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Full Length Research Paper

Upland rice response to nutrient application in Uganda

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Upland rice (*Oryza* spp.) yields are low in Uganda, partly because of little fertilizer use. Three on-station trials and two clusters of on-farm trials were conducted in Uganda at approximately 1000 m elevation to determine: yield response to N, P and K application; economically optimal nutrient rates (EOR); and N use efficiency components. Mean grain yield, with hulls, was 1.3 and 3.7 Mg ha^{-1} with 0 and 100 kg ha^{-1} N applied, respectively. Grain yield response to applied P when compared with N was less, and mean yield was not increased with K application. Depending on fertilizer cost relative to grain price (CP), mean EOR ranged from 54 to 92 kg ha^{-1} N and 17 to 30 kg ha^{-1} P. Equations were determined for yield response, estimation of EOR, and the benefit: cost ratio (BC) for fertilizer N and P use. Grain N concentration and N harvest index at EOR were 1.55 and 0.55 kg kg^{-1}, respectively. Mean recovery efficiency, partial factor productivity and agronomic efficiency declined with increasing N rate and were 0.75, 41 and 28 kg kg^{-1}, respectively, at the EOR. Fertilizer N and P use can be highly and moderately profitable, respectively, for upland rice production in Uganda with high N recovery and agronomic efficiency. In maximizing net return on finance-constrained investment in fertilizer use, CP and investment capacity needs to be considered.

Key words: Economic, fertilizer use, nitrogen, phosphorus, potassium, use efficiency.

INTRODUCTION

Upland rice production is less important in Uganda when compared with maize (*Zea mays* L.) and sorghum (*Sorghum bicolor* L. Moench), but rainfed rice production has doubled (FAOSTAT, 2013) during the past decade due to high market value through expansion of area sown. Smallholders are more likely to apply fertilizer to rice when compared with other cereals because of the high market value of rice. However, levels of nutrient application are low, at least partly to high costs of fertilizer use relative to the value of rice, and mean grain yield in Uganda which is estimated to be 1.5 Mg ha^{-1} (Otsuka and Kalirajan, 2006). Inadequate control of numerous constraints may contribute to the low yield as found in Tanzania with biotic constraints, low input use and low availability of soil N and P constraining productivity (Mghase et al., 2010).

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Currently there are no fertilizer recommendations for upland rice production in Uganda. Upland rice is produced across diverse situations in Africa and research findings for nutrient application to upland rice are mostly quite recent and indicate situation specificity but also some opportunity for generalization. Research in Uganda showed that upland rice grain yield can often be increased by more than 100% with application of N and P, and the crop is responsive to *Azolla* spp. and to a preceding green manure crop of *Mucuna pruriens* L. (Kaizzi, 2002; Kaizzi et al., 2007). Yield in Uganda was increased by 2 to 3.5 Mg ha^{-1} in response to 120 kg N ha^{-1} (Onaga et al., 2012). Miyamoto et al. (2012) found that paddy yield could be increased by 46 kg kg^{-1} of applied N. In Benin, yields were less with no N when compared with N applied in diagnostic trials (Koné et al., 2009). In the Ivory Coast, yield was maximized with just 50 kg ha^{-1} of NPK blended fertilizer 12:24:18 or with 12 kg ha^{-1} urea-N applied with the low responsiveness attributed to soil water deficits during grain fill (Galabi et al., 2011). In Burkina Faso, Ethiopia, Ghana and Nigeria, upland rice response to applied N was generally curvilinear to plateau with, on average, about 90% of the grain yield increase with 100 kg N ha^{-1} occurring with the first 50 kg N ha^{-1} (Apaseku et al., 2013; Habtegebrial et al., 2013; Oikeh et al., 2008; Okonji et al., 2012). On the dry savannah land of northern Nigeria, however, rice grain yield increased linearly with N rates up to 90 kg N ha^{-1} (Kamara et al., 2010). Koné et al. (2011) attributed situations of reduced grain yield and root development with N application to mid-season soil water deficits. Oikeh et al. (2008) determined 60 kg N ha^{-1} to be optimal for upland rice production by smallholders in Nigerian forest agro-ecosystems.

A curvilinear to plateau response to applied P is also common with about 40 and 65% of the response to application of 40 kg P ha^{-1} occurring, on average, with the 10 and 20 kg P ha^{-1} rates (Bationo, 2008; Apaseku et al., 2013; Okonji et al., 2012). Upland rice yields were increased from 1.7 to 2.3 Mg ha^{-1} with a Bray-1 soil test P of 4 mg kg^{-1} with P application (Oikeh et al., 2010), and from 0.98 to 1.27 Mg ha^{-1} with Bray-1 of 2 to 3 mg kg^{-1} (Sahrawat, 2000) with 45 kg P ha^{-1}. Oikeh et al. (2008) determined 26 kg P ha^{-1} to be optimal for upland rice production in Nigerian forest agro-ecosystems.

Given the inadequate information base for maximizing profit from fertilizer use on upland rice for production in Uganda, research was conducted to: 1) quantify the yield response of upland rice to N, P, and K; 2) determine economically optimal nutrient rates for N, P and K (EONR, EOPR and EOKR) at different CP; and 3) evaluate efficiency of N use by upland rice in Uganda.

MATERIALS AND METHODS

Five N, P and K response trials were conducted in western Uganda across three cropping seasons from 2009 to 2010 (Table 1). The location and season combinations are referred to as site-seasons. Three site-seasons were conducted at the Bulindi Zonal Agricultural Research and Development Institute and two site-seasons were clusters of single sets of treatments evaluated on-farm with four or five farms per cluster providing replication. Research was carried out in the Western Mid-Altitude Farmlands (Wortmann and Eledu, 1999). Bulindi trial sites received >50 mm rainfall in the two weeks before sowing and received 430 mm or more of rainfall by 100 days after sowing (Figure 1). Rainfall was less in season 2010B when compared with other seasons with only 55 mm from 23 to 55 days after sowing.

The soils were Acric Ferralsols except for pre-dominantly Petric Plinthsol at the Kwera on-farm location (Table 1). Surface soil samples for the 0- to 20-cm depth consisting of 10 cores per site-season were collected before planting and fertilizer application to determine basic soil properties. Sand, clay and soil organic matter content ranged from 154 to 503, 298 to 506 (Bouyoucos, 1936), and 36 to 54 (Walkley and Black, 1934) g kg^{-1} soil, respectively. Mehlich-3 P (Mehlich, 1984) ranged from 3.7 to 7.9 mg kg^{-1} soil. Exchangeable K was always >130 mg kg^{-1}.

Site preparation at Bulindi included disk plowing at 15 to 20 cm depth followed by secondary disk tillage at 10 cm depth to reduce soil surface roughness. Land preparation for on-farm trials varied according to the practices of cooperating farmers but always included tillage. The previous crop and sowing dates varied (Table 1). Seeding rates were selected to achieve final plant populations of 50 plant m^{-2}, with two plants per point and a spacing of 20 by 20 cm. Weed control was done using hand hoes twice or thrice depending on weed intensity and labour availability. During the season, chloropyrifos 5% (Dursban^{TM}) was applied for control of the stem borer complex and the African rice gall midge (*Orseolia oryzivora* Harris & Gagné (Diptera: Cecidomyiidae).

The experimental design was a randomized complete block design. The nutrient rates evaluated were the following: 0 (N_0), 50, 100, and 150 kg N ha^{-1}; 0, 12.5, 25.0, and 37.5 kg P ha^{-1}; and 0, 30, 60, and 90 kg K ha^{-1}. The N-P-K treatments were 0-0-0, 50-0-0, 100-0-0, 150-0-0, 50-12.5-0, 100-12.5-0, 150-12.5-0, 50-25-0, 100-25-0, 150-25-0, 50-37.5-0, 100-37.5-0, 150-37.5-0, 50-12.5-30, 100-12.5-30, 150-12.5-30, 50-25-60, 100-25-60, 150-25-60, 50-37.5-90, 100-37.5-90, and 150-37.5-90. The incomplete factorial arrangement limited the number of treatment in consideration of Liebig's law of the minimum, proposed by J. von Liebig in 1840, expecting N and P to be the most and least limiting of the three nutrient deficiencies, respectively. The N_0 treatment occurred only with P_0 and K_0, and P and K effects were tested only with N applied. Similarly, no K was applied for the P_0 treatment. The K rates varied with P rates and the K effect was determined by subtracting at the plot level the P rate minus K treatments from the corresponding K-plus treatments after verifying that the P × K interaction was not significant.

Varieties were a sub-plot factor including Nerica-4 and Superica-1. Nerica-4 is a genotype derived from the interspecific hybridization of WAB 56-104 (*Oryza sativa*, tropical japonica type) and CG 14 [*Oryza glaberrima*]). Superica-1 (*O. sativa*) is a Ugandan release. These Uganda releases were not hybrids and each variety had a maturity period of 120 days. The plot size was 4 by 6 m.

The N, P and K sources were urea, triple super phosphate and potassium chloride, respectively. Fertilizer P was applied pre-plant. Fertilizer N and K were applied with 25% pre-plant, 25% at tiller formation and 50% at panicle initiation. The fertilizers were surface broadcast applied at planting and incorporated. The side dress application of N and K was band-applied to the side of the row and covered with soil.

The plants were cut at ground-level from the inner rows in a 1.5 x 2.0 m area and air dried for at least 3 days. The panicles were threshed and the harvested grain weight was determined. After adding the panicle remnants, the straw was weighed to determine the straw yield. The harvested grain was weighed and grain yield calculated. Grain yield was adjusted to 140 g kg^{-1} water content.

Table 1. Characteristics of research sites at Bulindi for three seasons, and for clusters of on-farm trials conducted at Kwera and Kiziranfumbi, to determine upland rice response to applied N, P and K in Uganda.

Site-season[†]	Soil properties						Previous crop	Sowing date	Harvest date
	Sand	Clay	OM	pH	P	K			
	g kg^{-1}				mg kg^{-1}				
Bulindi 2009B	154	506	46	5.9	4.9	418	FL‡	Sept. 18 -20	Jan. 12 - 15
Bulindi 2010A	326	505	44	5.9	4.6	235	CR	Mar. 17 - 19	June 8 - 21
Bulindi 2010B	168	550	49	6.1	7.9	385	CR	Aug. 23 - 25	Jan. 9 - 11
Kwera	405	406	36	6.0	6.2	231	CR	Sept. 1 - 10	Jan 15 - 30
Kiziranfumbi	503	298	54	7.1	3.7	132	CR	Sept. 1 - 10	Jan 15 - 30

[†]The rainfall is bimodal, with planting for seasons A and B occurring in March and April and in late August and September, respectively. The latitude, longitude and elevation of the research locations, respectively, were: Bulindi, 1°30'N, 31°29'E, 1021 m, Acric Ferralsol; Kwera, 1°49'N, 32°58'E, Petric Plinthsol; and Kiziranfumbi, 1°21'N, 31°12'E, Acric Ferralsol. CR = cereal, FL = fallow.

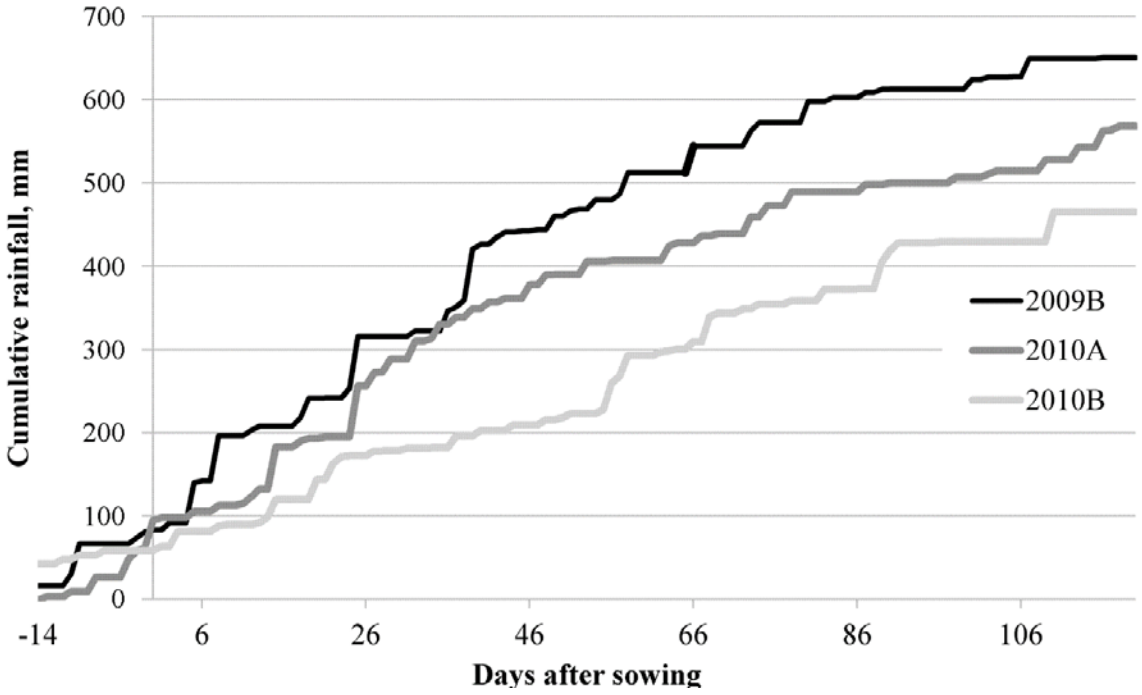

Figure 1. Cumulative rainfall for three cropping seasons at Bulindi, Uganda.

Grain and straw samples were oven-dried at 60°C, ground to pass a 0.5-mm sieve, and analyzed for total N in a single digest by a wet-ashing technique with colorimetric determination (Anderson and Ingram, 1993; Okalebo et al., 2002).

The data analyses were done by site-season using Statistix 9 (Analytical Software, Tallahassee, FL) with replications as random variables and varieties and nutrient rates as fixed variables. Analysis of variance combined across the Bulindi seasons was done to test for nutrient rate interactions with seasons after verifying homogeneity of variance. When significant nutrient rate effects occurred for a site-season, an asymptotic yield function was determined: grain yield (Mg ha^{-1}) = a - bc^n, where a was near maximum grain yield, b was the yield increase due to nutrient application, and c^n determined the shape of the curvilinear response where c was a curvature coefficient and n was the nutrient rate. Regression analyses by site-season and combined across site-seasons were done with plot data. Upland rice response to applied N was determined across all P levels after verifying a lack of N x P rate interaction, and response to applied P was determined with the zero N treatment omitted from the analysis.

The EONR and EOPR, or the nutrient application rates that gave the greatest net return ha^{-1} to fertilizer use, were calculated for a range of CP. A grain price of US$0.40 kg^{-1} (Uganda Sh. 2400 US$$^{-1}$) was used for the economic analysis. Equations were developed using non-linear regression analysis to relate EOR to CP.

The BC was considered to be the value of increased yield relative to cost of fertilizer use for the given application rate. Polynomial functions were determined for each crop-nutrient

Table 2. Nitrogen application effect on upland rice grain and straw yield in Uganda. The results are the means of two varieties as there was no variety by N rate or P x N rate interaction.

Site-season	N rate, kg ha^{-1}					N rate, kg ha^{-1}				
	0	50	100	150	Pr	0	50	100	150	Pr
	------- Grain yield, Mg ha^{-1} -------					------- Straw yield, Mg ha^{-1} -------				
Bulindi 2009B	0.75^{c†}	2.19^{b}	2.45a^{b}	2.48^{a}	*	2.74^{c}	5.26^{b}	6.06^{b}	7.04^{a}	***
Bulindi 2010A	1.00^{b}	2.56^{a}	2.55^{a}	2.62^{a}	*	3.37^{b}	5.98^{a}	5.99^{a}	5.78^{a}	**
Bulindi 2010B	0.99^{b}	3.27^{a}	3.56^{a}	3.36^{a}	***	4.01^{c}	7.20^{b}	8.25^{a}	8.20^{a}	**
Kwera	1.79^{c}	4.59^{b}	5.02^{ab}	5.18^{a}	***					
Kiziranfumbi	1.87^{b}	4.32^{a}	4.97^{a}	4.45^{a}	***					
Mean‡	1.42^{b}	3.39^{a}	3.71^{a}	3.62^{a}	***	3.37^{c}	6.15^{b}	6.77^{a}	7.01^{a}	***
SE	0.26	0.53	0.63	0.59		0.49	0.17	0.18	0.17	

† Different letters in a row under grain or straw yield indicate statistically significant differences at $\alpha \leq 0.05$. *, **, *** indicate statistical significance with P = 0.95, 0.99, and 0.999, respectively. The N rate x site-season interaction was significant for straw yield due to a relatively greater increase in straw yield in season 2010B compared with the other seasons.

Table 3. The coefficients for the upland rice grain yield response functions to applied N and the economically optimal N rates (EONR) for different cost of fertilizer N use to grain value ratios (CP).

Site-season	Response coefficients			EONR at five N:grain price ratios				
	--- Mg ha^{-1} ---			---------- \$ kg^{-1} (\$ kg^{-1})$^{-1}$ ----------				
	a†	b	c	2	4	6	8	10
Bulindi, 2009B	2.65	1.90	0.971	113	96	76	66	58
Bulindi, 2010A	2.59	1.56	0.945	67	55	47	43	38
Bulindi, 2010B	3.57	2.40	0.971	118	95	81	71	64
Kwera	5.59	3.44	0.979	113	106	92	85	80
Kiziranfumbi	4.85	2.81	0.956	150	132	114	101	91
Combined	3.67	2.40	0.958	92	76	66	60	54
SE	0.36	0.59	0.054					

†The response coefficients a, b, c are of the asymptotic function with yield = a -b*c^{n}; a is estimated yield at near plateau (Mg ha^{-1}), b is the yield (Mg ha^{-1}) increase due to nutrient application, c determines the shape of the response curve, and *n* (kg ha^{-1}) is the rate of applied nutrient.

combination to estimate BC with application rate and CP as independent variables. Differences and relationships were considered significant at $P \leq 0.05$.

Nonlinear functions that related total N in the aboveground biomass at harvest (UN) to the N rate and grain yield were determined. Asymptotic regression analysis, using individual plot data, related NUE properties to N rate. Exceptions were for straw N concentration and uptake which had linear and quadratic relationships to N rate, respectively, and for RE and agronomic efficiency of N use (AE) which had linear and quadratic relationships to N rate, respectively.

The NUE parameters included grain N concentration and content, HI, NHI, internal efficiency (IE) of total plant N taken up from soil and fertilizer, partial factor productivity (PFP), and physiological efficiency (PE), RE and AE for fertilizer N use (Cassman et al., 2002). The NUE components were calculated as follows: IE = *Y*/UN (kg kg^{-1}) where *Y* is grain yield (kg ha^{-1}); PFP = *Y*/N rate; NHI = grain N/UN; RE = (UN_{+N} - UN_{N0})/N rate; PE = (Y_{+N} - Y_{N0})/(UN_{+N} - UN_{N0}); and AE = (Y_{+N} - Y_{N0})/N rate. The units for IE, PE, AE, and PFP were kg grain kg^{-1} N and kg N kg^{-1} N for NHI. The effects of NHI and grain N concentration on IE were determined using linear regression analysis.

RESULTS

The mean upland rice paddy grain yield at N_0 was 1.42 Mg ha^{-1} and was more in on-farm trials than at Bulindi (Table 2). The predicted mean maximum grain yield was 3.67 Mg ha^{-1} (Table 3), with no significant yield increase for >100 kg ha^{-1} N applied. The N × P rate and N rate × site-season interactions, and the interactions with variety, were not significant for grain yield but there was a relatively greater increase in straw yield in season 2010B due to N application when compared with the other seasons.

Upland rice grain yield increased in response to N application for all site-seasons (Tables 2 and 3). The predicted overall mean grain yield increase was 2.40 Mg ha^{-1}. The results indicate a very high probability of response to applied N but the magnitude of the response varies. Mean yield response was greater for on-farm when compared with on-station trials but the mean curva-

ture coefficients were similar. Grain yield was significantly increased by application of >50 kg ha^{-1} N for only two of the five site-seasons but this was not sufficient to result in an N rate x site-season interaction. Combined across all site-seasons, the grain yield response to applied N was

$$Y = 3.67 - 2.40(0.958^{N}) \quad (1)$$

$$Y = 3.39 - 2.12(0.968^{N}) \text{ with no P or K applied} \quad (2)$$

The b and c coefficients of Equations 1 and 2 were not significantly different at α = 0.05 using a z-test. Upland rice straw yield was increased by 50 kg N ha^{-1} for the three site-seasons where measured, with an additional increase by applying more N for two site-seasons.

Net returns to N application were positive for all site-seasons and all CPs, and the site-season EONR ranged from 38 to 150 kg ha^{-1} depending on the CP (Table 3). The mean EONRs determined from the analysis combined across all site-seasons ranged from 54 and 92 kg ha^{-1} with CPs of 10 and 2, respectively (Figure 2). Net returns to applied N were more sensitive to N rate as the CP increased. The mean EONR can be estimated from the CP according to:

$$EONR, kg\,ha^{-1} = 107.2 - 8.78CP + 0.339CP^{2} \quad (3)$$

The P × K rate interaction was not significant but the P rate × site-season interaction was significant for grain yield with P application, in the presence of applied N, resulting in increased upland rice grain yield for three of the five site-seasons (Table 4). Yield increased with up to 25 kg P ha^{-1} for two site-seasons. In the combined analysis, grain yield was increased by 0.38 Mg ha^{-1} with 12.5 kg P ha^{-1}. The yield response function from the combined analysis was:

$$Y = 3.79 - 0.556(0.947^{P}) \quad (4)$$

Applying Equation 4, EOPR was determined to be related to CP, with CP for fertilizer P ranging from 4 to 12, as:

$$EOPR, kg\,ha^{-1} = 53.4 - 4.69CP + 0.134CP^{2} \quad (5)$$

Straw yield was increased with 12.5 kg ha^{-1} P applied at Bulindi in the 2010B season only (Table 4). Mehlich-3 P was low, ranging from 4 to 8 mg kg^{-1}, but there was no indication of a relationship between soil test P and grain yield response to applied P. Grain and straw yield were not affected by K application for any site-season.

The BC of fertilizer use was related to nutrient application rate and CP for ≤100 kg N ha^{-1} and ≤40 kg P ha^{-1} as follows:

$$Nrate \le 100\,kg\,ha^{-1} : BC_{N} = 56.52 - 0.541N - 9.983CP + 1.83E10^{-4}N^{2} + 0.425CP^{2} + 0.0326NCP \quad (6)$$

$$P\,rate \le 40\,kg\,ha^{-1} : BC_{P} = 10.67 - 0.172P - 0.383CP + 1.01E10^{-3}P^{2} + 0.0513CP^{2} + 0.00854PCP \quad (7)$$

The BC of fertilizer use was greater with N compared with P application, and decreased as application rate and CP increased.

Plant UN ranged from 31 to 89 kg ha^{-1} for N_0 and with 150 kg N ha^{-1} and was 79 kg ha^{-1} at EONR (Table 5). Variation in UN accounted for 82 and 74% of the variation in biomass and grain yield, respectively. Mean grain yield was 14.7 kg kg^{-1} UN for N_0 and 27.6 kg kg^{-1} UN across all N rates.

Internal efficiency (IE), or the efficiency of converting UN to grain yield, is a function of NHI and grain N concentration. The linear effects of NHI and grain N concentration accounted for 72 and 11% of the variation in IE, respectively. Grain N concentration, NHI and IE at an EONR = 66 kg ha^{-1} for CP = 6 were 15.5 g kg^{-1}, 55% and 35.4 kg grain (kg UN)$^{-1}$, respectively, which were higher than for the 0N rate (Table 5). The IE decreased with increased N rates.

Mean PFP declined with increased N rate and was 41 kg kg^{-1} at EONR. Mean AE decreased with N rate and was estimated to be 28 kg kg^{-1} at EONR when compared with 52 and 64 kg kg^{-1} for sorghum and maize, respectively (Kaizzi et al., 2012a, b). Mean PE of fertilizer N was not affected by N rate which is consistent with the results of the sorghum and maize studies. The following equations, determined from plot data of the three site-seasons at Bulindi, represent the N rate effect on various components of NUE for upland rice in Uganda.

$$UN, kg\,ha^{-1} = 91.82 - 60.89(0.977^{N}) \quad (8)$$

$$Grain\ N\ concentration, kg\,kg^{-1} = 17.50 - 3.47(0.992^{N}) \quad (9)$$

$$GrainUN, kg\,ha^{-1} = 47.60 - 34.42(0.968^{N}) \quad (10)$$

$$Stover\ N\ concentration, kg\,kg^{-1} = 5.32 - 0.0060N \quad (11)$$

$$StoverUN, kg\,ha^{-1} = 17.73 - 0.356N - 0.00129N^{2} \quad (12)$$

$$HI, kg\,kg^{-1} = 0.303 - 0.691(0.613^{N}) \quad (13)$$

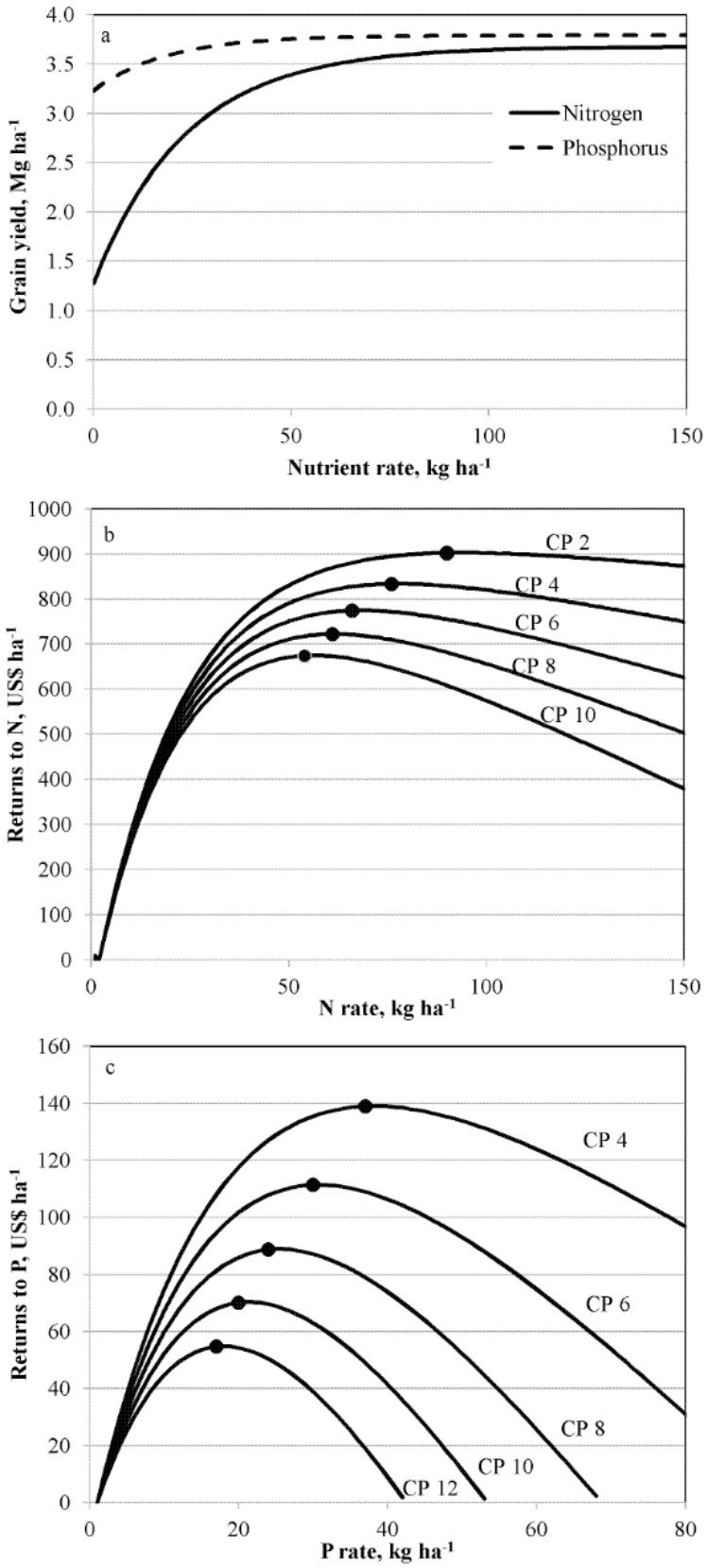

Figure 2. Upland rice yield response to applied N and P in Uganda with economically optimal N and P rates for different ratios of fertilizer use cost to grain price ratios (CP).

Table 4. Phosphorus application effect on upland rice grain and straw yield in Uganda. The results are the means of two varieties as there was no variety by N rate or P x N rate interaction.

Site-season	P rate (kg ha^{-1})					P rate (kg ha^{-1})				
	0	12.5	25	37.5	Pr	0	12.5	25	37.5	Pr
	------- Grain yield, Mg ha^{-1}-------					------- Straw yield, Mg ha^{-1} -------				
Bulindi, 2009B	2.21[†]	2.25	2.41	2.55	ns	5.81	6.04	6.22	6.23	ns
Bulindi, 2010A	2.36b	2.53b	2.50ab	2.80a	*	5.68	5.92	5.86	6.09	ns
Bulindi, 2010B	3.03b	3.29b	3.34ab	3.70a	*	7.14b	7.62ab	8.18ab	8.55a	*
Kwera	4.53	4.73	4.94	5.22	ns					
Kiziranfumbi	4.44b	5.66a	4.51b	4.75ab	*					
Mean[‡]	3.31c	3.69ab	3.67bc	3.88a	**	6.21	6.53	6.75	6.97	ns
SE	0.50	0.65	0.51	0.52						

[†]Different letters in a row under grain or straw yield indicate statistically significant differences at α ≤ 0.05. [‡]The P rate x site-season interaction was significant for grain yield with greater response to P rate for some site-seasons when compared with others. The grain yield response function to applied P was: Yield = 3.79 - 0.556*0.947^{P}.

Table 5. Mean effect of N rate on components of N use efficiency by upland rice averaged for 5 site-seasons in Uganda.

Component	Units	N rate, kg ha^{-1}				Pr	EONR[†]
		0	50	100	150		
Grain N concentration	g kg^{-1}	14.2	15.6	16.1	16.8	***	15.5
Straw N concentration	g kg^{-1}	5.60	5.32	6.08	6.05	***	5.6
Grain N content	kg ha^{-1}	13.2	41.4	46.7	47.9	***	43.6
Straw N content	kg ha^{-1}	18.0	32.3	41.6	41.2	***	35.6
Plant N content	kg ha^{-1}	31.2	73.7	88.3	89.2	***	78.7
Harvest index	kg kg^{-1}	0.24	0.31	0.30	0.30	***	0.30
N harvest index	kg kg^{-1}	0.44	0.57	0.54	0.54	***	0.55
Recovery efficiency	kg kg^{-1}		0.85	0.57	0.38	***	0.75
Agronomic efficiency	kg kg^{-1}		34.4	19.7	12.7	***	28.1
Internal efficiency	kg kg^{-1}	31.0	36.5	33.6	32.6	***	35.4
Partial factor productivity	kg kg^{-1}		52.8	28.9	18.9	***	41.4
Physiological efficiency	kg kg^{-1}		40.3	37.5	32.6	ns	

[†]The EONR was 66 kg ha^{-1} for a fertilizer N use cost to farm-gate grain price ratio of 6. ns, no significant effect at α ≤ 0.05. *** Significant effect at α ≤ 0.001.

$$NHI, kg\,kg^{-1} = 0.547 + 0.106 - 0.106(0.609^{N}) \quad (14)$$

$$IE, kg\,kg^{-1} = 23.63 + 0.0797N - 0.000570N^{2} \quad (15)$$

$$AE, kg\,kg^{-1} = 57.6 - 0.564N + 0.00177N^{2} \quad (16)$$

$$RE, kg\,kg^{-1} = 104.7 - 0.446N \quad (17)$$

$$PFP, kg\,kg^{-1} = 12.9 + 101(0.981^{N}) \quad (18)$$

DISCUSSION

The mean yield of 3.67 Mg ha^{-1} is not high relative to genetic potential of upland rice indicating the importance of other abiotic and biotic constraints in addition to deficiencies of N, P and K. Inadequate rainfall was not an apparent constraint to grain yield in the Bulindi trials as yield was highest in the 2010B season when rainfall was least and less well-distributed when compared with 2009B and 2010A. Variety performance across N rates was similar. This was in agreement with Onaga et al. (2012) who evaluated more varieties and reported a variety x N rate interaction but which accounted for less than 1% of the N rate main effect on variation in yield.

The indigenous soil N supply was apparently low as indicated by a mean grain yield of 1.42 Mg ha^{-1} and 31 kg ha^{-1} UN with N_0, even though SOM was always >36 g kg^{-1}. In comparison, mean grain yield was about 2 Mg ha^{-1} across several other studies in Africa (Apaseku et al., 2013; Bationo, 2008; Habtegebrial et al., 2013; Oikeh et al., 2008; Okonji et al., 2012). For further consideration of

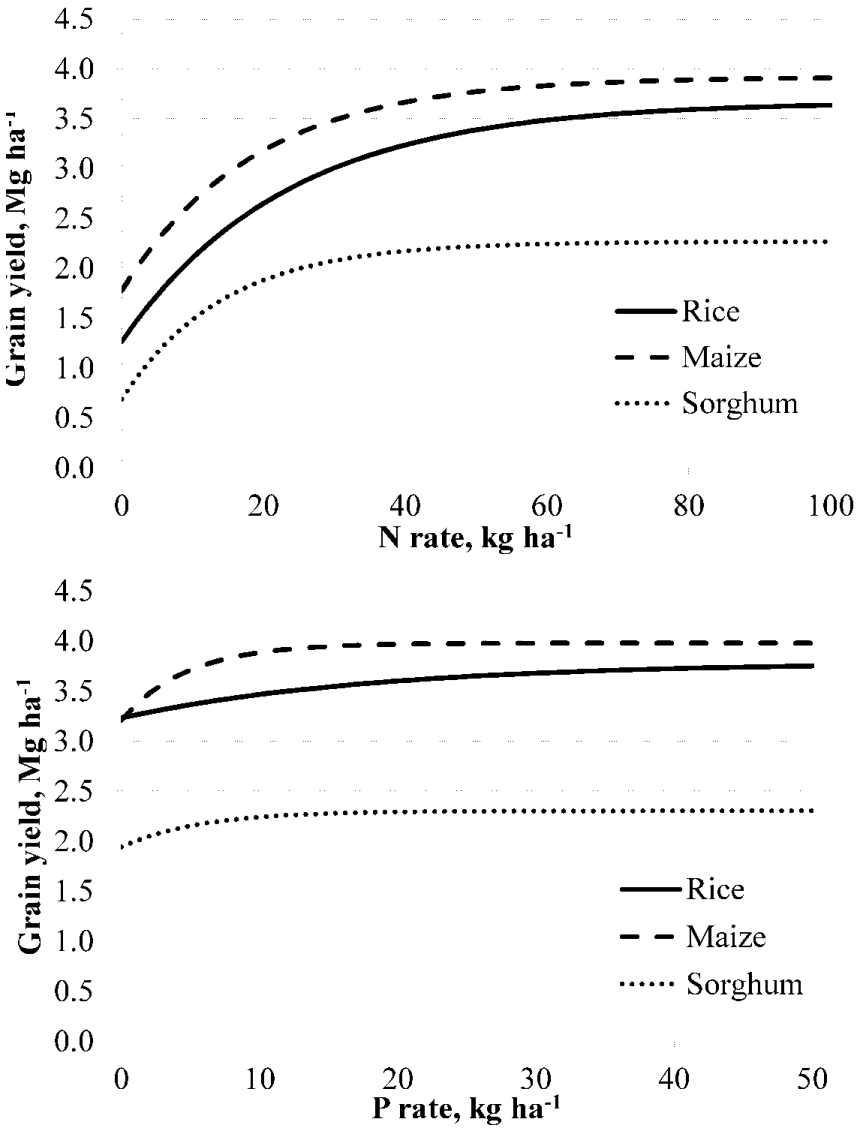

Figure 3. The N and P grain yield response functions of upland rice when compared with maize and grain sorghum in Uganda (Kaizzi et al., 2012a, b).

these agricultural soils of Uganda to supply N, UN with N_0 was 31 and 46 kg ha^{-1} by sorghum and maize, respectively (Kaizzi et al., 2012a, b).

The large response of upland rice grain yield to N application was consistent with earlier results from Uganda (Kaizzi, 2002; Kaizzi et al., 2007; Onaga et al., 2012), and with the responses of maize and sorghum in Uganda (Figure 3) (Kaizzi et al., 2012a, b). The response to applied N was about twice the mean for that of several studies cited above (Apaseku et al., 2013; Habtegebrial et al., 2013; Oikeh et al., 2008; Okonji et al., 2012). Yield with N applied and at N_0 was more and less for maize and sorghum, respectively, when compared with upland rice. The coefficient values for *b* of 2.14 and 2.40 and for *c* of 0.94 and 0.96 for maize and upland rice, respectively, were not significantly different resulting in similar curve shape although a higher yield plateau for maize when compared with upland rice.

Upland rice response to applied P, with N applied, was near linear and to a higher P rate when compared with maize and sorghum which had little response beyond 10 kg ha^{-1} P. The significant but small increase in upland rice yield to P application, with N applied, was generally consistent with the responses reported by Oikeh et al. (2008) and Sahrawat (2000) but low and high when compared with results of Bationo (2008) and Apaseku et al. (2013), respectively.

Depending on CP, the mean EONR and EOPR for upland rice ranged from 54 to 92 and 17 to 37 kg ha^{-1}, respectively. Finance-constrained farmers commonly do not have enough money to apply fertilizer at EOR to maximize net returns ha^{-1} for all their cropland. Optimi-

zing the choice of crop-nutrient-rate combinations, in consideration of CPs, is needed to maximize net returns on their constrained investment. Considering 14 other crop nutrient combinations in Uganda using common CP values, Kaizzi et al. (2012c) found that the decreasing order of BC for fertilizer nutrients applied at EOR was bean (*Phaseolus vulgaris* L.) N > groundnut (*Arachis hypogaea* L.) P = maize N = soybean (*Glycine max* L.) P > sorghum N > groundnut K > maize P > bean P > soybean K and > K applied to maize or sorghum. The BC for N applied to upland rice at EOR is greater than for any of the above and the BC for applied P to upland rice is less and more when compared with soybean P and sorghum N. This order could change with changes in relative CP due to changed grain values or nutrient costs. The upland rice EONR allows for BC >2, but BC >2 at EOPR only if CP ≤4 for fertilizer P. The results demonstrate that N application to upland rice can increase farm productivity with high profitability and should have priority over the 14 other crop-nutrient response functions evaluated here and in Kaizzi et al. (2012a, b, c) when fertilizer use is financially constrained. Phosphorus application for upland rice can be profitable when CP is not too high and/or when applied as less than EOPR.

Recovery of applied N in the aboveground upland rice biomass was 75% at EONR which is intermediate between the RE reported for maize and sorghum (Kaizzi et al., 2012a, b). The RE of sorghum was >100% as sorghum performance was poor at N_0 and applied N apparently boosted plant vigor and root growth to recover nitrate-N that was otherwise lost to leaching beyond the root zone. Other fertilizer N use efficiency components were low at EONR for upland rice as compared to maize and sorghum including AE and PFP, but this is largely due to the higher EONR of upland rice associated with the higher market value of rice when compared with maize and sorghum. Agronomy efficiency for upland rice from this Uganda study with 50 kg N ha^{-1} applied was 34.4 when compared with a mean of 18 kg kg^{-1} calculated from several other studies (Apaseku et al., 2013; Habtegebrial et al., 2013; Oikeh et al., 2008; Okonji et al., 2012) implying relatively high efficiency and potential for high net returns to N applied to upland rice in Uganda.

Conclusion

Upland rice yield increased by 178% with N applied at the EONR for a CP of 6. Yield can be further increased with P application but the results demonstrate that N deficiency is much more limiting than P or K deficiency. The results indicate that N application for upland rice production is highly profitable and a priority fertilizer application option relative to 14 other crop-nutrient options of finance-constrained smallholder farmers in Uganda. Application of P is also likely to be profitable when applied at EOR, but less so compared to N, while K application is not likely to be profitable. The recovery and agronomic efficiencies of applied N are high for EONR or lower rates, implying little residual effect for the following crop but also little loss to the environment.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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Abbreviations: AE, Agronomic efficiency of N use; **BC,** benefit/cost ratio; **CP,** fertilizer use cost to grain price ratio; **EONR,** economically optimal nitrogen rate; **EOPR,** economically optimal P rate; **EOKR,** economically optimal K rate; **EOR,** economically optimal rate; **HI,** harvest index; **IE,** internal efficiency; **K_0,** no K applied; **NHI,** N harvest index; **NUE,** N use efficiency; **N_0,** no N applied; **PE,** physiological efficiency; **PFP,** partial factor productivity; **P_0,** no P applied; **RE,** recovery efficiency; **SOM,** soil organic matter; **UN,** total N in the aboveground biomass at harvest.

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Foliar anatomy of some species of Asteraceae in South Western Nigeria

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The foliar anatomy of 12 species of Asteraceae around Ile Ife in South Western, Nigeria was described. The distinguishing characteristics of taxonomic value include; venation patterns, areole shapes, number of veinlet endings, trichome types, arrangement of vascular bundles. Venation types are actinodromous, craspedodromous or camptodromous, the presence of rectangular areoles are predominant in the species studied and this show family characteristics. However, the presence of crystal druses in the areoles of *C. odorata* is of diagnostic importance. The type of vascular bundle in the leaf midribs is classificatory as it divided the taxa studied into two groups; vascular bundles are amphicribal in *Bidens pilosa*, *Chromolaena odorata*, *Launaea taraxacifolia*, *Crassocephalum crepidiodes*, *Tridax procumbens* and *Vernonia cinerea* and bicollateral in *Ageratum conyzoides*, *Aspilia africana*, *Emilia praetermissa*, *Synedrella nodiflora*, *Tithonia diversifolia* and *Vernonia amygdalina*.

Key words: Asteraceae, foliar, taxonomic, venation, areole.

INTRODUCTION

Asteraceae (Compositae) is a very large cosmopolitan family whose members are highly advanced. It belongs to the sub-class Asteridae in the order Asterales. Asteraceae is the second largest family in the division Magnoliophyta with 1,100 genera and over 20,000 recognized species (Ming, 1999). The majority of Asteraceae species are herbaceous although an important component of the family consists of shrubs or even trees, many plants in the family Asteraceae are economically important as weeds, ornamentals, medicinals and green vegetables (Olorode, 1984).

The angiosperms are endowed with macromorphological characters of significant taxonomic values which can be easily observed with the naked eye or simple hand lens. Morphological attributes of vegetative organs have often constituted the mainstay of taxonomic studies in plants (Polhill, 1968; Pilbeam and Bell, 1979; Adedeji, 2005) and are very important in classification.

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Table 1. IFE Herbarium (Osun state, Nigeria) voucher specimen number for the twelve Asteraceae species studied.

Species	Voucher specimen numbers
Ageratum conyzoides Linn.	IFE 16884
Aspilia africana (Pers) C.D. Adams.	IFE 16882
Bidens pilosa Linn.	IFE 16887
*Chromolaena odorata (*L.) Kings & Robinson	IFE 16881
Crassocephalum crepidioides (Benth) S. Moore	IFE 16880
Emilia praetermissa Milne Redhead.	IFE 16874
Launaea taraxacifolia Willd.	IFE 16877
Synedrella nodiflora Gaertn	IFE 16886
Tithonia diversifolia (Hemsl) A. Gray	IFE 16875
Tridax procumbens Linn.	IFE 16876
Vernonia amygdalina Del. Cent	IFE 16885
Vernonia cinerea Linn.	IFE 16883

The use of anatomical methods in taxonomic investigations cannot be over emphasized. Although no character is absolutely immutable, but some are more fixed than the others and it is on those that are less plastic that the systematic anatomist rely. Taxonomic decision based on epidermal studies are quite reliable because they are not really affected by environmental conditions (Barthlott, 1981), thus comparative plant epidermal studies have been found to be reliable in taxonomy and systematics (Stace, 1969; Ogunkunle and Oladele, 2000; Metcalfe and Chalk, 1950, 1979; Naik and Nigrude, 1981; Palmer and Tucker, 1981; Adedeji, 2004; Adedeji and Illoh, 2004) all the authors have all stressed the taxonomic importance of anatomical features which along with other characters form taxonomic, identification and classification of plants.

The present study reported the use of foliar anatomy in establishing the taxonomic relationships between twelve species of Asteraceae.

MATERIALS AND METHODS

Leaf clearing (for venation studies)

Sizeable portions of the matured leaves of each species were taken from the standard median portion (midway between the tip and the base) of *Ageratum conyzoides* Linn*., Aspilia africana* (Pers) C.D Adams, *Bidens pilosa* Linn*., Chromolaena odorata* (Linn.) king & Robinson*, Crassocephalum crepidioides* Benth S. Moore*, Emilia praetermissa* Milne-Redhead*, Launaea taraxacifolia* Willd*, Synedrella nodiflora* Gaertn*, Tithonia diversifolia (*Hemsl) A. Gray*, Tridax procumbens* Linn*., Vernonia amygdalina* Del. Cent and *Vernonia cinerea* Linn*.* were obtained from the median parts of well expanded leaves.

These portions were decolorized by boiling in 90% ethyl alcohol to remove chlorophyll, and treated in 2% sodium hydroxide solution over night. They were rinsed in water thrice and transferred into 5% domestic bleach (JIK). They remained in the bleach until they became completely white, then rinsed in water thrice and preserved in 50% ethanol as described by Olatunji (1983). These were stained in 1% aqueous solution of Safranin O and mounted on a clean slide in 25% glycerol and the edges of the cover slips sealed with nail vanish*.*

Sectioning

Transverse sections of the leaf were cut at 20 µ thickness using Reichert Sledge Microtome and best sections preserved in 50% ethanol. The sections were stained in 1% aqueous solution of Safranin O for 5 min washed in 3 changes of water to remove excess stain and counterstained in 1% solution of Alcian blue for 5 min then washed in three changes of water and dehydrated by passing through series of ethyl alcohol: 50, 70, 80, 90 and 100% with two changes in 100% alcohol (dehydration process), and excess stain (differentiation process). The dehydrated and differentiated sections were cleared in xylene to remove last trace of water, to clear the sections (making it more transparent) and to remove last traces of ethanol and since xylene is the solvent of the mountant (DPX) used, it prevent cloudiness of the slide. The sections were mounted in DPX mountant according to Akinloye et al. (2012). Photomicrographs of the leaf sections of the twelve species were taken with Amscope digital camera attached to a light microscope. All microscopic measurements were made with the aid of an ocular and stage micrometer. The drawings were prepared in the scientific illustration unit of the National History museum, Obafemi Awolowo University by the museum's scientific illustrator with about twenty eight years experience in scientific illustrations. The slides were observed directly under LEICA DM500 binocular light microscope using different magnifications, the drawings were prepared without the aid of Camera Lucida but with painstaking replication to scale of the arrangement of the cells and structures in pencil line drawings before being transferred unto tracing films using Rotring pen size 0.1 with black Rotring ink. The drawings were later scanned into the computer for insertion into the appropriate sections in the text.

RESULTS

Herbarium survey

All plant species used in this work were collected authenticated in the Ife Herbarium and voucher specimens deposited in the same herbarium (Table 1).

***Ageratum conyzoides* Linn**.

Venation is actinodromous, perfect, marginal, basal. Areoles arrangement is random. The shape is triangular, quadrangular and pentagonal; 38.4 - 86.3±1 µm long and 24.9 - 92.3±1 µm wide. The veinlet endings are singly divided and number ranges between 0 - 2. Lamina (Plate 1A and B)**:** Epidermis uniseriate, cells rectangular. The cuticle on both surfaces are not prominent and where they are seen, they are non-striated. Palisade mesophyll one layer thick, consisting of cylindrical shaped parenchyma cells that are closely packed. Mean length of palisade mesophyll is up to 28.9±1.80 µm. Spongy mesophyll consist of parenchyma cells that are largely irregular in shape, irregularly arranged with intercellular spaces. Simple uniseriate multicellular trichome present. Midrib (Plate 1C and D): Epidermis uniseriate, the epidermal cells are irregular, occasionally polygonal. Vascular bundle, 1, bicollateral. Simple uniseriate multicellular trichome present.

***Aspilia africana* (Pers). C.D. Adams**

Venation is actinodromous, basal to suprabasal. Areoles shape is rectangular and quadrangular; 36.2 - 92.7 µm ± 1 long and 49.7- 102.6 µm ± 1 wide. The veinlet endings are bifurcated number ranges between 0 - 2. Lamina (Plate 2A and B): Epidermis uniseriate, cells rectangular, cuticles are not prominent and non-striated. Palisade mesophyll one layer of cells thick consisting of tightly packed elongated cells. Mean length of palisade mesophyll up to 34.5±1.80 µm. Spongy mesophyll consist of parenchyma cells that are largely irregular in shape, cells irregularly arranged with intercellular spaces. Midrib (Plate 2C and D): Epidermis uniseriate, epidermal cells are largely irregular in shape, occasionally polygonal varying in size and arrangement. Vascular bundles 5, crescentiform with dissected xylem, bicollateral. Trichome present, largely simple uniseriate bicellular.

***Bidens pilosa* Linn.**

Venation is pinnate, craspedodromous, that is, secondary veins terminating at the margin. Areoles are variables in size, polygonal to rectangular in shapes; 35.2 - 82.3 ± 1 µm long and 38.4 - 98.8 ± 1 µm wide. Veinlet endings are singly divided, number ranges between 1-3. Lamina (Plate 3A and B): Epidermis uniseriate, cells rectangular shaped. Not prominent and non-striated cuticles on both surfaces. Palisade mesophyll one layer thick, consisting of tightly packed elongated shaped parenchyma cells. Mean length of palisade mesophyll up to 29.8±1.70 µm. Spongy mesophyll consist of loosely packed varied, irregularly shaped parenchyma cells with intercellular spaces. Midrib (Plate 3C and D): Epidermis uniseriate, epidermal cells are irregular polygonal varying in sizes and arrangements. Vascular bundle 3, trapezoid amphicribal type. Simple uniseriate multicellular trichome present.

***Chromolaena odorata* Linn.**

Venation is actinododromous, that is, 3 or more primary veins diverging radially from a single point, suprabasal. Areole shapes are rectangular, polygonal and triangular; 79.1 - 149.2±1 µm long and 64.4 - 93.8±1 µm wide. Veinlet endings are singly divided, bifurcated and number ranges between 1 to 3. Crystal druses are present and prominent. Lamina (Plate 4A and B): Epidermis uniseriate, cells rectangular. The cuticles on both surfaces are not prominent, where they seen, they are non-striated. Palisade mesophyll one layer thick consisting of tightly packed cylindrical elongated parenchyma cells. Mean length of palisade mesophyll up to 27.5±1.80 µm. Spongy mesophyll consist of parenchyma cells largely irregular in shape, irregularly arranged with intercellular spaces. Simple uniseriate bicellular and multicellular trichomes are present. Midrib (Plate 4C and D): Epidermis uniseriate, epidermal cells are irregular variable in size and arrangement. Vascular bundle, 1, amphicribal, shield shaped. Trichome: Simple uniseriate multicellular trichome are present.

***Crassocephalum crepidioides* (Benth) S. Moore**

Venation is pinnate, craspedodromous, that is, secondary veins terminating at the margin. Areole shapes are rectangular to quadrangular; 19.8 - 83.2 ± 1 µm long and 40.7 - 87.9 ± 1 µm wide. Veinlet endings are singly divided, bifurcated and number ranges between 0 - 2. Lamina (Plate 5A and B): Epidermis uniseriate, epidermal cells are irregular in sizes and arrangement. Cuticles are not prominent and non-striated on both surfaces. Palisade mesophyll one layer thick consisting of tightly packed cylindrical elongated parenchyma cells. Mean length of palisade mesophyll up to 29.7±1.80 µm. Spongy mesophyll consist of parenchyma cells largely irregular in shape, irregularly arranged with intercellular spaces. No trichome present. Midrib (Plate 5C and D): Epidermis uniseriate, epidermal cells are polygonal to rectangular shaped varying in size and arrangement. Vascular bundle, 2, amphicribal, dissected xylem. No trichome present.

***Emilia praetermissa* Milne-Redhead**

Venation is pinnate, camptodromous, that is, secondary veins freely ramified towards the margin. Areoles are rectangular and hexagonal; 42.9 - 89.3±1 µm long and

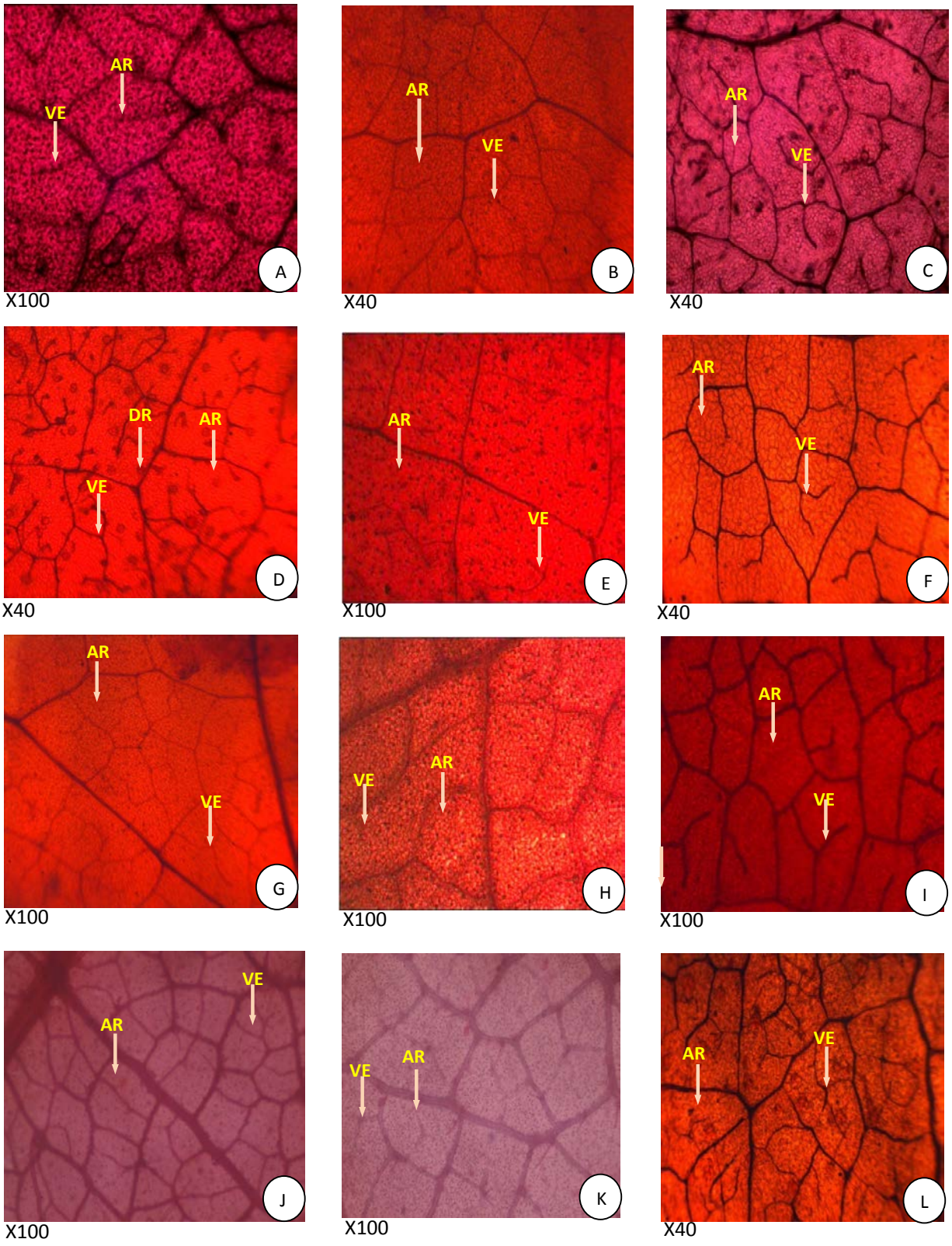

Plate 1. Venation patterns in the species studied. A, *Ageratum conyzoides;* B, *Aspilia africana;* C, *Bidens pilosa;* D, *Chromolaena odorata*; E, *Crassocephalum crepidiodes;* F, *Emilia praetermissa;* G, *Launaea taraxacifolia;* H, *Synedrella nodiflora*; I, *Tithonia diversifolia*; J, *Tridax procumbens*; K, *Vernonia amygdalina;* L, *Vernonia cinerea*; AR, Areole; DR, crystal druses; VE, veinlet endings.

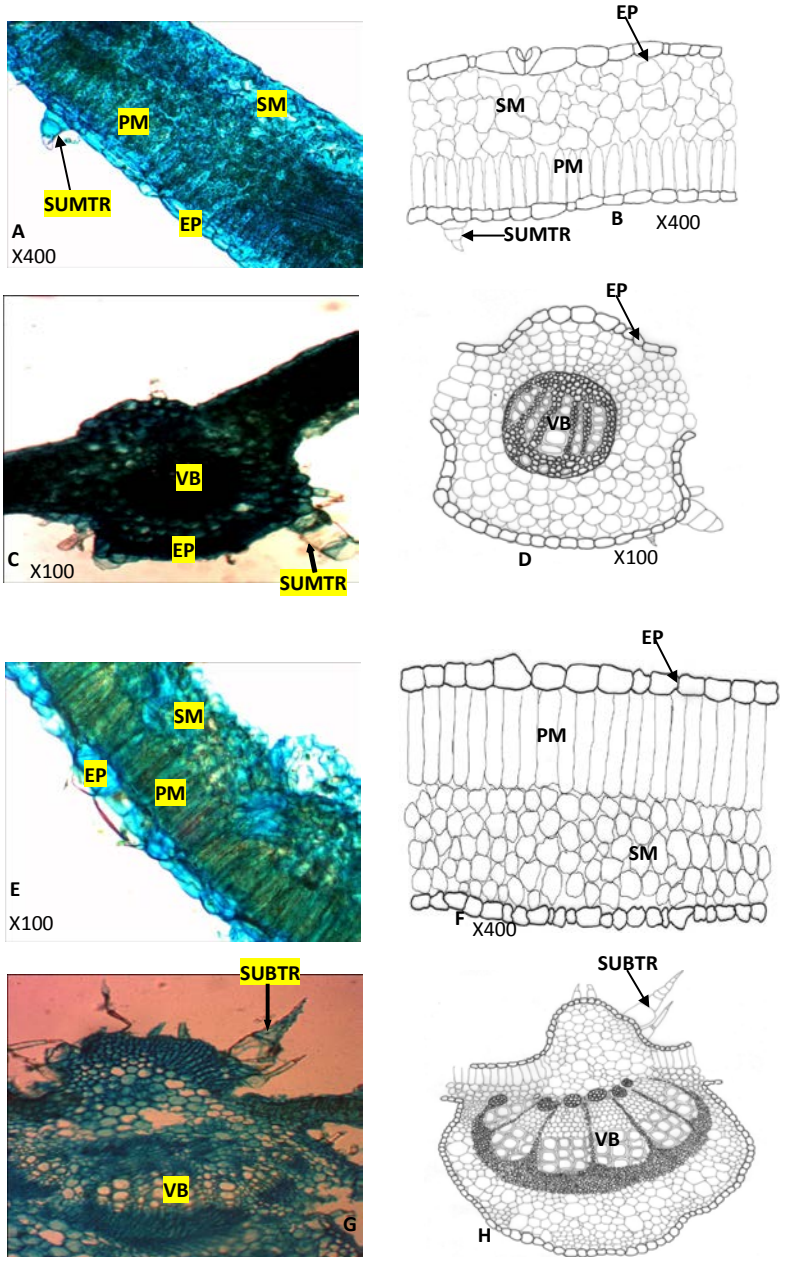

Plate 2. (A-D): Leaf anatomy in *Ageratum conyzoides;* (A and B): Transverse section leaf (lamina); (C and D): Transverse section leaf (midrib). BTR- Bicellular trichome, EP- Epidermis, PM-Palisade mesophyll, SM- Spongy mesophyll, ST- Stomata, SUBTR- Simple uniseriate bicellular trichome, SUMTR-Simple uniseriate multicellular non -glandular trichome, VB- Vascular bundle. (E-H): Leaf anatomy in *Aspilia africana* (E and F): Transverse section leaf (lamina); (G and H): Transverse section leaf (midrib). BTR- Bicellular trichome, EP- Epidermis, PM-Palisade mesophyll, SM- Spongy mesophyll, ST- Stomata, SUBTR- Simple uniseriate bicellular trichome, SUMTR-Simple uniseriate multicellular non -glandular trichome, VB- Vascular bundle.

36.2 - 68.9±1 µm wide. Veinlet endings are singly divided, bifurcated, number ranges between 0 - 2. Lamina (Plate 6A and B): Epidermis uniseriate, epidermal cells are rectangular shaped. Cuticles are not prominent and non-striated. Palisade mesophyll one layer thick consisting of tightly packed elongated cylindrical cells. Mean length of palisade mesophyll is up to 23.6±1.60 µm. Spongy mesophyll consist of parenchyma cells

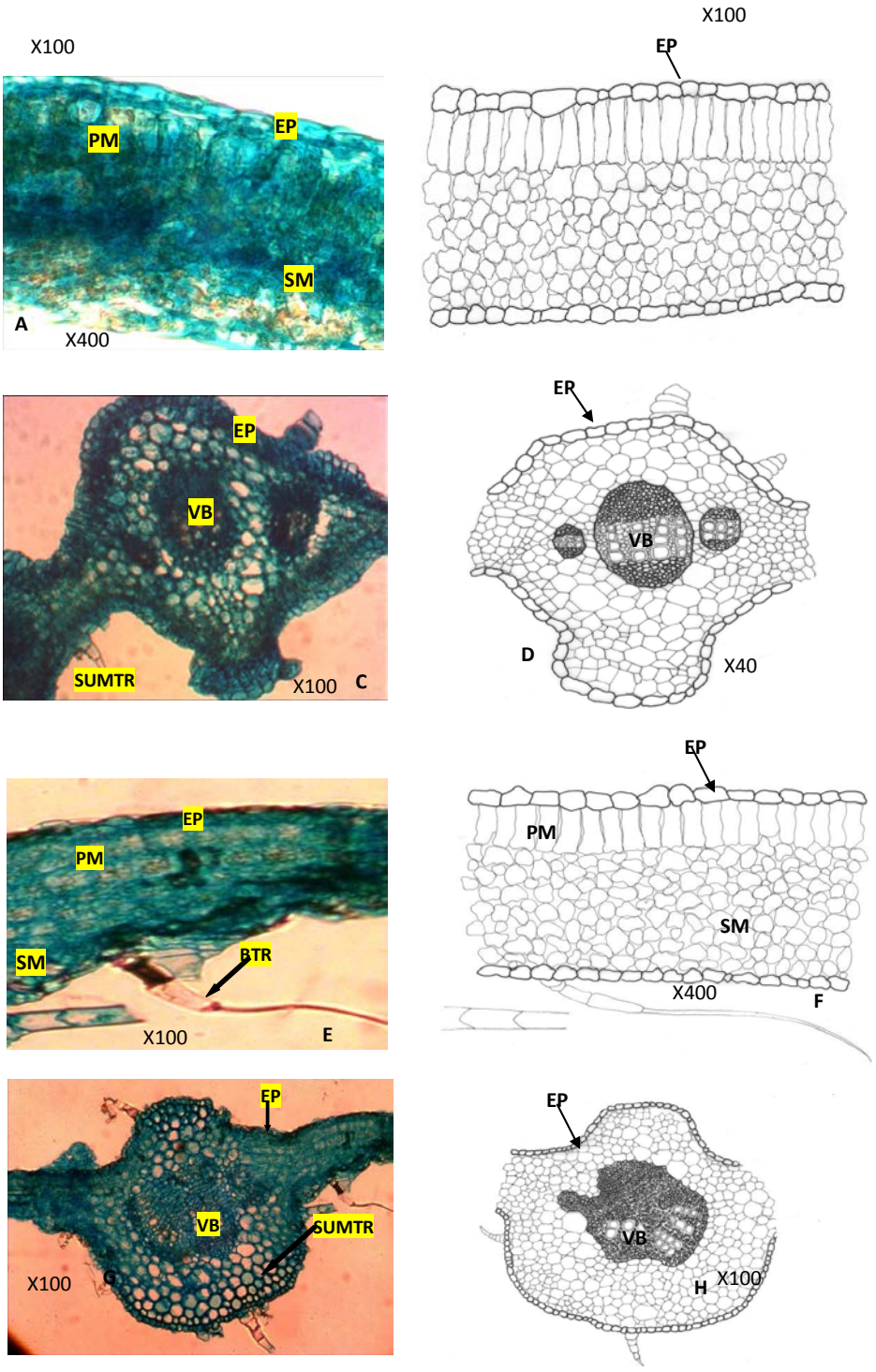

Plate 3. (A-D): Leaf anatomy in *Bidens pilosa* (A and B): Transverse section leaf (lamina); (C and D): Transverse section leaf (midrib). BTR- Bicellular trichome, EP- Epidermis, PM-Palisade mesophyll, SM- Spongy mesophyll, ST- Stomata, SUBTR- Simple uniseriate bicellular trichome, SUMTR-Simple uniseriate multicellular non -glandular trichome, VB-Vascular bundle. (E-H): Leaf anatomy in *Chromolaena odorata* (E and F): Transverse section leaf (lamina); (G and H): Transverse section leaf (midrib). BTR- Bicellular trichome, EP- Epidermis, PM-Palisade mesophyll, SM- Spongy mesophyll, ST- Stomata, SUBTR-Simple uniseriate bicellular trichome, SUMTR-Simple uniseriate multicellular non -glandular trichome, VB- Vascular bundle.

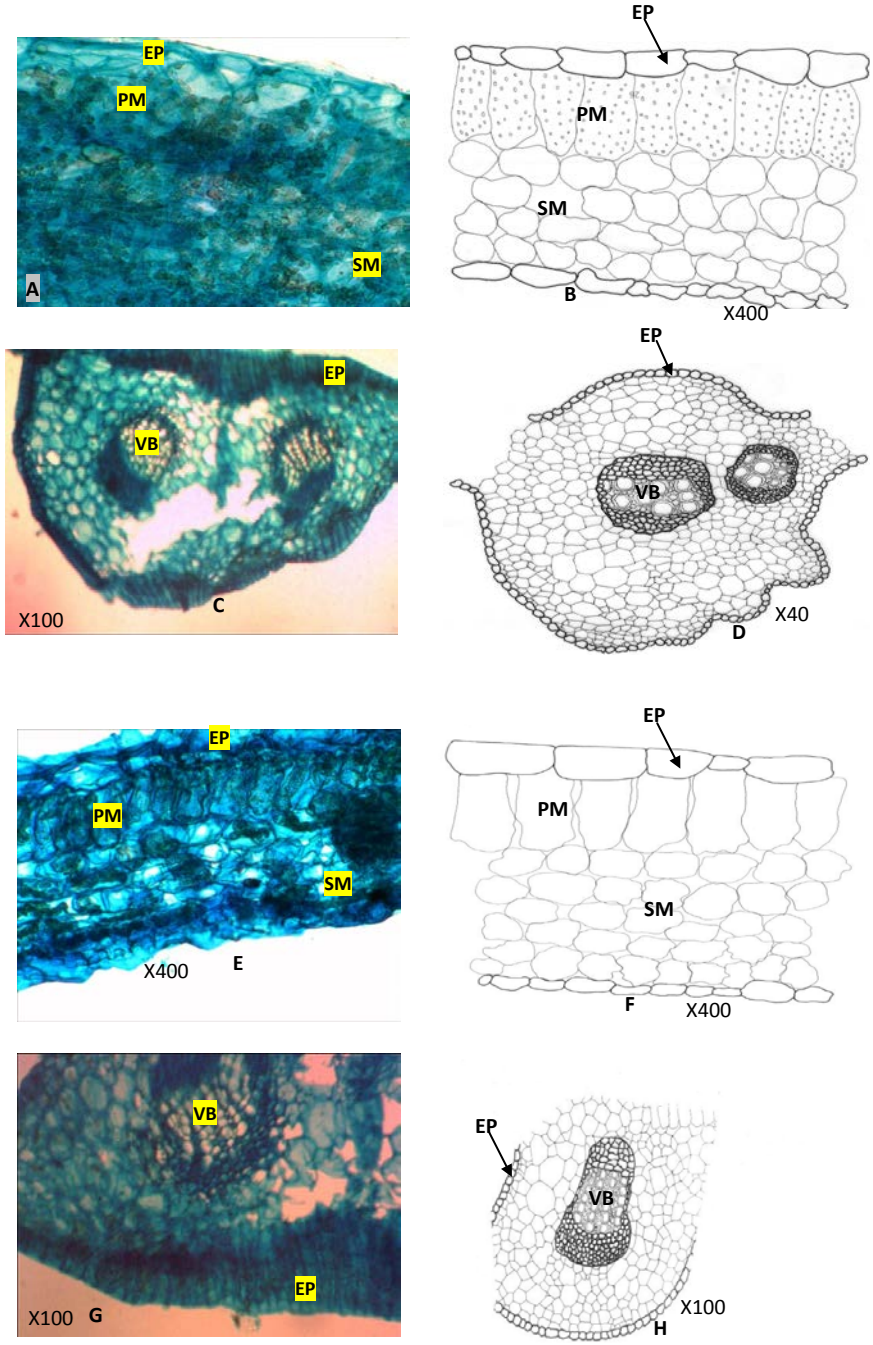

Plate 4. (A-D): Leaf anatomy in *Crassocephalum crepidioides;* (A and B): Transverse section leaf (lamina); (C and D): Transverse section leaf (midrib). BTR- Bicellular trichome, EP- Epidermis, PM-Palisade mesophyll, SM- Spongy mesophyll, ST- Stomata, SUBTR- Simple uniseriate bicellular trichome, SUMTR-Simple uniseriate multicellular non -glandular trichome, VB- Vascular bundle. (E- H): Leaf anatomy in *Emilia praetermissa.* (E and F): Transverse section leaf (lamina); (G and H): Transverse section leaf (midrib). BTR- Bicellular trichome, EP- Epidermis, PM-Palisade mesophyll, SM- Spongy mesophyll, ST- Stomata, SUBTR- Simple uniseriate bicellular trichome, SUMTR-Simple uniseriate multicellular non -glandular trichome, VB- Vascular bundle. BTR- Bicellular trichome, EP- Epidermis, PM-Palisade mesophyll, SM- Spongy mesophyll, ST- Stomata, SUBTR- Simple uniseriate bicellular trichome, SUMTR-Simple uniseriate multicellular non -glandular trichome, VB- Vascular bundle.

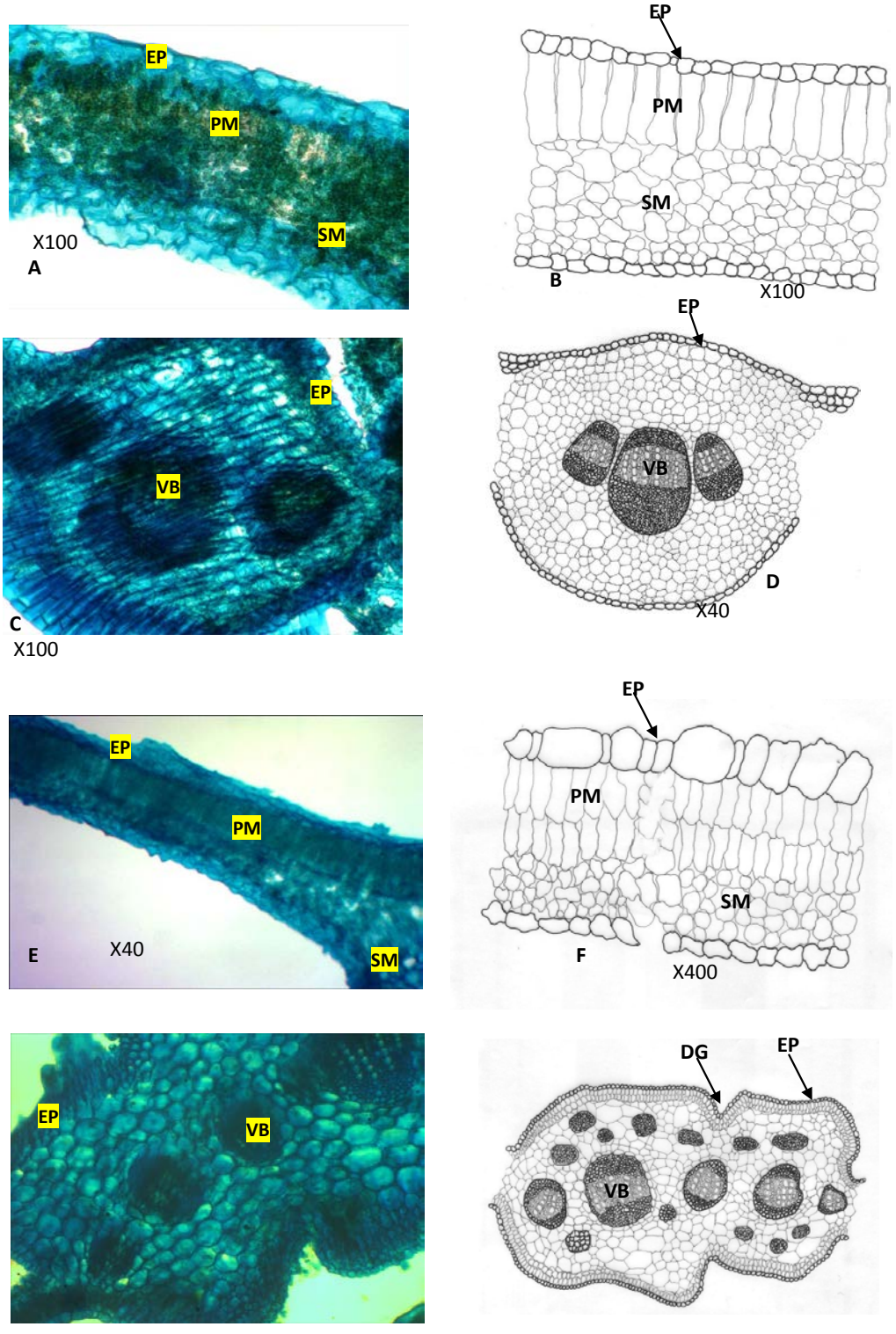

Plate 5. (A-D): Leaf anatomy in *Launaea taraxacifolia;* (A and B): Transverse section leaf (lamina); (C and D): Transverse section leaf (midrib). EP- Epidermis, PM- Palisade mesophyll, SM- Spongy mesophyll, ST- Stomata, SUMTR- Simple uniseriate multicellular non -glandular trichome, VB- Vascular bundle. (E-H): Leaf anatomy in *Synedrella noduflora*. (E and F): Transverse section leaf (lamina); (G and H): Transverse section leaf (midrib). EP- Epidermis, PM-Palisade mesophyll, SM- Spongy mesophyll, ST- Stomata, SUMTR-Simple uniseriate multicellular non -glandular trichome, VB-Vascular bundle

largely irregular in shape, irregularly arranged with intercellular spaces. No trichome present. Midrib (Plate 6 C and D): Epidermis uniseriate, epidermal cells are polygonal to rectangular in shape variable in size and arrangement. Vascular bundle, 1, bicollateral. No trichome present.

***Launaea taraxacifolia* Willd**

Venation is pinnate, craspedodromous, that is, secondary veins terminating at the margin. Areole shapes are rectangular to polygonal; 50.9 - 99.2±1 µm long and 36.2 - 88.9±1 µm wide. Veinlet endings are bifurcated and number ranges between 0 - 2. Lamina (Plate7A and B): Epidermis uniseriate, epidermal cells more or less rectangular. Cuticles are not prominent and non-striated. Palisade mesophyll one layer of cells thick consisting of tightly packed elongated cylindrical parenchyma cells. Mean length of palisade mesophyll is up to 26.8±1.60 µm. Spongy mesophyll consist of parenchyma cells enclosing intercellular spaces. No trichome present. Midrib (Plate 7C and D): Epidermis uniseriate, epidermal cells are rectangular. Vascular bundle 3, bicollateral. No trichome present.

***Synedrella nodiflora* Benth**

Venation is pinnate, actinodromous; that is, 3 or more primary veins diverging radially from a single point, perfect, marginal, basal. Areole shape is quadrangular; 31.6- 106.2±1 µm long and 28.7 - 96.7±1 µm wide. Veinlet endings are singly divided and number ranges between 0 - 2. Lamina (Plate 8A and B): Epidermis uniseriate, epidermal cells are largely irregular. Not prominent, non-striated cuticles. Palisade mesophyll two layers of cells thick, tightly short or elongated packed cylindrical parenchyma cells. Mean length of palisade mesophyll up to 26.8±1.65 µm. Spongy mesophyll consist of loosely packed cells enclosing large intercellular spaces. No trichome present. Midrib (Plate 8C and D): Epidermis uniseriate, epidermal cells are largely irregular in sizes and arrangements, occasionally polygonal. Vascular bundle 17, bicollateral. No trichome present.

***Tithonia diversifolia* Hemsl A. Gray.**

Venation is actinodromous, that is, 3 or more primary veins diverging radially from a single point, basal. Areole shape varies from rectangular to polygonal; 55.4 - 94.9±1 µm long and 45.2 - 87.9±1 µm wide. Veinlet endings are singly divided and number ranges between 0 - 2. Lamina (Plate 9A and B): Epidermis uniseriate, cells are irregular varying in sizes and arrangement. Cuticles are not prominent, non-striated. Palisade mesophyll one layer thick, tightly packed, cylindrical elongated cells. Mean length of palisade mesophyll up to 27.7±1.70 µm. Spongy mesophyll layer consist of tightly packed cells enclosing intercellular spaces. No trichome present. Midrib (Plate 9C and D): Epidermis uniseriate, cells are irregular, cuticle not distinct. 2 large, 4 small, bicollateral bundles. Simple uniseriate multicellular trichome present.

***Tridax procumbens* Linn**

Venation is pinnate, camptodromous, cladodromous, that is, secondary veins freely ramified towards the margin. The shape varies from rectangular to polygonal; 54.5 - 97.7±1 µm long and 46.8 - 82.5±1 µm wide. Veinlet endings are singly divided and number ranges between 0 - 2. Lamina (Plate 10A and B): Epidermis uniseriate, cells more or less elongate or cylindrical. Cuticles are not prominent and where observed, they are non-striated. Palisade mesophyll one layer of cells thick, tightly packed, cylindrical and elongated cells. Mean length of palisade mesophyll up to 28.7±1.60 µm. Spongy mesophyll consist of loosely packed irregular cells enclosing intercellular spaces. No trichome present. Midrib (Plate 10C and D): Epidermis uniseriate, cells are irregular vascular bundle, 1, amphicribal. No trichome present.

***Vernonia amygdalina* Del. Cent**

Venation is pinnate, camptodromous, brochidodromous, that is, secondary veins joined together in a series of prominent arches. The areoles are variable in size and shape rectangular to quadrangular in shape; 54.5 - 103.7±1 µm long and 35.6 - 97.4±1 µm wide. Veinlet endings are singly divided and number ranges between 0 - 2. Lamina (Plate 11A and B): Epidermis uniseriate, cells polygonal to irregular, variable in size and arrangement. Cuticles not prominent and non-striated. Palisade mesophyll, one layer of cells thick, tightly packed. Mean length of palisade mesophyll up to 29.6 ± 1.50 µm. Spongy mesopyhll cells consist of loosely packed irregular cells enclosing intercellular spaces. No trichome present. Midrib (Plate 11C and D): Epidermis uniseriate, epidermal cells variable in sizes and arrangement. Vascular bundle, 5, bicollateral. No trichome present.

***Vernonia cinerea* Linn**

Venation is pinnate, camptodromous, cladodromous, that is, secondary veins freely ramified towards the margin. Areoles are variables in size. The shapes vary from polygonal to rectangular; 65.5 - 118.7±1 µm long and 48.6 - 81.4±1 µm wide. Veinlet endings are simple, bifurcated and number ranges between 0 - 4. Lamina

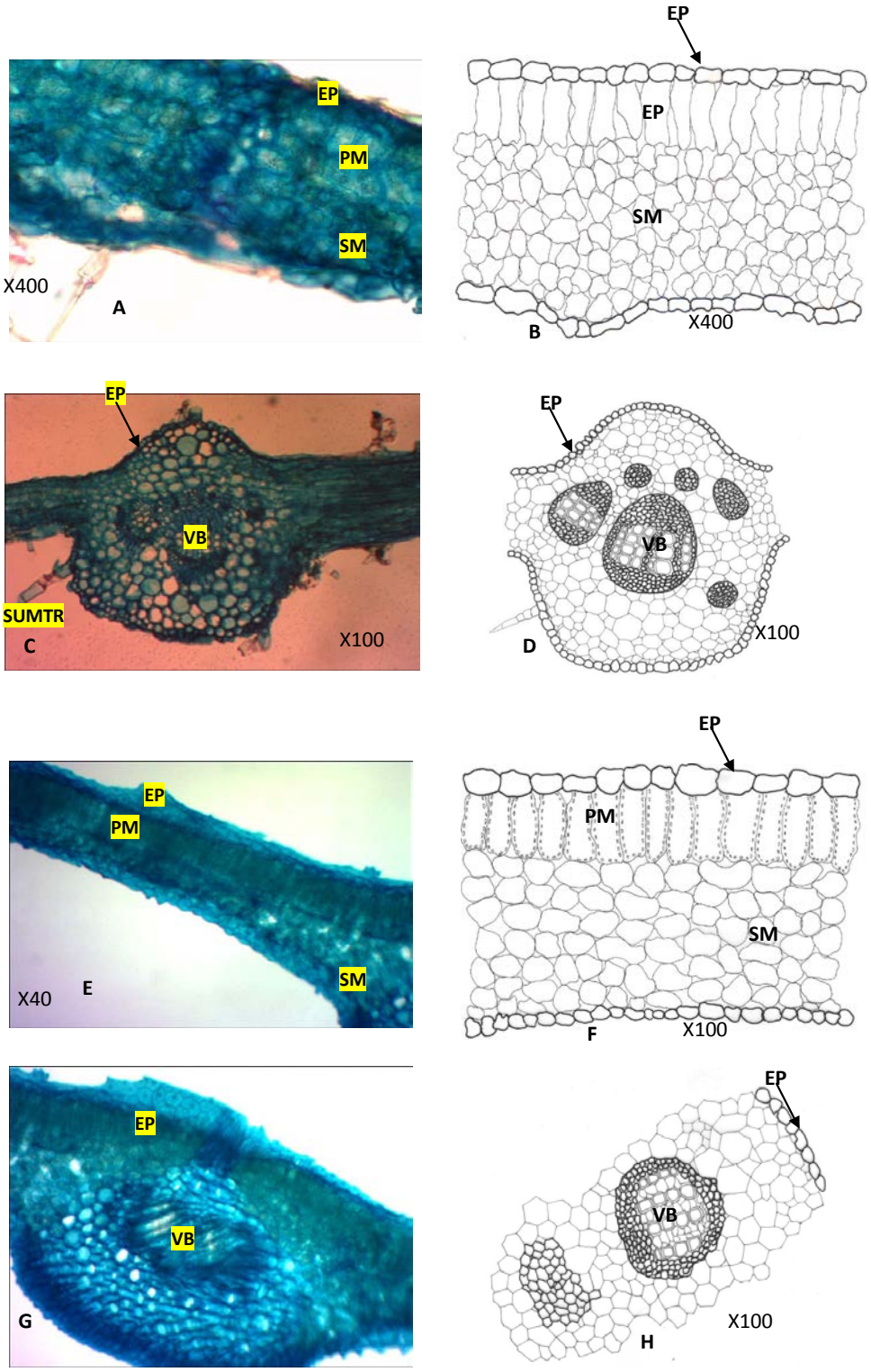

Plate 6. (A-D): Leaf anatomy in *Tithonia diversifolia;* (A and B): Transverse section leaf (lamina); (C and D): Transverse section leaf (midrib). EP- Epidermis, PM-Palisade mesophyll, SM- Spongy mesophyll, ST- Stomata, SUMTR-Simple uniseriate multicellular non -glandular trichome, VB-Vascular bundle. (E-H): Leaf anatomy in *Tridax procumbens;* (E and F): Transverse section leaf (lamina); (G and H): Transverse section leaf (midrib). EP- Epidermis, PM-Palisade mesophyll, SM- Spongy mesophyll, ST- Stomata, SUMTR-Simple uniseriate multicellular non -glandular trichome, VB-Vascular bundle

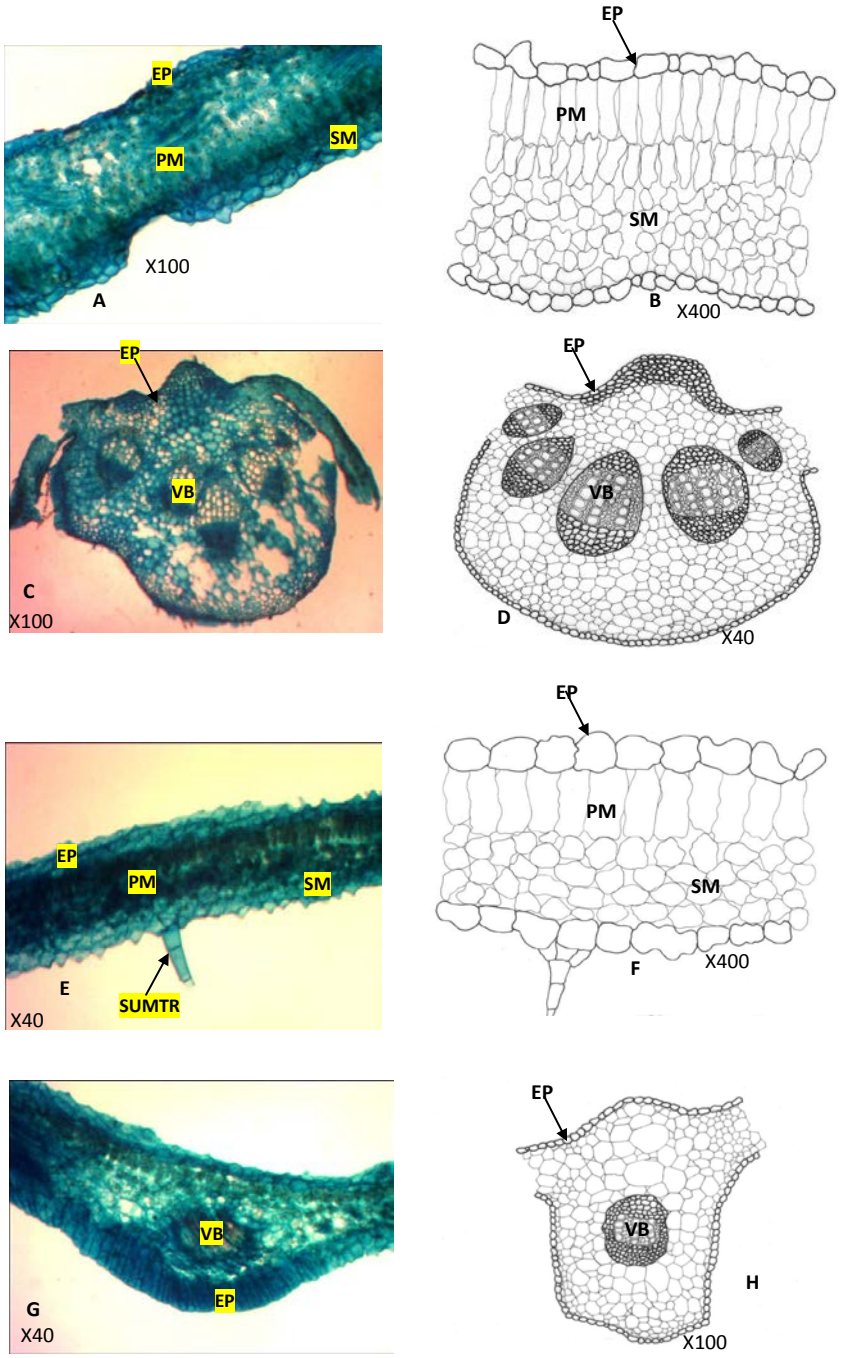

Plate 7. (A-D): Leaf anatomy in *Vernonia amygdalina;* (A and B): Transverse section leaf (lamina); (C and D): Transverse section leaf (midrib). EP- Epidermis, PM-Palisade mesophyll, SM- Spongy mesophyll, ST- Stomata, SUMTR-Simple uniseriate multicellular non -glandular trichome, VB-Vascular bundle; (E- H): Leaf anatomy in *Vernonia cinerea*; (E and F): Transverse section leaf (lamina); (G and H): Transverse section leaf (midrib). EP- Epidermis, PM-Palisade mesophyll, SM-Spongy mesophyll, ST- Stomata, SUMTR-Simple uniseriate

(Plate 12A and B): Epidermis uniseriate, cells are irregular to polygonal, variable in size and arrangement. Cuticles are not prominent and non-striated. Palisade mesophyll, one layer thick consisting of tightly packed elongated cylindrical parenchyma cells. Mean length of palisade mesophyll up to 28.4±1.70 µm. Spongy

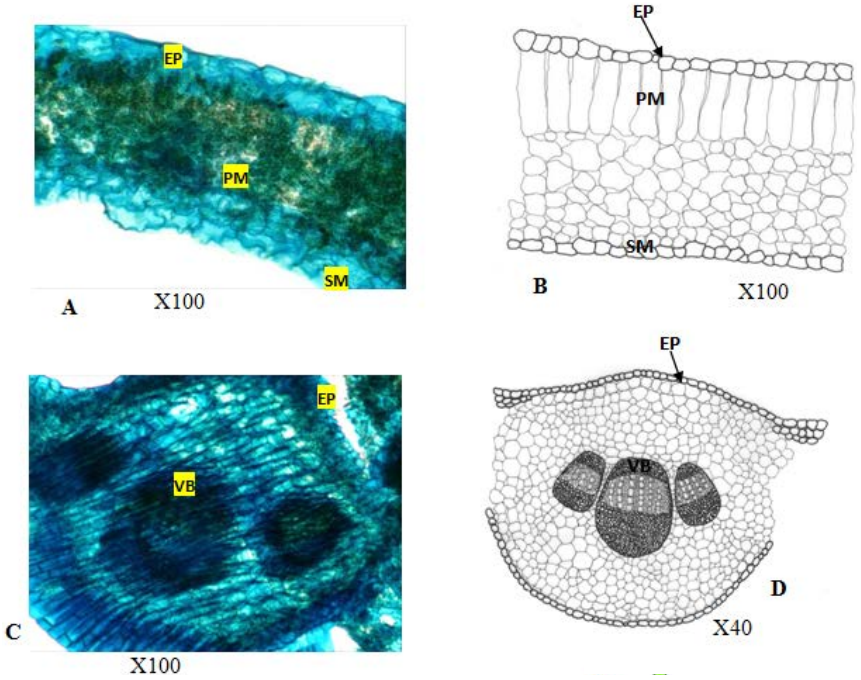

Plate 8. Leaf anatomy in *Launaea taraxacifolia.* (A and B): Transverse section of leaf (lamina); (C and D): Transverse section of leaf (midrib). EP- Epidermis, PM- palisade mesophyll, SM- spongy mesophyll, ST- Stomata, SUMTR- simple uniseriate multicellular non-glandular trichome, VB- vascular bundle.

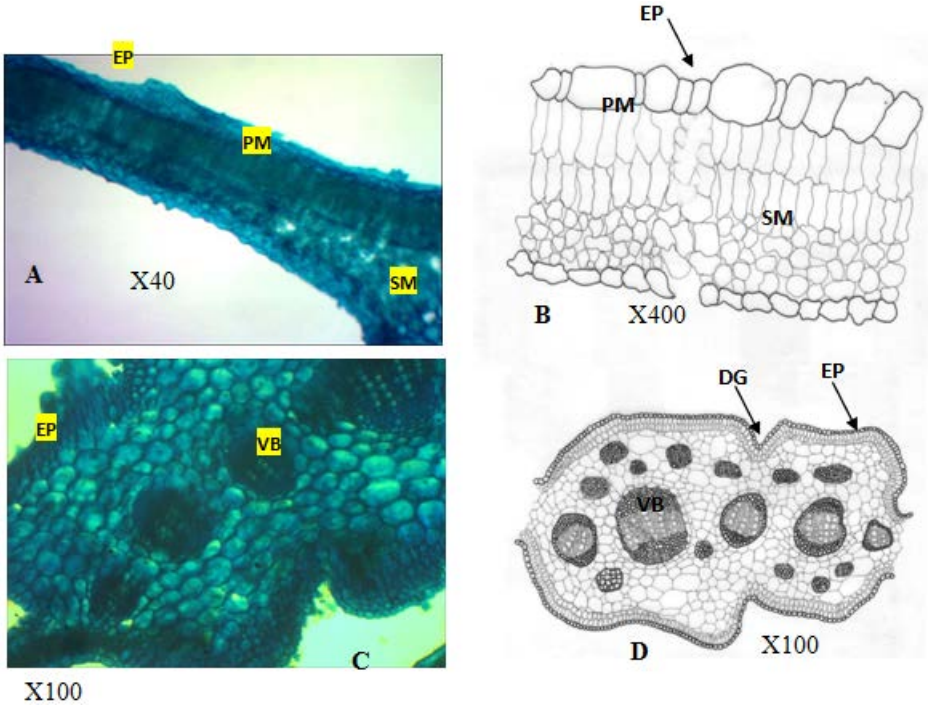

Plate 9. Leaf anatomy in *Synedrella noduflora.* (A and B): Transverse section of leaf (lamina); (C and D): Transverse section leaf (midrib). EP- Epidermis, PM- palisade mesophyll, SM- spongy mesophyll, ST- Stomata, SUMTR- simple uniseriate multicellular non-glandular trichome, VB- vascular bundle.

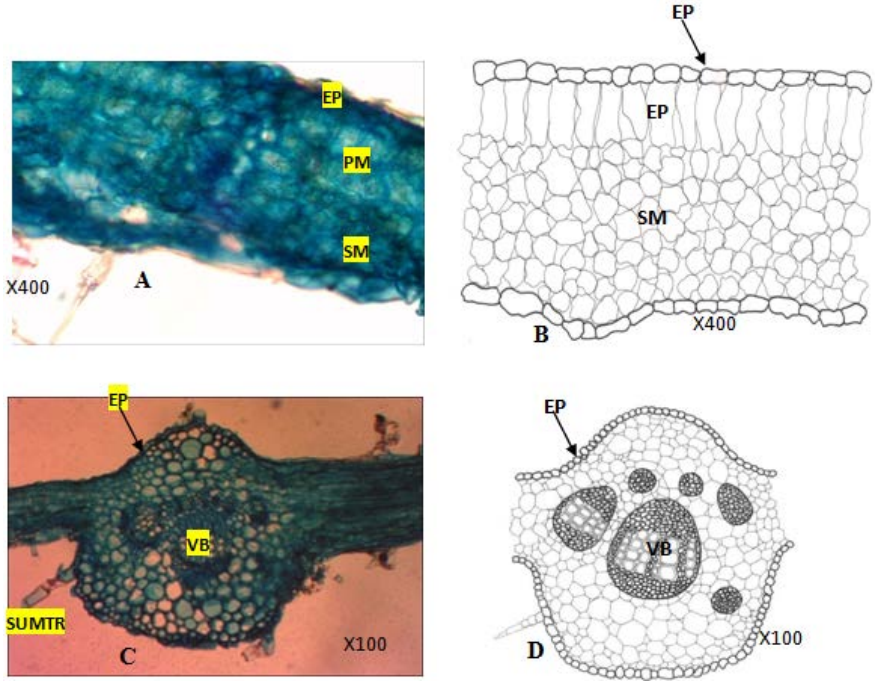

Plate 10. Leaf anatomy in *Tithonia diversifolia.* (A and B): Transverse section of leaf (lamina); (C and D): Transverse section of leaf (midrib). EP- Epidermis, PM- palisade mesophyll, SM- spongy mesophyll, ST- Stomata, SUMTR- simple uniseriate multicellular non-glandular trichome, VB- vascular bundle.

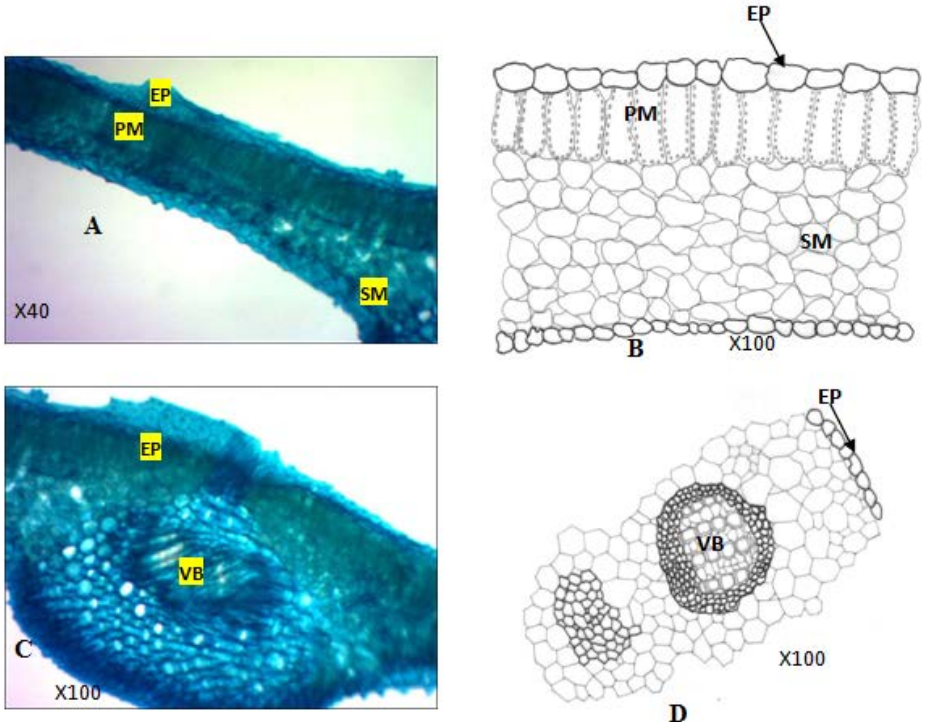

Plate 11. Leaf anatomy in *Tridax procumbens.* (A and B): Transverse section of leaf (lamina); (C and D): Transverse section of leaf (midrib). EP- Epidermis, PM- palisade mesophyll, SM- spongy mesophyll, ST- Stomata, SUMTR- simple uniseriate multicellular non-glandular trichome, VB- vascular bundle.

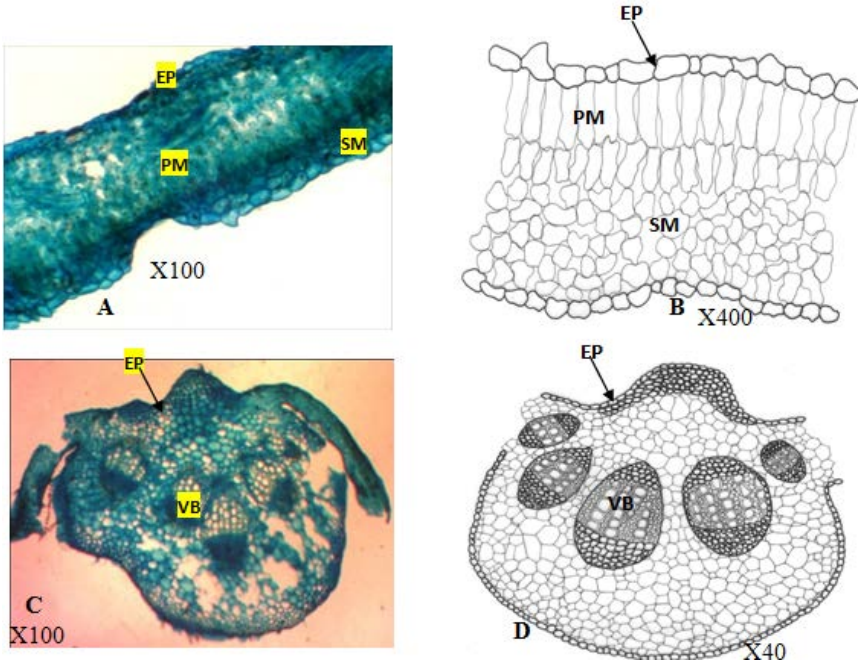

Plate 12. Leaf anatomy in *Vernonia amygdalina*. (A and B): Transverse section of leaf (lamina); (C and D): Transverse section of leaf (midrib). EP- Epidermis, PM- palisade mesophyll, SM- spongy mesophyll, ST- Stomata, SUMTR- simple uniseriate multicellular non-glandular trichome, VB- vascular bundle.

mesophyll cells consist of irregular cells enclosing intercellular spaces. No trichome present. Midrib (Plate 12C and D): Epidermis uniseriate, cells are irregular in shape, thin cuticle. Vascular bundle, 1 amphicribal. No trichome present.

DISCUSSION

A survey of literature of leaf anatomy shows that data obtained from it can be used amply for the clarification of taxonomic and phylogenetic relationships. The commonly used characters like palisade and spongy features, types of vascular bundles, trichome types were largely employed in this study. In all the species studied, the major or primary veins are pinnate. Venation types were actinodromous, craspedodromous or camptodromous, the presence of rectangular areoles were predominant in the species studied and this show family characteristics. However, the presence of crystal druses in the areoles of *C. odorata* is of diagnostic importance. The veinlet terminating end per areole also varies from one species to another, ranges from 0-2 in *A. conyzoides*, *A. africana*, *C. crepidioides*, *E. praetermiissa L. taraxacifolia*, *S. nodiflora* and *V. amygdalina*. For these taxa, number of veinlet endings is classificatory though there is an overlap. The veinlet endings divided singly or bifurcated. The general significant difference observed in the quantitative characters may be used to separate the species. *C. odorata* has a higher areolar area than the other species while *V. cinerea* has a higher number of veinlet endings than others (Plate 13A, B, C and D).

Leaf anatomy according to Carlquist (1961) provides a variety of features that could be used for taxonomic purposes. Many researchers have utilized leaf anatomical features for taxonomic consideration in many species of plants. These include the works of Naik and Nigrude (1981) on *Chlorophytum*, Ogundipe and Olatunji (1991) on *Cochlospermum*, Illoh (1995) on *Celosia*, Adedeji (2004) on *Emilia* and Adedeji and Illoh (2004) on *Hibiscus*. Though there is uniformity in the anatomy of the leaf in the species studied, characters like the shape of palisade and spongy mesophyll cells, non-prominence and non-striation of the cuticles and types of vascular bundles could be used to classify members of the family. The type of vascular bundle in the leaf midribs is classificatory as it divided the taxa studied into two groups; vascular bundles are amphicribal in *B. pilosa*, *C. odorata*, *L. taraxacifolia*, *C. crepidiodes*, *T. procumbens* and *V. cinerea* and bicollateral in *A. conyzoides*, *A. africana*, *E. praetermissa*, *S. nodiflora*, *T. diversifolia* and *V. amygdalina*. Similarly, the shape of vascular bundle is classificatory in those with dissected xylem, that is, *A. africana* and *C. crepidiodes* and those without dissected xylem, this delimit the two from other species studied. Among all the taxa studied, *S. nodiflora* is quite distinct in the surface view of the epidermis by having deep groove

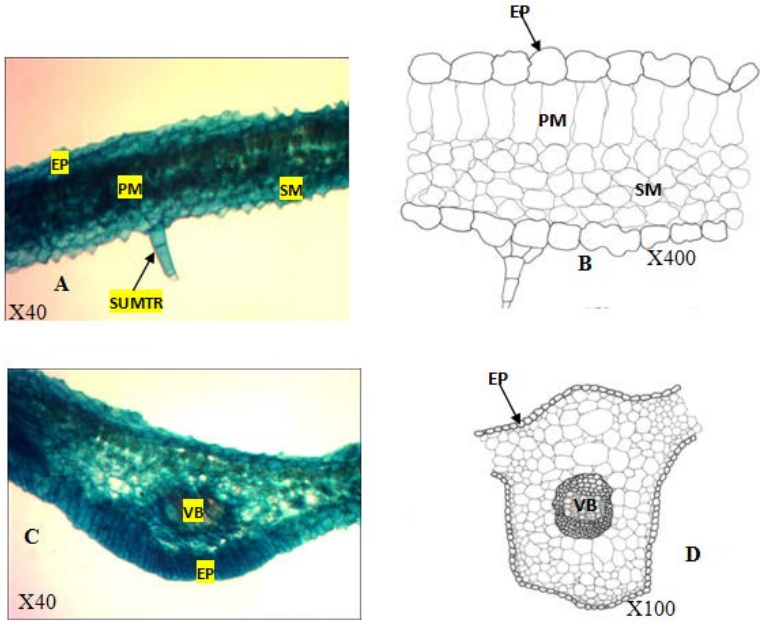

Plate 13. Leaf anatomy in *Vernonia cinerea*. (A and B): Transverse of section leaf (lamina); (C and D): Transverse section of leaf (midrib). EP- Epidermis, PM- palisade mesophyll, SM- spongy mesophyll, ST- Stomata, SUMTR- simple uniseriate multicellular non-glandular trichome, VB- vascular bundle.

and numerous number of bundles.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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The use of ecological methods in vegetative studies of plant species and abundance in south-eastern Nigeria

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The study on ecological survey of plant species biodiversities and abundance in Southeastern Nigeria was conducted between January 2012 and 2013 at Anambra State, Nigeria to determine the plant species biodiversities and abundance in three forest fringes viz: community, shrine and reserves using measures based on floristic, stratified random sampling, plotless measure and point centre quarter methods, respectively. Shannon-wienners index of diversity analysis proved that Umunze community forest was highest in biodiversity (0.95) with *Afzelia africana* as the most dorminant species (148.93) while *Pterocarpus* sp. has the highest importance value index (25.36). Achala forest reserve has *Chlorophora exdelsa* as the most dominant (686.09), while *Tectora grandis* recorded the highest importance value index (62.53). The dominant species in Iyiocha forest was *Pterocarpus* species (451.31), while *Newbouldia levis* has the highest importance value index. Regression analysis showed that at P<0.05, there is a significant relationship between species abundance and species diversity.

Key words: Ecology, survey, plants, biodiversities, abundance, Anambra.

INTRODUCTION

The scientific study of forest species and their interaction with the environment is referred to as forest ecology, while the management of forests is often referred to as forestry (Padoch et al., 1985). Primack (1991) noted that forest management has changed considerably over the last few centuries, with rapid changes from the 1980s onwards culminating in a practice now referred to as sustainable forest management. Forest ecologists

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concentrate on forest patterns and processes, usually with the aim of elucidating cause and effect relationships. Foresters who practice sustainable forest management focus on the integration of ecological, social and economic values, often in consultation with local communities and other stakeholders (Primack, 1991).

Forests can be found in all regions capable of sustaining tree growth, at altitudes up to the tree line, except where natural fire frequency or other disturbance is too high, or where the environment has been altered by human activity.

Anthropogenic factors that can affect forests include logging, urban sprawl, human-caused forest fires, acid rain, invasive species, and the slash and bum practices of swidden agriculture or shifting cultivation (Momberg, 1992). The loss and re-growth of forest leads to a distinction between two broad types of forest, primary or old-growth forest and secondary forest. FAO (1991) reported that, there are also many natural factors that can cause changes in forests over time including forest fires, insects, diseases, weather, competition between species, etc. In 1997, the World Resources Institute recorded that only 20% of the world's original forests remained in large intact tracts of undisturbed forest.

However, Agbelusi and Afolayan (1987) stated that, over the past decades, the annual increase in timber exports by value, mostly from West Africa has exceeded 12%, and local and external demands are certain to increase further. The availability of commercial timber in forests at present in use is diminishing but there are possibilities of bringing untapped areas into production. Sustainable management of the forests that can meet the standard of the Millennium Development Goals is therefore sacrosanct.

More than 75% of these intact forests lie in three countries - the Boreal forests of Russia and Canada and the rainforest of Brazil. In 2006, this information on intact forests was updated using latest available satellite imagery (Ramesteiner, 1998).

Old-growth forest contains mainly natural patterns of biodiversity in established seral patterns, and they contain mainly species native to the region and habitat. The natural formations and processes have not been affected by humans with a frequency or intensity to change the natural structure and components of the habitat. Secondary forest contains significant elements of species which were originally from other regions or habitats.

In the United States, most forests have historically been affected by humans to some degree, though in recent years improved forestry practices has helped regulate or moderate large scale or severe impacts. However, Leakey and Newton (1994) stated that, the United States Forest Service estimates a net loss of about 2 million hectares (4,942,000 acres) between 1997 and 2020; this estimate includes conversion of forest land to other uses, including urban and suburban development, as well as afforestation and natural reversion of abandoned crop and pasture land to forest. However, in many areas of the United States, the area of forest is stable or increasing, particularly in many Northern states. The opposite problem from flooding has plagued national forests, with loggers complaining that lack of thinning and proper forest management has resulted in large forest fires (Leakey and Newton, 1994).

Momberg (1992) posited that, the concept of sustainable forest management has continued to evolve since 1992 through international forest policy dialogue within the Intergovernmental Panel on Forests (IPF), the Intergovernmental Forum on Forests (IFF) and the United Nations Forum on Forests (UNFF) and through a large number of country led eco-regional initiatives aimed at translating the concept into practice. These include the development of criteria for and indicators of sustainable forest management supported by international organizations including FAO, the International Tropical Timber Organization (ITTO), the United Nations Environment Programme (UNEP) and other members of the Collaborative Partnership on Forests (CPF) (Momberg, 1992). This work is set to determine the species richness, species abundance, species diversities of the sampled forests and thus provide an inventory of the available species in this basin; also to determine the current status of forest resources of Anambra State (a) categories, (b) size, (c) composition, (d) degree of deforestation and finally determine the extent to which these forest resources have been sustainably managed with particular reference to timber product extractions.

METHODOLOGY

The study area

The first thematic step taken to carry out this research was a preliminary/reconnaissance survey of the forests to be sampled. This entails a careful study of the areas in question to determine the heterogeneity and understand the techniques to be used in sampling the areas. Anambra State has five (5) forest zones which are based on the five agricultural zones of the state. They include: Awka zone, Nnewi zone, Abagana zone, Otuocha zone and Onitsha zone. Out of these five zones, three zones were selected for sampling. Egboka (1993) stated that, Anambra is a state in the south-eastern Nigeria. °Its name is an anglicized version of the original 'Oma Mbala', the name of the river now known as Anambra River which the state is named after. The state derives its name from the Anambra River, the largest, most southerly, left bank tributary of the River Niger.

With a total land area of 4,416 sq km, Anambra State is situated on a generally low elevation on the eastern side of the River Niger, shares boundaries with Kogi, Enugu, Imo, Abia, Rivers, Delta and Edo states. It lies within the following geographical locations: 5° 4S1N to 6° 4S1N and 6° 361E to 7° 081E (Egboka, 1993). It is bordered in the West by Delta State, on the North by Kogi State, on the east by Enugu State and on the South by Imo State.

The climate

The climate of Anambra State is an equatorial tropical rain forest type. It is characterized by two main seasons viz: the rainy (wet) season and the dry season. The rainy season is characterized by heavy thunder storms and occurs between the months of April and October, while the dry season extends from November to March annually (Nwosu, 2003).

The intensity of the rainfall is generally heavy during the rainy season, except in the month of August where there is a noticeable drop in rainfall, he asserted. This phenomenon is normally referred to as the August break and hence the double maxima of rainfall which is the characteristic of this pattern of rainfall. This rainy season is characterized by high temperature (25 to 33°C), and high relative humidity (85%) (Nwosu, 2003). In the course of his work, Nwosu (2003) also observed that the dry season is characterized by chilly and dry North east Monsoon or hamattan winds. This lowers temperature appreciably especially in the months of December and January. He noted that its main features are: excessive evaporation, low relative humidity, and general dry weather which results in the drying and loss of vegetal cover. He also noted that in most part of Anambra State, temperature is usually high over the year.

Thus, the average minimum and maximum temperatures are about 25 and 32°C, respectively, while the annual rainfall is also very high with a mean of about 200 mm.

Distribution and geographical ranges of forests

Forests can be found in all regions capable of sustaining tree growth, at altitudes up to the tree line, except where natural fire frequency or other disturbance is too high, or where the environment has been altered by human activity.

The latitudes 10° north and south of the Equator are mostly covered in tropical rainforest, and the latitudes between 53°N and 67°N have boreal forest. As a general rule, forests dominated by angiosperms (broadleaf forests) are more species-rich than those dominated by gymnosperms (conifer, montane, or needlleaf forests), although exceptions exist (Michon and Bompard, 1987).

Forests sometimes contain many tree species only within a small area (as in tropical rain and temperate deciduous forests), or relatively few species over large areas (e.g., taiga and arid montane coniferous forests). Aumeerudy (1993) noted that, forests are often home to many animal and plant species, and biomass per unit area is high as compared to other vegetation communities. Much of this biomass occurs below ground in the root systems and as partially decomposed plant detritus. The woody component of a forest contains lignin, which is relatively slow to decompose when compared with other organic materials such as cellulose or carbohydrate.

Measures based on floristic

The species composition of each sampled forest was assessed floristically; this was accompanied by the amount or abundance of each species present at a site. It is useful to distinguish between abundance and richness, the latter being the number of species present on a particular area. However, the forest area was marked out and stratified, and then species measurement by girth was made of trees above one meter in height.

Stratified random sampling

This method of sampling, according to Moore and Chapman (1986) has been extensively used in disciplines other than ecology. They noted that it involves subdividing the field of study into relatively homogeneous parts and then sampling' each subdivision according to its area, or some other parameters.

Plotless measures

The use of plotless method was employed to estimate the density of the species. This design could also be used for collecting information on the species composition, growth and environmental factors. The type of plotless method that was employed is the point center quarter method.

The point center quarter method

In the point center quarter method, four distances were measured at each sampling point. Four quarters were established at the sampling point through a cross formed by two lines. One line is the compass direction and the second line running perpendicular to the compass direction through the sampling point. The line cross can also be randomly established by spinning a cross over each sampling point. The distance to the mid-point of the nearest tree from the sampling point is measured in each quarter.

The four distances of a number of sampling points are averaged and when squared are found to be equal to the mean area occupied by each tree. Cottam and Curtis (1956) tested the reliability of this method on several random populations by checking the result with the plot method. The estimates of the correct mean area per tree (MA) were found to apply to each of the different sets of mean distance. Therefore no correction factor is needed when the four quarter distances are averaged: $MA = D^2$, where D = the mean distance of four points to the nearest tree distances taken in each of four quarters. The mathematical prove of the workability of this method has been given by Morisita (1954). According to Cottam and Cuttis (1954), the accuracy increases with the number of sampling points and a minimum of 20 points is recommended.

Newsome and Dix (1968) noted that one of the limitations of this method for field application is that an individual must be located within each quarter and an individual must not be measured twice. After sampling, the species diversity was calculated using the data that accrued from the sampling of the forests.

Shannon-Winner index of diversity was used to analyze and determine the species diversity of each forest.

RESULTS

From Table 1, *Tectonia grandis* recorded the highest importance value index (63.53) while *Milletia zechiana* recorded the least important value index (6.38). *T. grandis* therefore becomes the abundant species of Achala forest reserve.

From Table 1, *Newbouldia levis* recorded the highest importance value index (45.99) while *Dialum guineense* recorded the least importance value index (2.77). *N. levis* therefore becomes the abundant species of Iyi-Ocha Shrine Forest.

From Table 1, *Pterocarpus* spp. has the highest importance value index (25.36) while *Buchholzia coriaceae* has the least importance value index (2.75).

Table 1. Species abundance of Achala Forest Reserve in Anambra Basin.

Species	Frequency	Relative frequency	Density	Relative density	Dominance	Relative dominance	IVI
Tectonia grandis	90	25.35	1.28	30	239.53	7.18	62.53
Gmelina aborea	60	16.9	0.8	18.75	216.48	6.49	42.14
Chlorophora excels	25	7.04	0.27	6.25	686.09	20.56	33.85
Tetrapleura tetraptera	25	7.04	0.27	6.25	588.09	17.62	30.91
Adansonia digitata	20	5.63	0.21	5	471.66	14.13	24.76
Irvingia gabonensis	30	8.45	0.32	7.5	252.13	7.55	23.5
Ceiba pentandra	20	5.63	0.21	5	339.52	10.17	20.81
Vitex doniana	25	7.04	0.27	6.25	170.16	5.1	18.39
Daniella oliveri	25	7.04	0.27	6.25	138.3	4.14	17.44
Draecena arborea	15	4.23	0.16	3.75	71.39	2.14	10.11
Milletia thonningii	10	2.82	0.11	2.5	128.87	3.86	9.18
Milletia zechiana	10	2.82	0.11	2.5	35.55	1.07	6.38
Total	355	99.99	4.27	100	3337.76	100.01	300

Pterocarpus spp. therefore becomes the abundant species of Unenzu Community Forest.

DISCUSSION

In assessing the species abundance of Achala Forest Reserve, it was discovered that *T. grandis* was the most frequent (90%) followed by *Gmelina aborea* (60%). *T. grandis* was also found to be more dense (1.28) than others. However, the most dominant was *Chlorophora excelsa* (686.09) followed by *Tetrapleura tetraptera* (588.09). When the importance value index was determined, it was observed that *T. grandis* recorded the highest importance value index (62.53), while *Milletia zachiana* recorded the least importance value index (6.38). It becomes clear that *T. grandis* was the most abundance species in Achala Forest Reserve.

However, from Table 2, Iyiocha Shrine Forest had *N. levis* as the most frequent (65%) followed by *Pterocarpus* species (35%) and *Delonix regia* (35%) respectively. Density followed the same pattern, *N. levis* (0.5), *Pterocarpus* species (0.3) and *D. regia* (0.3). The most dominant species was *Pterocarpus* species (451.31) while the least was *Dialum guineense* (7.37). Also from the table, *N. levis* recorded the highest importance value index (45.99) while *D. guineense* recorded the least importance value index. It became glaring that it is the most abundance species in Iyiocha Shrine Forest.

The species abundance of Unenzu Community Forest in Table 3 showed that *Pterocarpus* species and *N. levis* were the most frequent with 30% frequency each respectively. Records also showed that the most dense of the species in this forest was *Pterocarpus* species followed by *N. levis* and *Dacroydes edulis* with 0.07 density each. The most dominant of the species was *Afzelia africana* (148.95) followed by *Pterocarpus* species (110.08). Also, records showed that *Pterocarpus* species has the highest importance value index (25.36) while *Buchholzia coriaceae* has the least importance value index (2.75). It was clear that *Ptericarpus* species was the most abundance species in Unenzu Community Forest. However, Wright (1991) observed that abundance is contrasted, but typically correlate to incidence, which is the frequency with which the species occur in a sample. In his work to determine the abundance of species in the Nature Reserve Wisconsin, he noted that oak tree, gopherwood and *Virgilia* dominated the forest more than other species, though their quantitative measurements were not given. Damgaard (2009) have also worked extensively on species abundance on different forest resources and agreed that some species are actually more in abundance than others. They noted that one of the factors that could account for this was probably because majority could withstand extreme environmental condition as well as sustainable exploitation of the species. This is in agreement with the finding of this work in the sense that some of the dominant species have been sustainably exploited especially in the government regulated areas like the Forest Reserves.

Barfet et al. (2001) have also worked extensively on species abundance on different forest resources and agreed that some species are actually more in abundance than others. They noted that one of the factors that could account for this was probably because majority could withstand extreme environmental condition as well as sustainable exploitation of the species. This is in agreement with the finding of this research work in the sense that some of the dominant species have been sustainably exploited especially in the government regulated areas like the Forest Reserves.

Colwell and Coddington (1994) also in their work on

Table 2. Species Abundance of Iyi-Ocha Shrine Forest.

Species	Frequency	Relative frequency	Density	Relative Density	Dominance	Relative Dominance	IVI
Newbouldia levis	65	16.67	0.5	17.5	373.59	11.83	45.99
Pterocarpus spp.	35	8.97	0.29	10	451.31	14.29	33.26
Delonix regia	35	8.97	0.25	8.75	190.25	6.02	23.75
Dacroydes edulis	25	6.41	0.18	6.25	290.38	9.19	21.85
Chlorophora excelsa	15	3.85	0.11	3.75	393.38	12.45	20.05
Nauclea latifolia	25	6.41	0.18	6.25	132.55	4.2	16.86
Irvingia gabonensis	20	5.13	0.14	5	173.58	5.49	15.62
Ficus exasperate	20	5.13	0.14	5	64.17	2.03	12.16
Syzigium guineense	15	3.85	0.11	3.75	118.93	3.76	11.36
Chrysophyllum albidum	10	2.56	0.07	2.5	184.15	5.83	10.89
Parkia biglobosa	15	3.85	0.11	3.75	94.77	3	10.6
Spondias mombin	15	3.85	0.11	3.75	70.49	2.23	9.83
Borassus aetheopicum	15	3.85	0.11	3.75	68.26	2.16	9.76
Aubrrevillea kerstingii	15	3.85	0.11	3.75	59.67	1.89	9.49
Hildegardia barteri	10	2.56	0.07	2.5	98.21	3.11	8.17
Piptadeniastrium africanum	10	2.56	0.07	2.5	84.99	2.69	7.75
Anthocleista djalonensis	10	2.56	0.07	2.5	63.41	2.01	7.07
Adansonia digitata	5	1.28	0.04	1.25	129.47	4.1	6.63
Ficus carpensis	10	2.56	0.07	2.5	36.8	1.16	6.23
Monodora myrtstica	5	1.28	0.04	1.25	38.86	1.23	3.76
Enantia chlorantha	5	1.28	0.04	1.25	18.84	0.6	3.13
Bukholtzia coriaceae	5	1.28	0.04	1.25	15.51	0.49	3.02
Dialum guineense	5	1.28	0.04	1.25	7.37	0.23	2.77
Total	390	99.99	2.86	100	3158.95	99.99	300

Table 3. Species abundance of Unenzu Community Forest.

Species	Frequency	Relative frequency	Density	Relative Density	Dominance	Relative dominance	IVI
Pterocarpus spp.	30	7.59	0.08	8.75	110.08	9.01	25.36
Newbouldia levis	30	7.59	0.07	7.5	64.15	5.25	20.35
Afzelia Africana	15	3.8	0.03	3.75	148.95	12.19	19.74
Dacroydes edulis	30	7.59	0.07	7.5	54.63	4.47	19.57
Irvingia gabonensis	25	6.33	0.05	6.25	67.59	5.53	18.11
Anacardium occidentalis	25	6.33	0.05	6.25	37.8	3.09	15.67
Spondias mombin	20	5.06	0.04	5	41.07	3.36	13.43
Daniella oliveri	15	3.8	0.03	3.75	69.83	5.72	13.26
Vitex doniana	15	3.8	0.03	3.75	49.16	4.02	11.57
Prosopis Africana	15	3.8	0.03	3.75	44.4	3.64	11.18
Nauclea latifolia	10	2.53	0.02	2.5	55.74	4.56	9.6
Ficus exasperate	15	3.8	0.03	3.75	18.92	1.55	9.1
Ceiba pentandra	10	2.53	0.02	2.5	48.96	4.01	9.04
Anthocleista djalonensis	10	2.53	0.02	2.5	46.15	3.78	8.81
Delonix regia	10	2.53	0.02	2.5	44.37	3.63	8.66
Chrosophyllum albidum	10	2.53	0.02	2.5	37.91	3.1	8.13
Ficus carpensis	10	2.53	0.02	2.5	24.77	2.03	7.06
Dialium guineense	10	2.53	0.02	2.5	24.34	1.99	7.02
Dracena arborea	10	2.53	0.02	2.5	24.17	1.98	7.01

Table 3. Contd.

Species	Frequency	Relative frequency	Density	Relative Density	Dominance	Relative dominance	IVI
Borassus aethiopicum	10	2.53	0.02	2.5	16.13	1.32	6.35
Datariun microcarpium	10	2.53	0.02	2.5	14.01	1.15	6.18
monodora myristica	10	2.53	0.02	2.5	10.48	0.86	5.89
Elaeis guineensis	10	2.53	0.02	2.5	8.98	0.73	5.77
Chlorophora excelsa	5	1.27	0.01	1.25	38.8	3.18	5.69
Pentaclethra macrophylla	5	1.27	0.01	1.25	27.04	2.21	4.73
Rauvolfia vomitoria	5	1.27	0.01	1.25	26.37	2.16	4.67
Tetrapleura tetraptera	5	1.27	0.01	1.25	26.27	2.15	4.67
Hildegardia barteri	5	1.27	0.01	1.25	14.87	1.22	3.73
Parkia biglobosa	5	1.27	0.01	1.25	12.6	1.03	3.55
Milletia zechiana	5	1.27	0.01	1.25	10.11	0.83	3.34
Buchholzia coriaceae	5	1.27	0.01	1.25	2.91	0.24	2.75
Total	395	100	0.88	100	1221.54	100	299.99

Table 4. Species diversity of Achala Forest Reserve.

Species	n	N	Pi	ln(pi)	pi*ln(pi)	-Σ(pi)*ln(pi)
Adansonia digitata	4	80	0.05	-2.99573	-0.14979	H^1=2.16962
Chlorophora excels	5	80	0.0625	-2.77259	-0.17329	H_{max}= Ins (ln12)
Ceiba pentandra	4	80	0.05	-2.99573	-0.14979	2.48491
Daniella oliveri	5	80	0.0625	-2.77259	-0.17329	
Draecena arborea	3	80	0.0375	-3.28341	-0.12313	
Gmelina aborea	15	80	0.1875	-1.67398	-0.31387	Equitability=
Irvingia gabonensis	6	80	0.075	-2.59027	-0.19427	(H^1/H_{max})=
Milletia thorningii	2	80	0.025	-3.68888	-0.09222	0.87312
Milletia zechiana	2	80	0.025	-3.68888	-0.09222	
Tectonia grandis	24	80	0.3	-1.20397	-0.36119	
Tetrapleura tetraptera	5	80	0.0625	-2.77259	-0.17329	
Vitex doniana	5	80	0.0625	-2.77259	-0.17329	
Total					-2.16962	

species abundance observed that the vast areas of flat or gently sloping land in the hot deserts of North America were dominated by a single species of shrub-like tree, Larrea tridentate, while grasses and forbs grow in the spaces between these trees. Their work contrasted heavily with these findings because despite the fact that a particular tree or two were more in abundance, yet most other trees could be seen juxtaposed within the forests. The observed difference could stem from the fact that their research was conducted in the desert while this research was conducted in a forested area.

Species diversities

The analysis of the species diversities using Shannon Wiener index of diversity proved that Achala forest reserve has the diversity of 0.87; Iyiocha shrine forest has the diversity of 0.91, while Unenzu community forest has 0.94 species diversity. Unenzu community forest recorded highest in species diversity. Records also showed that Unenzu community forest has the highest number of tree species (32) as against 23 and 12 of Iyiocha and Achala, respectively (Table 4, 5 and 6).

The regression analysis proved that the t-value of the coefficient of the number of species is significant ($P<0.05$) indicating a significant relationship between number of species and species diversity. The coefficient of (0.005) implies that a percentage point increase in the number of species increase species diversity by 0.005. The number actually explains about 48.0% of species diversity. The p-value of the f-statistics is significant ($P<0.05$) indicating that the model is a good fit. Jost (2007), Tuomisto (2010) and Krebs (1999) all agreed that the observed species diversity is affected by not only the number of individual species, but also by the heterogeneity of the sample. They were also of the opi-

Table 5. Species diversity of Iyi-Ocha Shrine Forest.

Species	n	N	Pi	ln(pi)	pi*ln(pi)	-Σ(pi)*ln(pi)
Adansonia digitata	1	80	0.0125	-4.38203	-0.054775333	$H^{1=}$2.89221
Aubrevillea kerstingii	2	80	0.025	-3.68888	-0.092221986	
Anthocliesta djalonensis	2	80	0.025	-3.68888	-0.092221986	H_{max}= Ins (ln24)
Borassus aetheopicum	3	80	0.0375	-3.28341	-0.123128038	3.17805
Buchholzia coriaceae	1	80	0.0125	-4.38203	-0.054775333	
Chlorophora excels	3	80	0.0375	-3.28341	-0.123128038	
Chrysophyllum albidum	2	80	0.025	-3.68888	-0.092221986	Equitability=
Dacroydes edulis	5	80	0.0625	-2.77259	-0.173286795	(H^1/ H_{max})=
Delonix regia	7	80	0.0875	-2.43612	-0.213160192	0.91006
Dialum guineense	1	80	0.0125	-4.38203	-0.054775333	
Enantia chlorantha	1	80	0.0125	-4.38203	-0.054775333	
Ficus carpensis	2	80	0.025	-3.68888	-0.092221986	
Ficus exasperate	4	80	0.05	-2.99573	-0.149786614	
Hildegardia barteri	2	80	0.025	-3.68888	-0.092221986	
Irvingia gabonensis	4	80	0.05	-2.99573	-0.149786614	
Monodora myrtstica	1	80	0.0125	-4.38203	-0.054775333	
Nauclea latifolia	5	80	0.0625	-2.77259	-0.173286795	
Newbouldia levis	14	80	0.175	-1.74297	-0.305019628	
Parkia biglobosa	3	80	0.0375	-3.28341	-0.123128038	
Piptadeniastrum africanum	2	80	0.025	-3.68888	-0.092221986	
Pterocarpus spp	8	80	0.1	-2.30259	-0.230258509	
Spondias mombin	3	80	0.0375	-3.28341	-0.123128038	
Syzigium guineense	3	80	0.0375	-3.28341	-0.123128038	
Total					-2.892209253	

Table 6. Species diversity of Unenzu Community Forest.

Species	N	N	Pi	ln(pi)	pi*ln(pi)	-Σ(pi)*ln(pi)
Afzelia Africana	3	80	0.0375	-3.28341	-0.12313	H^1 =3.25857
Anacardium occidentalis	5	80	0.0625	-2.77259	-0.17329	
Athocleista djalonensis	2	80	0.025	-3.68888	-0.09222	H_{max}= Ins (ln31)
Borassus aethiopicum	2	80	0.025	-3.68888	-0.09222	3.43399
Buchholzia coriaceae	1	80	0.0125	-4.38203	-0.05478	
Chlorophora excels	1	80	0.0125	-4.38203	-0.05478	
Chrosophyllum albidum	2	80	0.025	-3.68888	-0.09222	Equitability=
Cieba pentandra	2	80	0.025	-3.68888	-0.09222	(H^1 / H_{max})=
Dacroydes edulis	6	80	0.075	-2.59027	-0.19427	0.94892
Dalium guineense	2	80	0.025	-3.68888	-0.09222	
Daniella oliveri	3	80	0.0375	-3.28341	-0.12313	
Datariun microcarpum	2	80	0.025	-3.68888	-0.09222	
Delonix regia	2	80	0.025	-3.68888	-0.09222	
Dracena arborea	2	80	0.025	-3.68888	-0.09222	
Elaeis guineensis	2	80	0.025	-3.68888	-0.09222	
Ficus carpensis	2	80	0.025	-3.68888	-0.09222	
Ficus exasperate	3	80	0.0375	-3.28341	-0.12313	
Hildegardia barteri	1	80	0.0125	-4.38203	-0.05478	
Irvingia gabonensis	5	80	0.0625	-2.77259	-0.17329	
Milletia zechiana	1	80	0.0125	-4.38203	-0.05478	

Table 6. Contd.

Species	N	N	Pi	ln(pi)	pi*ln(pi)	-Σ(pi)*ln(pi)
Monodora myristica	2	80	0.025	-3.68888	-0.09222	
Nauclea latifolia	2	80	0.025	-3.68888	-0.09222	
Newbouldia levis	6	80	0.075	-2.59027	-0.19427	
Parkia biglobosa	1	80	0.0125	-4.38203	-0.05478	
Pentaclethra macrophylla	1	80	0.0125	-4.38203	-0.05478	
Prosopis Africana	3	80	0.0375	-3.28341	-0.12313	
Pterocarpus spp	7	80	0.0875	-2.43612	-0.21316	
Rauvolfia vomitoria	1	80	0.0125	-4.38203	-0.05478	
Spondias mombin	4	80	0.05	-2.99573	-0.14979	
Tetrapleura tetraptera	1	80	0.0125	-4.38203	-0.05478	
Vitex doniana	3	80	0.0375	-3.28341	-0.12313	
Total					-3.25857	

nion that increasing the area sampled increases observed species diversity both because more individuals get included in the sample and because large areas were environmentally more heterogeneous than small areas. Their observation tallies with the present research work because virtually all the sampled forests were highly diverse. The discrepancies in the species diversities could also be attributed to environmental factors, forest management or soil conditions which were not measured.

Connell (1978) in one of his researches noted that a rich plant life forms cover organ Pipe National Monument in Southern Arizona. He observed that growth of *Ocofillo* species consisting of several slender branches 2 to 3 m tall springing from a common base, there was also Palo Verde trees with green bark and tiny leaves. According to Connell (1978), the most abundant was the *Saguaro*, a massive cactus that towers over all the other plants species. This agrees with this present research. Different plants species were observed though some were more in abundance and more frequent than others.

Whicker and Defling (1988) has been able to explain much of the variation in woody plant diversity and dominance by some tree species across Sonoran Desert landscapes by differences in soil age, frequency of land disturbance caused by soil erosion and soil depth. The key point here is that communities generally consist of many species that potentially interact in all the ways with one another.

Bush et al. (1989) concurred that species diversity increases with environmental complexity or heterogeneity. They however noted that an aspect of environmental structure important to one group of organisms may not have a positive influence on another group. Consequently, one must be acquainted with the ecological requirements of species to predict environmental structure that affects the diversity. Conclusively, this ecological survey showed that there is variance in plant species composition, abundance and biodiversity between the areas studied and thus suggest there is a declining rate of these plant species which portend great importance to man and animals in our societies today and thus provide a baseline studies on the various status of these plant species population studies.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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Influence of medium type and growth regulators on *in vitro* micropropagation of pineapple (*Ananas comosus* (L.), var. Smooth cayenne)

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Aseptic cultures of pineapple 'smooth cayenne' were established from shoot tip explants. They were initiated on Murashige and Skoog (MS) basal medium with vitamins supplemented with various combinations of 6-benzylaminopurine (BAP) (0, 0.1, 0.2, 0.3 and 0.4 mg L^{-1}) and Gelrite [1.0 g L^{-1} (semi-liquid), 1.5 g L^{-1} (semi-solid) and 2.0 g L^{-1} (solid)]. Explants were transferred five weeks after *in vitro* initiation to semi-liquid medium containing higher concentrations of BAP (0, 0.5, 1.0, 1.5 and 2.0 mg L^{-1}) for proliferation. Four sub-cultures were made at five weeks interval for twenty weeks in the proliferation medium. Established shoots were introduced into rooting medium containing either full (4.4 g L^{-1}) or half (2.2 g L^{-1}) strength MS basal medium with vitamins supplemented with 0, 0.5 mg L^{-1} BAP alone or in combination with 0, 0.9 and 1.8 mg L^{-1} α-naphthaleneacetic acid (NAA). Semi-liquid MS basal medium supplemented with 0.1 and 0.3 mg L^{-1} BAP gave the best regeneration results, producing the highest average shoot length (34.6 mm) and average number of shoot buds (2.4) per explants. Significant difference ($p \leq 0.05$) in average shoot number per explant was observed with semi-liquid MS basal medium supplemented with 1.5 mg L^{-1} BAP, producing the highest average shoot number (6.1) per explant at 5 weeks and this increased to 167.7 after 20 weeks. The half-strength MS basal medium without growth regulators or with 0.9 mg L^{-1} NAA gave the highest average root length (29.3 mm) and root number per shoot (7.9). The semi-liquid MS basal medium supplemented with low BAP (1.5 mg L^{-1}) is a cost-effective method for *in vitro* propagation of pineapple. Half strength MS basal medium without hormones or with low NAA are also cost-effective methods for inducing roots in pineapple.

Key words: Gelrite, growth regulators, medium type, pineapple, propagation.

INTRODUCTION

Pineapple [*Ananas comosus* (L.) Merr] is a tropical fruit of great economic importance with a lot of health benefits (Duval et al. 2001). It is considered to be an exotic fruit, used for dessert due to its attractive flavor and nutritive value (Khan et al., 2004). Pineapples are commonly consumed as fresh fruits or processed into canned fruit,

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juice and jam for export (Roostika and Mariska, 2003). Nigeria is the largest producer of pineapples in Africa and the 8th largest producer in the world; with 'smooth cayenne' being the most commonly cultivated variety in Nigeria (Odusote, 2013).

Large number of healthy pineapple planting materials are required (planting density of 60,000 per hectare) in order to meet the demand of both processing and an expanding fresh-market sector (Danso et al., 2008). Conventionally, pineapple is propagated vegetatively through suckers, slips or crowns (Khan et al., 2004), but this has limitations such as low multiplication rate, transmission of diseases and lack of uniformity (Lieu et al. 2004). *In vitro* propagation of pineapple shoots is proposed as a means of addressing these problems because it allows for efficient and rapid multiplication of disease-free pineapple plantlets in a relatively shorter period independent of the season (Firoozabady and Gutterson, 2003; Sether et al., 2001). The success of an *in vitro* or a micropropagation procedure with regards to the survival rate and performance of the plants depends on several factors including explants source, type of growth hormones, gelling agent, carbon source, pH of the medium and condition of the growth room during the *in vitro* growth process (Zuraida et al., 2011).

Micropropagation of shoot tips and buds from crown have been reported in pineapple (Al-Saif et al., 2011; Hammad and Taha, 2008; Kiss et al., 1995). A commonly used method was a combination of 6-benzylaminopurine (BAP) and naphthalene acetic acid (NAA) (Firoozabady and Gutterson, 2003), indole acetic acid (IAA) (Hamad and Taha, 2008), indole butyric acid (IBA) (Boxus et al. 1991) or 2,4-dichlorophenoxy acetic acid (2,4-D) (Liu et al., 1989). Although *in vitro* micropropagation of pineapple with BAP alone has been reported (Almeida et al., 2002; Be and Debergh, 2006), the use of BAP alone or at low concentrations for rapid *in vitro* multiplication of pineapple will be a cost-effective method.

Liquid culture medium is known to promote faster rates of growth of *in vitro* plantlets because there is rapid absorption of the nutrients by the plantlets during continuous agitation of the medium on a shaker (Danso et al., 2008; Firoozabady and Gutterson, 2003). The cost of production using this technique can be high particularly in developing countries like Nigeria with unstable power supply, which depend on alternative power backup. Testing the culture medium for regeneration and proliferation of pineapple using different of levels Gelrite (gelling agent) in combination with BAP and or NAA may result in the production of a cost-effective and efficient method for *in vitro* multiplication of pineapple. This study was therefore carried out to determine the concentration(s) of Gelrite and BAP that promotes maximum rate of multiplication of pineapple var. smooth cayenne with minimal input of chemical materials.

The effect of full and half strength MS supplemented with or without selected growth regulators on root induction was also studied.

MATERIALS AND METHODS

Plant material and sterilization

Crowns from freshly harvested pineapple 'smooth cayenne' were used as the explant source. Leaves were removed from crowns by gentle peeling, leaving about five primordial leaves and the base intact (Khan et al., 2004). Afterwards, the peeled crowns were thoroughly washed under running tap water for 60 min before transferring to the Laminar Flow Hood for surface sterilization. Surface sterilization was done by sequential immersion in 70% (v/v) ethanol for 5 min with gentle swirling, 20% sodium hypochlorite solution (v/v) is containing three drops of Tween 20 for 10 min and finally, 15% sodium hypochlorite solution (v/v) containing 3 drops of Tween 20 for 15 min. This was followed by three rinses in sterile distilled water to remove all traces of sodium hypochlorite and other chemicals.

Regeneration medium and culture conditions

Under aseptic conditions, the shoot tip with one to two leaf primordial (approximately 1 cm^3), was inoculated into freshly prepared Murashige and Skoog (MS), (1962) basal medium with vitamins in a test tube. This medium was supplemented with 30.0 g L^{-1} sucrose, BAP at 0, 0.1, 0.2, 0.3, and 0.4 mg L^{-1} and Gelrite at 1.0, 1.5, and 2.0 g L^{-1}; which constitutes semi-liquid, semi-solid and solid media; respectively. Prior to use of Gelrite, the pH of the medium was adjusted to 5.8 using 0.1 M sodium hydroxide (NaOH) or 0.1 M hydrochloric acid (HCl). The medium was dispensed at 15 ml to each test tube of size (24 x 145 mm) and sterilized by autoclaving at 15 psi and 121°C for 15 min. The cultures were maintained in the growth room at 26 ± 2°C under white fluorescent bulbs controlled with automatic timer to supply 16 h photoperiod (40 µmoles photons m^{-2} s^{-1}). A treatment consisted of eight test tubes arranged in a completely randomized design with three replications according to Snedercor and Cochran (1980). The best regeneration medium was used in subsequent studies.

Data were collected on the number of shoot buds, and the shoot length (mm) per tube for a successive period of 5 weeks.

Multiplication medium

Regenerated shoots obtained from the shoot-tip cultures were used as explants for this experiment. These were planted on freshly prepared multiplication medium. The multiplication medium consisted of MS basal salt with vitamins supplemented with 3% sucrose, BAP (0.5, 1.0, 1.5 and 2.0 mg L^{-1}) and Gelrite (1.0 g L^{-1}). A treatment consisted of eight culture jars arranged in a completely randomized design with three replications. Successive subcultures were carried out for up to 4 cycles with 5-weeks interval per cycle. The cultures were maintained in the growth room under the same condition as the regeneration stage. The number of shoots produced per initial explants in each jar was counted at 5 weeks interval for 20 weeks.

Rooting medium

Proliferated shoots were separated, and each single shoot was

Table 1. Effect of different combinations of Gelrite and BAP concentrations on *in vitro* regeneration of shoot-tips of pineapple after 5 weeks of initiation[a].

Gelrite (g L^{-1})	BAP (mg L^{-1})	Average no. of shoot buds/explants	Average shoot length (mm) explants
	0.0	1.0 ± 0.0	24.3 ± 2.2
	0.1	2.0 ± 0.3	34.6 ± 2.7
1.00	0.2	1.8 ± 0.3	30.4 ± 1.6
	0.3	2.4 ± 0.3	32.3 ± 2.4
	0.4	1.9 ± 0.3	28.0 ± 2.7
	0.0	1.0 ± 0.0	21.0 ± 1.6
	0.1	1.8 ± 0.3	23.5 ± 1.6
1.50	0.2	1.8 ± 0.3	24.8 ± 2.8
	0.3	1.5 ± 0.2	27.6 ± 1.4
	0.4	1.6 ± 0.3	26.8 ± 2.3
	0.0	1.0 ± 0.0	20.9 ± 1.8
	0.1	1.0 ± 0.0	23.4 ± 1.7
2.00	0.2	1.0 ± 0.0	28.1 ± 2.9
	0.3	1.0 ± 0.0	24.5 ± 2.4
	0.4	1.0 ± 0.0	29.9 ± 1.1
LSD		0.6	5.4

[a]Results are mean values ± standard error.

transferred to a rooting medium in test tube, for root induction and elongation. The rooting medium was composed of full-strength (4.4 g L^{-1}) or half-strength (2.2 g L^{-1}) MS basal medium with vitamins, supplemented with or without growth hormones. Medium supplemented with hormones had varying concentrations; BAP (0, and 0.5 mg L^{-1}) and NAA (0, 0.9 and 1.8 mg L^{-1}). The hormones were added alone and in combination.

Data on number of roots per shoot, number of days to first root emergence and root length were recorded for six weeks. Rooted plantlets were subsequently rinsed free of culture medium and transferred to black polythene bags containing sterile top soil. These were maintained under shade for 40 days before they were transferred to the field.

Statistical analysis

Data were subjected to analysis of variance (ANOVA) using the GLM procedure of SAS (Statistical Analysis Software, 2008). Means were separated using Fisher's LSD at $p \leq 0.05$.

RESULTS AND DISCUSSION

Shoot regeneration

Experiments were carried out to determine the best medium composition for shoot regeneration from shoot-tip cultures under controlled environment. The results showed that all the medium solidification types supplemented with BAP promoted regeneration of shoot buds but the semi-liquid medium (1.0 g L^{-1} Gelrite) gave the best regeneration in terms of the average number of shoot buds produced per explant (2.4) and average shoot length (34.6 mm) (Table 1). The highest average shoot length (34.6 mm) was observed in the medium with 0.1 mg L^{-1} BAP and the highest average number of shoot buds (2.4) was observed in 0.3 mg L^{-1} BAP. This result agrees with previous report (Firoozabady and Gutterson, 2003), which shows that BAP (0.1 to 0.5 mg L^{-1}) added to MS medium is essential for the regeneration of plants from shoot apices of pineapple. Paiva et al. (1998) obtained best results in the shoot induction of pineapple, cv. Skay, with either 1.0 mg L^{-1} BAP or 0.1 mg L^{-1} TDZ. This is different from results of this study in which lower concentrations of BAP (0.1 and 0.3 mg L^{-1}) gave best results for shoot induction.

The differences between both results may be due to genotype effect. Al-Saif et al. (2011) reported that 2.0 mg L^{-1} BAP in solid medium (7.0 g L^{-1} solidified with agar) gave shoot length of 9.5 mm. Result of this study showed that lower concentrations of BAP (0.1 to 0.4 mg L^{-1}) in solid medium produced higher shoot length (20.9 to 29.9 mm) (Table 1). With the exception of 0.3 mg L^{-1} BAP, an increase in BAP concentrations from 0 to 0.4 mg L^{-1} in the solid medium (2.0 g L^{-1} gelrite) resulted in increased shoot length. This is not in agreement with report of Hamad and Taha (2009) who observed a decrease in shoot length as BAP concentration increased up to 0.5 mg L^{-1} in solid medium. The highest average number of shoot buds per explant and shoot length obtained in

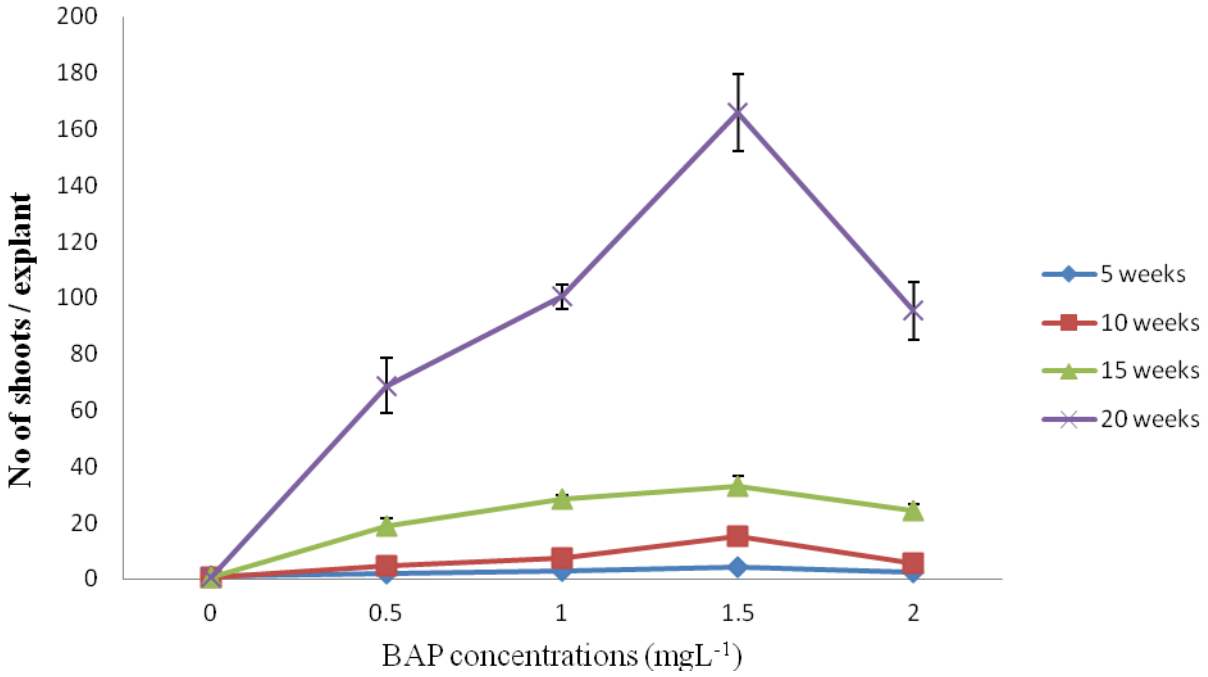

Figure 1. Effect of BAP concentrations on average shoot number of pineapple produced in a semi-liquid MS basal medium with vitamins ±SE.

semi-liquid medium (solidified with 1.0 g L^{-1} Gelrite) in this study maybe due to increased intake of the nutrients made possible by free movement of compounds or nutrients from the culture medium into the plant tissues. Gupta et al. (1981) showed that the use of liquid medium facilitates the nutrient uptake by the plant due to better distribution in the culture medium. Similar higher regeneration rates in liquid medium when compared with solid medium have been reported by Alvard et al. (1993).

Shoot proliferation

After the determination of the optimal Gelrite concentration for regeneration, the BAP concentration was increased from 0.5 to 2.0 mg L^{-1} with the aim of inducing more shoots in the semi-liquid medium (Figure 1). After five weeks of culture, the basal portions of pineapples consisting of proliferating buds (clumped explant) were observed to produce more shoots (Plate 1A and B). Medium supplemented with 1.5 mg L^{-1} BAP produced most average number of shoots (6.1) and this was significantly higher ($p \leq 0.05$) than that of other BAP concentrations evaluated (Figure 1). Zuraida et al. (2011) reported that explants cultured on liquid medium supplemented with 1.0 mg L^{-1} BAP produced the highest number of shoots (31) after 4 weeks when compared with 5 mg L^{-1} BAP which produced 14 shoots after four weeks of culture. This confirms the assertion that liquid culture explants usually display a higher frequency of growth rate when compared with solid or semi-liquid cultures (Gupta et al., 1981), and maybe as a result of continuous shaking of liquid cultures thus promoting increased intake of the nutrients in the medium. After a second sub-culture, a 3-fold increase in the average number of shoots (18.3) was recorded for 1.5 mg L^{-1} BAP at 10 weeks; and at 20 weeks a 28-fold increase was observed (167.6) (Figure 1, Plate 1C). Result of this study is similar to report of Almeida et al. (2002) in which MS medium supplemented with 1.5 mg L^{-1} BAP gave the best treatment at proliferation stage, producing an average of 701.1 shoots per explant in liquid medium after five subcultures. Hamad and Taha (2008) also reported that in 4 cycles of culture, shoot number was highest in BAP at 1.5 mg L^{-1}.

The proliferation induced by BAP corro-borates report of Kyte and Kleyn (1996) that a cytokinin is very crucial for cell division and axillary bud multiplication in plants. In this study, BAP (1.5 mg L^{-1}) alone produced average shoot number of 18.3 per clumped explant. This is higher than the average shoot number of 12.0 per explant observed in solid medium supplemented with BAP (2.0 mg L^{-1}) (Al-Saif et al., 2011); 9.0 shoots per explant obtained in response to 1.0 mg L^{-1} BAP (Be and Debergh, 2006). Barboza and Caldas (2001) used etiolated nodal segments for micropropagation of the pineapple hybrid PE X SC-52, and observed that BAP (2.0 mg L^{-1}) promoted the highest number of plants per

Plate 1. Different stages of *in vitro* propagation of smooth cayenne in semi-liquid medium. A = Regeneration stage with bud formation. B = Initial multiplication stage; C = advanced multiplication stage; D = rooting in half-strength MS basal medium with 0.9 NAA mg L^{-1}. E = rooting in half-strength MS basal medium without growth hormones.

shoot culture (10.4) and per nodal segment, when compared with kinetin (5.0 mg L^{-1}) or a combination of BAP (2.0 mg L^{-1}) and NAA (1.86 mg L^{-1}). Alvard et al. (1993) and George and Sherrington (1984) both reported higher rates of growth in liquid cultures when compared with solid medium. This may be attributed to the exposure to greater surface of the explants to a medium with uniform distribution of nutrients, and enhanced nutrient uptake. Medium containing BAP 1.0 mg L^{-1} which gave the second highest average number of shoots per explants (4.6) was observed to have a 2-fold increase in the average number of shoots per explant (9.5) after 10 weeks and a 25-fold increase in the average number of shoots (102.4) per explant after 20 weeks (Figure 1). Further increase in BAP concentration (2.0 mg L^{-1}) did not result in appreciable increase in the number of shoots. This may be due to inherent cytokinnin already present in the plant resulting to habituation decline.

Root induction

Medium with half-strength MS basal medium with vitamins (2.2 g L^{-1}) supplemented with 0.5 mg L^{-1} BAP + 0.9 mg L^{-1} NAA gave the lowest average days to root emergence (1.2) and the lowest average number of roots per shoot (approx. 0.3) (Table 2). This was followed by half- strength MS basal medium with vitamins supplemented with 0.9 mg L^{-1} NAA alone which gave average days to root emergence of (approximately 7.0) and the highest mean number of roots per shoot (approximately 7.9). There was no significant difference ($p \leq 0.05$) in

Table 2. Influence of MS strength and different growth regulator concentrations on root induction of pineapple cultured for 6 weeks[a].

MS strength	Plant growth regulators (mg L^{-1})		Average days to root emergence	Average no of roots/shoot	Average root length (mm)	Rooted plantlet (%)
	BAP	NAA				
Half strength	0.00	0.00	$7.33^{b} \pm 0.23$	$6.25^{b} \pm 0.29$	$29.3^{a} \pm 1.0$	$100^{a} \pm 0.0$
	0.00	0.90	$6.96^{b} \pm 0.19$	$7.88^{a} \pm 0.54$	$26.5^{b} \pm 0.8$	$100^{a} \pm 0.0$
	0.00	1.80	$7.46^{b} \pm 0.19$	$7.79^{a} \pm 0.40$	$28.7^{a} \pm 1.1$	$100^{a} \pm 0.0$
	0.50	0.00	-	-	-	-
	0.50	0.90	$1.17^{c} \pm 0.81$	$0.29^{d} \pm 0.20$	$1.0^{d} \pm 0.7$	$8^{c} \pm 0.06$
	0.50	1.80	-	-	-	-
Full strength	0.00	0.00	-	-	-	-
	0.00	0.90	-	-	-	-
	0.00	1.80	$10.71^{a} \pm 0.3$	3.46c ± 0.42	$6.4^{c} \pm 0.6$	$92^{b} \pm 0.06$
	0.50	0.00	-	-	-	-
	0.50	0.90	-	-	-	-
	0.50	1.80	-	-	-	-

[a]Means with same letter in the same column are not significantly different at $P \leq 0.05$ according to L.S.D.

the number of roots induced when NAA concentration was doubled from 0.9 NAA (7.9) to 1.80 mg L^{-1} NAA (7.8) in half strength MS medium. Contrary to result of this study, Hamad et al. (2013) reported that in liquid medium, shoots failed to form roots in half-strength MS basal medium containing NAA. Danso et al. (2008) reported that shoots cultured on 7.5 mg L^{-1} BAP and NAA concentrations (7.5 to 15.0 mg L^{-1}) did not result in any root formation in MD2 pineapple per shoot. Firoozabady and Gutterson (2003) obtained roots from liquid cultures of pineapple cultured on MS medium supplemented with 0.5 mg L^{-1} NAA and 0.5 mg L^{-1} IBA. NAA and IBA are root inducing growth regulators and have been used either alone or in combination for root induction in many cultures (Be and Debergh, 2006; Gupta et al., 1981). The average number of roots per shoot obtained with medium supplemented with 0.9 mg L^{-1} NAA (7.9) (Plate 1D) or 1.8 mg L^{-1} NAA (7.8) are higher than those obtained in medium supplemented with 1.0 mg L^{-1} IBA (5.0) (Khan et al, 2004); and when IBA was substituted by 1.0 mg L^{-1} NAA (3.6); this took between 8-15 days for induction of root.

The full or half-strength MS basal medium supplemented with 0.5 mg L^{-1} BAP did not produce any roots. This result agrees with the reports of Be and Debergh (2006), Danso et al. (2008) and Gupta et al. (1981) which showed that NAA, as an auxin, is important for root induction and regulation in plants. The half-strength MS basal medium containing no growth regulators had the highest root length (29.3 mm) (Table 2, Plate 1E) and the third highest mean number of roots (6.3). Almeida et al. (2002) also recorded success in rooting when shoots were transferred to MS medium with half the concentration of salts and no growth regulators for 30 days. The half-strength MS supplemented with 1.8 mg L^{-1} NAA gave the second highest average roots' length (28.7 mm, Table 2). Shoots cultured on half strength MS basal medium with vitamins and no growth regulators, 0.9 mg L^{-1} NAA or 1.8 mg L^{-1} NAA recorded 100% rooting. The full-strength MS medium with no growth hormones did not produce any root. Only full-strength MS supplemented with 1.8 mg L^{-1} NAA produced roots; the mean number of roots per explants and root lengths were 3.46 and 6.4 mm, respectively. These were significantly lower ($p \leq 0.05$) than results obtained from shoots cultured on half-strength MS medium.

Nearly all plantlets were successfully hardened in black polythene bags (survival rate of 85%) containing sterile top soil and placed under shade.

The results of this study demonstrates the efficient use of semi-liquid medium with low concentrations of BAP for *in vitro* regeneration and multiplication of pineapple, as well as the use of half-strength MS basal medium with vitamins and no growth hormones or low NAA concentration for root induction. The minimal use of materials and chemicals in tissue culturing of pineapple, directly translates to low cost of production. This economic approach to micro propagation enhances the availability and affordability of *in vitro* derived pineapple cv. smooth cayenne as quality planting materials to meet the ever increasing demand by farmers.

Conflict of interest

Authors declare that there is no conflict of interest.

whether financial or relevant interest that has influenced this study.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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