Journal of Underutilized Legumes
Vol. 1 (1): 34 - 55

Society for Underutilized Legumes
https://sulegumes.org/
e-ISSN: 2705-3776

Nitrogen assimilation in young African yam bean plant in a ferruginous ultisol

[1]Ikoughe G, [1,2]Ikhajiagbe B, [1]Ohanmu EO, [1]Ekhator PO, and [1]Okoye PC

[1]Environmental Biotechnology and Sustainability Research Group, Department of Plant Biology and Biotechnology, University of Benin, Nigeria
[2]Applied Environmental Biosciences and Public Health Research Group, Department of Microbiology, University of Benin, Nigeria

E-mail: ekhatorp3@gmail.com

Received November 25, 2018
Accepted for publication June 21, 2019
Published July 7, 2019

Abstract

The aim of the study was to investigate nitrogen assimilation in young African yam bean (*Sphenostylis stenocarpa*) plant in a ferruginous ultisol. Twenty (20) kg of the sun-dried top soil was measured into thirty (30) large experimental bags and arranged in the experimental farm following a completely randomized design. The ferruginous status of the soil was ascertained upon soil chemical analysis to content to be 1011.92 mg/kg. Four seeds each of nine accessions of viable African yam bean (TSs-141, TSs-142, TSs-143, TSs-144, TSs-145, TSs-146, TSs-147, TSs-148, and TSs-149) were sown in each bag. The experimental set up was monitored for 24 weeks and samples were taken to the laboratory for analyses while morphological growth parameters were measured onsite. The shoot of TSs-147 accession was lengthiest (49.84 cm) while 31.34 cm was the least measured for TSs-141. With regards to nitrogen content, TSs-147 (2.57 % N) and TSs-149 (2.52 % N) maintained higher nitrogen levels in their leaves than TSs-142 (1.96 % N) and TSs-145 (1.93 % N). With significantly higher NR activity as well as chlorophyll content, it is expected that TSs-147 would comparatively thrive better among the selected accessions if cultivated in areas with significant Fe concentration.

Keywords: Nitrogen assimilation, African Yam bean, Ferruginous ultisol.

Introduction

Iron is a vital element in life. The major scientific and medical interest in iron is as an essential metal, but toxicological considerations are important in terms of accidental acute exposures and chronic iron overload. With rare exceptions, virtually all live organisms are dependent on iron for survival. Despite the ubiquitous distribution and abundance of iron in the biosphere, iron-dependent life must contend with the paradoxical hazards of iron deficiency and iron overload, each with its serious or fatal consequences. Homeostatic mechanisms regulating the absorption, transport, storage, and mobilization of cellular iron are therefore of critical importance in iron metabolism, and a rich biology and chemistry underlie all of these mechanisms. A coherent understanding of that biology and chemistry is now rapidly emerging. Iron is a natural component of soils, but its concentration can be influenced by some industries. It has been reported that urban soils showed different heavy metal characteristics (Yang *et al.,* 2001). Apparent accumulations of Pb, Zn, Cu, and Ni were observed, of which Pb occurred mainly in conjunction with iron oxides, and Ni and Zn existed in residual forms. Cu showed the same importance of different chemical forms but for soluble forms. These metals affect the metabolism of essential nutrients like mitogen in plants.

Nitrogen is an essential element for all organisms, which composes proteins, nucleic acids and other important organic compounds. Nitrogen is often the most limiting elements that plants have to acquire from the soil

(Touraine *et al.,* 2001) and it often determines the potential yield of crops (Le Bot *et al.,* 1994; Touraine *et al.,* 2001). Nitrogen can be taken up by the plant roots either in the form of NO_3^- or NH_4^+.

AYB is one of Africa's under-exploited plant species that is likely to expand man's food base. This legume of minor or less importance has long been used as a traditional dual seed grain and tuber food crop in Africa and has other great potentials (Adewale and Dumet 2010).

The potential of AYB cannot be over emphasized. However, like many other crops in third world countries, it is still under-utilized due to inadequate information on its physiology, agronomy, lack of good planting material and improved varieties. Due to the restricted attention it receives in terms of production and research, it faces eminent danger of extinction/erosion. It is also losing out to major legume and tuber crops such as cowpea (*Vigna unguiculata*) and potato (*Solanum tuberosum*) that have been improved for better yield, quality, disease, and pest resistance. Like other under-utilised crops, its survival as a crop has largely been sustained through tradition and knowledge of the local growers. This is evident from the paucity of documented information on its culture in general.

Plants are exposed to a variety of environmental stressors due to human impact on the environment. Iron (Fe) is particularly cited in this study because most lands in Nigeria are ferruginous and farmers have reported decrease in plant production as well as yield due to Fe toxicity and previous studies have confirmed that high concentration of this metal in soil can hamper plant growth and development (Varroto *et al*., 2002). Effects of Fe toxicity on rice plant have been well researched; however, there is scarcity of information on how Fe iron toxicity affects the growth and development of African yam bean. Hence, the present investigation was embarked on so as to fill the gap in information concerning nitrogen assimilation in African yam bean in ferruginous soil. The study reported here was carried out in order to observe nitrogen assimilation in young African yam bean plant in a ferruginous utisol. Furthermore, the question the present study intended to answer was whether the presence of iron could hamper or stimulate increased nitrogen assimilation in young African yam bean plant and, if possible, could it be adjudged to be stress related?

Materials and Methods

The experiment was carried out in Plant Biology and Biotechnology (PBB) botanic garden. The study started on the 16th day of November, 2015 and lasted for 4 months. The experiment was conducted in a marked plot (7m by 5m) within the botanic garden. The site was dominated by annual grasses prior to clearing activity which took place during the preparatory stage of the experiment.

Preparation of the Experimental Plot

The plot (7m by 5m) in the botanic garden was manually cleared to ground level and the rubbles were carefully removed from the site. Instead of buckets, Bagco® sacks measuring 30 cm in diameter and 42 cm long were used because of size and easy usability. These were obtained from a nearby market and set in the cleared plot in readiness for soil collection.

Collection and Pollution of Soil

Top soil (0 - 10 cm) was gathered randomly from 10 locations in the garden and pooled together to obtain a composite sample. The choice of the spot was such that no experiments had been conducted in these plots especially those related to the use of any form of soil enhancers like fertilizers or contaminants. This was confirmed from the department’s chief laboratory technologist. Before proceeding with the experiment, a sample of the pooled soil was sent to the laboratory for physiochemical analysis in order obtain a baseline data. Twenty (20) kg of the sun-dried soil was measured into thirty (30) bags arranged in blocks.

Africa Yam Bean (AYB)

Selected accessions of AYB were ordered from Genetic Resource Centre of the International Institute for Tropical Agriculture (IITA) Ibadan. Seeds were certified 100% viable by IITA.

Husbandry

The research was carried out in the month of October 2016. As a result, the plants were faced with nearly scanty amount of rainfall. Consequently, each bag was carefully watered to prevent dehydration and risk of water stress. The soil moisture test described by FAO was utilized as a measure for ensuring that the soils were adequately moisturized. The experiment was allowed to progress under the prevailing environmental condition and husbandry adopted for the study. Several parameters were scored as soon as growth changes were observed in the set-up.

Staking of emerging seedling

After two weeks of sowing of AYB, two stakes of about 3m in height were attached to each side of the bags containing the emerging seedlings. This activity was done with regard to the fact AYB is a profusely climbing vine. Therefore, staking of the emerging seedlings was necessary to enhance lateral growth and reduce secondary branching.

Ascertaining the Ferruginous Status of Soil

Although soils within the study area had been termed ferruginous in previous studies because if the elevated Fe content (Ikhile, 2016), time to establish this assertion. Top soil was collected from within the experimental plot and random and pooled together to obtain composite sample. The soil sample was immediately taken to the laboratory for physicochemical analyses (see Table 1).

Soil Amendment

The soil originally obtained from Botanical Garden were measured and used to fill experimental bags at 20kg of sun-dried soil per bag. 30 bags were obtained all together.

Segregation into treatment

The 30 bags of soil obtained were divided into 2 groups of 15. The first group was labeled garden soil with no further addition of iron sulphate. Being 15 soil bags, they were further divided into 3 sub-groups of 5 each with the implication being that each sub-group with each of the 3 accessions of AYB in the experiment with 5 replications.

The second group of 15 bags were amended with iron sulphate at 2.5 trice the Environmental Bench Mark (EMB) of iron phytotoxicity (ESV of iron = 200mg/kg). Therefore, 500mg/kg was used. Since there were 20kg of soil per bag, each bag was amended with 10g of iron sulphate in solution. This was done first by mixing iron sulphate in 1.5 L of water and then using it to moisten each bucket. It should be noted that the choice of 1.5 L emanated from the predetermined water holding capacity of the soil which is 802ml/kg or 1.6 L per bag.

Seed Sowing

The 3 accessions of AYB were sown in both garden and iron – elevated soils and the plants were observed for the growth parameters including First day of emergence, time to 50 % emergence, height of emergent, dry weight of emergent, number of leaves per plant, leaflet area as well as some rooting parameters. Assessment of Plant Reproductive Capacity was measured as flowering duration, which was determined as days from the first flower until 50 % of the plants cease flowering; days to maturity, as well as number of pods per peduncle.

Enzyme Assay

The central portion of leaves was cut into discs with a diameter of 1.0 cm. The stems were cut transversally into 2 mm long pieces. Both tissues were assayed for their in vivo nitrate reductase activity (NRA). The basal assay procedure used was a modification of method described by Faleiros and Cazetta (1996), based on the incubation of fresh tissue (300 mg) in 10.0 cm^3 of a medium containing 25 mmol dm^{-3} phosphate buffer (pH 7.2), 25 mmol dm^{-3} KNO3 , 30 cm^3 dm^{-3} n-propanol, and 30 cm^3 dm^{-3} of triton X-100®. In order to induce anaerobic conditions in the incubation medium, the system was subjected to vacuum infiltration three times, as recommended by Klepper *et al*. (1971) to remove air from the tissue. The medium was maintained in the dark at 30°C for 60 minutes. The NO_2^- produced by action of the NR enzyme was determined by drawing an 0.5 cm^3 aliquot of the incubation medium, and treating this sample with 0.5 cm3 of 10 g dm^{-3} sulfanilamide in 3 mol dm^{-3} HCl and 0.5 cm3 of 0.2 g dm^{-3} N-(1-naphtyl)-ethylenediamine dihydrochloride. After 20 minutes, the solution was diluted to 4.5 cm^3 with deionized water, and the absorbance (540 nm) was measured using a 721G spectrophotometer.

Results and Discussion

Results

The present study examined nitrogen assimilation in young African yam bean plant after exposure to ferruginous ultisol. The physicochemical composition of the soil in the experimental site before contamination has been presented in Table 1. The results showed that the pH level of the soil was 5.97 prior to contamination with iron (Fe) while electric conductivity and total nitrogen content were 301.21μs/ cm and 0.18 % respectively. Presence of heavy metals was also revealed with iron (Fe) having the highest concentration (1011.92 mg/kg) followed by zinc (Zn) and manganese (Mn) at 30.12 mg/kg and 17.03 mg/kg correspondingly. Cd concentration in the experimental site was negligible at a concentration less than 0.001 mg/kg.

Table 2 shows the description of the accessions of African yam bean (AYB) that were used during the study. The AYB accessions ranged from TSs-141 to TSs-149. Although the name assigned to each accession indicates that the seeds differ physiologically, based on depiction by the International Institute of Tropical Agriculture, Ibadan, the GRIN taxa number is the same for all the accessions. Both the GRIN taxa and Taxonomy number were code named 352250 and 3602 respectively. The ID of the accessions differs by one unit from 96116 to 96124 while the storage was similar across the board. As shown in Table 2, collecting number was entirely different in all the accessions

Effects of elevated iron on selected growth parameters of African Yam Bean (AYB) at 50 days after sowing showed that there was an uneven response with respect to shoot length across the test accessions (Table 3). Results showed that TSs-147 had the highest shoot length at 49.84 cm while 31.34 cm was the least and attributed to TSs-141. Generally, it was observed that, out of the nine accessions AYB evaluated in the present study, five accessions including TSs-143, TSs-144, TSs-147, TSs-148, and TSs-149 hit a 40 cm mark in their respective shoot lengths, while the rest four accessions fall below the mark.

Observably, the shoot length in TSs-147 (49.84 cm) and TSs-149 (49.26) is closely matched with a slight difference of 0.58 cm. However, considering the number of leaves per plant, there was a shift in similarity record. TSs-149 maintained similarity with TSs-148 and TSs-142 on having 29 leaves per plant, while TSs-144 and TSs-146 with 30 and 31 leaves were fairly close respectively. Notwithstanding, TSs-147 with longest shoot length (49.84 cm) also showed highest number of leaves per plant with 39 leaves recorded for this accession in ferruginous utisol.

Upon measuring the leaflet area during the study, it was noticed that TSs-147 compared favourably with the rest of the accessions under the present investigation. It had a leaflet area of 32.06 cm while TSs-145 with a leaflet area of 23.78 cm compared unfavourably across the accessions investigated. Aside from TSs-142 and TSs-144, which hit a 30 cm mark for leaflet area alongside TSs-147, the rest of the accessions (TSs-149, TSs-146, TSs-143, TSs-141, and TSs-148) showed a leaflet area less than 30 cm in conjunction with TSs-145. Although TSs-141 had the highest root length (29.78 cm) while TSs-149 with a root length of 23.26 cm was shorter than other accessions, TSs-147 with second lowest root length (23.34 cm) had the highest number of root nodules per plant as 18 root nodules were recorded for the plant. TSs-142 had only 9 root nodules, whereas TSs-143, TSs-145, TSs-146, and TSs-148 are related in having 11 root nodules per plant. TSs- 144 followed by TSs-141 had the second and third highest number of root nodules at 14 and 13 nodules correspondingly.

Nitrate reductase activity in developing plants at 50 days after sowing has been presented in Figure 1. Results showed that the level of nitrate reductase activity differ across the nine accessions subjected to experimental conditions during the present study. For instance, at 15 days after sowing, nitrate reductase activity in TSs-145 and TSs-144 was low at 0.16 $\mu Mhr^{-1}g^{-1}$ and 0.2 $\mu Mhr^{-1}g^{-1}$ compared to TSs-146 and TSs-147 which showed high enzyme activity at 0.5 $\mu Mhr^{-1}g^{-1}$ and 0.48 $\mu Mhr^{-1}g^{-1}$ respectively. Under the same ferruginous condition, at 15 days after sowing, the activity of nitrate reductase remained similar in TSs-141 and TSs-148 (0.36 $\mu Mhr^{-1}g^{-1}$) while comparable enzyme kinetics was recorded for TSs-142 and TSs-149 (0.34 $\mu Mhr^{-1}g^{-1}$).

Based on the prevalent experimental condition, results showed that there was a change in enzyme activity with time. At 30 days after sowing, nitrate reductase activity in TSs-145 increased from 0.16 $\mu Mhr^{-1}g^{-1}$ to 0.52 $\mu Mhr^{-1}g^{-1}$ whereas TSs-146 showed a decrease from 0.5 $\mu Mhr^{-1}g^{-1}$ to 0.38 $\mu Mhr^{-1}g^{-1}$. Increase in enzyme activity was recorded for TSs-141, 142, and 148. There was an increase in nitrate reductase activity from 0.36 to 0.48 $\mu Mhr^{-1}g^{-1}$ in TSs-141 and 148, while TSs-142 had a slight increase of 0.08 $\mu Mhr^{-1}g^{-1}$. Observably, TSs-147 maintained slightly the same enzyme activity from onset of sowing to 30 days after sowing whereas TSs-144 shown an enhanced nitrate reductase activity between 15 and 30 days after sowing.

By the end of the next fortnight, nitrate reductase activity in TSs-148 had peaked at 1.6 $\mu Mhr^{-1}g^{-1}$. Enzyme activity in TSs-145 and TSs-146 remained unchanged when compared to the value obtained at 30 days after sowing. Overall, from the time of sowing until 45 days after sowing, nitrate reductase activity was highest in TSs-148 (1.6 $\mu Mhr^{-1}g^{-1}$) followed by TSs-141 (1.45 $\mu Mhr^{-1}g^{-1}$) and TSs-142 (1.42 $\mu Mhr^{-1}g^{-1}$). Lowest enzyme activity was observed in TSs-145 (1.1 $\mu Mhr^{-1}g^{-1}$) as compared across the board.

Figure 2 shows the percentage nitrogen content of leaves of African yam bean plants at 45 days after sowing. Results showed that the leaves of TSs-144 contained more nitrogen (2.78 %) compared to TSs-145 (1.93 %) which had the lowest concentration of nitrogen among all the accessions under investigation. From the results in Figure 2, it could deduced that apart from two accessions, TSs-145 and Tss-142, which showed percentage leave nitrogen content below 2 %, the rest of the accessions contained 2 % or more nitrogen in their leaves. Observably, under the condition of iron toxicity, there was a relationship with respect to total nitrogen content between TSs-147 and TSs-149 as well as between TSs-141 and TSs-143.

There was no significant difference in nitrogen content in TSs-147 (2.57 %) and TSs-149 (2.52 %) as the values are located in the same axis on the radar chart. Similar observation was recorded for TSs-141 (2.41 %) and TSs-143 (2.43 %). Overall, TSs-144 (2.78 %) showed high nitrogen content under ferruginous condition compared to the rest of the accessions under the present study.

At 45 days after sowing, plant samples were tested for nitrogen assimilation and results revealed that, in all accessions subjected to ferruginous condition, nitrogen uptake was more in the form of nitrate than ammonia (Figure 3). TSs-141 contained 22.5 ppm of ammonia nitrogen (AN) and 900 ppm of nitrate nitrogen (NN) thus it was adjudged as having the potential to assimilate more nitrate nitrogen under ferruginous condition compared to other accessions tested. The least concentration of both ammonia and nitrate nitrogen was recorded in TSs-142 which had 17 ppm of AN and 550 ppm of NN. Noticeably, nitrogen assimilation in the form of AN decreased from 22.5 ppm to 17 ppm as well as NN from 900 ppm to 700 ppm in TSs-141 and TSs-142 respectively. On the other hand, concentration of nitrate nitrogen was fairly maintained in TSs-142, TSs-143, and TSs-144 at 700 ppm, while similar relationship was recorded for TSs-145 and Tss-146 which assimilated 550 ppm of nitrate nitrogen.

Results also divulged the fact that TSs-145 which assimilated more ammonia nitrogen (27 ppm), compared to other accessions, had low concentration of nitrate nitrogen (548 ppm).

Comparative foliar chlorophyll contents of African yam bean plants at 45 days after sowing has been presented in Figure 4. Whereas TSs-148 had the highest concentration of chlorophyll-b at 0.375 U/mg, TSs-143 showed the lowest concentration at 0.075 U/mg followed by TSs-145 (0.0875 U/mg). In the same vein, TSs-141 comparatively had high concentration of chlorophyll-a (0.25 U/mg) while the lowest concentration was pinpointed in TSs-149 at 0.175 U/mg. It was observed that foliar chlorophyll-b content in TSs-142 and TSs-144 was similar at 0.32 U/mg. Generally, the concentration of chlorophyll-b was comparatively higher than chlorophyll-a in over 50 % of all the accessions under scrutiny in the present study. It was also recorded that of all the accessions considered during the present study, TSs-142 and TSs-144 showed closely related physiological characteristics under the prevalent condition. This speculation was based on the results in which the concentration of both chlorophyll-a and chlorophyll-b are exactly the same at 0.24 U/mg and 0.32 U/mg respectively. Thus, while TSs-142 and TSs-144 are second to TSs-141 in terms of highest concentration of chlorophyll-a, both accessions are also second to TSs-148 with respect to highest concentration of chlorophyll-b.

Discussion

Physicochemical Composition of the Soil

Prior to embarking on the present study, the soil was analyzed to ascertain its physicochemical properties before being incorporated in the study and it was established that the soil was highly ferruginous as it contained 1011.92 mg/kg of iron (Fe) in oxide form at a pH of 5.97. Apart from Zn and Mn whose concentration in the soil were 30.12mg/kg and 17.03 mg/kg respectively, the amount of other heavy metals such as Pb and Cd were negligible as well as below the specified ecological screening value (ESV). This implies that the effects of heavy metal on nitrogen assimilation in African yam bean (AYB) as documented in the present study can solely be attributed to the incidence of excess Fe in the soil. Ayeni *et al*. 2014 opined that at higher availability of iron, direct or indirect toxicity could be detected in plants. While direct iron toxicity arises when disproportionate uptake of iron (Fe) impairs cell structures, resulting to reduced plant growth and damage to foliage, indirect toxicity involves inhibition of nutrient uptake due to the damage inflicted on the epidermis surface of the roots (Jørgenson *et al*. 2013; Tripathi *et al*. 2014).

Effects of High Concentration of Iron on Growth Parameters of African Yam Bean

Iron stress triggered some morphological changes at varying degree on African Yam Bean (AYB) accessions considered during the present study. Shoot length deceased in some accessions such as TSs-141 and TSs-146 (31.34 cm and 34.57 cm respectively) but increased in others as recorded in TSs-149 and TSs-147 (49.26 cm and 49.84 correspondingly). This could be attributed to the reality that although a plant species may face an unfavourable condition, but when scrutinized on the basis of the cultivars or varieties, the potency to tolerate such environmental anomaly would differ (Table 4). The increase in shoot length observed in TSs-149 and TSs-147 as compared to their decreased counterparts showed that these AYB accessions had superior capacity to thrive in ferruginous utisol. Previous studies have revealed that different varieties of plants have varying tolerance for iron toxicity. Sahrawat (2004) stated that iron toxicity symptoms vary in rice cultivars, although texture, cation exchange capacity, and organic matter content influence the concentration of ferrous iron in soil solution, in which iron toxicity occurs. The study reported that toxicity symptoms commonly develop at the maximum tillering and heading growth stage, but may be observed at any growth stage of the rice crop. This supports the observation recorded for shoot length in the present study. Number of leaves reduced under the condition of iron toxicity.

Mehraban *et al.* (2008) established that high concentrations of Fe^{2+} in the substrate strongly reduced radicle development, supported on the dry weight, particularly when the Fe2+ doses exceeded 50 mg/L since elevated concentrations of the metal heightened the peroxidation of lipids, mainly in the radicle zone, which was followed by stunted growth of the plant part. Perhaps, this radicle activity affected the full development of the plants because reductions in radicle volume and losses of radicle hairs can stimulate a drastic decline in the relative content of water in plants (Dorlodot *et al.,* 2005). Resultantly, cascades of downregulation of genes that code for the cellular information for the development plant leaves may have been initiated in order to reduce transpiration under the prevalent stress condition because increase in number of leaves will ultimately enhance the rate at which the plant loses water (Monteiro *et al*. 2016). This explains the reduction in the number of leaves of AYB under iron toxicity as the plant adapted a shunt to check potential risk of death by dehydration; hence the decrease in the number of leaves so as to survive with the available radical hairs while minimizing the rate at which water is lost through transpiration of plant leaves. Poor development of radicle, as reported by Dorlodot *et al* (2005), may be the reason behind the reduction in root length documented in the present study. While some roots were shorter in some accessions like TSs-149 with root length of 23.26 cm, other accessions such as TSs-141 had longer roots (29.78 cm). Becker and Asch (2005) indicated that highly elevated concentrations of Fe^{2+} can lead to consequent decrease, and in some cases, complete cessation of growth in different organs of the plant. This assertion was supported by the findings of Peña-Olmos *et al*. (2014) as also documented in the present study.

Prominent decrease in the leaf area of the plants subjected to excess iron was observed in the present study. This implies that the continuous flow of the metal through the conducting tissues of the plants was amply powerful to initiate premature loss of the leaves in the plants studied. Possibly, this suggestion may have been as a result of the low levels of total soluble sugars, soluble protein, chlorophyll *a, b* and total chlorophyll, as induced by the Fe^{2+} (Mehraban *et al.*, 2008), which lessened foliar growth and development and the valuable leaf life, and, therefore, caused a decline in crop yield (Audebert, 2006b). Similarly, excess iron toxicity may have induced an increase in the lipids peroxidation of leaves, which affected the structure and process of cellular division, drastically decreasing foliar expansion (De Oliveira-Jucoski *et al.*, 2013). Iron toxicity inflicted variation in number root nodules in AYB accessions examined in the present investigation. Fifty percent (50 %) reduction in number of root nodules was observed in TSs-142 that had 9 root nodules compared to TSs-147 with 18 root nodules. The present study also recorded that the same TSs-147 with higher nodulation had the longest shoot length. This suggests that this accession has a physiological mechanism of tolerating iron toxicity and still take up the required amount of iron needed for the synthesis of multiple proteins such as two subunits of nitrogen-fixing enzyme, nitrogenase; plant and bacterial cytochromes required for respiration and energy metabolism; leghemoglobin to transport oxygen and buffer free molecular oxygen at nanomolar concentrations compatible with activity of oxygen-labile nitrogenase; and a variety of other Fe proteins such as ferredoxins and hydrogenases (Tang *et al.*, 1990; Brear *et al.*, 2013). This explains why TSs-147 thrived better than TSs-142 with reduced number of root nodules and ultimately showed weakened growth and development (O'Hara, 2001).

Effects of Iron Toxicity on Nitrate Reductase Activity in African Yam Bean (AYB)

The consequence of elevated iron (Fe) concentration on nitrate reductase (NR) activity was monitored in developing AYB plants at 50 days after sowing. Similar trend of varied response to Fe toxicity was observed with respect to NR activity. TSs-147 also showed high enzyme activity at 0.5 $\mu Mhr^{-1}g^{-1}$ compared to TSs-142 and TSs-149 (0.34 $\mu Mhr^{-1}g^{-1}$). High NR activity meant that the accession had sufficient pool of nitrogen despite the prevalent condition of Fe toxicity. This is tantamount to enhance protein synthesis and production of several biomolecules required for plant growth and development (Baruah and Bharali, 2015). Güsewell (2004) stated that homoeostatic regulatory mechanism is one of the strategies adopted by Fe-toxicity tolerant plant species to maintain stable nitrogen concentration. This line of action in these plant species is such that the activity of nitrate reductase enzyme is sustained as previous studies revealed that over 40 million tonnes of nitrogen are injected into the soil annually through the symbiotic relationship between the root of leguminous plant and nitrogen-fixing bacteria, which in turn are responsible for the production of nitrate reductase enzymes (Herridge *et al.*, 2008). These findings prompted the suggestion made in the present study in that, although trace element such as Fe inhibit nitrate reductase activity in acid soil according to Fu and Tabatabai (1989), plants that are tolerant to Fe-toxicity can thrive in elevated Fe soil while enhancing the NR activity. Thus, of all the accessions of AYB considered in the present study, TSs-147 showed higher potential to surviving and developing in ferruginous ultisol.

Percentage nitrogen (N) content of leaves of AYB was monitored after exposure to high Fe concentration. TSs-147 (2.57 % N) and TSs-149 (2.52 % N) maintained higher nitrogen content in their leaves than TSs-142 (1.96 % N) and TSs-145 (1.93 % N). The study suspects that Fe toxicity interfered with nitrogen in accessions that are intolerant of high Fe concentration. Perhaps, this may have been due to the fact that at high concentration, Fe can form complexes with O, N, S ligands which ultimately renders nitrogen inaccessible for normal physiological fucntions in the plant (Van Assche and Clijsters, 1990). According to Tamas *et al*. (1997) and Adhikari *et al*. (2006), this interference will manifest in the plant as impaired protein metabolism, membrane functions, water relations, and seed germination. This explains the hitch in growth and development in AYB accessions that were intolerant of Fe-toxicity as documented in the present study.

Effects of Elevated Iron Concentration on Chlorophyll Content and Nitrogen Assimilation in AYB

Samples of test plant were analyzed to determine nitrogen assimilation and ascertain in what form AYB took up nitrogen in ferruginous ultisol. All accessions examined absorbed nitrogen more in the form of nitrate than ammonium. Previous studies showed that plants, especially legumes, take up nitrogen in different form including nitrate, ammonium and atmospheric dinitrogen. However, irrespective of the form, the glutamine synthetase (GS)/ glutamate synthase (GOGAT) system incorporate the available nitrogen in form of ammonium (NH_4^+) which metabolic activities in the cytosol leverage on for the production of amino acids and amides (Schubert, 1995). The present study noticed that the concentration of nitrate and ammonium decreased under the condition of high Fe in soil. This occurred possibly because at high concentration, Fe which is needed at very small amount by plants turns out as an environmental stressor. As result, processes that facilitate N_2 fixation such Rhizobium prolifertation and nodulation may have been restricted. Damiani *et al*. 2016 demonstrated possible inhibition of nodulation by high N status which could lead to the production of reactive N species and similar activity was reported about high Fe concentration that triggered the generation of reactive oxygen species which hamper metabolic activities in plants (Kampfenkel *et al*. 1995). It was noteworthy that under ferruginous condition, AYB accessions behaved differently as some of them showed fairly similar concentration of nitrate nitrogen while others were noticeably affected. This observation was in agreement with the findings of Xu *et al*. (2009) whose study demonstrated the effects of iron plaque on nutrient uptake and translocation mechanisms in plants. The researchers concluded that plants that showed low tolerance to high Fe concentration showed severe symptoms of iron toxicity compared to high tolerant species.

Foliar parts of AYB accessions were closely monitored to ascertain the impact of high Fe-concentration on the chlorophyll content of the plants. It was noticed that foliar chlorophyll concentration decreased under Fe-stress. This, in all probability, could be attributed to the oxidative damage resulting from excess Fe which slowed or completely impeded chlorophyll biosynthesis (De Oliveira-Jucoski *et al*. 2013). Previous studies recorded that increase in Fe concentration resulted in risen pigment concentration to a certain level, however, changes in pigment ratio ensued. This suggested that plants only require optimum level of Fe in order to carry out normal metabolic functions. In the study, it was concluded that chlorophyll molecules are highly sensitive to Fe concentration (Nevona, 2016). Kampfenkel *et al.* (1995) confirmed that the rate of photosynthesis in conditions of saturated CO2 in leaves of *Nicotiana plumbaginofolia* decreased 40% after exposure to excess Fe2+ as a consequence of an increase in foliar Fe2+ content; which in turn caused a 25% decrease in the starch content of the studied leaves. Thus, the findings of the present study support the conclusion drawn by earlier researchers with respect to the effect of high Fe concentration on foliar chlorophyll content.

Conclusion

Nitrogen assimilation in young African yam bean plants in a ferruginous ultisol has been studied. The whole idea of the study was centred on the fact that the need to expand cultivation of utilization of AYB across agroecological zones became important, and elevated soil Fe content was a factor that affected crop development in most soils in the rainforest zones, Although Fe is required in minute quantities for some metabolic activities in plant, elevated levels of Fe in soil is detrimental to plant's metabolic and physiological functions as well as growth and development in general. Excess Fe concentration in the soil reduced shoot length in all African yam bean (AYB) accessions considered in the present study except TSs-147 and TSs-149. An important deduction made in this study was the fact that Fe toxicity is capable of impeding the use of certain legumes as source of nitrogen replenishment in the soil, particularly those that are intolerant to Fe stress. This may perhaps be one of many reasons why the crop is hardly grown in most iron-impacted soils in places like Benin City that is characterized by elevated Fe levels. The aforementioned inference was based on decrease in nitrate reductase activity due to high amount of Fe in soil, which ultimately led to low nitrogen assimilation in AYB accessions that were intolerant of Fe stress. Since TSs-147 seemed to thrive better than TSs-149, especially with regard to physiological assessments carried out such as foliar chlorophyll content and nitrate reductase activity, it can be concluded that AYB TSs-147 could be cultivated in areas with significant Fe concentration. This study encourages farmers to seek the assistance of seed scientists when acquiring seed for a farming season, while taking into consideration the nature of the soil. This will help to improve crop yield and prevent loss of farm produce.

Acknowledgements

The authors are greatful to the postgraduate and undergraduate students of "IB" who assisted with the study especially on the field

Conflict of Interests

None

Tables, Figures and Charts

Table 1: Physical and chemical properties of soil before contamination. These are background mean concentrations (n = 5)

Parameters	**Mean value (n = 5)**
pH	5.97 ± 0.67
Electric conductivity (µs/ cm)	301.21 ± 23.01
Total organic carbon (%)	0.49 ± 0.09
Total Nitrogen (%)	0.18 ± 0.06
Exchangeable acidity (meq/100g)	0.22 ± 0.08
Na (meq/100g)	10.90 ± 2.11
K (meq/100g)	1.48 ± 0.62
Ca (meq/100g)	14.32 ± 3.10
Mg (meq/100g)	12.01 ± 3.22
NO^-_2 (mg/kg)	16.43 ± 2.03
NO^-_3 (mg/kg)	30.01 ± 4.28
Soil texture	
Clay (%)	5.43 ± 0.88
Silt (%)	7.36 ± 1.74
Sand (%)	84.81 ± 12.12
Heavy metals	
Fe (mg/kg)	1011.92 ± 73.38
Cd (mg/kg)	<0.001
Mn (mg/kg)	17.03 ±3.22
Pb (mg/kg)	0.03 ± 0.01
Cu (mg/kg)	3.93 ± 0.01
Zn (mg/kg)	30.12 ± 3.06

Table 2: Description of accessions collected from the IITA, Ibadan

Accession name	ID	Country of origin	GRIN taxa	Taxonomy number	Storage	Collecting number
TSs-141	96116	Nigeria	GRIN:35250	3602	Seed collection;Medium term	ABG 98-4-2
TSs-142	96117	Nigeria	GRIN:35250	3602	Seed collection;Medium term	ABG 98-4-3
TSs-143	96118	Nigeria	GRIN:35250	3602	Seed collection;Medium term	AGB 98-4-9
TSs-144	96119	Nigeria	GRIN:35250	3602	-	AGB 98-4-10
TSs-145	96120	Nigeria	GRIN:35250	3602	-	AGB 98-4-12
TSs-146	96121	Nigeria	GRIN:35250	3602	-	AGB 98-4-15
TSs-147	96122	Nigeria	GRIN:35250	3602	Seed collection;Medium term	Enugu 98-4-1
TSs-148	96123	Nigeria	GRIN:35250	3602	Seed collection;Medium term	Enugu 95-3
TSs-149	96124	Nigeria	GRIN:35250	3602	Seed collection;Medium term	Enugu 95-4

Table 3: Effects of elevated iron on selected growth Parameters of African Yam Bean at 50 days

Plant Accessions	Shoot length (cm)	No. Leaves/Plant*	Leaflet Area (cm^2)[1]	Root length[1] (cm)	No. of root nodules per plant*
TSs-141	31.34	33	25.32	29.78	13
TSs-142	39.01	29	30.00	24.42	9
TSs-143	43.40	32	26.38	27.51	11
TSs-144	47.72	30	31.74	28.80	14
TSs-145	38.72	36	23.78	25.23	11
TSs-146	34.57	31	26.40	26.41	11
TSs-147	49.84	39	32.06	23.34	18
TSs-148	41.62	29	24.37	20.86	11
TSs-149	49.26	29	26.51	23.26	12

*reported to the nearest whole number

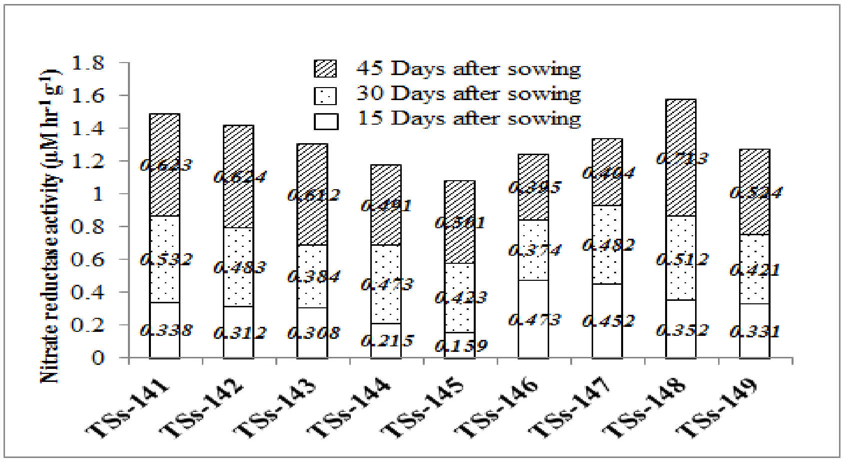

Figure 1: Nitrate reductase activity in developing plants at 50 days

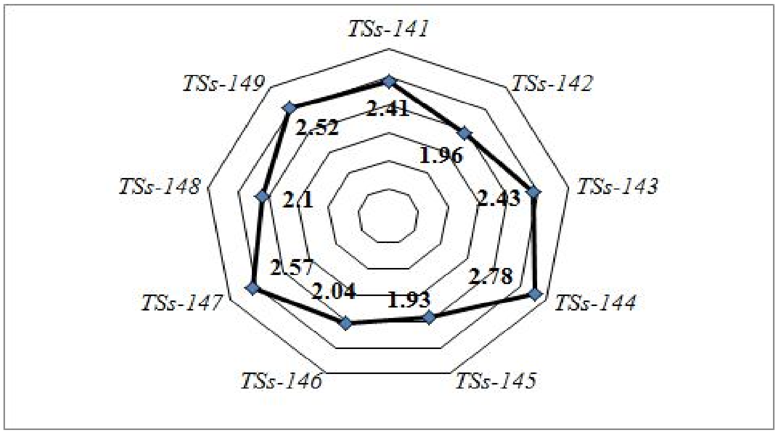

Figure 2: Nitrogen contents (%) of leaves of African yam bean plants at 45 days after sowing

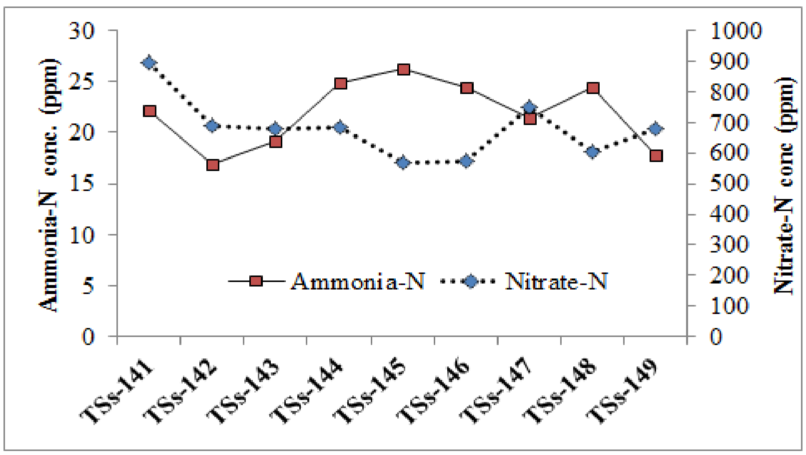

Figure 3: Nitrogen assimilated by leaves of African yam bean plants at 45 days after sowing

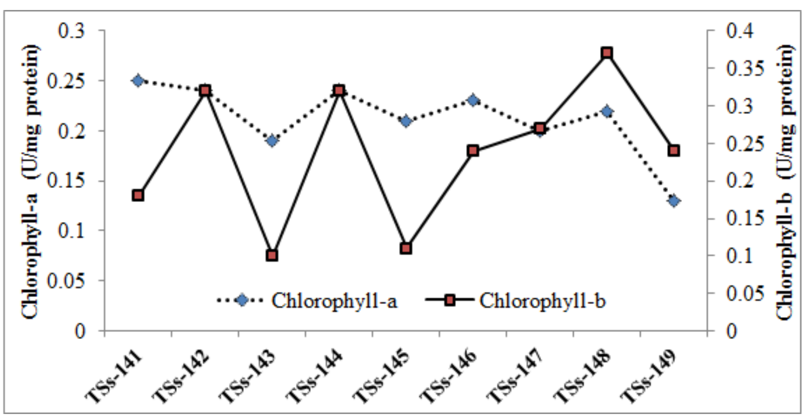

Fig. 4: Comparative foliar chlorophyll (U/mg protein) contents of African yam bean plants at 45 days after sowing.

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