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African Journal of Plant Science

Full Length Research Paper

Biochar as an alternative growth medium for tree seedlings in the Guinea Savanna Zone of Ghana

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A good planting medium is required for raising tree seedlings in the nursery, however Guinea savanna soils are generally poor in nitrogen and organic matter. This poses a challenge in accessing fertile soil for tree nurseries in Northern Ghana. The experiment was conducted in the Nyankpala campus to explore the potential of plant biochar as growth media for raising tree seedlings. Growth media prepared from six different biochar formulations (Groundnut Husk Biochar; Rice Husk Biochar; Wood Biochar; Groundnut Husk Biochar + Soil; Rice Husk Biochar + Soil; Wood Biochar + Soil) and control (untreated topsoil) were each replicated in three seed boxes. Seed boxes were arranged in a Completely Randomized Design with 50 *Khaya senegalensis* seeds sown in each box. Percentage seed emergence did not vary significantly between treatments ($p > 0.05$) although Groundnut Husk Biochar recorded a marginally higher emergence (65.71%). Similarly, Groundnut Husk Biochar recorded a significantly higher plant height (10.23 cm) in the second week after planting ($p < 0.05$) as well as mean number of leaves (6.02) in the sixth week after planting ($p < 0.05$). In general, Groundnut Husk Biochar had the greatest effect on initial growth performance of *K. senegalensis* and could therefore be explored as a growth medium for raising tree seedlings in Northern Ghana.

Key words: Biochar, growth media, *Khaya senegalensis*, plant height, soil.

INTRODUCTION

Biochar is a carbon-rich product produced through thermal decomposition of biomass in a closed container with limited or no supply of oxygen at a temperature below 700°C (Nartey and Zhao, 2014; Lehmann, 2007). Biochar may be produced from a wide range of organic feedstock materials such as plant residue, wood biomass, and organic waste from municipal and industrial sources (Břendová et al., 2012). But, the quality and quantity of biochar produced can be influenced by feedstock properties (Zhao et al., 2013) and conditions of the carbonization process (Steiner, 2016; Gaskin et al., 2008). For instance, higher lignin content of feedstock is associated with higher biochar yield. Biochar is well known for its potential contribution to climate change mitigation due to the high residence time of the carbon in biochar (Hammes et al., 2009). This makes it a good reservoir of carbon lost in decomposition (Laird et al., 2010). Biochar has also been identified as an effective adsorbent of organic pollutants in waste water (Liu and Zhang, 2009). Aside the environmental benefits, the high

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stability and water retention capacity of biochar makes it suitable for soil amendment (Lehmann, 2007). It improves both physical and chemical properties of soils when used as soil amendment (Jha et al., 2010; Chan et al., 2008; Glaser et al., 2002).

The incorporation of biochar has been reported to influence soil structure, texture, porosity, particle size distribution and density thereby enhancing its water holding capacity (Amonette and Joseph, 2009). Bulk density and water holding capacity of sandy soils can be enhanced by the micro and macropores in biochar structure (Novak et al., 2012). Biochar can convert the labile carbon into aromatic structures of relatively low decomposition rates (Kuzyakov et al., 2009). This carbonized form makes biochar suitable for addressing nutrient leaching and carbon depletion in poor soils (Ding et al., 2016).

Despite the potential of biochar as a soil amendment (Thies and Rillig, 2009) few trails have been conducted in the savanna to ascertain its ability to improve soil condition for raising seedlings which is a great challenge to nursery managers in northern Ghana. Savanna soils are generally low in organic matter and nitrogen (FAO, 2005) which limits its ability to support the growth of tree seedlings in the nursery requiring management options to improve soil fertility (Predotova et al., 2010). This study explored the possibility of using biochar produced from plant residues as growth media for tree seedlings in the Guinea savanna zone of Ghana. This will provide valuable information to nursery managers in the zone who are expected to produce large quantities of seedlings to feed the Ghana government reforestation programme.

MATERIALS AND METHODS

Study area

The experiment was conducted at the tree nursery of the Faculty of Natural Resources and Environment (FNRE) of the University for Development Studies in the Tolon District (Figure 1). The site is located at latitude 9°25' N to 10° 40' N and longitude 0° 58' N to 1° 12' W with an altitude of 183 m above sea level (SARI, 1997). The area falls within the Guinea savanna agro-ecological zone which records a unimodal rainfall pattern with a mean annual rainfall of 1,034.4 mm (SARI, 2004). The mean maximum temperatures (35°C) are recorded in March and April whilst the lowest temperatures (22°C) often occur in December (SARI, 2016). The vegetation is generally grassland interspersed with some woody species. Among some of the dominant woody species indigenous to the Guinea savanna include *Vitellaria paradoxa* (shea), *Adansonia digitata* (baobab), *Parkia biglobosa* (dawadawa), *Pterocarpus erinaceus* (rosewood) and others (SARI, 2004).

Biochar production

Rice husk and groundnut husk feedstock were obtained from a commercial rice mill at Nyankpala. However, wood shavings feedstock was obtained from the Nyankpala market for the experiment. The various feedstocks were charred in a modified oil barrel following the procedure described in Steiner et al. (2018). The biochar was produced on a simple top-lit updraft gasifier where a modified oil barrel was perforated with holes at the bottom to facilitate the free flow of primary air from the bottom. Also, larger L-shaped holes of 6 × 6 cm were perforated at the top sidewalls of the barrel which enabled the flow of secondary air. After which a cut was made on the top of the lid to create an opening of 20 cm with a 1 m tall chimney attached to the lid. Gasifiers were then produced in triplicates for the carbonization of the three different feedstocks. The biochar of the different plant residues was each ground into finely uniform particles before they were used as growth media.

Seed viability test

Khaya senegalensis seeds obtained from the Tamale Forestry Services Division (FSD) were sorted and tested for viability prior to the experiment. One hundred seeds were randomly selected for the viability test using the floating test (Baatuuwie et al., 2019). The seeds recorded a percentage germination of 85%.

Experimental design

Six different biochar formulations were used as experimental treatments with untreated topsoil as control; 100% groundnut husk biochar (GHB), 100% rice husk biochar (RHB), 100% wood biochar (WB), GHB + soil at a ratio of 1: 1, RHB + soil at a ratio of 1:1, WB + soil at a ratio of 1:1. Soil was collected from the FNRE mango plantation. Each treatment was replicated in three plastic seed boxes of sizes 0.45 × 0.25 m half-filled with the treatment growth medium and arranged in a complete randomized design, fifty (50) *K. senegalensis* seeds were then broadcasted in each seed box, after which they were gently covered with a thin layer of the same treatment growth medium. The seed boxes were kept under a shade net in the FNRE tree nursery. Each seed box was watered twice daily (morning and evening) with 1500 ml of water from the day of sowing to the end of the experimental period (six weeks). Weeds were removed regularly by hand to prevent competition.

Seed emergence was recorded by counting the number of seeds germinated daily per seed box for a period of thirty days after sowing. Growth parameters and chlorophyll content were recorded once every two weeks (second, fourth and sixth weeks after planting). However, plant girth was not recorded in the second week after planting because seedlings were still fragile. A TYS-B portable chlorophyll meter was used for measuring the leaf chlorophyll content, measuring tape for plant height and calipers for collar diameter (girth). Leaves were counted manually.

Data analysis

All data collected were recorded and classified in Microsoft Office Excel 2007. Mean plant height, leaf chlorophyll content, number of leaves and plant girth were subjected to one-way analysis of variance using Minitab version 17. Means were separated using Fishers least significant differences (LSD) and considered significant at $\alpha = 0.05$.

RESULTS AND DISCUSSION

Effect of biochar on *K. senegalensis* seed emergence

Percentage seed emergence did not vary significantly between treatments (P = 0.65), but seed emergence under GHB was marginally higher (65.71%) than all other

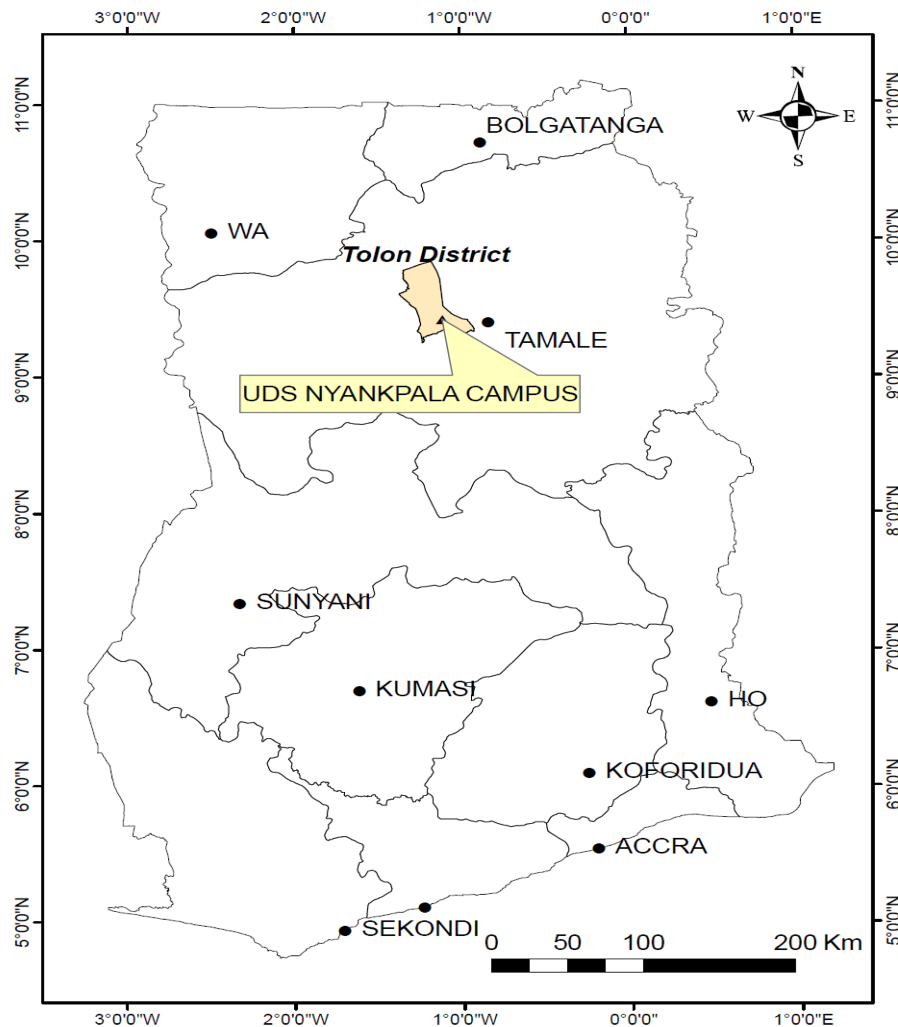

Figure 1. Map of the study area.
Source: Baatuuwie et al. (2019).

treatments. The RHB recorded the least percentage seed emergences (46.67%) (Figure 2). The relatively higher percentage emergence (65.71%) recorded under GHB than all other treatments might be due to the presence of Karrikins in GHB. Karrikins in biochar is reported to trigger germination of dormant seeds and regulate plant development (Kochanek et al., 2016). Biochar is also known to enhance soil pH (Novak et al., 2009), therefore the ability of a plant biochar to positively influence the pH of a growth media could aid in overcoming the inhibitory factors to germination.

Effect of biochar on initial growth performance of *K. senegalensis*

There was a significant difference in plant height ($P = 0.001$) between treatments in the second week after planting. However, plant height did not differ significantly between treatments in the 4th ($P = 0.18$) and 6th ($P = 0.49$) weeks after planting (Table 1). GHB recorded a marginally higher mean seedling height (10.32 cm) among all other treatments whilst the least (8.26 cm) was recorded in the control at the end of the experiment.

The control recording the least plant height (4.7 cm) in the sixth week after planting (Table 1) affirms the fact that savanna soils are poor in nitrogen and organic matter which barely support plant growth without major amendments (FAO, 2005). Therefore, biochar associated growth media might have some elevated levels of nitrogen and other nutrients for seedling growth. This is an indication that biochar properties might have influenced physical and chemical properties of the growth media providing favorable conditions for the *K. senegalensis* seedlings. On the contrary, plant height did not differ significantly in the 6th week after planting but

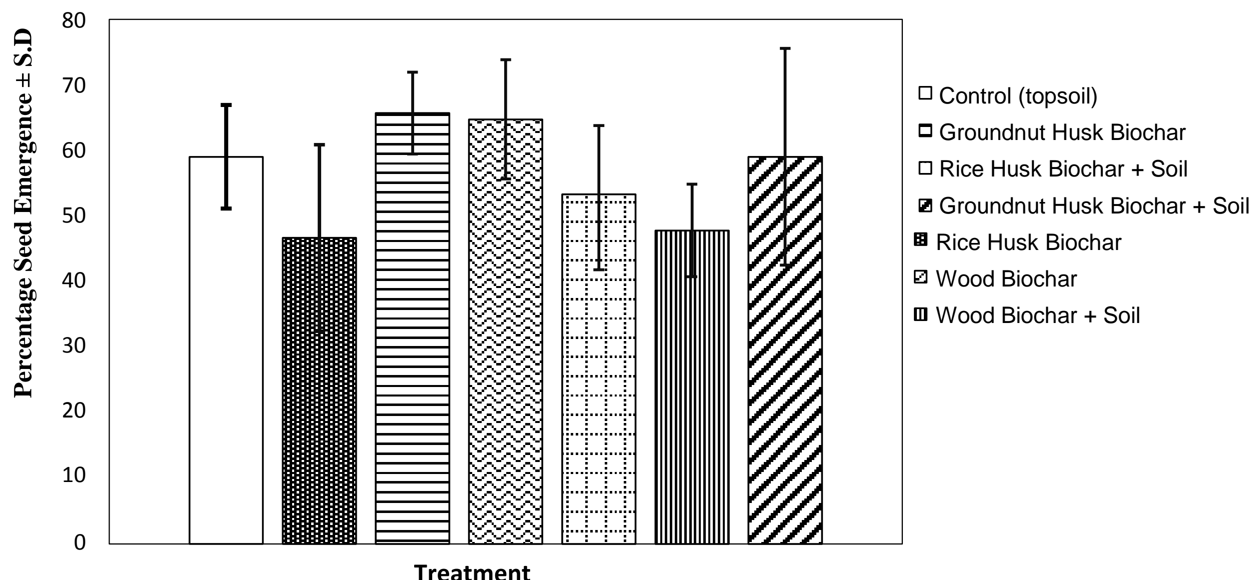

Figure 2. Emergence of *K. senegalensis* seeds under different biochar treatments.

Table 1. Mean height (cm) of *K. senegalensis* seedlings under different biochar treatments.

Treatment	Plant height (cm) ± S. D		
	Week 2	**Week 4**	**Week 6**
Control	4.7 ± 0.31^{d}	7.23 ± 1.33^{b}	8.26 ± 1.53^{a}
RHB	6.35 ± 0.45^{a}	8.72 ± 0.72^{ab}	9.09 ± 0.99^{a}
GHB	6.29 ± 0.36^{a}	9.72 ± 0.16^{a}	10.32 ± 1.19^{a}
WB	5.71 ± 0.81^{ab}	8.93 ± 1.10^{ab}	9.51 ± 1.78^{a}
GHB+Soil	5.42 ± 0.05^{bc}	8.32 ± 1.05^{ab}	9.54 ± 0.82^{a}
RHB+ Soil	5.41 ± 0.14^{bcd}	8.40 ± 1.31^{ab}	10.15 ± 1.18^{a}
WB+ Soil	4.78 ± 0.08^{cd}	8.34 ± 0.65^{ab}	9.79 ± 0.83^{a}
P - value	0.001	0.184	0.497

Means sharing a common superscript in a column are not significantly different at α = 0.05.

GHB recorded a marginally higher plant height (10.32 cm) than all other treatments. This could be an outcome of the high adsorption capacity of GHB which enabled accumulation of soil ions for plant root access (Saleh et al., 2011). Mean number of leaves per plant did not differ significantly between treatments in the second (P = 0.47) and fourth weeks (P = 0.84) after planting (Table 2). However, there was a significant difference between treatments (P = 0.047) in the sixth week after planting with seedlings growing on GHB recording the highest mean (6.02) number of leaves whilst RHB + Soil recorded the least (4.97).

The significantly higher number of leaves (6.02) recorded on GHB in the sixth week after planting might be attributed to the porous nature of GHB which created an atmosphere for microbial colonization and perhaps facilitated nutrient absorption and adsorption. GHB is reported to exhibit both monolayer and multilayer adsorption properties which enhances the adsorption of chemical elements such as ammonium ions (Clough et al., 2013). This makes the GHB surface heterogeneous in nature with the ability to adsorb all kind of plant nutrient elements. Lee et al. (2016), equally indicated the porous nature of GHB as a property through which it adsorbs dissolved ions such as calcium.

Plant girth did not differ significantly between treatments (P = 0.730) in the sixth week after planting although RHB had the largest plant girth (1.99 ± 0.1210) (Table 3). Contrary to the fact that most growth parameters were higher under GHB, the largest plant girth was recorded under RHB (1.99 cm). This could be due to the high ash content of rice husk (60 to 70%) attributed to the active uptake of silicon in rice (Carter et al., 2013). Again, rice husk provides a readily soluble

Table 2. Mean number leaves per plant under different biochar treatments.

Treatment	Number leave ± S. D		
	Week 2	**Week 4**	**Week 6**
Control	2.22 ± 0.21[a]	4.39 ± 0.39[a]	5.22 ± 0.73[abc]
RHB	2.38 ± 0.33[a]	4.23 ± 0.42[a]	4.65 ± 0.56[c]
GHB	2.28 ± 0.14[a]	4.85 ± 0.26[a]	6.02 ± 0.47[a]
WB	2.45 ± 0.21[a]	4.62 ± 0.43[a]	5.48 ± 0.35[ab]
GHB+SOIL	2.39 ± 0.32[a]	4.58 ± 0.20[a]	5.13 ± 0.26[bc]
RHB+SOIL	2.47 ± 0.12[a]	4.66 ± 0.89[a]	4.97 ± 0.48[bc]
WB+SOIL	2.10 ± 0.17[a]	4.31 ± 0.32[a]	5.48 ± 0.34[abc]
P - value	0.470	0.842	0.047

Means sharing a common superscript in a column are not significantly different at α = 0.05.

Table 3. Mean girth (cm) of *K. senegalensis* seedlings under different biochar treatments.

Treatment	Plant girth (cm)	
	Week 4	**Week 6**
Control (topsoil)	1.24 ± 0.10[b]	1.77 ± 0.273[a]
RHB	1.25 ± 0.056[ab]	1.99 ± 0.121[a]
GHB	1.39 ± 0.055[a]	1.63 ± 0.237[a]
WB	1.30 ± 0.048[ab]	1.83 ± 0.163[a]
RHB+SOIL	1.27 ± 0.029[ab]	1.87 ± 0.326[a]
GHB+SOIL	1.29 ± 0.035[ab]	1.77 ± 0.391[a]
WB+SOIL	1.25 ± 0.169[ab]	1.75 ± 0.1434[a]
P-value	0.355	0.730

Means sharing a common superscript in a column are not significantly different at α = 0.05.

Table 4. Mean chlorophyll index (mg m^{-2}) ± S.D under different biochar treatments.

Treatment	Chlorophyll content ± S. D		
	Week 2	**Week 4**	**Week 6**
Control (Topsoil)	30.40 ± 13.16[a]	44.62 ± 10.24[a]	49.92 ± 6.26[a]
RHB	18.32 ± 2.34[b]	26.52 ± 1.77[bc]	25.07 ± 1.45[c]
GHB	17.11 ± 0.91[b]	21.4 ± 2.93[c]	27.91 ± 6.99[bc]
WB	16.01 ± 3.79[b]	23.88 ± 2.87[bc]	24.57 ± 0.15[c]
RHB+SOIL	22.72 ± 6.05[ab]	31.98 ± 3.06[b]	31.74 ± 1.60[b]
GHB+SOIL	19.01 ± 1.51[b]	27.32 ± 4.17[bc]	31.15 ± 2.35[bc]
WB+SOIL	15.89 ± 3.01[b]	28.81 ± 5.89[bc]	27.97 ± 1.16[bc]
P – value	0.092	0.002	0.0001

Means sharing a common superscript in a column are not significantly different at α = 0.05.

form of lime (Nattaporn et al., 2013) which can increase nutrient availability for plant root uptake facilitating stem development.

Chlorophyll content of *K. senegalensis* leaves did not vary significantly between treatments in the second week after planting (P = 0.092). However, chlorophyll content (mg m^{-2}) differed significantly between treatments in the 4th (P = 0.002) and 6th (P = 0.0001) weeks after planting. Generally, in the 6th week after planting, seedlings of the control had the highest chlorophyll index (49.92 ± 6.26) whilst the WB recorded the least (24.57 ± 0.15) chlorophyll content (Table 4).

Chlorophyll content deviated from the trend for all other measured parameters with control (topsoil) recording a significantly higher chlorophyll content (49.92 ± 6.26) than all other treatments (P = 0.0001) in the 6th week after planting (Table 4). Perhaps the black colour of biochar served as a “black body” which absorbed sunlight

thereby reducing the amount of solar radiation available for chlorophyll formation.

Conclusion

Biochar prepared from different plant residues have varied effects on seed emergence and plant growth when used as growth media. GHB had the greatest effect on seedling emergence, plant height and number of leaves. However, in terms of plant girth, RHB mixed with soil produced seedlings with the largest stems. The topsoil (control) without any amendment had a unique influence on *K. senegalensis* seedlings, producing seedlings with high chlorophyll content. In general, GHB had the greatest effect on plant growth and could be used by nursery managers for raising tree seedlings in the Guinea savanna zone of Ghana.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Lead-induced changes in germination behavior, growth and inhibition of δ-aminolevulinic acid dehydratase activity in *Raphanus sativus* L.

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The seed germination test under lead (Pb) stress could be a quick test to understand plant tolerance to this heavy metal. The aim of this study was to compare the germination behavior of *Raphanus sativus* L. germinated in solution with different increasing concentration of lead, also to assess its accumulation and toxicity. The test was carried out in an incubator at 25 ± 1°C for 7 days and in a greenhouse for 11 weeks. Lead caused significant germination behavioral disturbances by changing the velocity coefficient and germination kinetics, with reducing the rate of early and final germination, however, the duration of germination was lengthened. Pb reduced the levels of the chlorophyll, delta-aminolevulinic acid dehydratase (ALAD) activity and growth. It increased lipid peroxidation and induced a significant accumulation of proline, positively correlated with Pb accumulation. Pb has a depressive effect on germination and causes disruptive disturbances of *R. sativus L* revealed by changes in non-enzymatic antioxidants, ALAD activity and growth. Radish has a capacity to accumulate Pb. The present results provide a model for detecting natural compounds able to improve seed germination of radish and counteract the harmful effects of lead.

Key words: Radish, concentration, rate, accumulation, proline, lipid peroxidation, chlorophyll.

INTRODUCTION

Lead is one of nature's most polluting heavy metals; it has harmful consequences on human and animal health and also on plants. It is the most encountered and the most toxic among plant pollutants. The plant accumulates lead in its various parts after absorption, Pb is not essential for its growth and its effect on the plant is different depending on the nature of the soil, the plant species and also its concentration (Sharma and Dubey, 2005; Patra et al., 2004).

In the plant cell when lead successfully penetrates, it increases the production of reactive oxygen species (ROS), which causes oxidative stress in different parts of the growing plant and leads to cell damages (Verma and Dubey, 2003). Lead has negative effects on the germination and growth of plants; it inhibits the photosynthesis process and reduces mineral nutrition

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and enzymatic activity (Mishra and Choudhari, 1998; Sharma and Dubey, 2005).

The leaves are the most important parts of the plant due to their role in producing food for the plant by capturing solar energy in the photosynthesis process. One of the first committed enzymes in the chlorophyll biosynthetic pathway is δ-aminolevulinic acid dehydratase (ALAD), which catalyzes the union of two δ-aminolevulinic acid (ALA) molecules into the monopyrrole porphobilinogen (PBG) (Jordan and Seehra, 1980). The cotyledons and leaves have a high chlorophyll content, they were used to measure the δ-aminolevulinic dehydratase activity and the chlorophyll content in order to determine the effect of lead on these two parameters. The purpose of our work was to evaluate the effects of lead on radish submitted at different concentrations, by analyzing plant tolerance, plant development, lead accumulation capacity and biochemical responses.

MATERIALS AND METHODS

The biological material that we used during the experiment is radish (*Raphanus sativus* L). The test was carried out in an incubator at 25 ± 1°C for 7 days for a seed germination test in laboratory of experimental bio-toxicology, bio-depollution and phytoremediation, then in the greenhouse of the University of Oran 1 Ahmed Ben Bella, Algeria for 11 weeks for a plant test.

Seed germination and plant growth

To prepare the seeds for germination, they were sterilized with 2% sodium hypochlorite for 10 min, then they were washed several times with distilled water to remove all traces of sodium hypochlorite. Seed germination was tested in Petri plates containing two Whatman filter papers. Ten seeds of radish were placed in each dish and moistened with 5 ml of either 0, 100, 250, 500 or 1000 mg l^{-1} of Pb solution. Eight replications were used in the experimentation for each concentration. The Petri plates were closed and maintained at 25 ± 1°C for 7 days in the incubator. Seed germination in each group was noted daily and the observations were performed for 7 days and germination rate was calculated. Two of the seedlings from each previous replication were planted in clean plastic pots filled with 400 g of sandy soil (2 V of sand / 1 V of compost) in a greenhouse.

Every week the replicates of each treatment were watered twice with lead acetate solution at different concentration and one time with Hoagland's solution for 11 weeks. Eight replications were used in the experimentation in the greenhouse for each concentration.

Determination of seedling growth and biomass

The length of seedlings was measured with a ruler (data expressed in millimeters) and the seedlings biomass was determined using a digital balance (the results expressed in grams).

Germination parameters

Early germination

This is the rate of first sprouts observed in the time interval between planting the seeds and their germination.

Estimation of germination rate (GR)

GR = (NI / NT) × 100

where NI: the total number of germinated seeds, NT: the number of seeds used in our experiment for each treatment (Li, 2008).

Mean daily germination

MDG = FGP / D

where FGP: final germination percent, D: test time -days (Osborne et al., 1993).

Kinetics of germination

Kinetics of germination is the evolution of the germination rate curve over a period of 7 days calculated according to the number of newly germinated seeds in each observation (Hajlaoui et al., 2007).

Mean time of germination (MTG) and coefficient of velocity (CVG)

It is an index of seed germination speed and velocity calculated by the following formula of Kotowski (1926).

$$CVG = (S_1 + S_2 + ... + Sn) / 【(S_1T_1) + (S_2T_2) + ... + (SnTn)】 \times 100$$

$$MTG = 【(S_1T_1) + (S_2T_2) + ... + (SnTn)】 / (S_1 + S_2 + ... + Sn)$$

where S_1: Number of seeds germinated at time T_1 and Sn: at time Tn.

Biochemical estimations

Determination of lead concentration

One to two grams of dry samples (plant and soil) was calcined at 450°C in a muffle oven for 4 h and they were digested with aqua regia (25% HNO_3 and 75% HCL) in the beakers. The beakers were heated until the contents had evaporated. The drops that remain in the beakers were dissolved in 10 ml of 5% HCL, filtered and then completed at 20 ml with 5% HCL. Lead was determined by atomic absorption spectroscopy (AAS) using SHIMADZU AA6600 apparatus (Tauzin and Juste, 1986).

Determination of total chlorophyll, chlorophyll a, chlorophyll b and carotenoids

100 mg of leaves cut into small fragments were placed in glass test tubes containing 10 ml of 95% acetone, then the tubes were placed in the cold at 4°C and in the dark for 48 h. The values of the assimilatory pigments levels were calculated according to the following formula (Lichtenthaler, 1987).

1) Total Chlorophyll: (µg. ml^{-1}) = 7.15 DO_{663} + 18. 71. DO_{647}
2) Chlorophyll *a*: (µg. ml^{-1}) = 12.25. DO_{663} – 2. 79. DO_{647}
3) Chlorophyll *b*: (µg. ml^{-1}) = 21.5. DO_{647} – 5. 10. DO_{663}
4) Carotenoids: (µg. ml^{-1}) = (1000 DO_{470} – 1.82 Chl *a* – 85.02 Chl *b*) / 198.

Determination of ALAD activity

100 mg of leaves were ground in a volume of 100 ml of Tris-HCl buffer (0.05 M, pH 8.75) which contains 10 mM $MgCl_2$ and 0 to 10 mM β-mercaptoethanol. The resulting mixture was filtered and centrifuged at 15000 g for 20 min.

The resulting supernatant was precipitated with 55% ammonium sulfate $(NH4)_2SO_4$ and then centrifuged at 15000 g for 30 min. The protein pellet was then dissolved in a minimum volume of the grinding buffer and used to measure the ALAD activity (Hault et al., 1987). The porphobilinogen (PBG) first monopyrole, the precursor of tetrapyrroles was determined using the Balance protocol (Balange, 1982). The activity of the ALAD was expressed in arbitrary units (DO at 560 nm).

Determination of lipid peroxidation

The accumulation of lipid peroxides in tissues was presented by the malondialdehyde (MDA) content using the term of thiobarbituric acid reactive substances (TBARS) (Velikova and Loreto, 2001). 1 g of samples was ground in 1 ml of ice-cold trichloroacetic acid (TCA), then it was centrifuged at 12000 g for 20 min. The supernatant was used for the determination of the TBARS in the samples using extinction coefficient of 155 mM^{-1} cm^{-1} (at wavelength of 532 nm).

Extraction and proline assay

100 mg of dry samples was ground with 1.25 ml of 95% ethanol, followed by three rinses with 1.25 ml of 70% ethanol each time, and then taken in test tubes. After 1 h, 2.5 ml of the upper phase was mixed with 1 ml of chloroform and 1.5 ml of distilled water. After stirring, the solution was placed in the cold for 48 h to obtain a good separation. The upper phase was used to determine the proline levels using the protocol of Bergman and Loxley (Bergman and Loxley, 1970). A standard curve was produced to evaluate proline concentration at 515 nm.

Statistical analysis

Data were subjected to one-way analysis of variance (ANOVA), a statistical package available from SPSS. Post-hoc testing was carried out using the LSD test. A significant level of 0.05 was used for all statistical tests. Results for each measured parameter were expressed as Mean ± Standard of Error (SE), n = 8 replicates.

RESULTS AND DISCUSSION

The root cells are responsible for the transport of lead from the external medium to the interior of the cell using the cation channels of the plasma membrane, in particular the Ca^{2+} channels (Seregin and Kozhevnikova, 2008). Penetrated lead can be accumulated or transferred to different parts of the plant. The results showed that the radish had a capacity to accumulate lead in these parts after exposure; this accumulation was significant when the Pb concentration was high. The accumulation was higher in the fibrous roots and taproot compared to the aerial parts (Figure 1A and B). These results concurred with many previous researches as has been reported *Nicotiana tabacum* (Gichner et al., 2008).

The effects of lead on seedling growth were not similar between plant species and between different parts of the same plant (Sharma and Dubey, 2005). The toxic effects of Pb depended on the dose and time of exposure; the development and germination of seedlings were severely limited by exposure to metal (Gichner et al., 2008).

The present results, as have been mentioned earlier, indicate that exposure to lead has reduced the fresh weight of radish at two periods (Figure 2A and B), which reveal a depressive effect of lead on plant development as compared to the control. Indeed, the biomass was reduced when the level of lead increased. Similar observations (Figure 2) have also been reported by Kibria et al. (2010) who noticed that a high concentration of Pb reduced the fresh and dry weight of the roots and even the aerial parts. The reduction of biomass could be the result of a direct effect of Pb on photosynthesis or on the physiological processes of the plant (Sharma and Dubey, 2005). Eun et al. (2000) reported that decreased root growth was the main effect of Pb, this inhibition can be correlated with the higher content of lead (Liu et al., 2008). This is in agreement with our results on the important decrease of roots length compared to the aerial parts for treatment of 7 days with a strong accumulation of lead. For treatment of 11 weeks, root elongation was significantly affected only at the dose of 1000 mg l^{-1} and the lengthening of the aerial part was not affected at the dose of 500 mg l^{-1} compared to the control (Figure 2C and D).

In Figure 3, at the macroscopic scale, lead caused adverse effects on plants at the germination stage, Pb inhibited germination and growth of seedlings and reduced length and the dry mass (Mishra and Choudhuri, 1998).

The results showed that tested seeds presented significant germination disturbances by reducing the rate of early germination (Figure 3A), mean daily germination (Figure 3B), coefficient of velocity of germination (Figure 3C) and final germination rate (Figure 3D) compared to controls. This depressive effect on the germination behavior was significant statistically, with increasing lead levels in the medium.

MTG was affected by lengthening when the medium of lead acetate concentration increases, 4 days for the control and 5 days for group treated with 1000 mgl^{-1} of Pb. For all groups that were treated with Pb, the rate of germination increased statistically not significant for the treated group at 100 mgl-1 in the 5, 6 and 7th day, but statistically highly significant for the groups receiving a treatment of 250, 500, or 1000 mgl-1 for all days compared to the control (Figure 3E). Our results coincided with Sengar et al. (2009) who observed a decline in seed germination rate.

It was noted that the process of photosynthesis was altered by Pb, explained by the decrease in the levels of

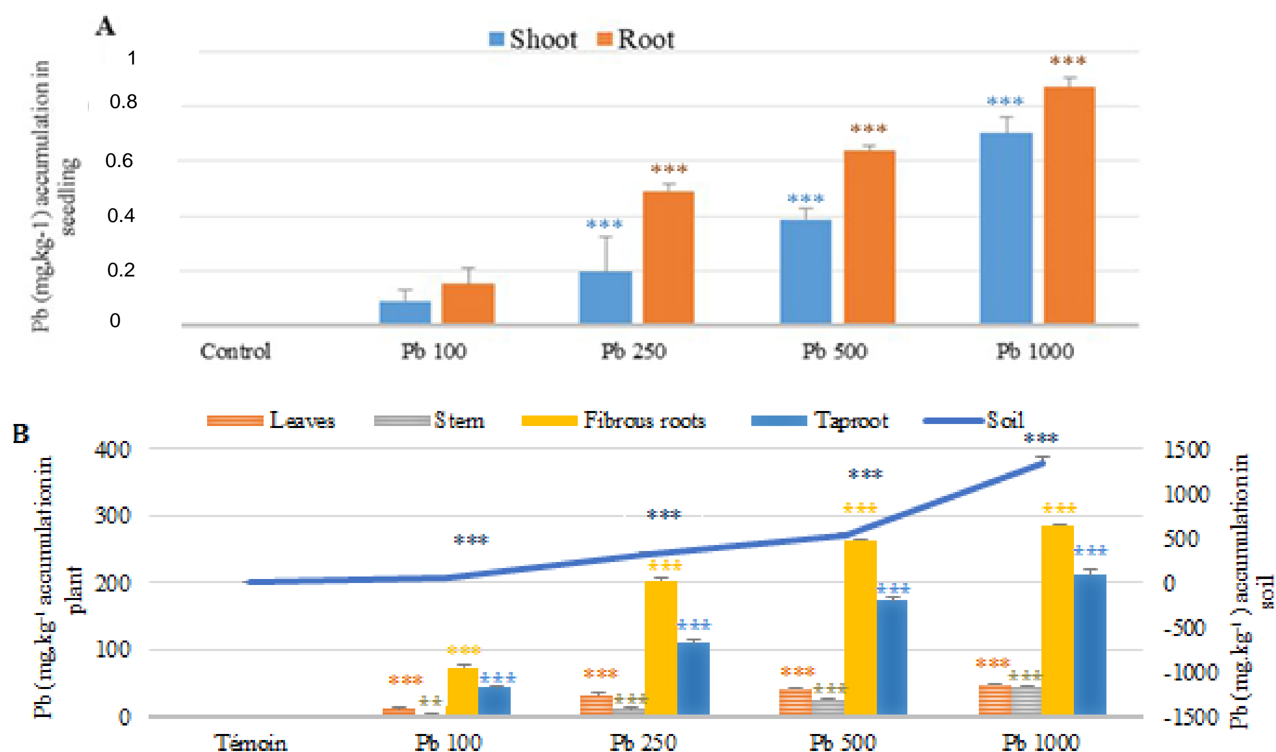

Figure 1. Accumulation of Pb (A) in shoot and root treated with Pb for 7 days, (B) in soil, leaves, stem, fibrous roots and taproot treated with Pb for 11 weeks of *Raphanus sativus* L. All values are expressed as Mean ± S.E, n= 3. Asterisks indicate significant differences between the treatments and the control (**P < 0.01; ***P < 0.001).

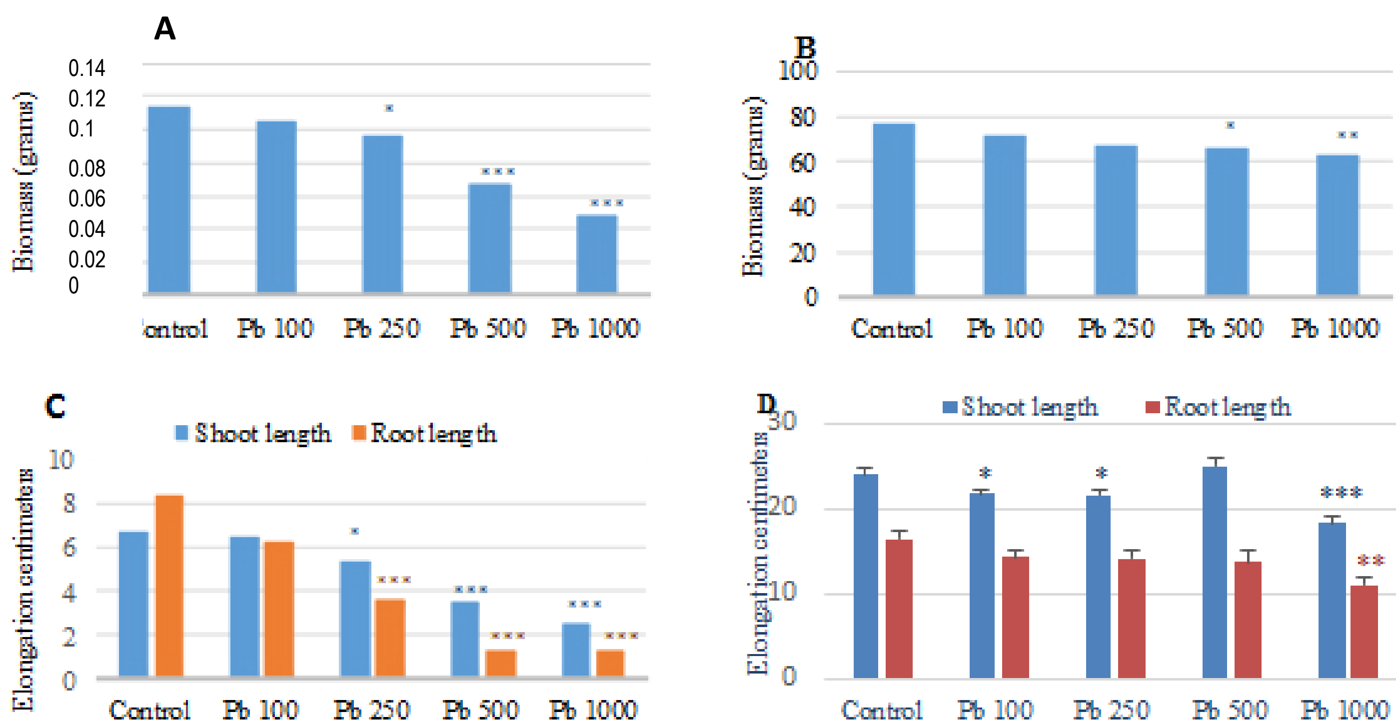

Figure 2. Effect of lead on (A) fresh weight for 7 days, (B) fresh weight for 11 weeks, (C) seedling length for 7 days and (D) plant length for 11 weeks of *Raphanus sativus* L.. All values are expressed as Mean ±SE, n = 8. Asterisks indicate significant differences between the treatments and the control (*P < 0.05; **P < 0.01; ***P< 0.001).

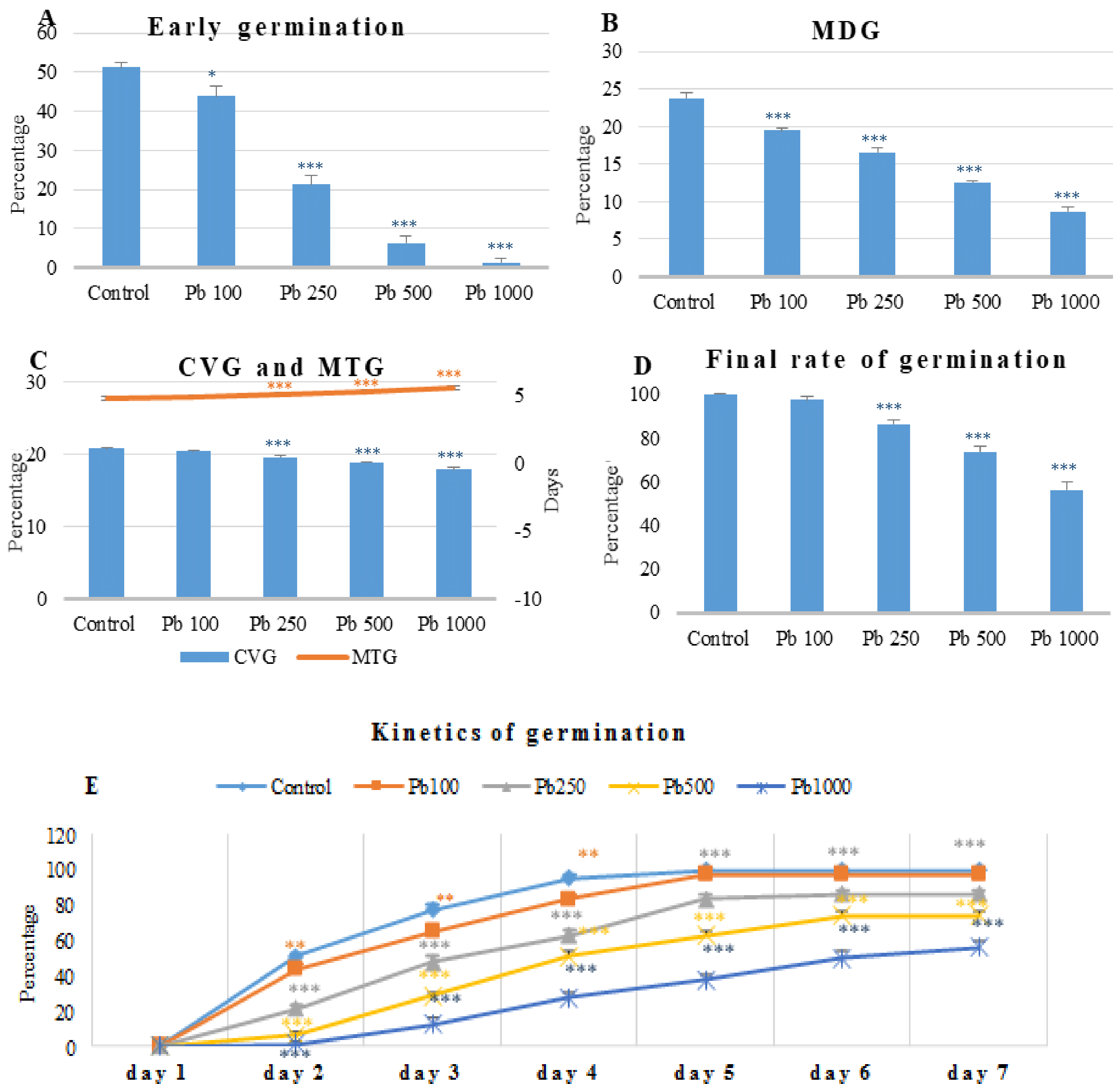

Figure 3. Effect of lead on (A) early germination, (B) mean daily germination (MDG), (C) coefficient of velocity of germination (CVG) and mean time to germination (MTG), (D) final rate of germination and (E) kinetics of germination of *Raphanus sativus* L. at 25°C ± 1 for 7 days. All values are expressed as Mean±S.E, n = 8. Asterisks indicate significant differences between the treatments and the control (*P < 0.05; **P < 0.01; ***P< 0.001).

chlorophylls and carotenoids. Chlorophyll a was more affected compared to chlorophyll b for treatment of 7 days. For treatment of 11 weeks, the carotenoid levels were significantly reduced for all treated groups; however, the chlorophyll b was not affected (Figure 4A and B). This reduction could be explained by the negative effect of Pb on the absorption of essential nutrients for plants such as Magnesium (Mg) and Iron (Fe) which results in the inhibition of photosynthesis or the closure of the stomata which results in a reduction in the amount of

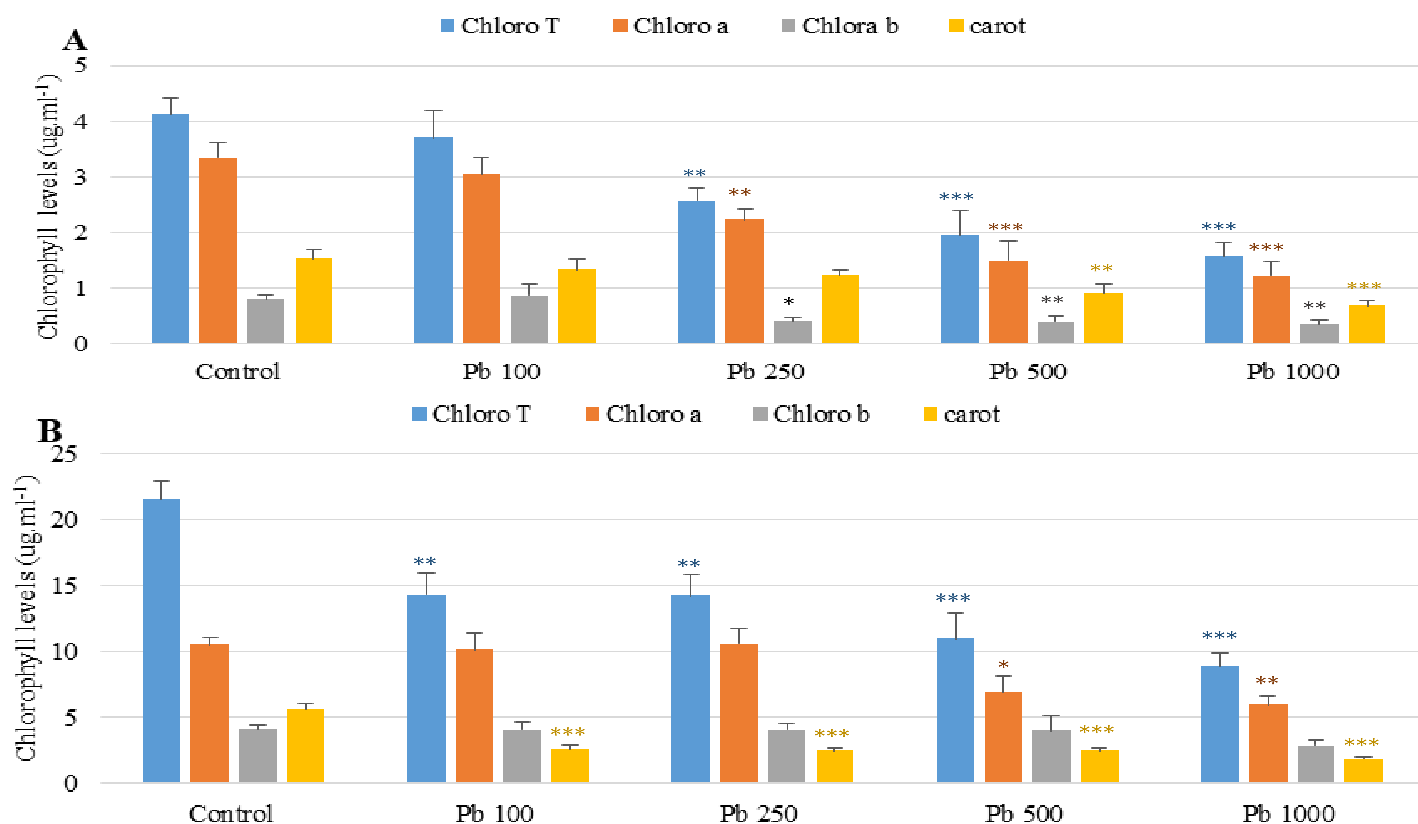

Figure 4. Effect of lead on levels of the total chlorophyll, a, b and carotenoids of *Raphanus sativus* L. for treatment of (A) 7 days and (B) 11 weeks. All values are expressed as Mean ± S.E, n = 8. Asterisks indicate significant differences between the treatments and the control (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$).

CO_2 (Burzynski, 1987; Sharma and Dubey, 2005). Pb could also alter the enzymatic activity such as the delta-aminolevulinic acid dehydratase which is the essential enzyme for photosynthesis (Cenkci et al., 2010).

The level of peroxidation content increased significantly in leaves, stem, fibrous roots and tap root during two periods with increasing lead concentration. The proline content was also significantly increased and ALAD activity was not affected for 7 days period, while it was significantly decreased for the 11 weeks period (Table 1). The absorption of heavy metals by plants has led to the generation of oxidative stress (Clemens, 2006b) and to the product of a large amount of ROS. MDA content is considered as an indicator of lipid peroxidation after abiotic stress (Ding et al., 2004) and biomarker of oxidative stress. Our results show that Pb causes increased level of lipid peroxidation in different parts of a plant after exposure. These results are similar to other published results, where an accumulation of lipid peroxidation content after exposure to Pb was also reported (Souza et al., 2012).

One of the first enzymes involved in the chlorophyll biosynthetic pathway is δ-aminolevulinic acid dehydratase (ALAD). At a high Pb concentration, our results showed a weak ALAD activity correlated with a decrease in the chlorophyll content, this disturbance could be caused by the accumulation of lead in the leaves.

At different concentrations of lead, ALAD activity was reduced. It seems to be more sensitive at the period of 11 weeks, while at the period of 7 days it is less sensitive.

Similar results that indicate inhibition of ALAD activity by Cd in soybean which were obtained by Noriega et al. (2007).

Proline accumulation is one of the methods by which plants can combat the toxic effect of heavy metals. The accumulation of proline contributes to the protection of enzymes, to the detoxification and chelation of metals, to the trapping of reactive oxygen species and to the stabilization of the protein synthesis (Sharmila and Pardha, 2002). Proline is synthesized from glutamic acid via 1-pyrroline-5-carboxylic acid (P5C), but also via arginine and ornithine (Lignowski and Splittstoesser, 1971). Moreover, glutamate and 2-oxoglutarate are the precursors of ALA in higher plants. Our results showed a large accumulation of proline correlated with a decrease in chlorophyll pigments (Table 2). This correlation is negative one for these two parameters. These results

Table 1. Effect of lead on levels of malondialdehyde, proline and ALAD activity of *Raphanus sativus* L. (A for treatment of 7 days and B 11 weeks).

Parameter	MDA (nM/g fresh tissue weight)				Proline (µg/ml^{-1})				ALAD arbitrary unit
	Leaves	Stem	Root		Leaves	Stem	Root		Leaves
Control	1.357±0.18	1.535±0.45	1.034±0.16		10.97±0.58	6.70±0.43	5.88±0.28		0.0256±0.007
Pb100	2.289±0.46^{NS}	2.810±0.58^{NS}	2.172±0.27^{NS}		17.25±0.92^{NS}	8.46±1.34^{NS}	8.67±0.50*		0.0198±0.005^{NS}
Pb250	3.803±0.54***	4.92±0.47***	4.085±0.69**		29.38±1.83***	11.78±1.30**	12.27±0.91***		0.0193±0.002^{NS}
Pb500	5.053±0.42***	4.836±0.41***	5.153±0.92***		34.93±4.57***	17.72±0.72***	15.93±0.87***		0.0189±0.002^{NS}
Pb1000	6.272±0.31***	6.701±0.65***	5.254±0.65***		40.71±0.71***	22.42±1.11***	20.29±1.06***		0.0174±0.002^{NS}
	Leaves	**Stem**	**Fibrous roots**	**Taproot**	**Leaves**	**Stem**	**Fibrous roots**	**Taproot**	**Leaves**
Control	5.10±1.07	6.40±1.14	8.92±0.69	11.39±1.89	177.47±36.91	161.78±36.34	55.15±8.58	68.05±13.55	0.280±0.016
Pb100	9.92±0.89^{NS}	7.31±0.762^{NS}	12.80±2.49^{NS}	13.95±2.39^{NS}	326.27±41.70^{NS}	199.50±20.18^{NS}	107.41±24.62^{NS}	121.20±35.60^{NS}	0.261±0.015^{NS}
Pb250	14.46±2.48***	10.00±1.58^{NS}	22.83±3.02**	25.73±2.47**	788.66±120.28***	234.05±35.30^{NS}	303.46±39.44***	395.22±60.17**	0.228±0.017**
Pb500	15.79±2.17***	11.36±1.76^{NS}	22.84±5.62**	28.31±5.63**	821.38±94.52***	435.04±67.88***	393.31±54.70***	405.49±76.74**	0.215±0.003**
Pb1000	15.08±1.10***	15.02±3.32**	29.47±2.59***	34.85±2.27***	1161.90±164.78***	496.79±69.09***	400.15±48.10***	431.79±96.01***	0.205±0.06***

All values are expressed as Mean ± E.S.M, n= 8. (NS not significant, *p < 0.05; **p < 0.01; ***p < 0.001).

Table 2. Spearman correlation matrix between lead shoot (Pb sh), total chlorophyll (Chlo T), chlorophyll a (Chlo a), chlorophyll b (Chlo b), carotenoid (carot), proline, lipid peroxidation (MDA) and ALAD activity in leaves (A for 7 days and B for 11 weeks).

Parameter	Pb sh	Chlo T	Chlo a	Chlo b	Carot	Proline	ALAD	MDA
A								
Pb sh	1							
Chlo T	-0.683**	1						
Chlo a	-0.668**	0.942**	1					
Chlo b	0.835**	0.771**	0.747**	1				
Carot	-0.604**	0.872**	0.864**	0.595**	1			
Proline	0.945**	-0.603**	-0.651**	-0.661**	-0.526**	1		
ALAD	0.007	-0.013	0.070	-0.037	-0.077	-0.126	1	
MDA	0.926**	-0.541**	-0.619**	-0.510**	-0.424**	0.731**	-0.047	1
B								
Pb sh	1							
Chlo T	-0.479	1						
Chlo a	-0.193	0.856**	1					
Chlo b	0.046	0.828**	0.798**	1				
Carot	-0.543*	0.864**	0.692**	0.647**	1			
Proline	0.573*	-0.555**	-0.377*	-0.245	-0.583**	1		
ALAD	-0.482	0.379*	0.312	0.242	0.375*	-0.569**	1	
MDA	0.675**	-0.521**	-0.320**	-0.273	-0.456**	0.669**	-0.344**	1

suggest there is a link between the biosynthetic pathways of chlorophyll pigments and proline. The competition between these two compounds on their common precursor, glutamate, can be at the origin of this response.

Conclusions

It is clear from the current study that lead has inhibitory effects on radish seed germination capacity, seedlings and plants growth and leads to the generation of oxidative stress. Radish can withstand high concentrations of lead and it has the power to accumulate it in its different parts. Also, we suggest using natural compounds that can improve the germination and development of radish and counteract the harmful effects of lead to increase its capacity to resist lead buildup.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Effects of sowing depth on seed germination and seedling growth of *Aframomum citratum* (Pereira) K. Schum

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Series of investigations have been carried out on the effect of sowing depths on seed germination and early seedlings growth of *Aframomum citratum* and has shown that sowing depth influences germination and early growth of most plant species. Thus, the importance of varying planting depths has received much attention from researchers for some time now. The depths studied in this research were 0, 3 and 6 cm, respectively. Using a split plot complete randomized design (CRD) in a non-mist propagator, a total of 864 seeds (432 fresh and 432 dry) of *A. citratum* were sown with three replicates for each soil type in prepared polyethylene bags. Germination was monitored daily for a period of six weeks while data for germination parameters was collected. Early growth parameters such as average number of leaves (NL), average leaf surface area (SA) and average height of seedlings, (SH) were measured every week for two months. Results revealed that germination started 2 months after fresh seeds were sown in all soil types. Dried seeds being treated with 50% dilution of concentrated sulphuric acid for 20 min did not germinate during the germination period that ranged from 2 to 7 months. Sowing depths significantly affected the cumulative germination percentage and early growth ($p<0.05$). Thus, the highest percentage of seedlings was produced at 0 cm sowing depth, followed by 3 cm sowing depth and the least was at 6 cm sowing depth. Germination of *A. citratum* seeds can be done based on the information given in this study.

Key words: *Aframomum citratum*, seeds, sowing depths, germination, early growth.

INTRODUCTION

The genus *Aframomum* K. Schum belongs to the Zingiberaceae family and is represented in Cameroon by over 20 species of rhizomatous herbs (Anjah et al., 2015a). It can be propagated by seed and vegetative parts (rhizomes), but by seed is the better way, as virus diseases are not transmitted through seeds (Dawid et al., 2014).

Aframomum citratum is a perennial herb with underground rhizome and stems up to 4 m high which are red at the young stage of growth. Its leaflets have

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petioles of about 2 cm long oblong linear blade and an asymmetric rounded base with inflorescences that originate at the base of the stem and having reddish ovate bracts up to 6 cm wide. It has characteristically trimmer flowers, tubular calyx, oblique truncates, and up to 7.5 cm in length and possess 3-lobed tubular corolla spreading oval with sharp edges, measuring about 7 cm x 5 cm. Further, it has ovoid fruits with capsule 3 cm in diameter, extended by the long tube of the calyx; seeds are small (± 3 mm in diameter), numerous, sub-globulous, contained in a white pulp (MINFOF, 2007); and produces purple coloured flowers which develop into pods that can be as long as 8 cm and about 3 cm wide, with each pod containing numerous reddish brown seeds (can be as many as 300 seeds in one pod).

The works of Lekané (2009) show that the leaves of *Aframomum* spp thanks to their particular aroma are used in packaging of the different traditional dishes; the fruits are sold to generate income both nationally and internationally; and are also being eaten fresh by men, monkeys and rodents. The seeds are used as spices in kitchens for various dishes in Bassa communities in Cameroon (Vivien and Faure, 1995). They are important for soil conservation through their rhizomes and large leaves that protect soils against erosion (Eyob et al*.,* 2008). Nelson et al. (2010) demonstrated that seed extract of this plant is a potential antifeedant, which can be used as an environmentally friendly insecticide. Aframomum seed is used in traditional pharmacology to cure cough. They also serve as a thickening agent in medicinal preparations (Laird, 2000). Some local populations in Kenya use stem juice extract and its leaves to treat wounds and intestinal infections (Akendengue, 1994). *A. citratum* plants are exploited for the use of their aromatic essential oils (Amvam et al*.,* 2002). Socio-culturally, the uses of the fruits and plants of Aframomum enter into the rites of enthronement of the traditional chiefs, of succession, and rites of initiation in the secret societies. These seeds are crushed and mixed with a white powder and then used during the ceremonies of valorization of twins. Ingram and Schure (2010) scored *A. citratum* in Cameroon three at a marketable value, indicating that the species is at large-scale trade. According to Tabuna (1999), the fruits of this species were sold in Africa and Europe. *A. citratum* has been designated a non-forest timber product (NTFP) in the Congo Basin and the part exploited are the seeds and leaves (FAO, 2004).

Despite the socioeconomic importance that *A. citratum* represents, it still presents a slow germination process of seeds and seedling development, which can be influenced by a series of factors such as sowing depth, agronomic cultivar and edapho-climatic conditions. The depth of sowing is one of the most important factors with influence on the seed germination of many plant species. Too shallow sowing results in poor germination due to inadequate soil moisture at the top soil layer. On the other hand, deep sowing can also significantly reduce crop emergence and yield. The deeper the seeds are placed, the more time it will take for them to emerge from the soil. The seeds sown deeper should produce shorter seedlings than the seeds sown at recommended depth of the crop (Raju et al*.,* 2017). The germination of *A. citratum* can be slow, surpassing 2 months, which leads to higher susceptibility to pathogens and stresses caused by the environment.

Multiplication through seeds of *Aframomum* spp is very difficult, and this can be related to factors including the soil retention capacity of water and the ability of the embryo to absorb water (Anjah et al., 2015a). Also, the ability of a soil to retain water depends on its texture and topography (Assie et al., 2010).

Another problem with these species is seed dormancy which may be general for all *Aframomum* species since in Cameroon, Anjah et al. (2015b) reported that seeds of dry *Aframomum melegueta*, have trouble sprouting. Dawid et al. (2014) also reported same for *Aframomum cororima*. Although several pretreatments exist, works on the elevation of the dormancy of seeds of *A. citratum* are very rare if not non-existent.

Aframomum spp regenerates easily by rhizome fragments with or without stem. This method of regeneration remains critical because it does not assure the sustainable survival of the species (Eyob et al., 2006), yet regeneration of plant species through vegetative propagation makes it possible to bypass the multiple problems posed by the seed (WAC, 2003).

Irrespective of the very low populations of *Aframomum* species, *A. citratum* in particular is getting to extinction, largely due to difficulties of sustainable harvesting, the economically unviable commercialization, and the lack of biological and ecological information on many other potential uses of the species (Sunderland et al., 2001; Van den Berg et al., 2001; Hyacinthe, 2015).

It is therefore necessary that further studies be carried out to determine the effect of seed sowing depth on the germination and early growth of *A. citratum*. Hence, it is necessary to stimulate public interest on their conservation, sustainable exploitation and possibly cultivation.

MATERIALS AND METHODS

Soil sample collection

This study was carried out in two phases. The first involved the collection of soils from three regions of Cameroon on a one hectare land each.: Buea situated in the Southwest region in latitude 4º10′0″N, longitude 9° 14′ 0″ E and altitude 870 m; Bamenda situated in the Northwest region in latitude 5º 57’ N, longitude 10º 10’ E and altitude 1,614 m and Dschang situated in the West region in latitude 5° 26' N, longitude 10° 26' E and altitude 1,400 m. The zigzag sampling method was used to collect soils (FAO, 2004).

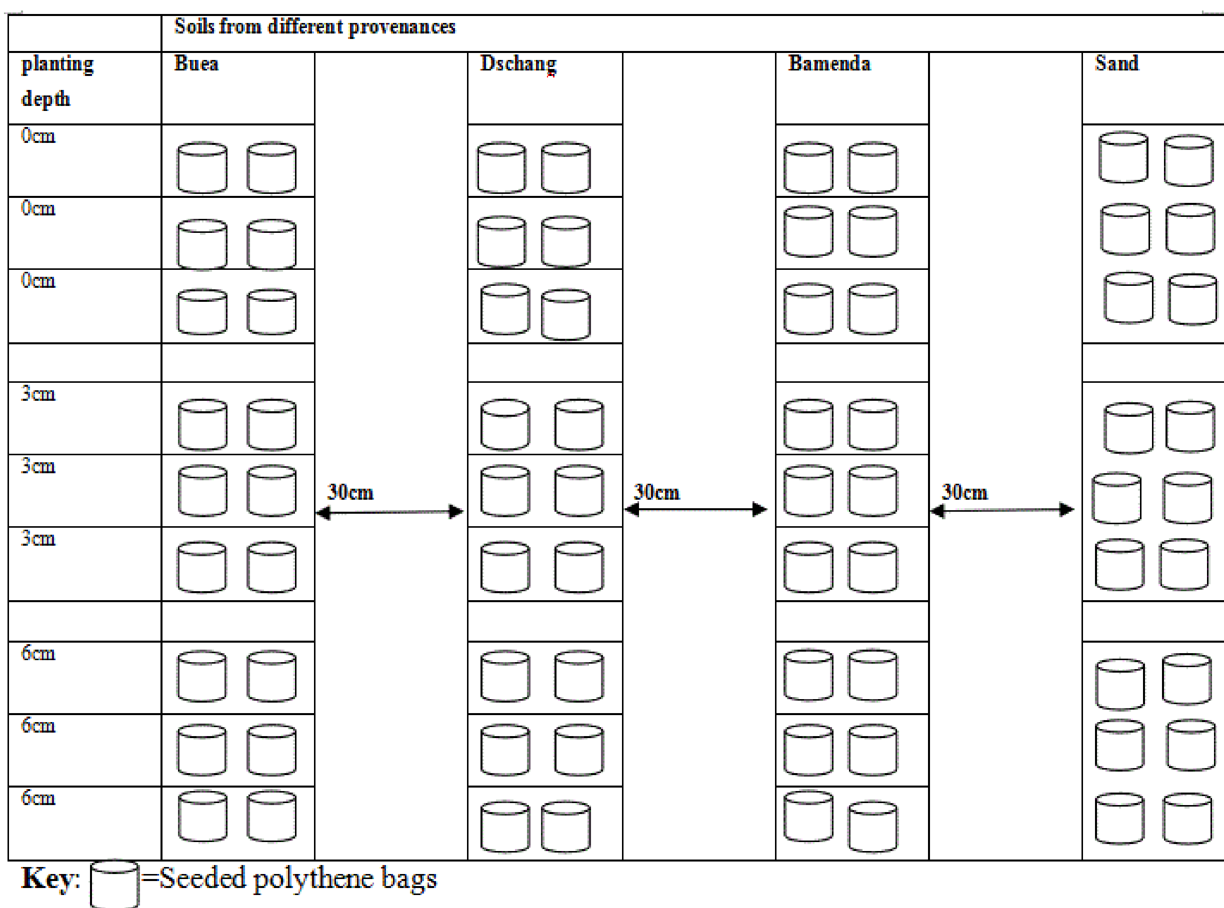

Figure 1. Experimental design for seed germination and seedling growth.

From each site, soils were dug from 13 different points using a soil auger at a depth of 0-20 cm. The soils of all the points were put into a bucket and mixed to have a homogenous mixture, and then put into labelled polythene bags.

The second phase involved observation and monitoring of germination and early growth parameters, by the establishment of a non-midst propagator. The study was conducted in the Nursery Department of Forestry, FASA located in the University of Dschang, Cameroon.

Source of *Aframomum citratum* seeds

Seeds were collected from the natural habitat of Bali Nyonga (Northwest region). The collection was done in May 2016 and kept in paper bag fully labelled.

Germination procedure

Floatation test was done to estimate the percentage of viable seeds in a seed lot. Both fresh and dried seeds of *A. citratum* were soaked into separate containers of water for 5 days. The seeds that floated were not viable while those that settled at the bottom of the container were considered viable to germinate. The germination experiment was conducted in the Nursery of the Department of Forestry, FASA located in the University of Dschang, Cameroon.

The seeds were sown in perforated polythene bags at different depths of soil, that is, 0, 3 and 6 cm, respectively. A total of 864 seeds (432 fresh and 432 dry) of *A. citratum* were used for this research with six seeds sown in each polythene bag randomly. Four replicates were used for each treatment. A total of 144 polythene bags (each batch containing 36 pots) were filled per soil origins and same for sand. After sowing of seed, they were covered with the soil of same composition to the level of required thickness for each treatment. Regular watering was done to maintain the proper moisture content. During the experiments, number of days taken for the emergence of first seedling and completion of germination were recorded in all the treatments. The polythene bags were laid out in a randomized complete block design inside a non-mist propagator of 4 m × 2 m (Figure 1). The 144 pots filled were treated with fungicides (Furaplants) to prevent fungal attack. Each batch of 36 pots filled was then divided into two groups of 18 pots each, in which were sown 108 fresh seeds and 108 dry seeds of *A. citratum.* Using a ruler and the edge of an HB pencil, the depths were determined. The ruler was placed close to the pencil, and the different sowing depths marked on the pencil. Seeds were sown in prepared polyethylene bags, which were treated with a systemic fungicide and nematicide prior to sowing of seeds. At the end of experiment, the following germination and early growth

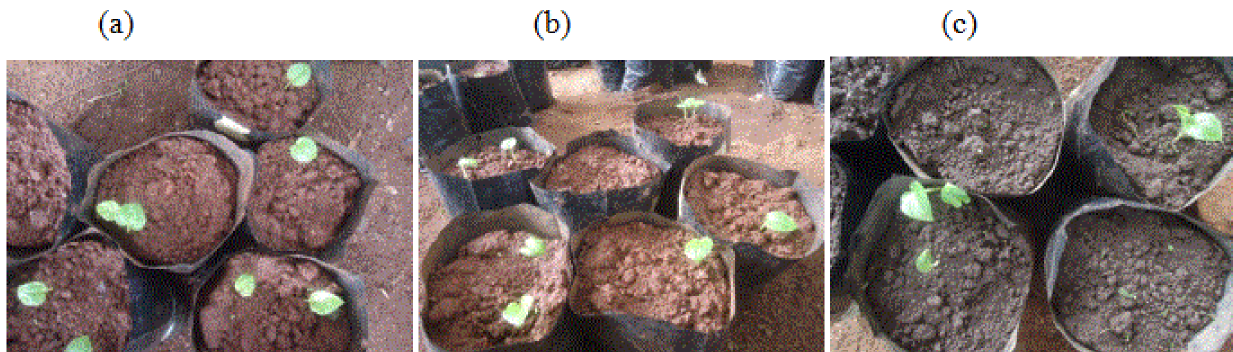

Figure 2. Early stage of germination: (a) Bamenda soil; (b) Dschang soil; (c) Buea soil.

parameters for each sowing depth were monitored and calculated every week: shoot height was measured from the base of the plants to the apex, while number of leaves was counted on the stem, germination percentage and leaf surface area.

Data collection

$$\text{Germination percentage (GP)} = \frac{\text{No. of seeds that germinated}}{\text{Total no. of seeds that were planted}} \times 100$$

(Niang et al., 2010).

$$\text{Germination speed (GS)} = \frac{n1}{1} + \frac{n2}{2} + \frac{n3}{3} + \cdots \frac{nx}{x}$$

(Keshava et al., 2014)

Where $n_1 \ldots n_x$ = number of seed germinated per day and 1, 2, 3…x = number of days.

Since all seeds did not germinate at the same time, averagely 2 seedlings of *A. citratum* emerged from each germination bag, having similar morphological characteristics which were selected to study early growth parameters. Early growth parameters such as average number of leaves, average leaf surface area and average height of seedlings, were measured and calculated as follows:

i) Shoot height (SH) was measured from the base of the plants to the apex.
ii) Number of leaves (NL) was counted on the stem every week.
iii) Leaf surface area (SA) = L × W × 2/3 (Raunkiaer, 1934), where L: length of leaf blade and W: width of leaf blade.

Data analysis

The data were subjected to analysis of variance (ANOVA) using XLSTAT software and the treatment means separated using Duncan Multiple Range Test.

RESULTS

Few of the seeds started germinating seven weeks after the day of sowing (Figure 2) and germination was monitored for the next 46 days. Observation and measurements of early growth parameters began 7 months after day of sowing. Out of the 432 fresh seeds and 432 dry seeds sown in the germination test, it was observed that only 200 fresh seeds germinated and dry seeds which were pre-treated for 20 min in concentrated sulphuric acid diluted at 50% did not germinate at all. For this research work, germination was defined as the point at which a seedling appeared at the soil surface.

Sowing depths and germination of *A. citratum* seedlings

The results show that germination was affected negatively by increase in sowing depth, since germination speed was highest at sowing depth of 0 cm (4.43) and lowest at sowing depth of 6 cm (0.97). Also, the number of seeds that germinated decreased with increase in sowing depth, hence germination percentage. As shown in Figure 3, germination percentage decreased with increased sowing depth (Figure 3).

Effects of sowing depth on early growth parameters of *A. citratum* seedlings

Effects of sowing depth on number of leaves

There was a fluctuation in seedling number of leaves throughout the 8 weeks of observation at different sowing depths. At the end of this study, the highest number of leaves were produced by seedlings in Buea soil at 0 cm (11 leaves) while the lowest number of leaves was produced by seedlings in Buea soil at 6 cm (2 leaves). Generally, the seeds sown at depths of 0 cm produced seedlings with highest number of leaves, whereas seeds sown at depths of 6 cm produced seedlings with lower

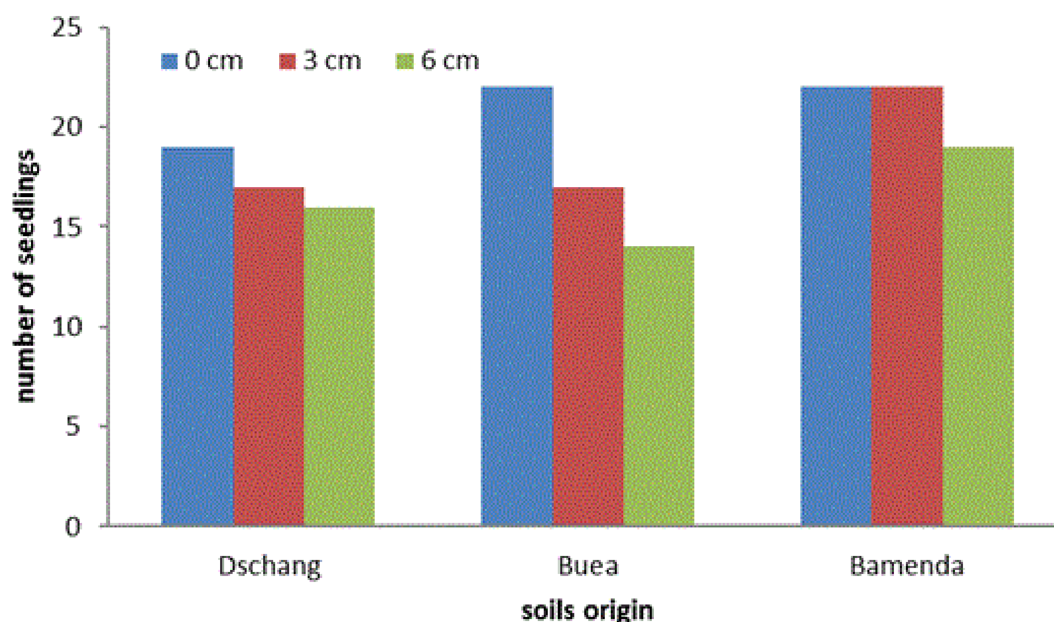

Figure 3. Number of seedlings, sowing depths and soils origin.

Table 1. Effect of different sowing depths on the mean number of leaves.

Sowing depth	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8
Buea (0 cm)	6.92±1.00[a]	6.92±1.00[ab]	7.50±1.78[a]	7.67±1.67[a]	8.00±1.65[a]	8.42±1.68[a]	8.92±1.68[a]	9.08±2.11[a]
Bamenda (0 cm)	7.42±1.16[a]	7.58±1.16[a]	7.33±1.44[a]	6.83±1.19[ab]	6.92±1.44[ab]	7.50±1.62[ab]	7.50±1.57[ab]	7.58±1.68[abc]
Dschang (0 cm)	5.58±1.16[d]	7.50±0.90[a]	7.17±1.11[a]	6.83±1.53[ab]	7.17±1.34[ab]	7.83±1.40[ab]	7.42±1.56[ab]	8.00±2.13[ab]
Dschang (3 cm)	6.75±0.62[abc]	7.33±0.89[a]	7.25±0.75[a]	6.67±1.44[ab]	7.08±1.31[ab]	7.58±1.68[ab]	7.42±2.07[ab]	7.92±2.15[ab]
Bamenda (3 cm)	6.75±1.36[ab]	7.17±1.03[a]	6.75±1.29[ab]	6.75±1.22[ab]	6.92±1.38[ab]	7.25±1.60[ab]	7.33±1.50[b]	7.67±1.67[abc]
Buea (3 cm)	5.75±1.22[cd]	6.17±0.94[bc]	5.92±1.83[bc]	5.92±1.83[bc]	6.42±1.88[bc]	7.08±1.38[ab]	7.33±2.10[b]	8.25±2.38[ab]
Dschang (6 cm)	5.83±1.40[bcd]	6.83±0.94[ab]	6.58±0.90[ab]	6.33±1.23[bc]	6.42±1.31[bc]	6.75±1.14[bc]	6.83±1.47[bc]	6.92±1.24[bcd]
Bamenda (6 cm)	4.83±1.03[d]	5.33±1.44[c]	5.33±1.44[c]	5.33±1.44[c]	5.42±1.24[cd]	5.75±1.76[cd]	5.75±1.71[cd]	6.25±1.29[cd]
Buea (6 cm)	3.33±1.15[e]	3.75±1.36d[d]	3.92±1.24[d]	3.92±1.24[d]	4.25±1.54[d]	5.00±1.04[d]	5.00±1.71[d]	5.67±1.61[d]

Means in the same week followed by the same letter in the row and column do not differ significantly by Duncan test at 5%.

number of leaves. Statistically, with respect to the different sowing depths, a significant decrease ($p \leq 0.05$) occurred for each soil type as sowing depth increased (Table 1).

Effect of planting depth on plant height

Depth of sowing is an important factor in maximizing the potential of shoot height. The effect of depth of sowing on plant height of *A. citratum* is represented in Table 2. The highest seedling height resulted from Dschang soil at 0 cm (24.03±7.36 cm) while the shortest shoot height (2.42±0.67 cm) resulted from Buea soil at 6 cm. For each soil type, shoot height of seedlings at 0 cm were longer than shoot height of seedlings at 6 cm. There was a significant difference ($p<0.05$) in the decrease of seedling shoot height as sowing depth increased. Meanwhile, seedling shoot height increased throughout the 8 weeks of growth observation (Table 2).

Leaf surface area

From Table 3, it was observed that the leaf surface area showed significant differences (0<0.05) for seedlings at different sowing depths. The leaf surface area for seedlings in Buea soil at 0 cm had the largest surface

Table 2. Effects of different sowing depths on the height of *A. citratum* seedlings in different soil types.

Sowing depth	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8
Dschang (0 cm)	11.00±2.83^{a}	12.92±3.42^{a}	15.08±4.48^{a}	16.96±5.33^{a}	19.00±6.12^{a}	22.08±6.89^{a}	22.50±7.06^{a}	24.03±7.36^{ab}
Bamenda (0 cm)	11.00±4.00^{a}	12.00±4.11^{ab}	13.50±4.56^{a}	15.50±5.49^{a}	17.92±6.65^{a}	20.10±7.73^{ab}	20.78±7.68^{ab}	21.78±8.07^{abc}
Buea (0 cm)	8.17±3.61^{bc}	10.17±3.61^{bc}	12.17±3.61^{ab}	14.25±3.54^{ab}	17.79±4.83^{a}	20.63±6.10^{ab}	22.84±5.74^{a}	24.49±5.68^{a}
Dschang (3 cm)	10.00±2.95^{ab}	11.83±3.04^{ab}	13.58±3.29^{a}	15.46±3.71^{a}	17.13±4.13^{ab}	18.79±3.95^{abc}	20.15±4.27^{ab}	22.13±4.14^{a}
Bamenda (3 cm)	9.58±3.65^{ab}	10.75±3.62^{ab}	12.25±4.49^{ab}	13.78±5.28^{ab}	15.28±6.10^{abc}	17.23±6.09^{bcd}	18.06±6.63^{abc}	19.46±6.50^{bcd}
Buea (3 cm)	6.25±2.22^{cd}	7.92±2.31^{cd}	9.67±2.61^{bc}	11.54±2.55^{bc}	13.25±3.37^{bcd}	15.22±4.22^{cde}	17.51±5.94^{bc}	18.68±5.16^{cd}
Dschang (6 cm)	4.58±2.23^{de}	6.42±2.68^{de}	8.33±2.93^{cd}	10.04±2.93^{c}	11.53±3.08^{cd}	13.13±3.45^{de}	13.89±3.78^{cd}	15.62±3.80^{de}
Bamenda (6 cm)	4.00±2.04^{de}	5.25±2.34^{e}	6.58±2.47^{d}	8.21±2.87^{c}	9.71±2.91^{d}	10.78±3.80^{e}	11.34±3.79^{d}	12.77±4.11^{e}
Buea (6 cm)	2.42±0.67^{e}	2.58±1.00^{f}	2.92±1.51^{e}	3.85±1.37^{d}	4.22±1.43^{e}	4.92±1.95^{f}	4.67±1.89^{e}	5.42±2.51^{f}

Means in the same week followed by the same letter in the row and column do not differ significantly by Duncan test at 5%.

Table 3. Effects of different sowing depths on the leaf surface area of *A. citratum* seedlings.

Sowing depth	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8
Bamenda (0 cm)	24.94±11.90^{a}	27.67±13.05^{a}	30.13±13.44^{a}	31.91±13.75^{a}	34.54±15.72^{a}	36.80±16.46^{a}	38.17±15.87^{ab}	40.97±15.53^{a}
Buea (0 cm)	20.62±8.43^{ab}	23.08±7.83^{ab}	24.72±7.99^{ab}	26.79±8.57^{ab}	32.39±9.55^{ab}	36.20±9.46^{a}	39.40±10.53^{a}	41.41±11.31^{a}
Dschang (0 cm)	20.69±9.95^{ab}	23.05±9.98^{ab}	25.33±9.48^{ab}	30.18±10.26^{a}	33.22±11.66^{a}	35.51±12.51^{ab}	37.84±13.11^{ab}	39.85±13.26^{a}
Dschang (3 cm)	18.64±4.10^{b}	21.54±4.27^{ab}	23.85±5.57^{ab}	25.40±5.85^{ab}	28.50±5.00^{abc}	30.70±5.00^{ab}	32.15±5.37^{ab}	34.51±5.46^{ab}
Buea (3 cm)	16.68±6.90^{bc}	17.80±7.00^{bc}	19.09±7.06^{bc}	20.34±7.44^{bc}	23.00±8.51^{cde}	29.45±12.78^{abc}	32.07±13.93^{ab}	34.68±14.59^{ab}
Bamenda(3 cm)	16.11±5.63^{bc}	17.68±5.41^{bc}	19.51±6.71^{bc}	21.52±7.12^{bc}	24.92±9.13^{bcd}	27.22±10.00^{bc}	29.84±10.13^{bc}	33.01±10.58^{ab}
Dschang (6 cm)	11.60±3.34^{cd}	13.69±3.94^{cd}	15.70±3.68^{cd}	17.54±3.80^{cd}	20.34±3.02^{de}	21.19±3.62^{cd}	23.36±4.08^{cd}	26.52±4.08^{bc}
Bamenda (6 cm)	8.00±4.13^{de}	9.43±4.98^{de}	11.15±4.84^{d}	13.53±4.49^{d}	15.64±4.20^{e}	17.13±4.93^{d}	18.25±4.44^{d}	20.88±6.38^{c}
Buea (6 cm)	2.78±1.90^{e}	3.81±2.52^{e}	4.73±3.59^{e}	5.61±3.63^{e}	5.81±3.85^{f}	6.41±4.01^{e}	7.83±5.68^{e}	9.82±6.33^{d}

Means in the same week followed by the same letter in the row and column do not differ significantly by Duncan test at 5%.

area (41.41±11.31 cm) and seedlings in Buea soil at 6 cm had the smallest leaf surface area (9.82±6.33 cm). During this research, leaf surface area decreased with increase in sowing depth and increased from one week to the next (Table 3).

DISCUSSION

In order for established plants to maintain a positive yield, crop seedling germination and growth should be high. Hence, knowing the sowing depth is a useful information for attaining an appropriate stand density and resultant optimum crop performance. The results of the experiments conducted indicate that seeds of *A. citratum* had a very low germination percentage when they were sown at sowing depths of 6 cm. Similar results were observed by Mohammed et al. (2004). Germination speed reduced from a mean value of 4.43 at the 0 cm planting depth to a mean value of 0.97 at the 6 cm.

The mass yield of germination decreases whenever small seeds are sown at deep depths. This was the case during our study, in which we observed that *A. citratum* seeds performed better when seeds were sown at 0 cm and 3 cm, meanwhile the lowest means were recorded when seeds were sown at 6 cm. The negative effect of sowing depth was reported by other researchers who found that seedling emergence of cotton seed decreased with increased depth (Nabi et al*.,* 2001). Hojjat (2011) reported that the germination parameters were significantly related by seed weight and large seeds germinated early, showing better germination than small seeds of lentil genotypes. An interaction between seed size and depth of planting indicated that the number of germinated seeds was greatly reduced with increased depth of planting; too shallow sowing results in poor germination due to inadequate soil moisture at the top soil layer while deep sowing can also significantly reduce crop emergence and yield (Aikins et al., 2006).

The observed results with regard to germination percentage are in concurrence with findings of Roozrokh et al. (2005) on chick pea. The negative effect of deep sowing depth was reported by Nabi et al. (2001) who found that seedling emergence decreased with increased

sowing depth in cotton. The deeper the seed is sown the more strength it needs to push its shoots above the soil surface. It is suggested that with similar seeds, shallow sowing depths are best. Supporting evidences were also reported by Singh et al. (2017) in *Cinnamomum tamala Nees*.

Conclusion

The results obtained during these studies have greatly shown that the germination of seeds depends on the planting depth. Germination and emergence reduce with increase of planting depth. The seeds that were planted at deeper depths e.g. 6 cm were able to germinate last, as opposed to the ones that were planted at lower depths (0 cm and 3 cm). The number of leaves, shoot height and leaf surface area increased with the number of days when counting was done. Therefore, farmers planting *A. citratum* are advised to have moderate depths for planting. This is to ensure strong anchorage to the soil, and also to ensure faster germination of the seeds.

Recommendation

It is recommended that famers try to sow *A. citratum* seeds at a depth not exceeding 6 cm, possibly between 0 and 3 cm followed by a stable watering regime to obtain good seed emergence and germination in all agricultural undertakings, minimize loss and increase yield. Silviculturalists and famers should be educated on the appropriate agricultural practices regarding suitable depths and how to grow this plant; the farmers should also be advised on how to choose the right soil for growing media and proper watering techniques to ensure high yield.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Morphological and molecular characterization of cultivated yam (*Dioscorea* species) in selected counties in Kenya

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This study was conducted to characterize *Dioscorea* spp. in Kenya using morphological and molecular characteristics. Data on 22 morphological traits were subjected to cluster analysis and multivariate analysis using principal component (PCA). The dendrogram of cluster analysis revealed three main groups: Species distribution based on PC-1 and PC-2 showed the distantly related species in each quarter; *D. alata* L. (1st quarter), *D. bulbifera* L. (2nd quarter), *D. cayenensis* Lam. (3rd quarter) and *D. minutiflora* Engl. (4th quarter). In molecular characterization, one sub-cluster grouped *D. minutiflora* Engl. and *D. burkilliana* J. Miege as one genetic group. However not all *D. minutiflora* Engl. species were in one specific cluster showing that there may be variation within the species. *D. alata* L. and *D. bulbifera* were seen to be potentially related because they shared a common origin. *D. bulbifera* L. and *D. cayenensis* Lam. genotypes clustered together, indicating that the species might be closely related. Generally, the *rbcL* marker demonstrated the phylogeny of Kenyan *Dioscorea* spp L. Comparison of morphological and molecular data analysis gave almost similar results. From the study, the phylogenetic relationships of Kenyan *Dioscorea* spp. were established and morphological and molecular characterization was efficient in establishing species relatedness among *Dioscorea* spp.

Key words: *Dioscorea* spp., *rbcL*, principal component analysis, molecular characterization, morphological characterization, yams.

INTRODUCTION

Yams (*Dioscorea* spp.) are important monocotyledonous tuberous plants belonging to the order Dioscoreales, family Dioscoreaceae and the genus *Dioscorea* (Tamiru et al*.,* 2008; APG III, 2009). The genus contains about 644 species distributed throughout the tropics in West Africa, South East Asia and Tropical America (Asiedu and Sartie, 2010; Couto et al., 2018). More than 8 species are important staples *D. rotundata* Poir. (White yam), *D. alata* L. (Water yam), *D. cayanensis* Lam. (Yellow yam), *D. bulbifera* L. (Aerial yam), *D. dumetorum* (Kunth) Pax. (Trifoliate yam), *D. esculenta* (Lour) Burk. (Chinese yam) *D. nummularia* Lam., *D. pentaphylla* L., *D. hispida* Dennst. and *D. trifida* L. (Ihediohanm et al*.,* 2012). They are annual or perennial herbaceous vines*,*

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with edible underground and aerial tubers (either stem or root depending on species) and are the world's second most significant tuber crop.

Yams are essential sources of food consumed as vegetables boiled, baked or fried. Yams bring food security to about 300 million people in Africa, Asia, parts of South America, Caribbean and the South Pacific Islands (Nanbol and Namo, 2019). Some species contain medicinal components useful in the pharmaceutical industries. For example, *D. nipponica, D. alata* L. and *D. zingiberensis* contain diosgenin helpful in relieving arthritis and muscle pain and lowers cholesterol levels (Chandrasekara and Kumar, 2016; Jesus et al., 2016). Purple yam contains anthocyanin that slows down lipid peroxidation and prevents the onset of cardiovascular disease (Blesso, 2019; Reis et al*.,* 2016).

In Kenya, the diversity of yams has been evolving over the years as numerous generations in many parts of the country select and domesticate different species and types independently according to their local cultivation practices and needs. In a recent report by the Kenya National Strategy on Genetic Resources (2016-2020), yams were listed among the underutilized and neglected crops in the country. The cultivated yams in Kenya include *D. rotundata* Poir., *D. minutiflora* Engl*., D. bulbifera* L., *D. dumetorum* (Kunth) Pax*., D. alata* L. and *D. cayenensis* Lam. They are mainly cultivated by elderly farmers basically for food in counties of Eastern, Central, Western and Coastal regions of the country (Muthamia et al., 2013).

Molecular studies done on Kenyan yams have been minimal. Previous studies have investigated the genetic diversity using polymorphic Simple Sequence Repeats (SSR) markers that distinguished the landraces Muthamia et al. (2013) and ploidy levels; this revealed variable ploidy levels among the local yam landraces (Muthamia et al., 2014) and not the phylogeny of the species. Both studies recommended further work on the phylogeny of Kenyan yams. Other studies have solely utilized morphological characters to infer relationships within and between the *Dioscorea* species in Kenya (Mwirigi et al*.,* 2009). This study aims to establish the relationships of Kenyan *Dioscorea* species using morphological and molecular characterisation, taking into account the recommendations of previous research.

MATERIALS AND METHODS

Study area

The study was conducted in Meru, Embu, Taita Taveta, Busia and Bungoma counties. These counties were selected based on information gathered from the Kenya Agricultural and Livestock Research Organization on where *Dioscorea* species are mainly grown. *Dioscorea* specimens were collected from six farms from three sub-counties in Meru county; Imenti North (N 00° 4´32.43684´; E 37° 38´54.29688´), Imenti Central (S 0° 1´34.56264´; E 37° 38´37.65588´) and Tigania Central (N 00° 8´35.1168´; E 37° 50´52.20852´). Specimens were also collected in Embu county (S 00° 27´53.36388´; E 37° 29´56.65272´), Taita-Taveta county (S 03° 24´2.46888´), Busia county (N 00° 29´47.86548´; E 34° 12´7.0272´) and Bungoma county (N 00° 34´10.300´; E 34° 33´31.1536´). Purposive sampling was used to select representative study sites with respect to the potential of yam production. This was done with the help of agricultural officers in each county who identified farmers farming yams.

Collection of *Dioscorea* specimens

Leaves and voucher specimens of *Dioscorea* species were collected from the various geographical regions of Kenya in the year 2018 (September to November). Collected specimens were identified and voucher specimens deposited in Kenyatta University Herbarium. Silica-gel dried leaves were collected for each sample for molecular characterization.

Morphological characterization

Twenty-four yam specimens were used for this study. Morphological data were observed directly on living plants under field conditions from farms where yams were grown. Twenty-two characteristics obtained from the International Plant Genetic Resources Institute's (IPGRI) descriptors of yam (Dioscorea species) were considered (IPGRI, 1997) (Table 1).

DNA extraction

DNA was extracted from 0.2 g silica-gel dried leaves obtained from 17 randomly selected representative specimens and collected into Eppendorf tubes. Normal saline was added, and centrifuged. 400 µl lysis buffer was added and incubated for 1 h at 55-60°C with occasional mixing. The specimens were crushed and incubated again at 37°C for 3-4 h to deactivate lysozyme in the lysis buffer. They were cooled for 30 min, and afterwards centrifuged at 13,000 revolutions per minute for 5 min; an equal amount of chloroform was added gently and mixed thoroughly. The specimens were centrifuged again at 13,000 revolutions per minute for 8 min, using a large-bore pipette. The supernatant was transferred to another labelled Eppendorf tube, 600 µl isopropanol was added and mixed gently until the DNA was precipitated. The specimens were kept at -4°C for 20 min to precipitate the DNA further, centrifuged at 12,000 revolutions per minute for 5 minutes and the supernatant was discarded. The DNA pellets were washed by adding 70% ethanol and centrifuged again at 13,000 revolutions per minute for 2 min. The supernatant was discarded and the pellets were air-dried at room temperature. DNA yield was checked by running 3 µl of freshly extracted DNA specimens on 1% agarose gel stained with 3 µl loading dye and 1µl SYBR® green stain; it was visualized under an ultraviolet transilluminator at the Kenyatta University Tissue Culture laboratory. The quality and concentration of all DNA specimens were determined using Agarose gel electrophoresis.

PCR and sequencing

PCR was achieved using *rbcl* marker (H1f F: CCACAAACAGAGACTAAAGC and Fofana R: GTAAAATCAAGTCCACCGCG (Fofana et al., 1997) and synthesized from Inqaba Biotec East Africa (IBEA), SouthAfrica. This primer marker was selected as a result of ease of PCR amplification and discriminatory power among yam species (Girma et al., 2015a). *rbcl* codes for ribulose 1, 5 bisphosphate carboxylase/oxygenase. This was carried out in a 25 µl reaction

Table 1. Character and character states scored for morphological studies.

Character	Character state
Twining direction	1-Clockwise (climbing to the left) 2-Anticlockwise (climbing to the right)
Stem colour	1-Green; 2-purplish green; 3-brownish-green; 4-dark brown; 5-purple and 6-other.
Absence/presence of spines	Absent/ Present
Absence/presence of wings	Absent/ Present
Wing position	At the base/ Above base
Spine shape	1-Straight; 2-Curved upwards; 3-Curved downwards
Leaf colour	1-Yellowish; 2-Pale green; 3-Dark green; 4-Purplish green; 5-Purple; 6-Other
Leaf margin colour	1-Green; 2-Purple; 3-Other
Vein colour	1-Yellowish; 2-Green; 3-Pale purple; 4-Purple; 5-Other
Position of leaves	1-Alternate; 2-Opposite; 3-Alternate at base/opposite above; 4-Other
Leaf type	Simple/ Compound
Leaf margin	Entire/ Serrate
Leaf shape	1-Ovate; 2-Cordate; 3-Cordate long; 4-Cordate broad; 5-Sagittate long; 6-Sagittate broad; 7-Hastate; 8-Other
Leaf apex shape	1-Obtuse; 2-Acute; 3-Emarginate; 4-Other
Petiole colour	1-All green with purple base; 2-All green with purple leaf junction; 3-All green with purple at both ends; 4-All purplish-green with purple base; 5-All purplish-green with purple leaf junction; 6-All purplish-green with purple at both ends; 7-Green; 8-Purple; 9-Brownish green; 10-Brown; 11-Dark brown; 12-Other
Flowering	1-No flowering; 2-Flowering in some years; 3-Every year
Flower colour	1-Purplish; 2-White; 3-Yellowish; 4-Other
Inflorescence type	1-Spike; 2-Raceme;3- Panicle
Aerial tuber shape	1-Round; 2-Oval; 3-Irregular (not uniform); 4-Elongate
Skin colour	1-Greyish; 2-Light brown; 3-Dark brown; 4-Other
Surface texture	1-Smooth; 2-Wrinkled; 3-Rough
Flesh colour	1-White; 2-Yellowish white or off-white; 3-Yellow; 4-Orange; 5-Light purple; 6-Purple; 7-Purple with white; 8-White with purple; 9-Outer purple/inner yellowish; 10-Other

volume containing 2.5 µl of 10x standard Taq, reaction buffer; 0.5 µl of 10 mM dNTPs; 0.5 µl of 10 µM primer H1F; 0.5 µl of 10 µM primer Fofana; 1 µl of template DNA, 0.125 µl of Taq, DNA polymerase, 19 µl nuclease-free water and 0.5 µl of Triton X.

The PCR reaction was carried out in Techgene thermocycler FTGENE5D model (Techne- UK). The PCR reaction conditions for amplification consisted of initial denaturation at 94^{0}C for 2 min followed by 35 cycles (denaturation at 94°C for 30 s, primer annealing at 46°C for 30 s, extension at 72^{0}C for 90 s) and a final extension at 72°C for 7 min. The PCR products were stored at 4°C until used. PCR products were stained with SYBR green and separated by gel electrophoresis in 1% (w/v) agarose gel in 0.5X TBE buffer at 80 V for 30 min. After gel electrophoresis, the PCR products were visualized using an Ultra-violet trans-illuminator lamp. One hundred base pair (100bp) ladder was used for estimation of the molecular sizes of the bands. Gels were photographed using a Samsung digital camera. PCR products were then sent to South Africa for bidirectional sequencing at Inqaba Biotec East Africa (IBEA).

Data analysis

Data analysis based on morphological data

Data on morphological characteristics from 24 specimens were coded into numerical values and used for cluster analysis. The dendrogram was drawn based on a hierarchical cluster analysis using single linkage (nearest-neighbour) procedure using DARwin computer software version 6. The dendrogram obtained was used in comparison with *rbcL* phylogenetic tree. Standardized data for qualitative characters were subjected to multivariate analysis and principal component analysis to identify the most discriminating morphological character using MVSP 3.2 and Conoco 5 software, respectively.

Phylogenetic analysis

The obtained sequences were exported to Finch TV Version 1.4.0 for base-calling. A consensus sequence was then created using DNA Baser Assembler v5.15.0; then a contig was created in comparison with the reference sequence using Gene studio Professional Edition. BLAST analysis was done to find identities that match the species. *rbcL* sequences were subjected to multiple alignments using the muscle alignment method in MEGA X to identify gaps and similar and mismatch regions among the two molecular characters. Maximum Likelihood (ML) and neighbour-joining algorithms were applied in phylogeny reconstruction. UPGMA was the statistical method used. The aligned sequences after subjection to the above parameters resulted in the construction of *rbcL* maximum likelihood phylogenetic trees.

Table 2. Eigenvalues.

Parameter	Axis 1	Axis 2	Axis 3	Axis 4
Eigenvalues	16.392	6.558	5.222	2.534
Percentage	46.451	18.583	14.798	7.181
Cum. Percentage	46.451	65.033	79.831	87.012

Table 3. PCA variable loadings.

Traits	PC 1	PC 2	PC 3	PC 4
A-Twining direction	0.073	-0.044	0.007	0.047
B-Stem colour	**0.111**	-0.007	**-0.102**	**0.223**
C-Spines	**0.113**	0.074	-0.071	-0.029
D-Spine shape	**0.259**	0.058	**-0.160**	-0.066
E-Spines on stem base	**0.579**	**0.273**	**-0.558**	**-0.351**
F-Wings	-0.094	0.024	-0.011	-0.044
G-Wing position	-0.094	0.024	-0.011	-0.044
H-Leaf colour	0.005	-0.003	-0.049	-0.096
I-Leaf margin colour	**0.115**	0.032	**-0.154**	**0.613**
J-Vein colour	-0.063	**-0.124**	0.056	-0.038
K-Leaf position	**0.103**	-0.070	0.010	**0.223**
L-Leaf type	0.000	0.000	0.000	0.000
M-Leaf margin	0.000	0.000	0.000	0.000
N-Leaf shape	**-0.225**	0.034	0.081	**-0.398**
O-Leaf apex shape	0.000	0.000	0.000	0.000
P-Distance between lobes	**0.559**	**0.270**	**0.767**	-0.013
Q-Petiole colour	**-0.350**	**0.902**	-0.032	**0.127**
R-Flowering	-0.063	0.039	-0.039	**-0.112**
S-Tuber shape	-0.029	0.022	-0.012	**0.104**
T-Skin colour	0.100	-0.053	-0.048	0.065
U-Surface texture	**0.115**	0.024	-0.068	0.068
V-Flesh colour	-0.087	-0.013	**0.114**	**-0.416**

RESULTS

Principal component analysis

The PCA results established that the first four principal components together described 87.01% of the overall variance present in the data set (Table 2). Scores on the first principal component (PC-1) which explained 46.45% of the total dissimilarity were vastly correlated to stem colour, presence of spines, spine shape, spines on stem base, leaf margin colour, leaf position, the distance between lobes and surface texture (Table 3).

The second principal component (PC-2) described 18.58% of the overall dissimilarity and was vastly correlated to spines on stem base, the distance between lobes and petiole colour (Table 3). The third component (PC-3) which described 14.78% of the dissimilarity was primarily related to the distance between lobes and flesh colour. The fourth principal component (PC-4) described 7.18% of the total distinction and was determined by leaf margin colour, stem colour, leaf position, petiole colour and shape of the tubers. The distribution of species based on the first and second principal components shows dissimilarity among the species and how extensively dispersed they are along both axes (Figure 1). The two components explain a cumulative variability of 65.03%. Based on the distribution of specimens in the first quarter, *D. alata* L. is the most distantly related to that group; whereas in the second quarter *D. bulbifera* L. is the least similar in the group. The most distant in the third quarter is *D. cayenensis* Lam. The last quarter is made up of a *D.minutiflora* Engl. that is least similar to the group (Figure 1).

Correlation between the variables related to the first and second principal components are presented in Figure 1. From the correlation circle in Figure 1, petiole colour has a significant effect on the variables as a result of the arrow being long. There is a positive correlation between

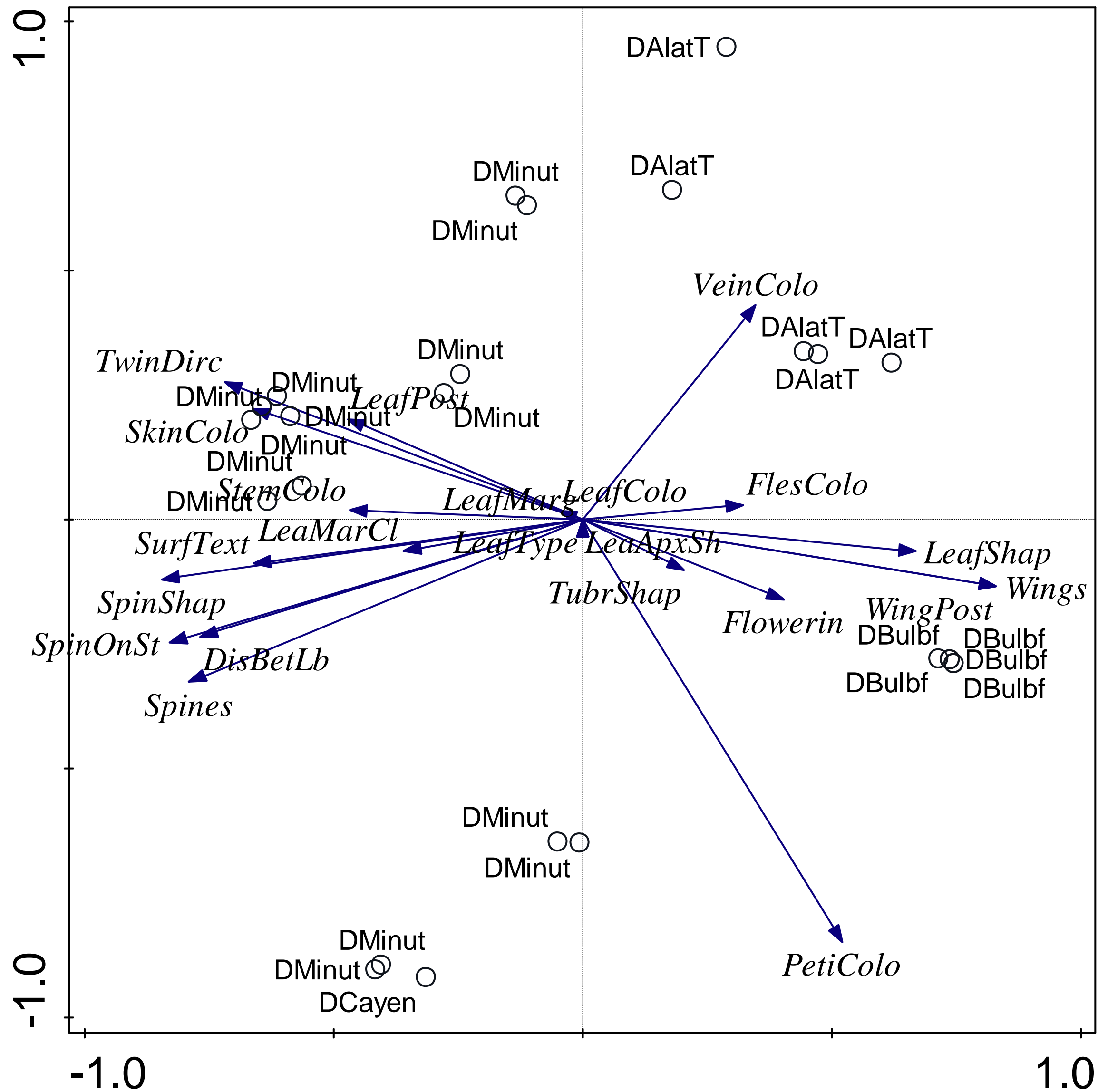

Figure 1. Correlation circle of the first two principal components (PC1 and PC2).

the shape of leaves and the presence of wings. However, there is a negative correlation between the shape of leaves and the presence of wings on one hand and twining direction and tuber skin colour. Petiole colour and spines are not correlated as well as petiole colour and vein colour.

Dendrogram based on morphological characters

The 24 yam specimens included in the morphological study were grouped into three clusters (Figure 2). Cluster 1 grouped *D. minutiflora* Engl. species collected from different areas; Teso North, Embu and Meru. This cluster had two sub-clusters; 1a and b respectively. Sub-cluster 1a is a group of *D. minutiflora* Engl. characterised with many spines on stem base, spines curved upwards, leaf veins yellow, yellow leaf margins, leaves alternate at base/ opposite above, green petioles and rough tuber surface texture. Sub-cluster 1b is a group of *D. minutiflora* Engl. with many spines on the stem base, spines curved downwards, vein green, leaf margin green, leaf position opposite, petioles all green with a purple base and tuber surface texture rough. This suggested a close relationship between the *D. minutiflora* Engl species collected from the different areas (Teso North, Embu and Meru) based on similar morphological traits.

Cluster 2 contained three sub-cluster groups Cluster 2(I), Cluster 2(II) and Cluster 2(III). Sub-cluster 2(I) is a group of *D. alata* L. species from Taita taveta and Busia

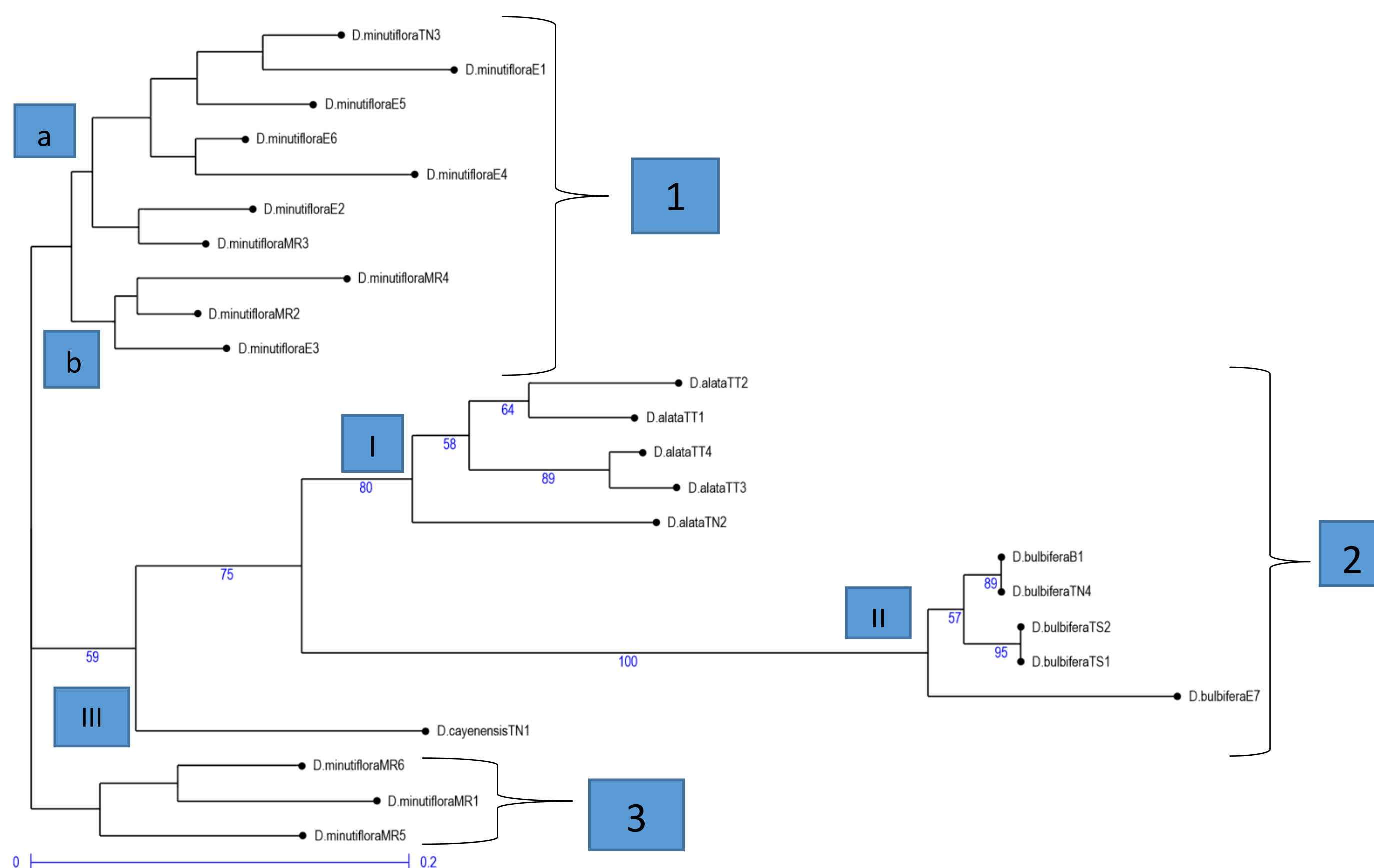

Figure 2. Dendrogram showing the relationship in yams (*Dioscorea* s) species based on morphological characteristics.

(Teso North) that twine to the right in an anticlockwise direction, stem purplish-green, leaf margin purple, leaf shape cordate long, petioles purplish-green with a purple base, tuber flesh purple and white. *D. alata* L. species collected from Teso North had sagittate long leaves, and tuber flesh purple in colour whereas *D. alata* L. tuber flesh colour from Taita Taveta was white.

Sub-cluster 2(II) is a group of *D. bulbifera* L. species from Bungoma, Busia (Teso North and South) and Embu counties with stem twining to the left in a clockwise direction, spines absent, wings present on the stem, flowering in some years and presence of aerial tubers. However, only *D. bulbifera* L. from Bungoma and Busia showed flowering and clustered together whereas that from Embu did not.

Sub-cluster 2(III) had *D. cayenensis* Lam. from Busia (Teso North) characterised by yellowish veins on the leaves, cordate broad leaf shape and a cylindrical tuber with tuber flesh colour yellow. Cluster 3 is a group of *D. minutiflora* Engl. from Meru County. Few spines on stem base, green stems, pale green and dark green leaves, brown leaf margins, green leaf veins, cordate leaves and white tuber flesh colour characterised this cluster. These characters were key in distinguishing this cluster from Cluster 1.

Molecular characterisation of Kenyan yam (Dioscorea species)

Six species' identities were used to construct the dendrogram among the 17 selected genotypes.

The dendrogram based on *rbcL* markers distinguished the seventeen yam genotypes into two main cluster groups (Figure 3)

Cluster 1 consisted of two main subclusters (a) and (b). Subcluster 1(a) and cluster 2 comprised *D. minutiflora* genotypes. This is similar to the cluster 1 and 3 of the morphological analysis which consists of *D. minutiflora* species clustered together (Figure 2). However, *D. minutiflora* genotypes were in different clusters; 1(a) and cluster 2, showing that there may be variation in the genotypes (Figure 3). Subcluster b(I) consisted of *D. alata* genotypes similar to the cluster 2(i) of the morphological analysis. Subcluster b(II) consisted of *D. bulbifera* and *D. cayenensis* genotypes, indicating that the two species might be closely related as shown in morphological studies in cluster 2(II and III) (Figure 3).

The results showed a high correlation between the morphological and molecular data in the study of Kenyan yams (Figures 2 and 3). The evolutionary history was inferred using the Neighbor-Joining method (Saitou and

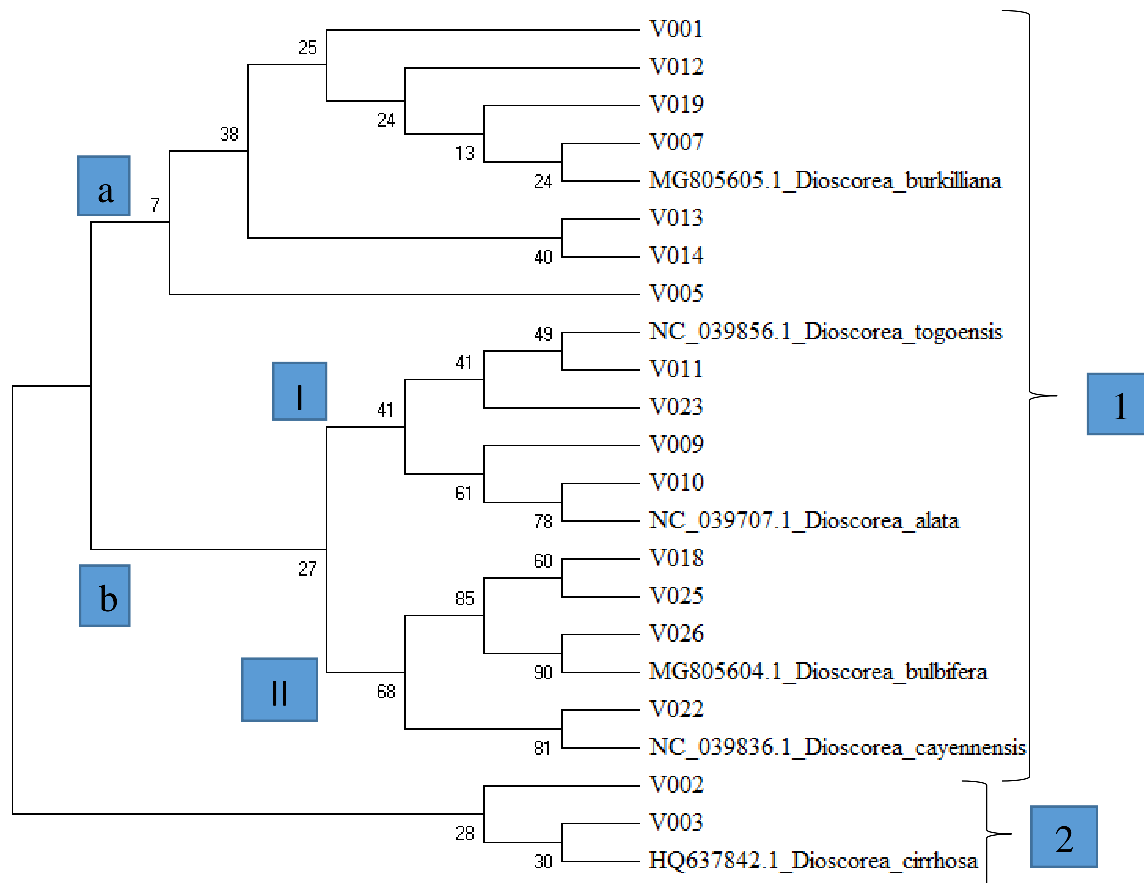

Figure 3. Evolutionary relationships of taxa.

Nei, 1987) (Table 4). The bootstrap consensus tree inferred from 1000 replicates is taken to represent the evolutionary history of the taxa analyzed (Felsenstein, 1985). Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches (Felsenstein, 1985). The evolutionary distances were computed using the Maximum Composite Likelihood method (Tamura et al., 2004) and are in the units of the number of base substitutions per site. The analysis involved 23 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions with less than 95% site coverage were eliminated. That is, fewer than 5% alignment gaps, missing data, and ambiguous bases were allowed at any position. There were a total of 593 positions in the final dataset. Evolutionary analyses were conducted in MEGA X (Kumar et al., 2018) (Table 5).

DISCUSSION

Morphological traits that had a paramount role in discriminating between the yam species in this study were stem colour, leaf margin colour, leaf position, the distance between lobes, petiole colour, tuber shape, tuber surface texture and tuber flesh colour. These results are in congruence with results obtained by Jyothy et al. (2017). They revealed that morphological variability score on the first principal component (PC-1) was highly correlated with characters related to tuber shape and tuber flesh colour. Similarly, Mwirigi et al. (2009) reported that PC-2, PC-3 and PC-4 were mainly correlated with characters related to leaf position and tuber flesh colour similar to the results of PC-4 and PC-3 from this study. Results obtained from Sheikh and Kumar (2017) revealed that variability scores on the first principal component (PC-1) were highly correlated with characters related to stem colour. This was also similar with the results obtained in this study on the first principal component (PC-1) being highly correlated with stem colour. From the dendrogram, morphological characterisation of Kenyan yams from 5 geographical regions indicated that most species from the Eastern area (Meru and Embu) are closely related despite their geographic location being widespread and some showing a few morphological variations. This is as a result *D. minutiflora* Engl. from the

Table 4. Gene bank species identities.

Lab designation	Species identification	The accession number of nearest neighbour	Percentage identity (%)
V005, V007	*Dioscorea burkilliana*	MG805605.1	98.83
V001,V012,V013, V014, V019	*Dioscorea togoensis*	NC_039856.1	98.83
V009,V010,V011, V023	*Dioscorea alata*	NC_039707.1	99.63
V018, V025, V026	*Dioscorea bulbifera*	MG805604.1	99.82
V002, V003	*Dioscorea cirrhosa*	HQ637842.1	98.83
V022	*Dioscorea cayennensis*	NC_039836.1	99.46

Table 5. Laboratory species identities.

	Lab designation	Species identities	Area of collection
1	V001	*Dioscorea minutiflora*	Meru
2	V002	*Dioscorea minutiflora*	Meru
3	V003	*Dioscorea minutiflora*	Meru
4	V005	*Dioscorea minutiflora*	Meru
5	V007	*Dioscorea minutiflora*	Meru
6	V009	*Dioscorea alata*	Taita-Taveta
7	V010	*Dioscorea alata*	Taita-Taveta
8	V011	*Dioscorea alata*	Taita-Taveta
9	V012	*Dioscorea minutiflora*	Embu
10	V013	*Dioscorea minutiflora*	Embu
11	V014	*Dioscorea minutiflora*	Embu
12	V018	*Dioscorea bulbifera*	Embu
13	V019	*Dioscorea minutiflora*	Embu
14	V022	*Dioscorea cayennensis*	Teso North
15	V023	*Dioscorea alata*	Teso North
16	V025	*Dioscorea bulbifera*	Teso North
17	V026	*Dioscorea bulbifera*	Bungoma

two regions clustering together. This indicates a likelihood of numerous exchange of planting materials among and between farmers from different zones. It is also likely that constant vegetative propagation and selection have contributed to the wide phenotypic variability of *D. minutiflora* Engl. (Mwirigi et al., 2009). However, there are four accessions of *D. minutiflora* Engl. in Meru and Embu distinguished by the size of the tuber and spiny stem base. It can be seen that *D.alata* L. (Taita Taveta and Busia) and *D. bulbifera* L. (Embu, Bungoma and Busia) are very closely related and distant to *D. cayenensis* Lam (Busia).

The dendrogram from molecular data was prepared by using the neighbour-joining method. In the cluster analysis *D. minutiflora* Engl. and *D. burkilliana* J. Miege from West Africa were grouped, indicating that they might be considered as one genetic group, as stated by Chaïr et al. (2005). In another study, Magwé-Tindo et al. (2018) identified Guinea Yam wild relatives using the whole plastome phylogenetic analyses which clearly showed that *D. minutiflora* Engl. and *D. burkilliana* J. Miege formed two strongly supported groups and clustered together. This is in agreement with results obtained by Ramser et al. (1997) who found them in the same habitat. Miège (1968), in his study, established *D. burkilliana* J. Miege and *D. minutiflora* Engl. as two morphologically similar species that differ only by the characteristics of their below-ground parts. These results are in agreement with the results of this study as a result of *D. burkilliana* J. Miege and *D. minutiflora* Engl. clustering together.

D. alata L. and *D.bulbifera* L. are seen to be potentially related from Figure 2 because they share a common origin. This, however, contradicts established taxonomy as well as earlier molecular studies involving both species stating that *D. alata* L. and *D.bulbifera* L. are not closely related (Malapa et al., 2005). On the other hand, the fact that some cultivars of *D. alata* L. produce aerial tubers may support the observed closeness of the species to *D. bulbifera* L. (Tamiru et al., 2007). The input of both morphological and molecular data is critical in

producing well-resolved species delimitation. In this study, results showed a correlation between morphological and molecular data analysis, indicating that molecular data supported morphological species delimitation. Caddick et al. (2008) in his study stated that higher sampling of taxa and morphological and molecular characters for Dioscoreales had produced resolved topologies that corroborate the circumscription that was proposed by APG (1998). His study also concluded that increased bootstrap support in analysis indicated high congruence between independent morphology and molecular data sets and demonstrated that both morphological and molecular data are essential in resolving the relationships within Dioscoreales.

Sartie et al. (2012) in their study on genotypic and phenotypic diversity of cultivated tropical yams using phenotypic and SSR markers established an improved understanding about the genetic and phenotypic relatedness among *D. rotundata* Poir., *D. cayenensis* Lam., *D. alata* L. and *D. dumetorum* (Kunth) Pax. This is similar to what was done in this study using phenotypic and molecular markers to establish phylogeny of *Dioscorea* in Kenya. Girma et al. (2015b) in their study of morphological and SSR analysis of *D. alata* L. indicated that combining SSR markers and phenotypic data were useful for identification of *D. alata* L. accessions likewise to combining morphological data and molecular markers in characterizing Kenyan *Dioscorea* species.

Conclusion

Dioscorea species grown in Kenya exhibited morphological variations. Phylogenetic relationships of Kenyan *Dioscorea* species were established with *D. alata* L. and *D. bulbifera* L. seen to be closely related and *D. minutiflora* Engl. and *D. burkilliana* J. Miege from West Africa grouping together as one genetic group. Molecular and morphological characterization was efficient in establishing species relatedness among *Dioscorea* species. Future studies should consider collections from other localities in addition to Meru, Embu, Taita-Taveta, Busia and Bungoma counties and more than one molecular marker should be used.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Evaluating the incidence and severity of rice yellow mottle virus and maize streak virus on rice (*Oryza sativa* L.) and associated insects in the Federal Capital Territory, Abuja, Nigeria

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Rice is an important staple food in Nigeria, affected by several diseases especially viruses. This study was carried out to evaluate the incidence and severity of two important viruses on rice plants and to identify associated insects in the Federal Capital Territory (F.C.T), Abuja in 2019. Field experiment was carried out from June to October, 2019 at the Teaching and Research Farm of the Faculty of Agriculture, University of Abuja, Nigeria, where ten rice varieties were assessed for incidence and severity. The seed and leaf samples were collected for serological indexing. Data collected was subjected to statistical analysis using SPSS and mean separation was done with Duncan Multiple Range Test. Of the 210 leaf samples collected, FARO 61 and 44 had the lowest incidence (19%), while FARO 65 recorded the highest (25.3%). FARO 52 recorded the highest severity (46%) while FARO 61 and FARO 60 had the lowest severity (30.1%) for rice yellow mottle sobemo-virus (RYMV) and maize streak geminivirus (MSV). Insects such as Spittle bug (*Locris rubens* and *Poophilus costalis*), Ladybird beetle (*Cheilomenes sulphurea*) and Groundhopper (*Paratettix* sp) were trapped on the field. All rice seed and leaf samples collected did not test positive to RYMV and MSV using Enzyme-Linked immunosorbent assay (ELISA). This study provides the first research work on rice viruses in the FCT and further studies are recommended.

Key words: Nigeria, rice, rice yellow mottle sobemo-virus (RYMV), maize streak geminivirus (MSV), incidence, severity, virus symptoms, enzyme linked immuno sorbent assay (ELISA).

INTRODUCTION

In several West African countries namely Guinea Bissau, the Gambia, Guinea, Sierra Leone, Cote d'Ivoire, Liberia and Nigeria, rice has become a major component of diet. In 2000, rice represented a third of the total

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Table 1. Ten varieties of rice planted on the experimental field at the Teaching and Research Farms, Faculty of Agriculture, University of Abuja, FCT

S/N	Variety	Type	Source	Type
1	Faro 61	Lowland	NCRI	Certified seeds
2	Faro 45	Upland	NCRI	Certified seeds
3	Faro 47	Upland	NCRI	Certified seeds
4	Faro 60	Lowland	NCRI	Certified seeds
5	Pac 832	Lowland	Premier seeds	Certified seeds
6	Faro 65	Upland	NCRI	Certified seeds
7	Faro 44	Lowland	NCRI	Certified seeds
8	Faro 52	Lowland	NCRI	Certified seeds
9	Faro 64	Upland	NCRI	Certified seeds
10	Faro 22	Lowland	NCRI	Certified seeds

cereal-derived calorie intake of the West African population (FAO, 2017). The increase in Nigeria's population has necessitated an increase in the level of food production, among which is rice. Rice (*Oryza sativa*) is one of the most widely grown crops in all parts of Nigeria with consumption per capita of 32 kg. In the past decade, consumption has increased by 4.7%, almost four times the global consumption growth, and 6.4 million tons in 2017, accounting for 20% of Africa's consumption (PwC, 2018). It is grown for market and home consumption. With the increased availability of rice, it has become part of the everyday diet of many in Nigeria.

In spite of the efforts made in increasing rice cultivation, yield has remained very low, thus, the production has not met the consumption level of the growing population (Ajala and Gana, 2015). Rice production in Nigeria is affected by pests, diseases and some other constraints. Some of the insect pests that usually affect rice are Planthoppers (*Laodelphax striatellus; Sogatella furcifera; Nilaparvata lugens*), Leafhoppers (*Nephotettix* spp), Beetles (*Cheilomenes* spp), Aphids (*Rhopalosiphum rufiabdominale*), Rice mealy bug (*Brevennia rehi*) and Stem borers (*Scirpophaga incertulas*) (Li et al., 2019; Hegde et al., 2016). Small holder farmers in Nigeria usually do not have access to low-interest credit facilities and this limits the rate of production of rice. Also, inadequate funding for research and extension in Africa especially Nigeria, is limiting the production of rice, as research and extension can be used to provide support for farmers to increase their production output (Osanyinlusi and Adenegan, 2016). Diseases such as: Fungus [Rice blast (*Magnaporthe grisea*) (Dean et al., 2005)], Nematodes [Stem nematode (*Ditylenchus dipsaci*), Root knot nematode (*Meloidogyne graminicola*)], Virus [Sobemovirus - Rice Yellow Mottle Virus (RYMV), Mastrevirus – Maize Streak Virus (MSV)], are also major constraints in the production of rice in Nigeria, causing low yield, low quality and sometimes yield loss. The viruses that have been reported to infect rice in West Africa and of economic importance are Grassy stunt disease (transmitted by *Nilaparvata lugens* Stal.), Orange leaf disease (transmitted by *Inazuma dorsalis* Motschulsky), Rice stripe necrosis virus (RSNV), Rice crinkle disease, Maize streak geminivirus (MSV), African cereal streak virus and RYMV (Abo and Sy, 1997). In Nigeria, RYMV has being the most prevalent causing a major challenge in rice production since 1976 when it was first reported (Rossel et al., 1982). Earlier studies done in the Southern part of Nigeria show the incidence of RYMV is up to 70% (Onasanya et al., 2011; Odedara et al., 2016). The adverse effect of viruses on rice production in Nigeria as well as limited information and literature on rice viruses in Abuja, necessitated this research with the objective to evaluate the incidence and severity of RYMV and MSV infecting rice and identify insects associated with rice production in Abuja.

MATERIALS AND METHODS

Research location

The field study was conducted between June to October, 2019 at the Teaching and Research Farm of the Faculty of Agriculture, University of Abuja, F.C.T, Nigeria. This location has Longitude and Latitude (8.9817° N, 7.1811° E) and an elevation of 273 m. The average annual rainfall in the FCT is 1350 mm (Balogun, 2001).

Samples

Six lowland rain-fed and four upland rice varieties obtained from National Cereal Research Institute (NCRI) Badeggi and Premier Seeds Nigeria Ltd were used for this study. The rice varieties were identified by their new name FARO (Federal Agriculture Research Oryza) (Table 1).

Experimental design

A Randomized Block Design (RBD) with three replications was used. The field was divided into three blocks with 10 plants of each variety. Plots size was 3 m × 3 m with plant spacing of 0.3 m × 0.3

m. Distance between replications was 1 m and distance between each plot was 0.5 m, with a total field size of 34.5 m × 11 m. Sowing was done by direct seeding with 3 seeds per hole.

Cultural practices

Layout and pegging of the field was done followed by land clearing; this was done with hand labor, and this involved removing of stumps, brush, stones and other obstacles from the field. This was followed by ploughing in order to break up the soil; harrowing and leveling was then done afterwards to produce fine tilth. The land preparation activities were done before the onset of the rain, in order to create a favorable environment for the rice plants to germinate. The rice plants were thinned from 3 plant stand per hole to 1 plant stand per hole and empty spaces were gap filled 3 weeks after planting (WAP), in order to allow the plants receive proper growth requirements and avoid competition. Selective herbicide (2,4-D) was used to control weeds on the field at 3, 6 and 9 WAP. Compound fertilizer (NPK 15-15-15) was added using basal application at 6 and 10 WAP respectively. A scarecrow was erected and catapult was used in order to control birds. Also, fence was erected with bamboo and chicken wire mesh round the field to control cattle, goats, rats and grass-cutters. Rogueing was also done by removing off-types and weeds between headings to harvesting. Harvesting was done at maturity (19-20 WAP) by cutting the rice stands close to the ground level with the use of a sickle, after the rice panicle changed color from green to yellow/brown and became hard.

Data collection

Data was collected on germination percentage, virus disease incidence and severity of 270 rice plants belonging to 10 varieties and 3 replicates as well as weather data for 2019.

Germination percentage

Germination percentage was estimated 3 weeks after planting, by counting the number of seeds that germinated and expressed as a percentage of the total number of seeds planted.

$$\text{Germination (\%)} = \frac{No\ of\ seeds\ that\ germinated}{Total\ no\ of\ seeds\ planted} \times 100$$

Assessment of incidence

Disease incidence was estimated by counting the number of plants that expressed virus-like symptoms by close observation and recording of each symptom type and expressed as a percentage of the total number of plants assessed.

$$\text{Disease incidence (\%)} = \frac{No\ of\ symptomatic\ plants}{Total\ no\ of\ plants\ sampled} \times 100$$

Assessment of severity

Symptom severity was done by scoring plants showing virus-like symptoms (7 per plot). This was done by visual assessment and using a modification of the Standard Evaluation System (SES) of International Rice Research Institute (1996) on a scale of 1-9. Where 1 = No symptom observed, 3 = Leaves green but with sparse streaks and less than 5% symptoms on leaves, 5 = Leaves green or pale green with mottling and 6-25% symptoms on leaves, 7 = Leaves pale, yellow and 26-75% symptoms on leaves, 9 = Leaves turn yellow or orange with more than 75% symptoms on leaves and some plants dead.

$$\text{Disease severity (\%)} = \frac{Sum\ of\ all\ disease\ ratings}{No\ of\ plants\ assessed} \times \text{maximum score (100)}$$

Weather data

The weather data (Temperature, Hours of sunshine, Relative humidity and Rainfall) for the year of experiment (2019) was gotten from the Nigeria Meteorological Agency (NiMet).

Insect trapping

Insects were trapped and collected on the field by using plastic bucket traps filled with one-quarter solution of water, 70% ethanol (as a preservative) and 1.5% teepol detergent. The trap was monitored periodically and the liquid in the trap containers were changed after every collection. Insects collected at the end of the experiment were sent to the Insect Museum, Department of Crop Protection, Ahmadu Bello University (ABU), Zaria, for identification.

Collection of samples

Seed samples

Rice seed samples of each variety planted were collected before planting. The samples were taken to the Virology and Molecular Diagnostic laboratory of the International Institute of Tropical Agriculture (IITA), Ibadan, for virus indexing using Enzyme Linked Immuno Sorbent Assay (ELISA).

Leaf samples

Young leaf samples were collected at 8 weeks after planting (WAP) from plants that showed typical virus-like symptoms and also from weed on and around the rice field. Collected leaf samples were placed in 25 ml plastic bottles containing Calcium Chloride ($CaCl_2$). Each sample was prevented from direct contact with the $CaCl_2$, with the placement of piece of non-absorbent cotton wool in between, before being grounded and later placed into vials which were analysed using ELISA.

Serological tests

Detection of virus in seed samples using antigen-coated plate enzyme linked immunosorbent assay (ACP-ELISA)

Ten rice seed samples (made up of husk, endosperm and embryo) were collected from the 10 different rice varieties before planting and indexed for viruses using ACP-ELISA as described by Afolabi et al. (2009). The rice seed samples collected were indexed for RYMV and MSV. The method and protocol used in detecting these viruses from samples using ELISA is as follows.

10 seed samples were drawn from each of the 10 rice varieties, weighed and subjected to the Antigen Coated Plate (ACP) form of Enzyme Linked Immunosorbent Assay (ELISA). The seed samples were ground with mortar and pestle in 2 ml of extraction buffer [8 g sodium chloride, 0.2 g monobasic potassium phosphate, 1.15 g diabasic sodium phosphate, 0.2 g potassium chloride, 0.2 g sodium azide dissolved in 900 ml H_20 adjusted to pH 7.4 with HCl to make

Table 2. Effect of varietal difference on germination percentage, plant height and number of leaves of rice during growing season 2019 in Abuja, Nigeria.

Variety	Germination %	Plant height (cm)			No. of leaves		
		Week3	Week6	Week9	Week3	Week6	Week9
FARO 61	90.33^{b}	15.61^{f}	31.41^{a}	56.12^{e}	5.23^{c}	7.52^{d}	10.04^{d}
FARO 45	91.66^{b}	17.14^{c}	37.50^{a}	80.55^{a}	5.80^{a}	8.42^{a}	11.57^{a}
FARO 47	91.33^{b}	16.69^{d}	39.44^{a}	65.19^{c}	4.95^{c}	7.66^{cd}	10.80^{bc}
FARO 60	69.66^{d}	18.18^{a}	23.79^{a}	51.47^{e}	5.19^{c}	8.00^{bc}	10.52^{bcd}
PAC 832	84.66^{c}	14.60^{h}	25.24^{a}	58.82^{e}	5.42^{b}	8.09^{ab}	11.14^{abc}
FARO 65	82.00^{c}	15.60^{f}	29.80^{a}	62.34^{d}	4.95^{c}	7.71^{bcd}	10.42^{cd}
FARO 44	80.33^{c}	15.84^{e}	32.62^{a}	61.08^{d}	5.42^{b}	8.09^{ab}	10.66^{bcd}
FARO 52	96.66^{a}	17.84^{b}	33.30^{a}	67.85^{b}	5.80^{a}	8.00^{bc}	10.90^{abc}
FARO 64	80.33^{c}	14.75^{g}	34.89^{a}	69.20^{b}	5.66^{a}	8.42^{a}	11.28^{ab}
FARO 22	72.00^{d}	14.72^{g}	29.73^{a}	55.62^{e}	5.19^{c}	7.76^{bcd}	10.95^{abc}

Means in the same column with different alphabets are significantly different at P ≤ 0.05.

up (1 l) + 0.5 ml Tween 20/L and 2% polyvinyl pyrollidone (PVP)]. The 96 polystyrene microtitre plates were labeled and coated with 100 µl of antigen 1/10 in coating buffer with 1% Dieca, covered and incubated for 1 hour at 37°C. The plates were removed, washed with PBS-Tween thrice at 3 min interval and tap dried. Blocking was done with 200 µl per well of 3% dried skimmed milk in PBS-Tween to trap the virus and incubated at 37°C for 30 min to enable binding. The plates were removed and washed as stated above. 100 µl of sap of healthy cowpea leaf samples mixed with pool of antibodies (21 different vegetable and cowpea antibodies were mixed together) was added into wells and incubated for 2 h at 37°C. Plates were removed and washed. Then, 100 µl enzyme goat anti-rabbit antibody diluted at 1:2000 was added to wells and incubated then washed as above. Substrate pNPP in substrate buffer at 3 mg in 30 ml was added into wells and kept for change in color. The optical density (OD) of the content of each well was subsequently read after 4 h and overnight respectively using ELISA reader (Diagnostic and Medical Solutions Micro Plate Read - ELISA Plate Analyzer) at a wavelength of 405 nm.

Detection of viruses in leaf samples using antigen-coated enzyme linked immunosorbent assay (ACP-ELISA)

A total of 210 rice leaf samples (7 per plot) were collected from the field and indexed for Rice Yellow Mottle Virus (RYMV) and Maize Streak Virus (MSV). 0.1g of leaf sample was weighed and grinded with mortar and pestle in 1 ml of extraction buffer [8 g sodium chloride, 0.2 g monobasic potassium phosphate, 1.15 g diabasic sodium phosphate, 0.2 g potassium chloride, 0.2 g sodium azide dissolved in 900 ml H_2O adjusted to pH 7.4 with HCl to make up (1 l) + 0.5 ml Tween 20/L and 2% polyvinyl pyrollidone (PVP)]. The 96 polystyrene microtitre plates were labeled and coated with 100 µL of antigen 1/10 in coating buffer with 1% Dieca, covered and incubated for 1 h at 37°C. The plates were removed, washed with PBS-Tween thrice at 3 min interval and tap dried. Blocking was done with 200 µl per well of 3% dried skimmed milk in PBS-Tween to trap the virus and incubated at 37°C for 30 min to enable binding. The plates were removed and washed as stated above. 100 µl of sap of healthy cowpea leaf samples mixed with pool of antibodies (21 different vegetable and cowpea antibodies were mixed together) was added into wells and incubated for 2 h at 37°C. Plates were removed and washed. Then, 100 µl enzyme goat anti-rabbit antibody diluted at 1:2000 was added to wells and incubated then washed as above. Substrate pNPP in substrate buffer at 3 mg in 30 ml was added into wells and kept for change in color. The optical density (OD) of the content of each well was subsequently read after 4 h and overnight respectively using ELISA reader (Diagnostic and Medical Solutions Micro Plate Read - ELISA Plate Analyzer) at a wavelength of 405 nm.

Detection of viruses in weed samples using antigen-coated enzyme linked immunosorbent assay (ACP-ELISA)

Ten weed samples were collected from within, inside and outside the field and indexed for RYMV and MSV. The ELISA protocol used for detection of RYMV and MSV as described for rice leaf samples above was used.

Data analysis

Rice virus disease incidence and severity was calculated based on the number of plants sampled on the field. Germination percentage, percentage disease incidence and mean severity were calculated. The data were subjected to statistical analysis using Statistical Package for the Social Sciences (SPSS) version 16.0. Mean separation was done with Duncan Multiple Range Test.

RESULTS

Growth of rice from the field study

Table 2 shows the effect of varietal difference on the growth of rice from the field study in Abuja in 2019. The variety FARO 52 recorded the highest germination percentage of seed (96.66%) while FARO 60 recorded the lowest germination percentage of seed (69.66%). At 3 weeks after planting (WAP), FARO 60 had the highest plant height (18.18 cm); while FARO 22 had the shortest plants (14.72 cm). At 6 WAP FARO 47 recorded the tallest plant height (39.44 cm) while FARO 60 recorded

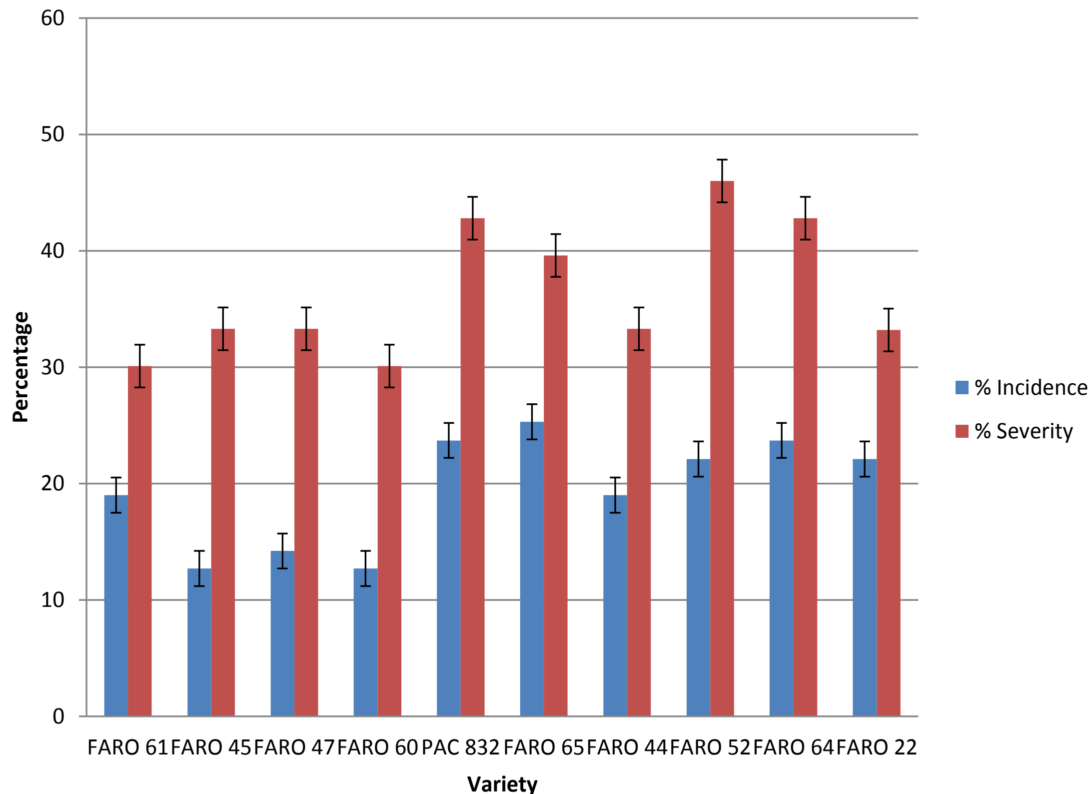

Figure 1. The mean incidence and severity of important viruses infecting rice (*Oryza sativa* L.) in Abuja, FCT in 2019.

the shortest plant height (23.79 cm). The variety FARO 45 recorded the highest plant height (80.55 cm) at 9WAP while the lowest plant height was recorded on FARO 60 (51.47 cm). After analysis, the number of leaves per plot at 3 WAP for FARO 45 and FARO 52 were the highest (5.80) while the lowest was recorded on FARO 47 and FARO 65 (4.95). At 6 WAP, FARO 45 and FARO 64 recorded the highest number of leaves (8.42) while FARO 61 recorded the lowest number of leaves (7.52). FARO 45 recorded the highest number of leaves (11.57) at 9WAP while FARO 61 had the lowest number of leaves (10.04).

Collection of leaf samples from field study

Five different virus-like phenotypic symptoms were expressed on leaf surfaces on the field in different proportions. The most common symptom observed was yellowing of leaves (Costa et al., 2018; Ndikumana et al., 2015) followed by necrosis, orange discolouration and dark patches.

Incidence and severity

From the 210 leaf samples collected from the field study and according to visual assessment of virus-like symptoms, FARO 61 and 44 recorded the lowest mean percentage incidence (19%); while FARO 65 recorded the highest mean percentage incidence at 25.3% (Figure 1). Results also showed that FARO 52 recorded the highest mean percentage severity (46%), while FARO 61 and FARO 60 recorded the lowest mean percentage incidence (30.1%).

Virus detection in rice seeds and leaves by ELISA

The seed samples of the ten rice varieties that were also indexed using ELISA did not test positive to RYMV and MSV. Also, all the leaf samples collected from the field study indexed for RYMV and MSV using ELISA did not test positive to RYMV and MSV (Figure 2). The absorbance values at spectrophotometric wavelength of 405 nm were not up to one and half times the values of the healthy controls after the one hour reading and overnight reading.

Virus detection in weeds by ELISA

The weed samples which were collected from the field study 8 weeks after planting rice were indexed for RYMV

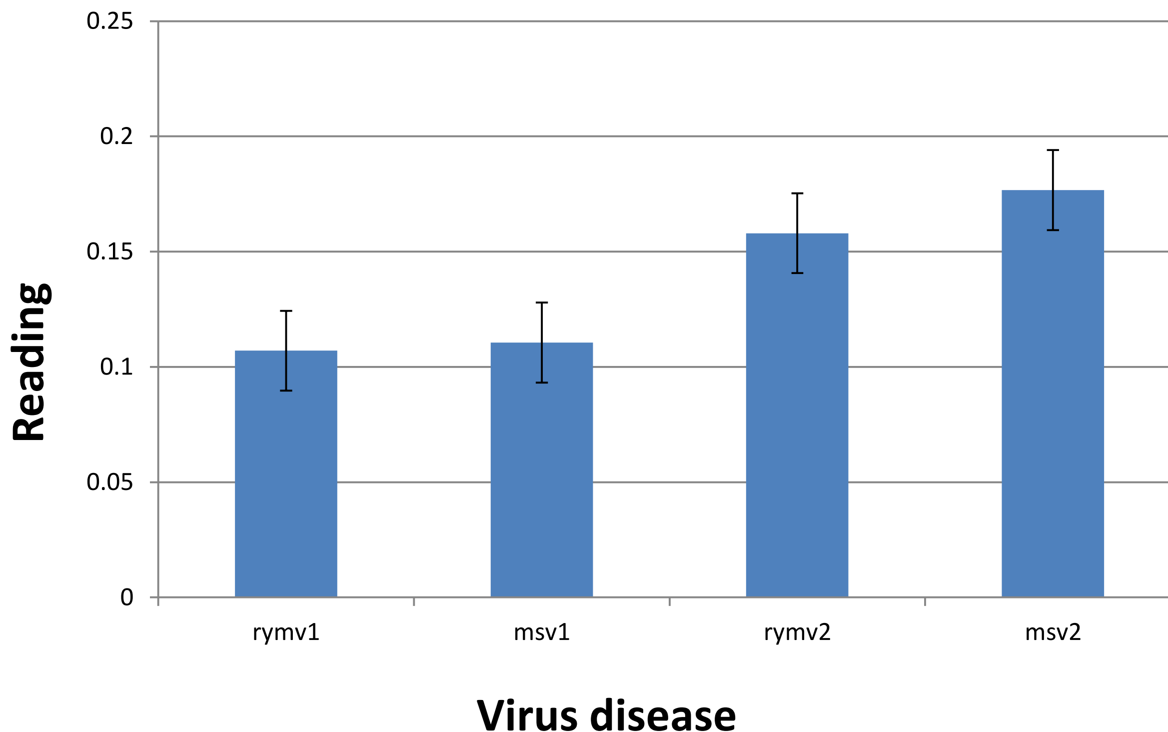

Figure 2. Result of Rice seed and leaf samples indexed for RYMV and MSV using ELISA. RYMV, Rice yellow mottle virus; MSV, maize streak virus; 1. One hour reading; 2, Overnight reading.

and MSV using ELISA did not test positive to RYMV and MSV (Figure 3). The absorbance values at spectrophotometric wavelength of 405 nm were not up to one and half times the values of the healthy controls after the 1 h reading and overnight reading.

Insects associated with rice production

From the field study, 5 insects were identified to be associated with rice production in the FCT (Table 3). The insects identified were Spittle bug (*Locris rubens* and *Poophilus costalis*), Ladybird beetle (*Cheilomenes sulphurea*), Groundhopper (*Paratettix sp*) and Grasshopper (*Catantops* sp). Ladybird beetle (*Cheilomenes sulphurea*) was found to occur more on the rice field. The two spittle bugs that were identified were of different family, genus and species but of same order (Homoptera). Furthermore, the grasshopper that was identified was at the nymphal stage.

Weather data for 2019 rice growing season in F.C.T

The weather report in 2019 indicated that mean temperature was highest (26.4°C) during the early month of rice planting in June and the end of the season in November. The remaining months (July to October) recorded relatively lower temperatures with the lowest mean temperature (24.6°C) recorded in September towards the ending of the growing season. The longest sunshine (8.3 h) was recorded in November while the lowest (2.1 h) was recorded in August. The months of June and July recorded same duration of sunshine (3.9 h) and a short decline in sunshine hours (4.6- 4.9 h) was indicated from September to October. The highest rainfall (243 mm) during the growing season was recorded in September. There was a steady increase in monthly rainfall from the beginning of the growing season in June till September (114.6-243 mm) with a sharp decline recorded between October and November (199.9 and 50.7 mm). Relative humidity was highest (88%) in August while the lowest (68%) was recorded in November. There was a decline in relative humidity from June to July (82 - 84%) and another decline was recorded from September to November (83 - 68%) (Table 4).

DISCUSSION

This study provides information on the incidence and severity of two important viruses infecting rice in Nigeria. The samples collected from the field experiment were indexed for RYMV and MSV. The serological diagnosis showed that rice seed samples from the 10 varieties planted on the field were not positive to RYMV and MSV.

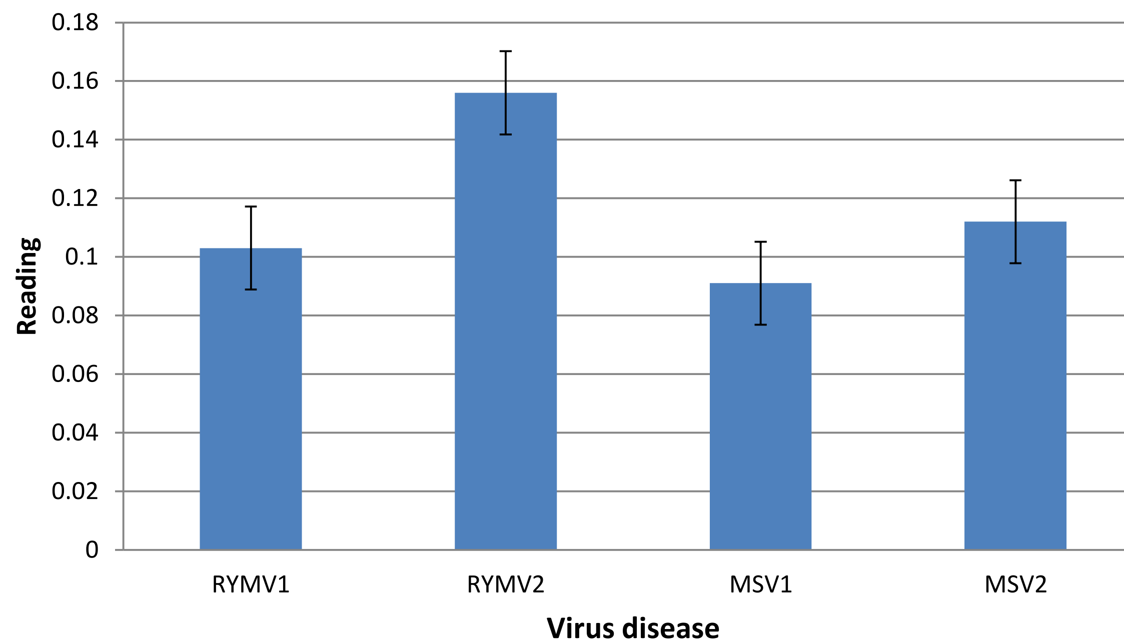

Figure 3. Result of weed samples indexed for RYMV and MSV using ELISA. RYMV, Rice Yellow Mottle Virus; MSV, Maize Streak Virus; 1, One hour reading; 2, Overnight reading.

Table 3. Identification of insect pest associated with Rice production from the field study in Abuja, F.C.T.

S/N	Order	Family	Common Name	Genus	Species	Author	Status
1	Homoptera	Cercopidae	Spittle bug	Locris	Rubens	Erichson	Vector
2	Coleoptera	Coccinellidae	Ladybird beetle	Cheilomenes	Sulphurea	Olivier	Vector
3	Orthoptera	Acrididae	Grasshopper	Catantops	sp. (Nymph)		Vector
4	Homoptera	Aphrophoridae	Spittle bug	Poophilus	Costalis	Walker	Vector
5	Orthoptera	Tetrigidae	Groundhopper	Paratettix	sp.		Vector
6	Coleoptera	Coccinellidae	Ladybird beetle	Cheilomenes	Sulphurea	Olivier	Vector

This could be due to the fact that the samples were certified seeds which met the required minimum standards of seed certification before they can be marketed as seeds (Seed Act, 2019). This can also be attributed to the non-transmission of these viruses through seeds as reported by Konate et al. (2001). The collected leaf samples showing virus-like symptoms of viral infection did not test positive to RYMV and MSV

Table 4. Weather data for 2019 during the rice growing season in Abuja.

Month	Mean temperature (°C)	Sunshine (h)	Rainfall (mm)	Humidity (%)
June	26.4	3.9	114.6	82
July	25.8	3.9	140.6	84
August	24.7	2.1	226.7	88
September	24.6	4.6	243	83
October	25.0	4.9	199.9	80
November	26.4	8.3	50.7	68

using ELISA. Although the symptoms observed on the field were the usual symptoms of RYMV and MSV which had been described by Thottappilly and Rossel (1993) and Fauquet et al. (1988), the observed symptoms could have been caused by abiotic factors often related to physical factors, environmental factors or cultural practices. The high and consistent rainfall recorded in 2019 rice growing season could have been responsible for the low vector population and absence of infection, as the vectors would have been washed away. The two viruses that were selected for detection have been reported in other West African countries as well as some parts of Nigeria (Sere et al., 2008; Banwo et al., 2004; Alegbejo, 2013; Oludare et al., 2015; Odedara et al., 2016; Onasanya et al., 2011). Odedara (2016) reported from surveys carried out in Ogun State in 2012 the presence of RYMV in 7.7% of leaf samples collected and tested from Ewekoro Local Government Area (LGA) and 13.3% from leaf samples collected and tested from Obafemi Owode LGA. Further survey in 2013 recorded the presence of RYMV in 30.0% of leaf samples collected and tested in Ewekoro LGA, while Obafemi Owode recorded 90.0%. Though there have been reports of alternate hosts like weeds, harbouring these viruses (Awoderu, 1991; Konate et al., 1997; Okioma et al., 1983), the weed samples collected from the field for detection also did not test positive to the rice viruses.

The symptoms observed on these plants may have been caused by other viruses that were not identified or yet to be identified. The absence of RYMV and MSV in this study could also be due to low concentration of virus in the samples (Lacroix et al., 2016). In addition, the results recorded in this research may be attributed to the level of resistance or susceptibility of the rice varieties tested to the virus diseases (Arli-Sokmen et al., 2016; Mwaipopo et al., 2017). Previous reports by Abo et al., (2005) and Salaudeen (2012) show that some rice varieties (such as FARO 52) possess resistance to RYMV, although further classification has been recommended to aid breeding for resistance.

Bakker (1970) reported that RYMV was sometimes transmitted by biting insects such as grasshopper (*Conocephalus*); but all leaf samples indexed were not positive to RYMV and MSV, this may be because the insects were not able to transmit any virus. Most of the identified insects (*Locris rubens*, *Poophilus costalis, Cheilomenes sulphurea* and *Paratettix* spp) have been reported by Koudamiloro et al. (2014) to be found on rice fields in Burkina Faso, Cameroon, Mali, Nigeria and other parts of West Africa. The insects have also been confirmed as vectors of viral diseases infecting rice and have the capacity to cause extreme damage to the rice plant (Koudamiloro et al., 2019; Asala and Alegbejo, 2016). This study provides the first research on rice viruses in the Federal Capital Territory, Abuja and since epidemiologically, viral diseases are not static, further field trials and survey studies are recommended.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Sensor based validation of nitrogen fertilizer for quality protein maize variety using a handheld normalized difference vegetative index sensor at Bako, Western Ethiopia

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Regardless of the huge yield potential and area under maize production, its current productivity in Ethiopia is by far below its potential. Declining of soil fertility and poor nutrient management is among the major factors limiting the productivity of the crop. As a result, an experiment was conducted at Bako, Ethiopia in 2016 to validate the N application and determine the best rate for side dressing using handheld Normalized Difference Vegetative Index (NDVI) sensor. The experiment was laid out in randomized complete block design in factorial arrangement with three replications. Three N levels (0, 25 and 50 kg N ha^{-1}) all applied at the time planting and four N rates (19, 38, 56 and 75 kg N ha^{-1}) for side dressing. Significant differences were observed between the applied N fertilizer for grain yield and yield components. Higher correlation coefficients (0.78) between grain yield, NDIV and INSEY at V_4 were observed. Application of 25 kg N ha^{-1} and 38 kg N ha^{-1} at planting and side dressing at 35 days after sowing correspondingly, gave higher grain yield for quality protein maize in the area. Further studies are required across various locations using different maize varieties to provide conclusive recommendations.

Key words: INSEY, maize, NDVI, nitrogen.

INTRODUCTION

Maize (*Zea mays* L.) is one of the main valuable crops worldwide because of its high value as stable food and as feed for animals and even for construction purposes (Zerihun et al., 2016). Ethiopia is the fourth largest producing country in Africa, and first in the East Africa region (FAO, 2012). Maize is leading all other cereal crops in terms of production and productivity, and second in area coverage next to teff. The total land areas of 10,219,443.46 ha (81%) was under cereals of which maize covered about 17% (2,135,572 ha) and 27% (78,471,746.57 quintals) grain yields (CSA, 2017). Despite the huge area under maize production, its current

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national average yield is about 3.7 t ha^{-1} (CSA, 2017) as compared to the world's average yield which is about 5.2 t ha^{-1} (FAOSTAT, 2012). Although several factors can contribute to big yield gaps, declining soil fertility and poor nutrient management are among the major factors contributing to low productivity of maize (Mourice et al., 2015; Chimdi et al., 2012; Vesterager et al., 2008).

Nitrogen (N) management in maize cultivation system is one of the major concerns since N is an essential plant nutrient and it is the most yield limiting factor in major hybrid maize production (Baral and Abhikari, 2015; Blumenthal et al., 2008). Many farmers refrain from applying sufficient amount of fertilizer due to its prohibitive prices, or lack of knowledge of which type and rates to apply (Hopkins et al., 2008). Conversely, excessive application is uneconomical, environmentally unsafe and potentially harmful to the crop (Guo et al., 2010). About 30 to 70% of the applied N may be lost as ammonia within 7 to 10 days after application and may lead to an elevated level of NO_3 in the soil and makes it susceptible to NO_3 loss through leaching, volatilization or surface soil (Canfield et al., 2010; Xue et al., 2010a). Since yield is likely to be low under N stress during tasselling and silking, coincidence of N availability in the soil solution and plant uptake are crucial to unlocking the potential of modern hybrids.

Maize breeders have been developed and released quality protein maize (QPM) hybrids. Thus, in addition to releasing QPM varieties, it would be very crucial to have site specific nutrient managements for the benefit of farmers in terms of nutrition, economy and reduced environmental hazards; it aims at "doing the right thing, at the right place and at the right time". When used in combination with information technologies it defines precision agriculture (Bongiovanni and Deboer, 2004). Precision agriculture is mainly used in large-scale commercial farmers using satellite and sensor-based technologies, which is not affordable by African smallholder farmers. Nevertheless, in the early 2000s, Trimble Company started manufacturing a hand-held Normalized Difference Vegetative Index (NDVI) sensor, a real pace towards popularizing precision agriculture regardless of farm-scale (Trimble, 2012). NDVI correlates with many variables such as crop N deficiency, final grain yield, weed species, and long-term water stress. Lukina et al. (2001) indicated that there is a high correlation (0.80 to 0.97) between percent vegetation cover and NDVI measurements. N use efficiency could be increased by the use of spectral radiance (Ngie et al., 2014; Li et al., 2009). Hence, the use of NDVI sensor brings precision agriculture to Ethiopian smallholder farmers, increasing yield and returns of N fertilizer, as well as minimizing the risk of ecological contamination. Farmers in the utmost part of Ethiopian often do not apply adequate amounts of N fertilizer as required by the crop. They only use basal application of N mostly once in a blanket recommendation, which causes loss of N and reduces yield. Moreover, much of the effort in making fertilizer recommendation with modern approach has not been investigated, yet there is the potential of using NDVI sensors to upturn economic and environmental wellbeing in the study area rather than blanket recommendation used as national level. Lately, there was an attempt in Western and rift valley parts of Ethiopia, on in-season N fertilizer calibration using handheld NDVI sensor (Tolera et al., 2015, 2014; Addis et al., 2015), which will be used by validating the results for maize production. Thus, the objective was to validate the calibrated N rates, and determine the optimum N rate for side dressing supported by handheld NDVI sensor for quality protein maize variety.

NDVI, land cover is one of the most important data used to demonstrate the effects of land use changes, especially human activities. Some studies have produced land cover maps of the controlled classification technique over Landsat satellite imagery. In addition, agriculture planning has many benefits in terms of the environment. For instance, it is used in making decisions about the future situation of agriculture land, and it is necessary to predict how the land has changed over time and the effects of natural factors and human activities on the land. Some of the studies show that weed invasion is a problem for the agricultural ecosystems in terms of production. It causes water stress, affects light and nutrients. Many studies represent that water stress is a problem for production as well as light. Moreover, many studies recently showed that weed is to be managed with feed management (Cetin et al., 2019; Kaya et al., 2019; Cetin, 2013).

MATERIALS AND METHODS

The experiment was conducted at Bako Agricultural Research Center in 2016 cropping season. Bako lies at an altitude of 1650 m.a.s.l and is situated at 9 E 6' N latitude and 37 E 09' E longitude. The area's mean annual rainfall is 1239 mm, with unimodal distribution and maximum precipitation being received in the months of May to August (MBARC, 2014). The experimental area is characterized by warm and humid climate with mean minimum and mean maximum air temperatures of 13.5 and 29.7, respectively (WWW.IQQO.ORG). The soil type is reddish-brown clay loam Nitosols (Mesfin, 1998). It is an acidic soil with a pH range of 4.5-5.6. The farming system of the surrounding area is a mixed farming and is one of the major maize (*Zea mays* L.) growing belts in the country; *teff* (*Eragrostis tef*), finger millet, sorghum and soybean are commonly cultivated there.

The experiment was laid out in a randomized complete block design with factorial arrangement in three replications. The plot size was 5.1 x 4.5 m. The treatments consisted of three N levels (0, 25, and 50 kg N ha^{-1}) applied at the time of planting, and four N rates (19, 38, 56 and 75 kg N ha^{-1}) for side dressing applied 35 days after planting, constituting a total of 12 treatments.

The experimental fields were plowed three times at different time intervals starting from end of April and leveled manually prior to field layout. Recommended phosphorus (20 kg P ha^{-1}) in the form of triple super phosphate was uniformly and equally applied to all experimental plots at the time of planting. N fertilizer in the form of Urea was applied at different rates as constituted in the treatments.

Table 1. Analysis of variance for yield and yield traits as influenced by nitrogen rates, and interaction effects at Bako, western Ethiopia.

Source of variation	F probability (p = 0.05)							
	D.f.	**PH**	**LA**	**LAI**	**GY**	**DB**	**HI**	**TKW**
Nitrogen at planting (N)	2	<.001	<.001	<.001	<.001	<.001	<.001	<.001
Nitrogen for side dressing (SD)	3	0.158	<.001	0.007	<.001	<.001	0.006	0.016
N * SD	6	0.466	<.001	0.008	<.001	0.039	<.001	0.015
Replication	2	0.002	0.293	0.529	<.001	<.001	<.001	0.072
Residual	22	–	–	–	–	–	–	–
Total	35							

d.f. = degree freedom, PH = Plant height, LA = Leaf area, LAI = Leaf area index , GY= Grain yield, DB= Above ground dry biomass, HI = Harvest Index and TKW = thousand kernel weight.

One medium maturing quality protein maize hybrid (BHQPY545) variety was used for the experiment. The variety was released by Bako National Maize Research Center in 2008. The cultivar is well adapted to altitude areas of 1000-1800 m.a.s.l and it requires an annual rainfall of 500-1000 mm with uniform distribution in its growing periods. Its yield potential ranges from 8.0-9.5 t ha^{-1} at research field and 5.5-6.5t ha^{-1} at farmers' field (Adefris et al., 2015). The trial was planted with inter-row of 75 cm and intra-row spacing of 30 cm. All other non-treatment management practices were applied as per recommendation for the variety to all experimental plots.

NDVI values were recorded from the central four rows using hand-held Green Seeker sensor at vegetative growth stage of leaf four (V4) and six (V6) of maize. The value of NDVI readings range from 0.00 to 0.99; higher reading leads to healthier plant, healthier crop canopy with a higher NDVI value (Lan et al., 2009). In-season estimation of yield (INSEY) was computed for the area as: INSEY= NDVI÷GDD, where, GDD is the number of Growing Degree Days greater than zero from seed emergence to sensing. The INSEY provides an estimate of daily biomass production or growth rate (Raun et al., 2005), and is therefore a vital determinant of final grain yield. GDD = [(daily maximum Temperature + daily minimum Temperature) ÷ 2] minus base temperature for maize (Lukina et al., 2001). The base temperature for maize is 10°C.

The maize was harvested from four rows by excluding two border rows from each side. A net plot size for each plot was 2.25 x 5.1 m (11.475 m^2). Stand counts per net plot were counted at the time of harvesting. Plant height, biomass yield, grain yield, harvest index, thousand kernel weight and other relevant agronomic traits were recorded at appropriate growth stages. Costs that vary among treatments were also assessed. The cost of Urea, the cost of labor required for the application, and cost for shelling were estimated by assessing the current local markets. The price of Urea (11200.00 ETB 100 kg^{-1}) and daily labors (35 ETB per man per day based on government's current scale in the study area), and the cost of maize shelling (100 ETB t ha^{-1}) were considered to get the total cost that varied among the treatments. On the other hand, non-varied costs were not included since all management practices were uniformly applied to each experimental plot. The grain yields harvested were adjusted down by 10% to reflect actual production environments. Gross revenue was calculated as adjusted grain yield multiplied by field price (7000.00 ETB t ha^{-1}) that farmers receive for the sale of the crop. The net benefit and the marginal rate of return were calculated as per standard manual (CIMMYT, 1988). Finally, combined analysis of variance was carried out using Gen Stat 15th Edition software, and Duncan's multiple range tests at $P < 0.05$ was used to compare treatment means (Duncan, 1955). Pearson's correlation analysis and regression were also performed to observe association and relationship between different variables as affected by different levels of N fertilizer applications.

RESULTS AND DISCUSSION

The combined analysis of variance revealed that applied N fertilizer at planting and side dressing significantly (P<0.01) affected grain yield, dry biomass, leaf area, harvest index and 1000 kernel weight (Table 1). There was also significant (P<0.05) difference between the applied N fertilizer on leaf area index. In addition, applied N at planting showed a highly significant (P<0.01) variation on plant height, leaf area, leaf area index, grain yield, dry biomass, harvest index and thousand kernel weight. Likewise, side dressing N rates showed significant (P<0.01) effect on leaf area, grain yield and dry biomass. Furthermore, applied N as side dressing significantly (*P<0.05*) affected leaf area index, harvest index and thousand kernel weight. On the contrary, the response of plant height to side dressing N rates did not show significant variations. NDVI values at V4 growth stage were significantly (P<0.01) affected by applied N rates at planting and side dressing (Table 2). Higher NDVI value at V4 was recorded when 25 and 75 kg N ha^{-1} was applied at planting and side dressing respectively, and it shows a decrease in NDVI value as N rate increased (Table 3).

The values of NDVI readings become greater while growth continues after V4, but it was small at the beginning (Figure 2a, b and Table 3). This was possibly low due to the initial growth stage/failure of canopy cover over the space and lack of early N stress. At later vegetative stages, the value improved most likely due to a more canopy cover. Increase in N level enhanced spectral vegetation indices which have been shown to be helpful for indirectly obtaining information such as photosynthetic efficiency and potential yield (Baral and Abhikari, 2015; Ngie et al., 2014).

A strong relationship between the NDVI values and grain yield of maize was observed (Figure 1). On the other hand, this shows the handheld sensor is one of the best instruments in indicating crop health and lack of

Table 2. Analysis of variance for NDVI value and INSEY under different levels of N fertilizer at Bako, western Ethiopia.

Source of variation	D.f.	F probability (p = 0.05)					
		NDVI at node			INSEY at node		
		V4	V6	V8	V4	V6	V8
Nitrogen at planting (N)	2	<.001	<.001	<.001	<.001	<.001	<.001
Nitrogen for side dressing (SD)	3	<.001	0.805	0.558	0.003	0.321	0.558
N * SD	6	<.001	0.881	0.910	<.001	0.109	0.910
Replication	2	0.085	0.110	<.001	<.001	0.249	<.001
Residual	22	–	–	–	–	–	–
Total							

d.f .= degree freedom, V4, V6 and V8 = vegetative growth stages at leaf four, six and eight, correspondingly.

Table 3. Effects of N fertilizer rate on leaf area, leaf area index, dry biomass, harvest index and thousand seed weight of quality protein maize at Bako, Ethiopia.

Nitrogen levels (kg ha-^{1})	Nitrogen rate for side dressing (kg ha-^{1})	Leaf area (cm^2)	Leaf area index	Dry biomass (t ha^{-1})	Harvest index (%)	Thousand kernel weight (g)
0	19	5207^{ef}	2.22^{d}	18.3^{f}	36.1^{de}	314.9^{e}
0	38	5078^{f}	2.21^{d}	20.5^{e}	38.0^{a}	330.0^{bcd}
0	56	5337^{de}	2.33^{cd}	21.7^{de}	37.5^{ab}	331.1^{bcd}
0	75	5163^{f}	2.28^{cd}	22.5^{cd}	36.0^{de}	322.5^{de}
25	19	5409^{d}	2.37^{c}	23.2^{bcd}	36.4^{d}	341.0^{a}
25	38	5636^{c}	2.51^{b}	24.2^{abc}	36.4^{cd}	335.0^{abc}
25	56	5797^{abc}	2.58^{ab}	24.5^{ab}	35.8^{de}	339.0^{ab}
25	75	5936^{a}	2.64^{a}	25.4^{a}	$37.2b^{c}$	327.1^{cd}
50	19	5792^{abc}	2.58^{ab}	23.8^{abc}	$36.2d^{e}$	322.2^{de}
50	38	5729^{bc}	2.55^{ab}	23.6^{bc}	36.4^{d}	326.3^{cd}
50	56	5860^{ab}	2.60^{ab}	24.1^{abc}	35.5^{e}	328.5^{cd}
50	75	5631^{c}	2.49^{b}	24.1^{abc}	36.5^{cd}	326.1^{cd}
LSD (5%)		153.8	0.11	1.57	0.71	9.1
CV (%)		1.6	2.7	4.0	1.2	1.6

stress that can give maximum yield, and minimize environmental contamination due to poor N management. Moges (2004); Raun et al. (2001) found that NDVI values were positively correlated with final grain yield. Further, Adis et al. (2015) indicated that there is a strong relationship between NDVI and grain yield of quality protein maize variety.

Likewise, a strong relationship between INSEY and grain yield was observed with 0.87 and 0.76 at V4 and V6 growth stages, correspondingly (Figure 2). Grain yield increased to 100 kg ha^{-1} N applied at V4; in a similar pattern the INSEY increased, and gradually results of both parameters declined. The smallest value of both grain yield and INSEY was recorded from the lowest N treatment at V4 growth stages (Table 4). As nitrogen level increased the NDVI values became higher at V4 and V6 growth stages. This result confirms that handheld NDVI sensor can predict grain yield with INSEY for maize. Stevens (2014) indicated that INSEY correlated with grain yield of maize.

There is also evidence of a strong relationship between wheat grain yield and INSEY (Raun et al., 2001). Furthermore, higher leaf area (5936 cm^2), leaf area index (2.64) and dry biomass (25.4 t ha^{-1}) were recorded from the use of 25 kg N ha^{-1} at the time of planting and 75 kg N ha^{-1} for side dressing 35 days after planting (Table 3). Higher thousand kernel weight (341 g) was attained from application of 25 and 19 kg N ha^{-1} at planting and side dressing, correspondingly. Conversely, lower leaf area (5078 cm^2) and leaf area index (2.21) were recorded from 38 kg N ha^{-1} treatments applied only as side dressing. Thousand kernel weight (314 g) and dry biomass (18.3 t ha^{-1}) were obtained from N applied at planting combined with 19 N kg ha^{-1} for side dressing. The lowest harvest index (35.5%) was, however, recorded from treatments receiving 50 and 56 kg N ha^{-1} at planting and side

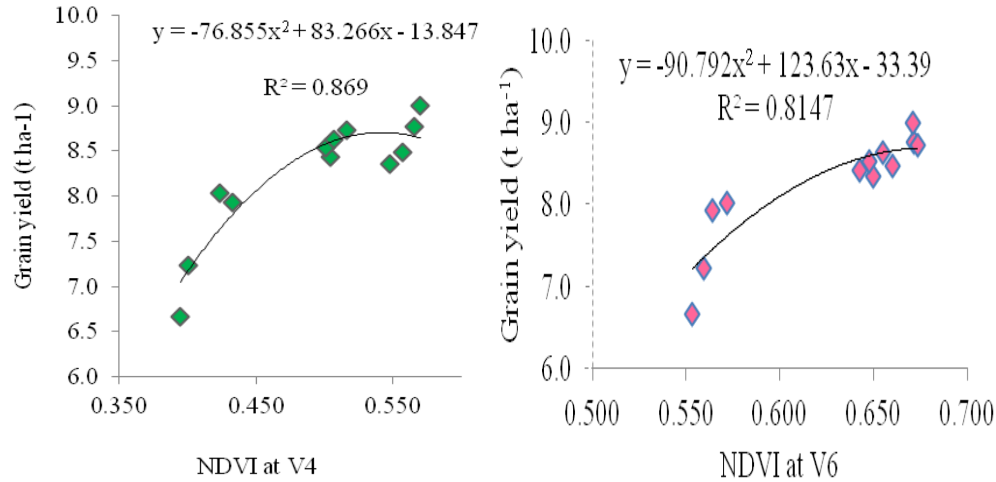

Figure 1. Grain yield of maize Vs. NDVI at V4 (left) and V6 (right) in 2016 at Bako, Ethiopia.

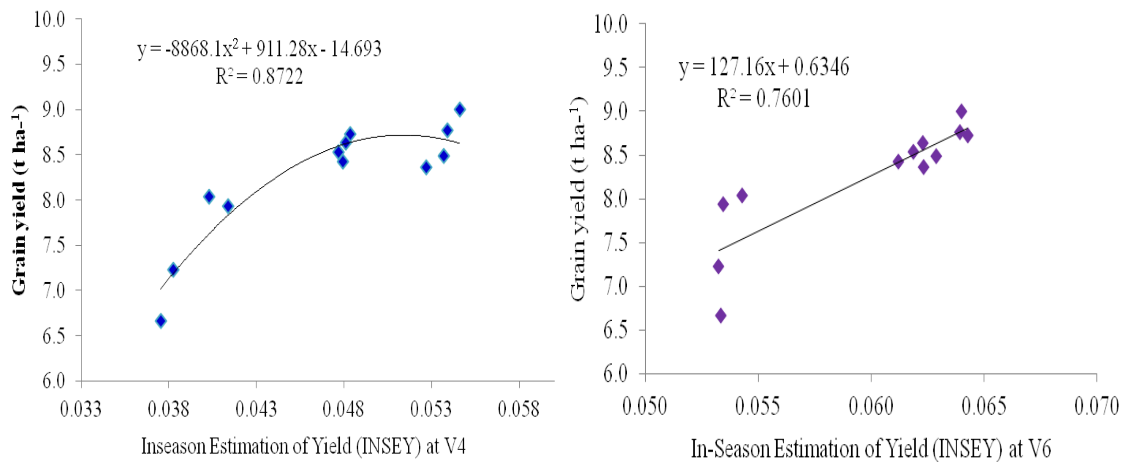

Figure 2. Grain yield Vs. INSEY at V4 (left) and V6 (right) stage of maize at Bako, Ethiopia.

dressing, respectively.

Applied N rates both at planting and side dressing also showed significant effects on mean grain yield of maize. The highest mean grain yields (9.0 t ha^{-1}) were obtained when 25 kg N ha^{-1} at planting with 75 kg N ha^{-1} for side dressing were used (Table 4). On the other hand, the lowest grain yield was recorded from plot receiving only 19 kg N ha^{-1} as side dressing compared to other treatment combinations.

Interrelationships between growth phenology and yield traits of maize

Applied N rates at planting were significantly associated with all growth phenology, yield and yield traits of maize except for TKW and HI which were non-significant and negatively associated (Table 5). On the other hand, side dressed N fertilizer showed a non-significant association with all yield traits except for grain yield. Significantly positive association (0.68, and 0.71) was also observed between applied N at planting, and NDVI at V4 and V6 growth stages. There is also higher correlation (0. 67 and 0.77) between applied N rates and INSEY at V4 and V6 growth stages. It means that, if the application of N rates is high, INSEY of yield at V4 and V6 growth stages of maize will increase. Likewise, significantly positive association (0.59, 0.48, 0.60, 0.77 and 0.76) was observed between applied N and PH, dry biomass, GY, LA and LAI correspondingly; whereas, N application at planting was negatively associated with HI of maize (-0.34). Moreover, the NDVI value and INSEY at V4, and

Table 4. NDVI reading, INSEY and grain yield of QPM in 206 at Bako, Ethiopia.

N rates at planting (kg ha-^{1})	Side dressing N rates (kg ha-^{1})	Grain yield (t ha-^{1})	NDVI at V4	NDVI at V6	INSEY at V4
0	19	6.7^g	0.395^f	0.55	0.038^d
0	38	7.2^f	0.401^f	0.56	0.038^d
0	56	7.9^e	0.433^e	0.56	0.041^c
0	75	8.0^{de}	0.423^e	0.57	0.040^c
25	19	8.4^{cd}	0.548^b	0.65	0.053^a
25	38	8.8^{ab}	0.566^a	0.67	0.054^a
25	56	8.7^{abc}	0.517^c	0.67	0.048^b
25	75	9.0^a	0.570^a	0.67	0.055^a
50	19	8.5^{bc}	0.557^{ab}	0.66	0.054^a
50	38	8.4^{bc}	0.504^{cd}	0.64	0.048^b
50	56	8.5^{bc}	0.501^d	0.65	0.048^b
50	75	8.6^{bc}	0.507^{cd}	0.66	0.048^b
LSD (5%)		0.30	0.013	0.044	0.0020
CV (%)		2.2	1.5	4.1	2.5

Table 5. Relationship between various growth phenological, grain yield and yield traits of maize at Bako in 2016, Ethiopia.

	N	SD	PH	DB	GY	TW	HI	LA	LAI	NDF	NDS	INF	INS
N		0.00	0.59**	0.48*	0.597**	0.063	-0.357*	0.774**	0.762**	0.68**	0.71**	0.67**	0.77**
SD			0.09	0.31	0.370*	0.014	0.051	0.192	0.229	-0.01	0.07	-0.03	0.06
PH				0.66**	0.700**	0.178	-0.454**	0.535**	0.483**	0.57**	0.65**	0.59**	0.56**
DB					0.897**	0.428*	-0.347*	0.613**	0.580**	0.61**	0.69**	0.64**	0.61**
GY						0.415*	-0.341*	0.765**	0.747**	0.78**	0.79**	0.78**	0.75**
TW							0.144	0.175	0.196	0.36*	0.40*	0.39*	0.39*
HI								-0.324*	-0.251	-0.20	-0.37*	-0.18	-0.29
LA									0.960**	0.79**	0.78**	0.75**	0.83**
LAI										0.78**	0.77**	0.76**	0.84**
NDF											0.87**	0.98**	0.93**
NDS												0.86**	0.91**
INF													0.91**
INS													

N= Nitrogen rate applied at planting; SD= Nitrogen applied as side dressing;PH= Plant height; DB= Above ground dry biomass; NDE= Normalized difference vegetative index at V8; INF= In season Estimation of Yield at V4; INS= In season Estimation of Yield at V6; INE= In season Estimation of Yield at V8, *and**= significant at 1 and 5 % probability level.

LAI and NSEY at V6 growth stage have significant correlation with grain yield (0.78 and 0.75). It means that, NDVI reading and calculated INSEY at V4, and LAI and NSEY at V6 vary together in the same direction for grain yield. The NDVI values at V4 with INSEY at V4 and V6 (0.98 and 0.93), and NDVI at V6 with INSEY at V4 and V6 growth stages (0.86 and 0.91) have higher correlation.

Partial budget analysis is indicated in Table 6. The highest net benefit ETB 53,590 ha^{-1} with marginal rate of return of 380 % and value to cost ration of ETB 29 per unit of investment were obtained when 25 and 38 kg N ha^{-1} were applied during planting, and side dressing (Table 6). The second higher net benefit ETB 51,590 ha^{-1} and marginal rate of return 363% with value to cost ration of ETB 39 per unit of investment were achieved when 25 and 19 N kg ha^{-1} were used during planting and side dressing, respectively. Conversely, minimum net benefit was attained from using only 19 kg ha^{-1} N applied as side dressing. The values to cost ratio ranged from ETB 14 to 68 per unit of investment of N application. Hence, the use of 25 kg N ha^{-1} at planting and 38 kg N ha^{-1} during side dressing was economically feasible for QPM production in the study area.

Conclusion

Determining the nitrogen status of the crop using NDVI

Table 6. Partial budget analysis for N fertilizer rates for QPM at Bako, Ethiopia.

Treatments NL (Kg ha^{-1})	Grain yield (t ha^{-1})	Adjusted grain yield (t ha^{-1})	Gross benefit (ETB)	TVC (ETB)	Net benefit (ETB)	Value to cost ratio	MRR (%)
0/19	6.7	6.0	42210	607.9	41602	68	
0/38	7.2	6.5	45360	1127.7	44232	39	510
25/19	8.4	7.6	52920	1330.5	51590	39	363
0/56	7.9	7.1	49770	1611.8	48158^{D}	30	
25/38	8.8	7.9	55440	1850.3	53590	29	380
50/19	8.5	7.7	53550	2065	51485^{D}	25	
0/75	8.0	7.2	50400	2119.7	48280^{D}	23	
25/56	8.7	7.8	54810	2334.4	52476 ^{D}	22	
50/38	8.4	7.6	52920	2584.8	50335 ^{D}	19	
25/75	9.0	8.1	56700	2842.3	53858	19	30
50/56	8.5	7.7	53550	3068.9	50481^{D}	16	
50/75	8.6	7.7	54180	3576.8	50603	14	

NL= nitrogen levels; TVC= Total Variable Costs; D= Dominated.

sensor is an effective way of managing N in maize producing farms. There were significant differences observed between applied N rates for various phenological growth, grain yield and yield traits of maize. Further, higher correlation coefficients (0.78) between grain yield, and NDIV and INSEY at V4 were observed. Moreover, significantly higher mean grain yield was obtained between 63 kg N ha^{-1} (25 kg N ha^{-1} during planting and side dressing with 38 kg N ha^{-1}) to 100 kg N ha^{-1} (25 kg N ha^{-1} at planting with 75 kg N ha^{-1} applied as side dressing). The NDVI sensor can be a very good indicator of N status of maize at early vegetative growth stage for N management in Bako. Application of 25 and 38 kg N ha^{-1} during planting and side dressing 35 days after sowing gave higher grain yield, and net benefit of ETB 53590 ha^{-1}. Thus, application of N fertilizer rate at 25 and 38 kg N ha^{-1} during planting and side dressing 35 days after sowing correspondingly is the best rate and is economically feasible to achieve best performance of the maize variety BHQPY545 in the study area. However, similar studies are required across various locations using different maize varieties to provide conclusive recommendations.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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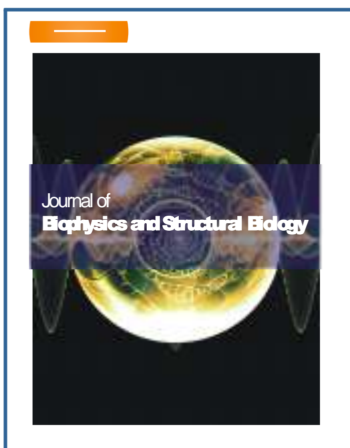

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