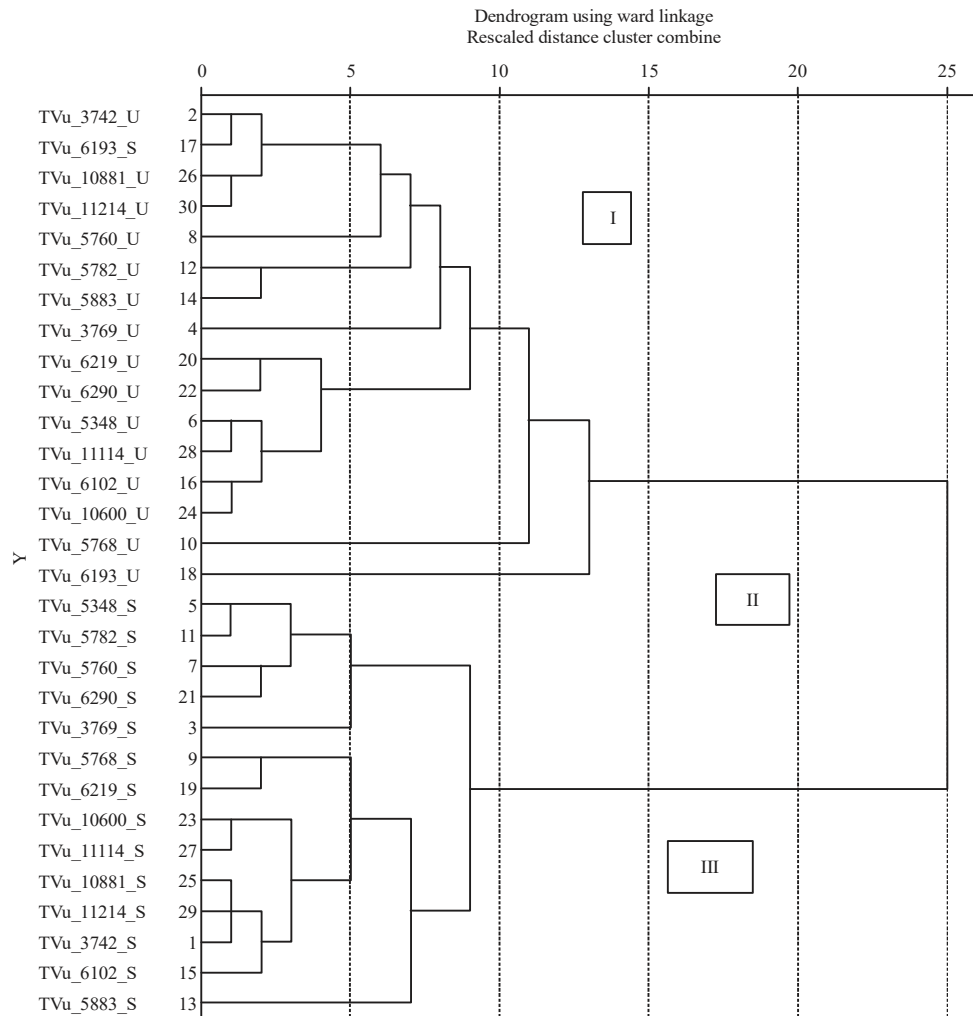


Fig. 4: Dendrogram from hierarchical cluster

Phaseolus vulgaris and reported a dose-dependent increase in the accessory pigments especially tocopherol. However, the effects of treatment on some pigmentation parameters of developed emergent of cowpea at 42 days after sowing showed no significant changes in plant chlorophyll which is inconsistent with the findings of Zengin and Munzuroglu³³. Without adequate iron levels, plants is expected to express apical leaf chlorosis and slower root growth but with various tolerance mechanisms, differential responses may not be unexpected.

The presence of free radicals and reacting oxygen species from excess iron levels may induce a number of strategies to keep iron levels at bay or to harvest and detoxify free radical produced³⁴. In the present study, no significant increased levels of antioxidant related to the chlorophyll apparatus (lycopene, carotenoids and tocopherol)

were reported, perhaps implying that plants may have adopted other measure for guarantying plant survival.

In an attempt to pin-point on very relevant parameters among the few assessed during the study, a principal component analyses was conducted³⁵. Results presented shows from a presented rotated component matrix, total carotenoids and total lycopene were identified (component-1) as well as chlorophyll-a (component-2). This is not unexpected as lycopene is an accessory pigment used by plants during stressed conditions when chlorophyll utilization is impaired^{34,36}. Though the results and discoveries of this study are in consistence with the findings of Gogile *et al.*¹⁷ and Akande *et al.*¹⁸, the discoveries of tolerant accessions to iron overload will be of better contribution to the cultivation of cowpea because they were planted in a field with similar conditions needed for their cultivation.

Notably, the control soil was ferruginous, with iron levels higher than 1 g kg⁻¹, evidence of iron toxicity measured by selected morphological growth responses was reported at different growth stages-during the vegetative growth and at reproductive stages, culminating in different yield dispositions.

CONCLUSION

This study therefore discovered the capacities of genotypes TVu-3742, 5768, 5782, 5883, 6193, 6219, 6290 and 10881 to maintain yield levels under further elevated iron conditions and thus suggesting possible iron tolerance for those accessions. It is recommended that these genotypes can be cultivated in iron-toxic soils for improved productivity of cowpeas although further studies into the hormonal and molecular mechanisms involved in their tolerance in the tolerant genotypes are needed as these will uncover insights into the management of cowpeas cultivated in elevated iron soils for improved yields.

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