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Pitout JDD, Church DL, Gregson DB, Chow BL, McCracken M, Mulvey M, Laupland KB (2007). Molecular epidemiology of CTXM-producing Escherichia coli in the Calgary Health Region: emergence of CTX-M-15-producing isolates. Antimicrob. Agents Chemother. 51: 1281-1286.

Pelczar JR, Harley JP, Klein DA (1993). Microbiology: Concepts and Applications. McGraw-Hill Inc., New York, pp. 591-603.

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Full Length Research Paper

Complete inhibition of mycelial growth of fungal pathogens of maize by botanicals

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Rice husk extract (RHE), bamboo extract (BE) and wood extract (WE) at different concentration levels (0.0, 0.1, 0.5, 1.0 and 1.5%) were evaluated (in completely randomized design) *in vitro* as an antagonist to mycelial growth of selected fungal pathogens of maize. Botanical preparations were utilized singly (rice husk, bamboo and wood extracts) and in combinations (RHE x BE, RHE x WE, BE x WE and RHE x BE x WE). RHE at 1.0% concentration and in combination- RHE x BE x WE at 1.5% concentration, completely inhibited mycelial growth of *Fusarium solani, Fusarium equiseti, Fusarium verticilloides* and *Macrophomina phaseolina*. Other botanical preparations, either singly or in combinations showed significant ($p<0.05$) reduction in mycelial growth of the fungal pathogens. Thus, these botanicals have phytofungicidal potentials towards controlling pathogenic fungi of maize, hence, could be useful in the control and management of maize diseases on large scale farming.

Key words: Rice husk, bamboo, wood, extract, maize, mycelial growth, fungal pathogens.

INTRODUCTION

Maize (*Zea mays* L.) is one of the most important cereals in the world after wheat and rice with regards to cultivation area and total production (Akinbode, 2010). Diseases have been a major constraint to maize production, it reduce the value and quality of maize grains produced (Lamprecht et al., 2008) and may definitely increase the cost of harvesting. There are diverse diseases of maize consisting of seed rots and seedling blights (Crous et al., 2006), Northern corn leaf blight, Anthracnose, *Pythium* and *Fusarium* root rot and Southern rust (Gautam and Stein, 2011) among others. Various approaches have been used over many decades to control maize diseases such as breeding for resistance and chemical pesticides (Tagne et al., 2008).

The problems of chemical pesticides are resistance, pest resurgence, environmental pollution and risks to human health. Most of the pesticides and inorganic fertilizers are not environmentally friendly, apart from the

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fact that health hazards may loom as a result of the consumption of their residues in food, these agrochemicals are expensive and may not be available for farmers use when needed (Oyekanmi et al., 2008). In view of this, national and international bodies have raised a global call to promote maize production through biological approaches, being environmentally friendly and cost-effective (Abiala et al., 2011).

Biological control is on the increase but the use of natural bioprotectants like botanical extracts has not really received significant attention. Therefore, encouraging the use of botanical extracts as a promising alternative is a good step towards controlling and managing fungal pathogens of maize in Nigeria. Rice husk, bamboo and wood extracts have been used singly and reported to be effective on mycelial growth of *Mycosphaerella fijiensis* Morelet (Abiala et al., 2011). Similarly, rice husk extract alone was also reported by Killani et al. (2011) to be effective in the laboratory and on the field on pathogenic fungi isolated from rhizosphere soil of cowpea. To further establish the activities of rice husk, bamboo and wood extracts, we therefore focused this research work on *in vitro* effects of these botanical extracts (singly and in combination) on mycelial growth of fungal pathogens of maize prior to field application.

MATERIALS AND METHODS

Source of fungal pathogens and botanical extracts

Fusarium oxysporum, Fusarium solani and *Fusarium equiseti* were obtained from Plant Pathology Unit, Department of Botany, University of Ibadan, while *Macrophomina phaseolina*, *Curvularia lunata*, *Drechslera* sp., *Fusarium verticilloides* and *Bipolaris maydis* were obtained from the Plant Pathology Unit of Institutes of Agricultural Research and Training, Ibadan. The botanical extracts: rice husk, wood and bamboo were obtained from Dr. H. Kikuno of Plant Physiology Unit, International Institute of Tropical Agriculture (IITA) Ibadan.

Evaluation of botanical extracts

One liter potato dextrose (PD) agar (39 g/l) was prepared in media bottle and dispensed at varying volumes of 100, 99.9, 99.5, 99.0 and 98.5ml into 250-ml sterile conical flasks. The contents were sterilized in the autoclave at a temperature of 121°C for 15 min at 1.2 bars. After autoclaving, the medium was allowed to cool to the temperature of 45°C. Equal volumes of rice husk, bamboo, and wood extracts were utilized in different treatment combinations: Rice husk extract (RHE) x bamboo extract (BE), RHE x wood extract (WE), BE x WE and RHE x BE x WE. Thereafter, 0.0, 0.1, 0.5, 1.0 and 1.5 ml of each of the botanical extracts singly or in combinations were aseptically pipetted with a calibrated 250 ml pipette into sterilized PDA medium to represent concentration of 0, 0.1, 0.5, 1.0, and 1.5%, respectively. These were slowly mixed together by rolling each bottle in the palm to allow homogenous mixture of medium and the extract. Fifteen milliliters (15 ml) of this mixture was poured into 9 cm sterile disposable Petri dishes and allowed to solidify at room temperature inside the laminar flow hood. Mycelial discs of young actively growing cultures of each pathogen was cut separately with a sterile cork borer and inoculated at the center of already prepared plates containing the mixture (botanical extracts + medium) and the control plates (medium alone). The experiment was carried out in three replicates. The plates were incubated at 28±2°C and periodically observed in 3 day intervals for nine days to allow antagonist-pathogen interactions.

Data collection

Laboratory data were collected on the 3rd, 6th and 9th day. The mycelial growth diameter (cm) of each pathogen was measured and the percentage of growth inhibition was calculated according to Odebode et al. (2004) as follows:

$$\text{Growth inhibition (\%)} = \frac{(D_o - D_t) \times 100}{D_o}$$

Where D_o = Diameter of mycelial growth of fungal pathogen in the control plates; D_t = diameter of mycelial growth of fungal pathogen in the treatment plates.

Statistical analysis

All statistical analyses were performed using SAS System for Windows Version 9.1 (2009). The data collected were analyzed using the analysis of variance (ANOVA) procedures and the least significant difference test (LSD) at p=0.05 was used to compare treatment means for each parameter.

RESULTS

RHE significantly ($p<0.05$) reduced the mycelial growth of *F. verticillioides*, *M. phaseolina*, *F. equiseti* and *F. oxysporium* in comparison with the control. At day 3, RHE at 1.5% concentration was less effective on mycelial growth of *B. maydis* as compared to other fungal pathogens that were completely inhibited, though varied at days 6 and 9 with respect to mycelial growth of *C. lunata* (1.33 cm) and *Dreschlera* sp. (5.13 cm) (Table 1). BE was not effective on all the fungal pathogens at both 0.1 and 0.5% concentration levels. Furthermore, their mycelial growth at 0.5 and 1.0% were almost the same with their control at days 3, 6 and 9 of observation. The effect of BE on *C. lunata* was extremely discouraging at concentration levels of 0.5, 1.0 and 1.5%. BE was only effective at higher concentration (1.5%) on mycelial growth of *Dreschlera* sp., *F. verticillioides*, *M. phaseolina*, *F. equiseti* and *F. solani* (Table 2). Similarly, WE was not effective on the fungal pathogens at lower concentrations (0.1, 0.5 and 1.0%). The effect of WE at 1.0% was not different from that of 1.5% concentration. Observation at day 6 revealed that there was no comparative effect of WE on the fungal pathogens. Even at day 9, WE had no significant ($p<0.05$) effect on *B. maydis*, *C. lunata, Dreschlera* sp., *M. phaseolina*, *F. solani* or *F. oxysporium* (Table 3).

Table 1. Effect of rice husk extract on pathogenic fungi of maize.

Days	Treatment	Mycelial mean growth (cm)				
		Concentration (%)				
		0.0	0.1	0.5	1.0	1.5
Day 3	*Bipolaris maydis*	3.23±0.58^{a}	2.10±0.00^{b}	1.97±0.06^{b}	0.53±0.46^{c}	0.53±0.46^{c}
	Curvularia lunata	3.33±0.06^{a}	2.03±0.12^{b}	1.87±0.15^{c}	0.00±0.00^{d}	0.00±0.00^{d}
	Dreschlera sp.	4.23±0.55^{a}	3.80±0.20^{a}	2.50±0.10^{b}	1.67±0.15^{c}	0.00±0.00^{d}
	Fusarium verticilloides	1.37±0.35^{a}	1.00±0.10^{b}	0.90±0.00^{b}	0.00±0.00^{c}	0.00±0.00^{c}
	Macrophomina phaseolina	2.03±0.06^{a}	1.83±0.06^{a}	1.60±0.00^{c}	0.00±0.00^{d}	0.00±0.00^{d}
	Fusarium equiseti	2.27±0.12^{a}	1.80±0.10^{b}	1.50±0.00^{b}	0.30±0.52^{c}	0.00±0.00^{c}
	Fusarium solani	0.87±0.12^{a}	0.23±0.40^{b}	0.00±0.00^{b}	0.00±0.00^{b}	0.00±0.00^{b}
	Fusarium oxysporum	2.23±0.56^{a}	1.97±0.58^{b}	1.77±0.06^{c}	0.90±0.00^{d}	0.00±0.00^{e}
	LSD	0.42	0.30	0.12	0.44	0.28
Day 6	*Bipolaris maydis*	5.63±1.39^{a}	3.20±0.00^{b}	2.90±0.10^{bc}	1.63±0.55^{c}	1.63±0.55^{c}
	Curvularia lunata	6.80±0.10^{a}	3.80±0.20^{b}	3.80±0.20^{b}	0.53±0.46^{c}	0.53±0.46^{c}
	Dreschlera sp.	7.97±0.42^{a}	7.67±0.32^{a}	5.27±0.12^{b}	4.17±0.12^{b}	2.27±0.15^{c}
	Fusarium verticilloides	4.90±0.10^{a}	3.47±0.06^{b}	1.77±0.21^{c}	0.00±0.00^{d}	0.00±0.00^{d}
	Macrophomina phaseolina	3.67±0.58^{a}	3.83±0.06^{b}	3.40±0.00^{c}	0.00±0.00^{d}	0.00±0.00^{d}
	Fusarium equiseti	4.37±0.06^{a}	3.23±0.23^{b}	2.73±0.12^{c}	1.83±0.21^{d}	0.00±0.00^{e}
	Fusarium solani	2.20±0.26^{a}	1.93±0.15^{a}	0.60±0.52^{b}	0.00±0.00^{c}	0.00±0.00^{c}
	Fusarium oxysporum	4.9±0.10^{a}	4.03±0.06^{a}	3.67±0.15^{b}	2.40±0.1.^{c}	0.00±0.00^{d}
	LSD	0.91	0.29	0.39	0.47	0.31
Day 9	*Bipolaris maydis*	7.13±0.35^{a}	4.83±0.06^{b}	4.83±0.47^{b}	2.70±0.78^{b}	2.70±0.78^{b}
	Curvularia lunata	8.50±0.00^{a}	6.27±0.21^{b}	4.83±0.47^{c}	1.03±0.06^{d}	1.33±0.49^{d}
	Dreschlera sp.	8.40±0.17^{a}	8.03±0.15^{a}	8.00±0.10^{a}	6.37±0.47^{b}	5.13±0.15^{c}
	Fusarium verticilloides	8.07±0.15^{a}	6.67±0.12^{b}	5.03±0.15^{c}	0.00±0.00^{d}	0.00±0.00^{d}
	Macrophomina phaseolina	6.10±0.20^{a}	5.93±0.06^{d}	5.47±0.06^{b}	0.00±0.00^{c}	0.00±0.00^{c}
	Fusarium equiseti	7.77±0.06^{a}	5.33±0.23^{b}	4.43±0.12^{c}	3.43±0.25^{d}	0.00±0.00^{e}
	Fusarium solani	3.07±0.12^{a}	2.80±0.44^{a}	2.27±0.35^{b}	0.00±0.00^{c}	0.00±0.00^{c}
	Fusarium oxysporum	8.07±0.15^{a}	7.70±0.15^{b}	6.50±0.10^{c}	4.97±0.15^{d}	0.00±0.00^{e}
	LSD	0.31	0.36	0.49	0.59	0.57

Data are presented as means for three replicates, followed by standard deviation within replicates. Values followed by the same letters in rows are not significantly different at P = 0.05. LSD = least significant difference.

Considering combinations of botanical extracts, there was no complementary effect of RHE x BE at 0.1 and 0.5% levels on mycelial growth of *M. phaseolina*, *F. equiseti* and *F. solani* in comparison with *B. maydis* and other fungal pathogens. At day 6, a clear distinction on the effectiveness of RHE x BE at 0.5 and 1.0% concentration with respect to mycelial growth of all the fungal pathogens was observed. Further observation showed that at day 9, RHE x BE at 1.5% concentration completely inhibited the mycelial growth of *C. lunata*, *F. verticillioides*, *F. equiseti* and *F. oxysporium* (Table 4). Activity of RHE x WE significantly ($p<0.05$) varied on the fungal pathogens most especially at 0.1, 0.5 and 1.0% concentration. RHE x WE at 1.5% concentration, completely inhibited the mycelial growth of all the fungal pathogens with the exception of *F. solani*. Observation also showed that, RHE x WE at 1.0% concentration consistently maintained complete mycelial growth inhibition of *Dreschlera* sp. and *F. equiseti* from days 3 to 9 (Table 5). The effect of BE x WE on *C. lunata* were significantly ($p<0.05$) similar at concentrations of 0.5, 1.0 and 1.5%. Observation at day 6 and 9 showed that *F. oxysporium* defiled BE x WE at all the concentration levels. However, an outstanding mycelial growth reduction was recorded for *C. lunata* (3.40 cm) as compared to the control (7.30 cm) (Table 6). The effect of RHE *x* BE *x* WE on the fungal pathogens was highly encouraging most especially at 1.0 and 1.5% concentration levels.

Table 2. Effect of bamboo extract on pathogenic fungi of maize.

Days	Treatment	Mycelial mean growth (cm)				
		Concentration (%)				
		0.0	0.1	0.5	1.0	1.5
Day 3	*Bipolaris maydis*	3.83±0.06^{a}	2.90±0.10^{b}	2.80±0.17^{b}	2.27±0.12^{c}	2.08±0.06^{d}
	Curvularia lunata	2.67±0.15^{a}	2.47±0.06^{b}	2.47±0.06^{b}	2.33±0.06^{bc}	2.23±0.06^{c}
	Dreschlera sp.	2.63±0.58^{a}	2.30±0.20^{b}	2.27±0.15^{b}	1.63±0.25^{c}	1.23±0.06^{d}
	Fusarium verticilloides	1.93±0.57^{a}	1.80±0.10^{b}	1.70±0.00^{b}	1.47±0.06^{c}	1.23±0.06^{d}
	Macrophomina phaseolina	2.40±0.10^{a}	2.33±0.21^{a}	2.00±0.06^{b}	1.87±0.06^{b}	1.53±0.21^{c}
	Fusarium equiseti	2.10±0.10^{a}	1.90±0.10^{b}	1.63±0.06^{c}	1.17±0.06^{d}	1.00±0.10^{e}
	Fusarium solani	2.33±0.56^{a}	2.37±0.06^{a}	2.33±0.21^{a}	2.01±0.20^{a}	1.53±0.12^{b}
	Fusarium oxysporum	2.87±0.06^{a}	2.63±0.06^{b}	2.60±0.00^{b}	2.47±0.12^{c}	2.23±0.06^{d}
	LSD	0.15	0.21	0.19	0.23	0.18
Day 6	*Bipolaris maydis*	5.70±0.17^{a}	5.07±0.25^{b}	4.73±0.12^{c}	4.43±0.06^{d}	3.60±0.10^{e}
	Curvularia lunata	4.70±0.20^{a}	4.77±0.15^{a}	4.03±0.12^{b}	3.97±0.12^{b}	3.97±0.15^{b}
	Dreschlera sp.	4.90±0.10^{a}	4.47±0.15^{b}	4.33±0.21^{b}	2.77±0.25^{c}	2.80±0.17^{c}
	Fusarium verticilloides	4.27±0.12^{a}	4.10±0.10^{ab}	3.80±0.34^{bc}	3.47±0.15^{c}	2.80±0.10^{d}
	Macrophomina phaseolina	4.57±0.21^{a}	4.47±0.25^{a}	4.30±0.87^{a}	4.17±0.06^{a}	3.73±0.12^{b}
	Fusarium equiseti	3.35±0.15^{a}	3.20±0.20^{ab}	2.97±0.06^{b}	2.43±0.12^{c}	1.90±0.30^{d}
	Fusarium solani	4.63±0.06^{a}	4.67±0.21^{a}	4.33±0.21^{ab}	4.27±0.15^{b}	2.83±0.21^{c}
	Fusarium oxysporum	4.93±0.06^{a}	4.83±0.06^{a}	4.60±0.00^{b}	4.37±0.12^{c}	3.97±0.15^{d}
	LSD	0.25	0.32	0.61	0.24	0.30
Day 9	*Bipolaris maydis*	7.87±0.25^{a}	7.10±0.17^{b}	6.80±0.10^{c}	6.13±0.00^{d}	2.57±0.15^{e}
	Curvularia lunata	6.40±0.10^{a}	6.43±0.49^{a}	5.57±0.15^{b}	5.37±0.12^{b}	5.23±0.06^{b}
	Dreschlera sp.	7.10±0.20^{a}	6.80±0.17^{a}	6.43±0.12^{b}	5.07±0.31^{c}	4.77±0.12^{c}
	Fusarium verticilloides	7.00±0.10^{a}	6.80±0.10^{a}	6.10±0.95^{ab}	6.07±0.49^{ab}	2.27±0.21^{b}
	Macrophomina phaseolina	7.80±0.20^{a}	7.57±0.25^{ab}	7.00±0.52^{bc}	6.63±0.06^{bc}	6.53±0.12^{c}
	Fusarium equiseti	5.80±0.10^{a}	5.63±0.15^{a}	5.43±0.06^{a}	4.43±0.12^{b}	3.10±0.50^{c}
	Fusarium solani	6.93±0.06^{a}	6.83±2.51^{a}	6.43±0.12^{b}	6.20±0.20^{b}	4.97±0.15^{c}
	Fusarium oxysporum	6.97±0.12^{a}	6.80±0.10^{b}	6.60±0.00^{c}	6.03±0.06^{d}	5.83±0.12^{e}
	LSD	0.27	0.42	0.86	0.46	0.38

Data are presented as means for three replicates, followed by standard deviation within replicates. Values followed by the same letters in rows are not significantly different at P = 0.05. LSD = least significant difference.

RHE x BE x WE completely inhibited all the fungal pathogens at both the lower (0.1 and 0.5%) and higher (1.0 and 1.5%) levels of concentration throughout the days of observation (Table 7).

Generally, the concentration levels (0.1, 0.5, 1.0 and 1.5%) of the botanical extracts were significantly ($p<0.05$) effective on the fungal pathogens in order of effectiveness RHE > BE > WE. Complete mycelial inhibition was recorded for RHE at 1.5% concentrations on *M. phaseolina*, *F. solani* and *F. verticillioides* (Table 8) and justified that RHE alone and in combinations: RHE x BE, RHE x WE and RHE x BE x WE were observed as the best botanical extracts.

DISCUSSION

Eco-friendly approaches for plant disease management have been exploited worldwide as observed in this study. The bio-assay test at different levels of concentration (0.1, 0.5, 1.0 and 1.5%) is not far-fetched from a number of reports showing the efficacy of botanical extracts (Joshi et al., 2011; Manasathein et al., 2011). This may as well be similar to the report of Odebode et al. (2004) that plants are known to produce a variety of secondary metabolites, which are bioactive and thus may have inhibitory effects on bacteria, fungi, insects and other microorganisms. The effectiveness of the botanical extracts was observed to be dependent on the concentration used. This agreed with the work of Anamika and Simon (2011) that showed botanical extracts were effective at higher concentrations on *Alternaria alternata* of *Aloe vera* dry rot.

The rice husk extract alone showed inhibitory effect on mycelial growth of the fungal pathogens even at low

Table 3. Effect of wood extract on pathogenic fungi of maize.

Days	Treatment	Mycelial mean growth (cm)				
		Concentration (%)				
		0.0	0.1	0.5	1.0	1.5
Day 3	*Bipolaris maydis*	3.13±0.06^{a}	3.10±0.10^{a}	2.57±0.06^{b}	2.47±0.06^{b}	2.23±0.06^{c}
	Curvularia lunata	2.97±0.15^{a}	2.90±0.10^{ab}	2.63±0.15^{bc}	2.43±0.21^{c}	2.07±0.21^{d}
	Dreschlera sp.	2.83±0.06^{a}	2.77±0.15^{a}	2.70±0.26^{a}	2.37±0.23^{b}	2.00±0.10^{c}
	Fusarium verticilloides	2.37±0.15^{a}	2.43±0.06^{a}	2.43±0.06^{a}	1.97±0.23^{b}	1.67±0.21^{c}
	Macrophomina phaseolina	2.63±0.06^{a}	2.53±0.15^{ab}	2.43±0.06^{b}	2.07±0.06^{c}	1.90±0.10^{c}
	Fusarium equiseti	2.33±0.06^{a}	2.17±0.06^{a}	2.17±0.06^{a}	1.90±0.10^{a}	0.70±0.66^{b}
	Fusarium solani	2.50±0.00^{a}	2.43±0.06^{a}	2.43±0.06^{a}	2.10±0.10^{b}	2.03±0.06^{b}
	Fusarium oxysporum	2.43±0.06^{a}	2.40±0.00^{b}	2.17±0.06^{b}	2.00±0.10^{c}	1.80±0.10^{d}
	LSD	0.15	0.17	0.20	0.26	0.44
Day 6	*Bipolaris maydis*	5.60±0.17^{a}	5.53±0.25^{a}	4.83±0.06^{b}	4.73±0.12^{b}	4.37±0.12^{c}
	Curvularia lunata	5.00±0.20^{a}	4.87±0.32^{ab}	4.77±0.15^{ab}	4.73±0.38^{ab}	4.37±0.32^{b}
	Dreschlera sp.	5.00±0.10^{a}	4.93±0.15^{a}	4.73±0.31^{ab}	4.47±0.23^{b}	4.07±0.15^{c}
	Fusarium verticilloides	5.93±0.21^{a}	4.20±0.00^{ab}	4.77±0.06^{b}	4.33±0.29^{c}	3.63±0.15^{d}
	Macrophomina phaseolina	5.57±0.12^{a}	5.47±0.21^{a}	5.37±0.06^{a}	4.80±0.00^{b}	4.20±0.30^{c}
	Fusarium equiseti	4.30±0.10^{a}	4.03±0.06^{a}	4.04±0.06^{a}	3.80±0.20^{a}	2.43±1.16^{b}
	Fusarium solani	5.90±0.00^{a}	4.47±0.21^{b}	5.37±0.06^{b}	4.37±0.12^{c}	3.90±0.10^{d}
	Fusarium oxysporum	5.27±0.12^{a}	5.20±0.00^{a}	4.97±0.06^{a}	4.03±0.55^{b}	3.30±0.10^{c}
	LSD	0.24	0.32	0.23	0.49	0.78
Day 9	*Bipolaris maydis*	7.70±0.17^{a}	7.70±0.20^{a}	6.87±0.06^{b}	6.43±0.12^{c}	6.03±0.06^{d}
	Curvularia lunata	7.23±0.31^{a}	7.17±0.32^{a}	7.10±0.17^{a}	7.07±0.25^{a}	6.07±0.84^{b}
	Dreschlera sp.	7.03±0.25^{a}	6.93±0.15^{a}	6.90±0.10^{a}	6.17±0.15^{b}	5.97±0.15^{b}
	Fusarium verticilloides	7.33±0.21^{a}	7.53±0.06^{a}	6.90±0.10^{b}	6.13±0.12^{c}	5.50±0.10^{d}
	Macrophomina phaseolina	7.87±0.06^{a}	7.67±0.21^{ab}	7.57±0.06^{b}	6.63±0.06^{c}	5.87±0.25^{d}
	Fusarium equiseti	7.57±0.49^{a}	6.67±0.12^{b}	6.53±0.06^{b}	5.77±0.15^{c}	4.57±0.61^{d}
	Fusarium solani	8.00±0.00^{a}	7.67±0.21^{b}	7.57±0.06^{b}	6.33±0.21^{c}	5.73±0.21^{d}
	Fusarium oxysporum	7.63±0.15^{a}	7.53±0.06^{a}	7.13±0.12^{a}	6.46±0.67^{b}	5.40±0.10^{c}
	LSD	0.43	0.32	0.17	0.48	0.68

Data are presented as means for three replicates, followed by standard deviation within replicates. Values followed by the same letters in rows are not significantly different at P = 0.05. LSD = Least Significant Difference.

Table 4. Combined effect of rice husk and bamboo extract on pathogenic fungi of maize.

Days	Treatment	Mycelial mean growth (cm)				
		Concentration (%)				
		0.0	0.1	0.5	1.0	1.5
Day 3	*Bipolaris maydis*	3.20±0.00^{a}	2.80±0.00^{b}	2.43±0.06^{c}	1.27±0.06^{d}	0.00±0.00^{e}
	Curvularia lunata	2.93±0.12^{a}	2.67±0.12^{a}	1.83±0.40^{b}	0.00±0.00^{c}	0.00±0.00^{c}
	Dreschlera sp.	3.03±0.25^{a}	2.90±0.17^{a}	2.10±0.10^{b}	1.47±0.15^{c}	0.00±0.00^{d}
	Fusarium verticilloides	2.80±0.10^{a}	2.87±0.12^{a}	1.73±0.38^{b}	0.00±0.00^{c}	0.00±0.00^{c}
	Macrophomina phaseolina	2.20±0.10^{a}	2.20±0.10^{a}	2.17±0.06^{a}	1.63±0.06^{b}	0.97±0.15^{c}
	Fusarium equiseti	2.17±0.06^{a}	2.13±0.12^{a}	1.77±0.15^{a}	0.27±0.46^{b}	0.00±0.00^{b}
	Fusarium solani	2.23±0.38^{a}	2.10±0.00^{a}	2.03±0.15^{a}	1.13±0.11^{b}	0.00±0.00^{c}
	Fusarium oxysporum	2.23±0.06^{a}	2.10±0.10^{b}	2.00±0.00^{b}	0.00±0.00^{c}	0.00±0.00^{c}
	LSD	0.30	0.18	0.37	0.31	0.09

Table 4. Contd.

Day 6	*Bipolaris maydis*	5.47±0.06^{a}	5.27±0.12^{a}	4.37±0.31^{b}	2.93±0.12^{c}	0.00±0.00^{d}
	Curvularia lunata	3.97±0.25^{a}	4.27±0.12^{a}	3.73±0.15^{b}	0.00±0.00^{c}	0.00±0.00^{c}
	Dreschlera sp.	5.23±0.25^{a}	5.13±0.32^{a}	4.20±0.10^{b}	3.43±0.15^{c}	0.67±0.61^{d}
	Fusarium verticilloides	6.10±0.26^{a}	5.70±0.17^{a}	4.67±0.15^{b}	1.40±0.69^{c}	0.00±0.00^{d}
	Macrophomina phaseolina	5.57±0.06^{a}	5.30±0.26^{ab}	4.97±0.25^{b}	4.10±0.10^{c}	3.17±0.15^{d}
	Fusarium equiseti	5.67±0.06^{a}	5.33±0.06^{a}	4.47±0.06^{b}	2.47±0.72^{c}	0.00±0.00^{d}
	Fusarium solani	5.97±0.55^{a}	5.33±0.67^{ab}	5.07±0.12^{b}	3.70±0.26^{c}	2.43±0.40^{d}
	Fusarium oxysporum	5.53±0.06^{a}	5.03±0.06^{b}	5.00±0.00^{b}	0.00±0.00^{c}	0.00±0.00^{c}
	LSD	0.44	0.50	0.26	0.65	0.46
Day 9	*Bipolaris maydis*	7.33±0.21^{a}	7.50±0.10^{a}	6.83±0.12^{a}	5.47±0.38^{b}	1.63±1.42^{c}
	Curvularia lunata	7.80±0.40^{a}	7.63±0.06^{a}	7.00±0.17^{b}	0.00±0.00^{c}	0.00±0.00^{c}
	Dreschlera sp.	7.30±0.20^{a}	6.87±0.45^{ab}	6.20±0.20^{b}	6.20±0.36^{b}	2.57±0.95^{c}
	Fusarium verticilloides	7.47±0.49^{a}	7.60±0.10^{a}	6.80±0.10^{a}	2.93±0.93^{b}	0.00±0.00^{c}
	Macrophomina phaseolina	7.37±0.06^{a}	7.30±0.10^{a}	6.87±0.32^{b}	6.07±0.12^{c}	4.90±0.20^{d}
	Fusarium equiseti	7.27±0.32^{a}	7.00±0.17^{a}	6.70±0.62^{a}	3.93±1.35^{b}	0.00±0.00^{c}
	Fusarium solani	7.67±0.72^{a}	7.37±0.67^{a}	7.30±0.26^{a}	5.93±0.42^{b}	4.37±0.50^{c}
	Fusarium oxysporum	7.30±0.10^{a}	6.70±0.10^{a}	6.60±0.10^{a}	1.17±1.01^{b}	0.00±0.00^{c}
	LSD	0.65	0.52	0.50	1.25	1.11

Data are presented as means for three replicates, followed by standard deviation within replicates. Values followed by the same letters in rows are not significantly different at P = 0.05. LSD = least significant difference.

Table 5. Combined effect of rice husk and wood extract on pathogenic fungi of maize.

Days	Treatment	Mycelial mean growth (cm)				
		Concentration (%)				
		0.0	**0.1**	**0.5**	**1.0**	**1.5**
Day 3	*Bipolaris maydis*	2.47±0.06^{a}	2.27±0.06^{a}	2.17±0.15^{b}	1.23±0.06^{b}	0.00±0.00^{c}
	Curvularia lunata	2.53±0.12^{a}	2.40±0.00^{a}	2.30±0.00^{a}	0.73±0.64^{b}	0.00±0.00^{c}
	Dreschlera sp.	2.63±0.06^{a}	2.23±0.12^{b}	2.23±0.06^{b}	0.00±0.00^{c}	0.00±0.00^{c}
	Fusarium verticilloides	2.17±2.07^{a}	2.07±0.06^{b}	0.00±0.00^{c}	0.00±0.00^{c}	0.00±0.00^{c}
	Macrophomina phaseolina	2.67±0.06^{a}	2.40±0.00^{b}	1.70±0.00^{c}	1.47±0.06^{d}	0.00±0.00^{e}
	Fusarium equiseti	3.17±0.15^{a}	2.83±0.1^{b}	0.30±0.52^{c}	0.00±0.00^{c}	0.00±0.00^{c}
	Fusarium solani	2.80±2.47^{a}	2.47±0.25^{b}	2.20±0.10^{c}	2.03±0.06^{c}	1.00±0.10^{d}
	Fusarium oxysporum	2.83±0.0^{a}	2.53±0.06^{a}	2.30±0.20^{a}	0.77±0.67^{b}	0.00±0.00^{c}
	LSD	0.15	0.21	0.36	0.57	0.06
Day 6	*Bipolaris maydis*	5.03±0.06^{a}	3.77±0.15^{b}	3.43±0.06^{c}	2.23±0.12^{d}	0.00±0.00^{e}
	Curvularia lunata	5.17±0.15^{a}	4.50±0.00^{b}	4.40±0.00^{b}	2.77±0.68^{c}	1.37±0.06^{d}
	Dreschlera sp.	5.43±0.06^{a}	4.87±0.06^{b}	4.57±0.12^{c}	0.00±0.00^{d}	0.00±0.00^{d}
	Fusarium verticilloides	4.67±0.15^{a}	4.40±0.10^{a}	2.27±0.21^{b}	1.50±0.87^{c}	0.00±0.00^{c}
	Macrophomina phaseolina	5.43±0.32^{a}	4.90±0.00^{b}	3.90±0.00^{c}	3.57±0.06^{d}	0.00±0.00^{e}
	Fusarium equiseti	5.83±0.32^{a}	5.37±0.15^{b}	1.63±0.32^{c}	0.00±0.00^{d}	0.00±0.00^{d}
	Fusarium solani	4.93±0.32^{a}	4.70±0.26^{a}	4.13±0.15^{b}	4.00±0.10^{b}	2.07±0.21^{c}
	Fusarium oxysporum	5.43±0.06^{a}	5.46±0.06^{a}	5.03±0.15^{a}	2.87±1.01^{b}	2.67±0.06^{b}
	LSD	0.34	0.22	0.28	0.92	0.14
Day 9	*Bipolaris maydis*	6.87±0.12^{a}	5.30±0.10^{b}	4.87±0.12^{c}	4.33±0.12^{d}	0.00±0.00^{e}
	Curvularia lunata	6.97±0.15^{a}	6.30±0.00^{b}	5.80±0.00^{c}	4.03±0.31^{d}	3.40±0.10^{e}
	Dreschlera sp.	7.83±0.06^{a}	6.73±0.12^{b}	6.07±0.12^{c}	0.00±0.00^{d}	0.00±0.00^{d}

Table 5. Cont.

Fusarium verticilloides	6.90±0.10[a]	6.47±0.06[a]	1.73±0.23[b]	0.90±1.01[b]	0.00±0.00[c]
Macrophomina phaseolina	7.10±0.10[a]	6.50±0.00[b]	5.43±0.06[c]	4.37±0.12[d]	0.00±0.00[e]
Fusarium equiseti	7.83±0.21[a]	7.10±0.26[b]	2.57±0.38[c]	0.00±0.00[d]	0.00±0.00[d]
Fusarium solani	7.00±0.10[a]	6.20±0.26[b]	5.73±0.21[c]	5.47±0.12[c]	2.93±0.35[d]
Fusarium oxysporum	7.10±0.10[a]	6.80±0.10[a]	6.30±0.20[a]	4.30±0.95[b]	4.33±0.12[b]
LSD	0.21	0.26	0.34	0.88	0.23

Data are presented as means for three replicates, followed by standard deviation within replicates. Values followed by the same letters in rows are not significantly different at P = 0.05. LSD = least significant difference.

Table 6. Combined effect of bamboo and wood extract on pathogenic fungi of maize.

Days	Treatment	Mycelial mean growth (cm)				
		Concentration (%)				
		0.0	**0.1**	**0.5**	**1.0**	**1.5**
Day 3	*Bipolaris maydis*	2.60±0.10[a]	2.56±0.15[a]	1.90±0.10[a]	1.43±0.12[b]	1.73±0.12[c]
	Curvularia lunata	2.43±0.12[a]	2.27±0.12[a]	1.50±0.17[b]	1.33±0.15[b]	1.36±0.06[b]
	Dreschlera sp.	3.47±0.12[a]	3.20±0.20[a]	2.37±0.06[b]	2.20±0.10[bc]	2.07±0.21[c]
	Fusarium verticilloides	3.73±0.12[a]	3.60±0.10[a]	2.33±0.21[b]	1.90±0.00[c]	1.17±0.12[d]
	Macrophomina phaseolina	2.30±0.10[a]	2.20±0.00[a]	2.80±0.20[b]	2.67±0.25[b]	1.77±0.06[c]
	Fusarium equiseti	2.80±0.10[a]	2.50±0.10[b]	2.40±0.10[b]	2.40±0.00[b]	1.43±0.06[c]
	Fusarium solani	2.60±0.10[a]	2.47±0.15[a]	2.10±0.10[b]	2.00±0.10[b]	1.73±0.12[c]
	Fusarium oxysporum	2.73±0.21[a]	2.57±0.12[a]	3.17±1.42[a]	3.20±1.48[a]	1.93±0.06[b]
	LSD	0.21	0.22	0.89	0.93	0.19
Day 6	*Bipolaris maydis*	4.70±0.10[a]	4.63±0.46[a]	3.90±0.30[b]	3.73±0.12[b]	2.90±0.20[c]
	Curvularia lunata	4.93±0.12[a]	3.73±0.01[a]	3.70±0.17[b]	3.33±0.15[b]	3.67±0.06[b]
	Dreschlera sp.	5.90±0.00[a]	5.87±0.90[b]	5.23±0.12[c]	5.07±0.15[c]	4.13±0.25[cd]
	Fusarium verticilloides	5.53±0.12[a]	4.57±1.01[a]	4.90±0.26[a]	4.33±0.15[ab]	3.03±0.15[c]
	Macrophomina phaseolina	5.53±0.06[a]	5.37±0.15[a]	5.17±0.21[a]	4.50±0.30[b]	3.80±0.44[c]
	Fusarium equiseti	5.70±0.10[a]	5.45±0.25[a]	5.37±0.06[a]	5.37±0.06[a]	3.20±0.26[b]
	Fusarium solani	5.27±0.06[a]	5.23±0.06[a]	4.90±0.26[ab]	4.73±0.23[bc]	4.47±0.25[c]
	Fusarium oxysporum	5.77±0.15[a]	5.67±0.83[a]	5.13±0.85[a]	5.48±0.96[a]	4.47±0.25[a]
	LSD	0.17	1.20	0.62	0.66	0.44
Day 9	*Bipolaris maydis*	6.90±0.10[a]	6.93±0.12[a]	5.87±0.31[b]	5.53±0.31[c]	5.43±0.15[c]
	Curvularia lunata	7.30±0.17[a]	5.63±0.15[b]	5.47±0.12[b]	5.13±0.15[c]	3.40±0.10[c]
	Dreschlera sp.	7.90±0.00[a]	6.57±0.25[b]	6.23±0.12[d]	6.90±0.36[b]	6.00±0.10[d]
	Fusarium verticilloides	5.57±0.06[c]	6.67±0.12[a]	6.27±0.23[b]	5.90±0.17[d]	4.97±0.15[e]
	Macrophomina phaseolina	7.23±0.12[a]	7.23±0.21[a]	7.03±0.15[ab]	6.43±0.15[b]	4.93±0.67[c]
	Fusarium equiseti	7.43±0.12[a]	7.33±0.12[a]	6.97±0.40[a]	7.10±0.10[a]	4.37±0.40[b]
	Fusarium solani	7.00±0.10[a]	6.97±0.06[a]	6.27±0.23[b]	6.00±0.02[b]	6.07±025[b]
	Fusarium oxysporum	7.40±0.10[a]	6.63±1.03[a]	6.37±0.95[a]	7.03±0.68[a]	6.03±0.38[a]
	LSD	0.18	0.68	0.70	0.48	0.57

Data are presented as means for three replicates, followed by standard deviation within replicates. Values followed by the same letters in rows are not significantly different at P = 0.05. LSD = least significant difference.

concentration. Similar observation of rice husk extract has been reported by Abiala et al. (2011) on *Mycosphaerella fijensis*. This suggests that the rice husk extract may contain some acidic compounds (Killani et al., 2011)

Table 7. Combined effect of rice husk, bamboo and wood extract on pathogenic fungi of maize.

Days	Treatment	Mycelial mean growth (cm)				
		Concentration (%)				
		0.0	0.1	0.5	1.0	1.5
Day 3	*Bipolaris maydis*	2.47±0.06^{a}	2.33±0.06^{b}	2.03±0.12^{c}	1.53±0.06^{d}	0.00±0.00^{e}
	Curvularia lunata	2.60±0.10^{a}	2.20±0.00^{b}	1.57±0.06^{c}	0.00±0.00^{d}	0.00±0.00^{d}
	Dreschlera sp.	2.53±0.12^{a}	2.13±0.12^{b}	1.90±0.10^{b}	0.17±2.25^{c}	0.00±0.00^{d}
	Fusarium verticilloides	2.33±0.06^{a}	2.06±0.12^{a}	1.73±0.31^{a}	0.83±0.76^{b}	0.00±0.00^{c}
	Macrophomina phaseolina	2.07±0.31^{a}	1.80±0.20^{a}	1.47±0.12^{b}	0.00±0.00^{c}	0.00±0.00^{c}
	Fusarium equiseti	2.93±0.12^{a}	2.67±0.12^{a}	1.70±0.20^{b}	0.83±0.72^{c}	0.00±0.00^{d}
	Fusarium solani	2.47±0.06^{a}	2.07±0.06^{b}	1.83±0.06^{c}	0.00±0.00^{d}	0.00±0.00^{d}
	Fusarium oxysporum	2.57±0.15^{a}	2.57±0.15^{a}	2.17±0.12^{b}	0.00±0.00^{c}	0.00±0.00^{c}
	LSD	0.25	0.19	0.27	0.66	0.00
Day 6	*Bipolaris maydis*	4.73±0.21^{a}	4.37±0.21^{b}	3.80±0.26^{c}	2.70±0.17^{d}	0.00±0.00^{e}
	Curvularia lunata	5.47±0.12^{a}	4.90±0.00^{b}	3.73±0.12^{c}	0.30±0.52^{d}	0.00±0.00^{d}
	Dreschlera sp.	5.10±0.17^{a}	4.67±0.12^{b}	3.90±0.10^{c}	3.30±0.36^{d}	0.00±0.00^{e}
	Fusarium verticilloides	3.53±0.06^{a}	3.27±0.15^{a}	2.97±0.15^{b}	2.03±0.25^{c}	0.00±0.00^{d}
	Macrophomina phaseolina	4.10±0.26^{a}	3.70±0.20^{a}	3.93±0.15^{a}	0.27±0.46^{b}	0.00±0.00^{b}
	Fusarium equiseti	4.60±0.10^{a}	4.23±0.15^{a}	2.63±0.60^{b}	1.87±0.06^{c}	0.00±0.00^{d}
	Fusarium solani	5.53±0.15^{a}	4.07±0.15^{b}	3.33±0.06^{c}	0.00±0.00^{d}	0.00±0.00^{d}
	Fusarium oxysporum	4.67±0.15^{a}	3.60±0.44^{b}	3.30±0.43^{b}	1.73±0.23^{c}	0.00±0.00^{d}
	LSD	0.28	0.37	0.51	0.53	0.00
Day 9	*Bipolaris maydis*	7.10±0.26^{a}	6.87±0.12^{a}	5.80±0.30^{d}	4.13±0.15^{c}	0.00±0.00^{d}
	Curvularia lunata	7.20±0.17^{a}	6.97±0.06^{a}	5.60±0.00^{b}	1.40±0.70^{c}	0.00±0.00^{d}
	Dreschlera sp.	7.20±0.17^{a}	6.17±0.12^{b}	5.93±0.12^{b}	5.00±0.36^{c}	0.00±0.00^{d}
	Fusarium verticilloides	6.00±0.10^{a}	5.77±0.15^{a}	5.40±0.20^{b}	2.97±0.31^{c}	0.00±0.00^{d}
	Macrophomina phaseolina	7.10±0.20^{a}	6.47±0.15^{a}	5.77±0.21^{a}	1.03±1.79^{b}	0.00±0.00^{b}
	Fusarium equiseti	6..73±0.15^{a}	6.40±0.20^{a}	4.03±0.90^{b}	2.83±0.12^{c}	0.00±0.00^{d}
	Fusarium solani	6.33±0.12^{a}	5.80±0.17^{b}	5.50±0.10^{c}	1.63±0.15^{d}	0.00±0.00^{e}
	Fusarium oxysporum	7.10±0.10^{a}	6.57±0.25^{b}	5.90±0.10^{c}	2.47±0.38^{d}	0.00±0.00^{e}
	LSD	0.29	0.28	0.62	1.24	0.00

Data are presented as means for three replicates, followed by standard deviation within replicates. Values followed by the same letters in rows are not significantly different at P = 0.05. LSD = least significant difference.

as reported by Yoshida et al. (2000) that it contains pyroligeneous acid which is the bioactive secondary metabolite that has inhibitory effects on fungal pathogens. The significant effect of the rice husk extract on different fungal pathogens is therefore an indication that the mechanisms of interaction of the pyroligeneous acid and the fungal pathogens should be given attention.

Bamboo and wood extracts showed the least inhibitory effects on all the fungal pathogens even at 1.5% concentration. This indicates that the effect of the botanical extracts as a phytofungicide also depends on the concentration levels used on the pathogenic fungi. Abiala et al. (2011) reported that bamboo and wood extracts completely inhibited mycelial growth of *Mycosphaerella fijensis* at higher (5%) concentration level as compared to mycelial growth reduction at low (1.5 and 2.5%) concentration levels. Correlating this with our study suggests that, increase in concentration levels may likely increase the effectiveness of bamboo and wood extracts on mycelial growth of fungal pathogens of maize. Also, the significant differences and variation in the effectiveness of bamboo and wood extracts may be as a result of their unknown active ingredient. This is in agreement with Maobe et al. (2013) that evaluated eight medicinal plants and proposed that the crude extracts may contain lots of phytochemical compounds that may be responsible for their effect on the clinical pathogens. With respect to this, the inhibitory activity of botanical extracts may vary with the virulence of the pathogens and most likely with the chemical components of the botanicals. This also conforms to the work of Odebode et al. (2004) carried out on two annonaceous plants *Isolana cualifora* verdc and

Table 8. Mean percentage growth inhibition of fungal pathogens at 1.5% concentration (day 9).

Pathogenic fungi	RHE	BE	WE	RHE *x* BE	RHE *x* WE	BE *x* WE	RHE *x* BE *x* WE
B. maydis	42.00	22.04	16.45	77.73	100.0	21.26	100.0
C. lunata	56.62	16.16	4.60	100.0	51.99	55.42	100.0
Dreschlera sp.	24.20	28.63	11.89	64.84	100.0	24.05	100.0
F. verticilloides	100.0	13.33	16.36	100.0	100.0	10.78	100.0
M. phaseolina	100.0	15.00	13.98	39.49	100.0	31.80	100.0
F. equiseti	55.80	23.57	16.02	100.0	100.0	41.25	100.0
F. solani	100.0	13.07	20.88	43.04	58.10	14.29	100.0
F. oxysporum	38.43	13.41	15.28	100.0	38.97	18.47	100.0

Cleistochlamys krikii Berth (Oliv), the crude extract and pure compounds isolated from both plants inhibited both bacterial and fungal pathogens.

Evaluation of botanicals individually has been the norm in plant pathology with respect to biological control of plant diseases. We hypothesized in this study that the significant effect of botanical extracts in their combinations on pathogenic fungi of maize is possible and may likely perform far better than individually. The combination of RHE x BE x WE, RHE x BE and RHE x WE extracts significantly inhibited the mycelial growth of the pathogenic fungi. Although, increase in concentration levels favours BE x WE combination. This conforms to the report of Webster et al. (2008) that crude extracts are generally a mixture of active and non-active compounds (crude fusions) and therefore higher minimum inhibitory concentration are expected. Observed variation of antifungal activities of these botanical extract combinations suggests that there may be differences in the nature and chemical composition of the plants.

The *in vitro* antifungal properties of the extract either singly or in combinations reveal its efficacy in the control of at least one of the pathogenic fungi. The rice husk extract at low concentration levels performed excellently well, followed by bamboo extract while the least was wood extract. The complete mycelial growth inhibition observed in this study may likely correlate with what is expected *in vivo*. Taking advantage of these botanical extracts most especially in their combinations will be of significant importance to sustainable crop production and thus, support ecofriendly-based agricultural management systems. Therefore, proper management coupled with good formulations of these botanicals will be of significant effect for total elimination of these fungal pathogens on maize field.

Conflict of interest

The authors did not declare any conflict of interest.

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Full Length Research Paper

Genetic analysis of groundnut rosette virus disease in groundnut (*Archis hypogaea* L.)

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Groundnut chlorotic rosette disease (GCRD) transmitted by the aphid, *Aphis craccivora*, is an important virus disease of groundnut in Africa. Breeding for host resistance remains the best strategy to minimize losses due to this disease. Nine cultivated groundnut genotypes with differential reaction to GCRD were crossed in an incomplete diallel mating design to determine the combining ability of GCRD resistance. The parents and 36 F_2 populations were inoculated with veruliferous *A. craccivora* at the seedling stage and evaluated for disease reaction at two locations in Nigeria in 2012. Disease incidence (based on visual symptoms) was recorded three times at fortnightly interval using area under disease progress curve. General combining ability (GCA) and specific combining ability (SCA) effects for GCRD resistance were highly significant ($P < 0.01$), indicating that both additive and non-additive gene effects governed the inheritance of GCRD resistance. The Baker ratio was low (0.3) for GCRD indicating that non-additive gene effects was more important than additive gene effects in controlling GCRD resistance in these crosses. As a result, progeny performance could not be adequately predicted from GCA effects alone. Therefore, effective selection of superior genotypes would be achieved at advanced generations when maximum homozygosity is attained.

Key words: Groundnut chlorotic rosette disease, area under disease progress curve, combining ability, additive and non-additive gene effects.

INTRODUCTION

Groundnut (*Arachis hypogaea* L.), is cultivated annually on about 24.63 million hectares worldwide with annual production of 41.27 million tons in shell with a productivity of about 1.85 t ha^{-1} (FAO, 2012). It is highly adapted to tropical and subtropical climates of the world and cultivated in nearly 100 countries. It is a key crop for small scale farmers especially in Africa and Asia where the crop serve as a valuable source of dietary protein, oil, and fodder for livestock. It contains 48-50% oil and 26-28% protein, and is a rich source of dietary fibre, minerals, and vitamins (Janila et al., 2013). In addition, groundnut has the ability to fix atmospheric nitrogen to

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the soil to help in the maintenance of soil fertility. In West Africa, Nigeria is the largest producer of groundnuts with a production of 3.07 million tons on about2.4 million hectares (FAO, 2012). Despite the economic, social and cultural importance of groundnuts, its productivity is severely constrained by several biotic and abiotic factors. Among them groundnut chloroticrosette disease (GCRD) causes severe crop losses The most serious yield losses were reported during the year 1975 when an epidemic in northern Nigeria destroyed approximately 0.7 million hectares of groundnut, with an estimated loss of US$250 million (Yayock et al., 1976).The GCRD is characterised by small, chlorotic, twisted and distorted leaflets with shortened internodes and thickened stems. Affected plants especially those infected at a young stage are severely stunted (Bock et al., 1990). The disease also affects both quality of the haulm and the pod.

Three agents *Groundnut rosette assistor virus* (GRAV), *Groundnut rosette virus* (GRV) and a Satellite-RNA (Sat-RNA) (Reddy et al., 1985; Murant et al., 1988; Taliansky et al., 2000) contribute to the etiology. The intimate interaction between GRAV, GRV, and sat-RNA is crucial to the development of the disease.

Host resistance is the most cost effective and environmentally friendly method to minimize losses due to GCRD. Several resistant varieties have been developed in West Africa Resistance to GCRD is not immunity and succumbs to high inoculum pressure and adverse environmental conditions (Bock et al., 1990). Breeding for resistance to groundnut rosette disease demands a good knowledge of the breeding methodologies as well as a good understanding of the disease and its causal organisms. Identification of sources of resistance and its efficient utilization require an understanding of the genetic control of resistance and knowledge of the amount of genetic variability available for selection. Determining the suitable parents to use for the development of resistant genotype is particularly important.Early genetic studies on groundnut rosette disease showed that resistance was effective against GRV and its sat-RNA and was governed by two independent recessive genes (de Berchoux, 1960). He also stated that resistant lines were not immune and that individual plants could become infected when subjected to inoculation by massive number of aphids. This resistance was reported to operate equally against both chlorotic rosette (de Berchoux, 1960) and green rosette (Harkness, 1977). He attributed the low recovery of resistant plants from Virginia x Spanish crosses to heavy inoculum pressure at an early stage of growth and suggested occurrence resistance breakdown from generation to generation. Bock and Nigam (1988) studied the inheritance of resistance to chlorotic rosette (GRV and its sat-RNA) in crosses involving botanical varieties of groundnut from Malawi and confirmed the findings of de Berchoux (1960) of two recessive genes governing the resistance in all the backgrounds. In resistant plants, the presence of GRAV was detected. Gene conferring resistance to GRV and its sat-RNA did not confer resistance to GRAV (Bock and Nigam, 1988; Bock *et al.,* 1990). Similar findings on the inheritance of resistance to green rosette using mixed infection in the field (GRV + and its sat-RNA + GRAV) and single GRV infection under greenhouse conditions were reported from Nigeria by Olorunju *et al.* (1992). There was an exception from the RMP12 x M124.781 crosses, where in F_2 generation, the plant segregated into 1 susceptible: 3 resistant, suggesting dominant gene action governing rosette resistance (Olorunju et al., 1992).Amin (1985) reported a high level of resistance to *A. cracivora* in some crosses under greenhouse conditions. Progenies of *A. chacoense* and *A. villas* interspecific derivatives with cultivated groundnut also showed high resistance to *A. crracivora*. Resistance to aphid vector identified in cultivated groundnut ICG 5240 [EC36892] (Padgham et al., 1990) was reported to be controlled by single a recessive gene (van de Merwe, 2001; Herselman et al., 2004).

Breeders have largely used the diallel mating scheme to estimate the potential value of genotypes, and their combining ability effects for resistance to foliar disease in groundnut from a fixed or randomly chosen set of parental lines (Adamu et al., 2008). The studies of combining ability provide a guideline for selecting elite parents or combiners which may later be hybridized to accumulate fixable genes through selection. Both SCA and GCA have been reported to be significant in conditioning resistance to foliar disease in groundnut (Vishnuvardhan et al., 2011). Pensuk et al. (2002), from a 6 x 6 diallel cross of resistance to peanut bud necrosis tospovirus (PBNV) reported highly significant GCA effects for PBNV incidence in F_2 and F_3 generations. SCA was also significant, but the relative contribution to variation among crosses was lesser than those of GCA effects. In an earlier study, Anderson et al. (1990) reported significant GCA and SCA effects for peanut stripe virus (PStV) and rust incidence from a study of diallel in groundnut. Makne (1992) found significant SCA for seed weight per plant, number of pods per plant and pod weight per plant and concluded that these traits were controlled by a non-additive gene action. Adamu et al. (2008) recommended that selection for pod yield and resistance to groundnut rosette disease should be done among progenies from RMP12/ICGV87281 and RMP12/ICGV87018 since they showed best general combiners for these traits. He also suggested that the significance of SCA mean squares for some of the traits is an indication that non-additive gene effects played an important role in their inheritance. SCA mean square was much smaller than GCA mean squares, which indicated that additive genetic variance was more important than non-additive genetic variance for these traits. Studies on combining ability in F_2 and F_3 crosses of Spanish and Virginia groundnut have shown that GCA and SCA were significant for almost all traits (Ali et al., 2001) with

Table 1. Pedigree, source, description and characteristics of parental genotypes used for population development.

Genotype	Pedigree	Source‡	Description
ICGX – SM 00020/5/9	ICG 12991 x ICGV-SM 95713	ICRISAT	Resistant to *A craccivora;* early maturing
ICGX – SM 00017/5/P_{10}/P_1	ICG 12991 x ICGV-SM 99529	ICRISAT	Resistant to *A craccivora;* early maturing
ICGX – SM 00020/5/P_4/P_1	ICG 12991 x ICGV-SM 99574	ICRISAT	Resistant to *A craccivora;* early maturing
ICGV IS 07890	ICG 12991 x ICGV-SM 95603	ICRISAT	Resistant to *GCRD;* early maturing
ICGV IS 07899	ICG 12991 x ICGV-SM 95603	ICRISAT	Resistant to *GCRD;* early maturing
ICIAR-19BT	KH 241D/ICGV 87922	ICRISAT	Resistant to *GCRD;* early maturing
SAMNUT 14	55 – 437 ex –Dakar	IAR	Susceptible to GCRD; late maturing
KWANKWASO	Local collection	SAMARU	Susceptible to GCRD; late maturing
MANIPENTA	Local collection	SAMARU	Susceptible to GCRD; late maturing

‡ICRISAT; IAR = Institute for Agricultural Research, Samaru, Zaria. Authors: for the" source "is give an asterisk.

preponderance of SCA which implies that selection for pod yield would be more effective in later generations. However, greater magnitude of GCA effect over SCA has been reported indicating the importance of additive genetic variance over non-additive variance. The mating designs have been used extensively to study the genetics of resistance to viral diseases in wheat, such as wheat soil borne mosaic virus, Barley yellow dwarf virus and wheat streak mosaic virus (Dubey et al., 1970; Cisar et al., 1982; Hakizimana et al., 2004).

From the available reports, it is evident that information on the precise nature of genetic control of GCRD in groundnut is still lacking. Appropriate experimental design that includes the GRD resistant lines should provide additional information on the gene action involved in the expression of resistance. The knowledge on combining ability and type of gene action responsible for regulation of expression of GCRD would certainly help in planning for appropriate breeding strategies. The objective of this study was to determine the mode of inheritance of resistance to GCRD.

MATERIALS AND METHODS

Population development and phenotype evaluation

The study involved the use of nine experimental lines comprising of three aphid resistant (ICGX – SM 00020/5/9, ICGX – SM 00017/5/P_{10}/P_1 and ICGX – SM 00020/5/P_4/P_1 and three GCRD resistant (ICGV IS 07890, ICGV IS 07899 and ICIAR-19BT) genotypes (these genotypes were previously evaluated for three years (2008 – 2010) at the Institute for Agricultural Research (IAR), Ahmadu Bello University (ABU),Zaria, Nigeria and were confirmed to have field resistance to aphids and GCRD)obtained from the International Crop Research Institute for Tropical Agriculture (ICRISAT) inMali. Three widely cultivated varieties (SAMNUT14, KWANKWASO, and MANIPENTA) were also included as parents in the population development. The pedigree descriptions of the nine genotypes are presented in Table 1. The genotypes were manually cross-pollinated in a half diallel mating scheme at the screen house of IAR, SamaruIAR) Samaru, (11°10.00"N and 7°38.00" E, 693 m), and ABU in 2011. Additional manual cross-pollinations were made at IAR during the 2011 rainy season. Seed limitations for multi-location evaluation were overcome by advancing F_1 seeds to next generation (F_2) as suggested by (Hallauer et al., 2008).

The nine parental lines along with the 36 F_2 progenies were evaluated for GCRD resistance using a 9 x 5 α − lattice design with two replications at two locations (Samaru, Kaduna state, and Lafia, Nasarawa state (8°32"N, 7°42"E) during the 2011/2012 growing seasons using an infector – row techniques (susceptible SAMNUT 14 genotype was planted in alternate rows with test materials) as described by Olorunju et al. (2001) at the two locations. The infector rows were planted 2 weeks prior to the test materials to allow the build-up of inoculum. Two row plots of 4.0 m in length with inter and intra-row spacing of 0.75 m x 0.25 m, respectively, were used.

Aphid and GCRD resistance evaluation

A. craccivora colonies were collected from infested cowpea *Vigna unguiculata* L., and groundnut *A hypogaea* plants at different locations in groundnut producing area in Nigeria to cover the different isolates that may be present in the country. The colonies (presumed to be viruliferous) were each maintained on susceptible groundnut genotype SAMNUT 14 in a screen house.

Two wingless (apterae) aphids were transferred onto 7 to 14-day-old seedlings of nine parental lines and their 36 F_2 progenies grown at IAR. Each genotype was observed for the presence or absence of aphid colonies (adults as well as nymphs) 7 days after infestation. Plants with no aphid colonies were re-infested with aphids seven days after the first infestation. It is rare to find plants without aphids in choice tests because the aphids are free to roam to find suitable plant hosts. Aphids that appeared to be transient, possibly probing for feeding sites, are often observed on resistant plants in choice tests, along with dead aphids. Sometimes several viviparous aptera, surrounded by a few nymphs, were observed on resistant plants without the development of established colonies. Rate of aphid infestation was evaluated using 0-4 scale, combined with GCRD incidencedeveloped by Mensah et al. (2005, 2008). Aphid infestation three time (at forthright interval) by a 0 = No aphid, 0.5 = fewer than 10 aphids per plant, no colony formed, 1.0 =11–100 aphids per plant, plants appear healthy, 1.5 = 101–150 aphids per plant, plants appear healthy, 2.0 = 151–300 aphids per plant, mostly on the young leaves or tender stems, plants appear healthy, 2.5 = 301–500 aphids per plant, plants appear healthy, 3.5 = 501–800 aphids per plant, young leaves and tender stems are covered with aphids, leaves appear slightly curly and shiny, 3.5 = more than 800 aphids per plant, plants appear stunted, leaves appear curled and slightly yellow, no sooty mould and few cast skins and 4.0 = more than 800 aphids per plant, plants appear stunted, leaves

appear severely curled and yellow and are covered with sooty mould and cast skins. An aphid damage index (DI) for each line was calculated by the following formula: DI = ∑ (scale value x no. of plants in the category)/ (4 x total no. of plants) x 100. The DI ranges between 0 for no infestation and 100 for the most severe damage (Mensah et al., 2005). The DI was used as an indicator of aphid resistance and was applied in the analysis. The disease severity was recorded as the amount of plant tissue that is diseased, chlorotic rosette.

Reaction to GCRD was recorded on a scale of 1 to 9 as described by GGP (2000) as follows: 1 = No apparent rosette symptoms, 3 = 10 – 20% rosette symptoms, 5 = 20 to 60% rosette symptoms, 7 = 60 – 80% rosette symptoms and 9 = 100% rosette symptoms. The results of these observations were transformed to compute infection responses as measured by area under disease progressive curve (AUDPC) based on Moldovan et al. (2005).

following thefunction: AUDPC = $\sum_{i=1}^{n-1}\left(\frac{y_i + y_{i+1}}{2}\right)\left(t_i + 1 - t_i\right)$

Where: y = disease severity at the i^{th} observation (transformed), t = time (days) of i^{th} observation, n = Total number of assessment time.

Agronomic data such as pod weight per plant (g), and sound kernel weight per plant (g) were measured on five randomly selected plants per plot.

Data analysis

Genetic analysis of resistance to GCRD

Analysis of the diallel for general combining ability (GCA)andspecific combiningability (SCA) for all traits were based on the Model I, method 2 proposed by Griffing (1956). Parents and one set of F_2's, excluding reciprocal F_2's, were included in the analysis combining abilities.

Trait values were predicted based on traits mean value to produce a balanced data set. Diallel data were analysed using the Diallel SAS-05 program (Zhang et al., 2005). GCA and SCA effects were determined for parents and the 36F_2's, respectively. The following linear mixed model was fitted to data to estimate variance components for single and multi-location diallel tests. The model for the analysis of variance for single location was: $Y_{ijk} = \mu + r_k + g_i + g_j + s_{ij} + e_{ijk}$ where μ is the mean, r_k is the replication effect, g_i and g_j are the GCA effects, s_{ij} is the SCA effect, and e_{ijk} is the experimental error for the Y_{ijk} observation (k =1…… 36, r = 2, i = j = 9.

Model for the analysis of variance for multi-location was:

$$Y_{ijklm} = \mu + L_i + r_{j(i)} + GCA_k + GCA_l + SCA_{kl} + L*GCA_{ik} + L*GCA_{il} + L*SCA_{ikl} + E_{ijklm}$$

Where, Y_{ijklm} is the m^{th} observation of the j^{th} replication for k^{th} cross in i^{th} location, μ is the overall mean, L the i^{th} fixed (location) effect, , $r_{j(i)}$ the fixed effect of the j^{th} replication within the i^{th} location, $j = 1-2$, GCA_k, GCA_l, was the random GCA effect of the k^{th} female or the i^{th} male ~Normally Independently Distributed (NID) (0, σ_G^2), SCA_{kl} was the random specific combining ability (SCA) effect of the k^{th} and the i^{th} parents~ (NID) (0, σ_S^2), $L*GCA_{ij}$ was the random GCA by location Interaction effect ~ (NID) (0, σ_{IG}^2), $L*SCA_{ikl}$ was the random SCA by location Interaction effect ~ (NID) (0, σ_{IS}^2) and E_{ijklm} was the randomerror term ~ (NID) (0, σ_E^2).

The variance explained by the general combining ability effects of parents (half-sibs) was half of a additive genetic variance that is $\sigma_{GCA}^2 = 1/2 * \sigma_A^2$ while the variance explained by the female and male interactions (specific combining ability) was equal to dominance genetic variance that is dominance genetic variance $\sigma_{SCA}^2 = \sigma_D^2$. Phenotypic variance was the sum of the observational components of variance

$$\sigma_{Ph}^2 = 2 * \sigma_{GCA}^2 + \sigma_{SCA}^2 + \sigma_E^2$$

Baker's ratio

Prediction of progeny performance based on GCA and SCA is carried out by the use of Baker's ratio, which was the ratio of combining ability variance component described by Baker (1978) as follows: $\left[(2\sigma_{GCA}^2)/(2\sigma_{GCA}^2 + \sigma_{SCA}^2)\right]$. The closer this ratio was to unity, the greater the predictability based on GCA alone.

RESULTS

General and specific combining ability for the traits

For the traits studied, the mean square (MS) values for both GCA and SCA were significant (P < 0.05) (Table 2). The SCA mean squares were higher than the GCA mean squares for DI and AUDPC (Table 2). The magnitude of GCA x location interaction for the majority of traits were relatively small compare to the GCA mean squares. Significant SCA × location interactions (p < 0.05) were observed for all traits. The GCA and SCA variance components were significantly different from zero for all the traits (Table 3). To understand the relative importance of general and specific combining abilities for DI and AUDPC, estimates of components of GCA and SCA that approximates variances estimated according to Bakers ratio (Baker, 1978) indicates a ratio of closer to unity (0.73) for aphid damage index and low value was

Table 2. Mean squares of combined ANOVA for half 9 x 9 diallel analysis for general and specific combining abilities and their interactions with location for ten morphological traits of groundnut evaluated at two locations in 2012.

Source of variation	Df	PWPT	SKWPT	DI	AUDPC
GCA	8	4578.12**	2132.30**	5197.60**	165.90**
SCA	36	1194.09**	282.54**	578.97*	652.03**
GCA x Location	8	478.98**	67.13**	530.68**	440.04**
SCA x Location	36	459.05**	10.50**	239.59**	67.57**
ERROR	88	997.02	698.01	2670.79	190.74

*and ** significant at P < 0.05 and P < 0.01 levels of probability, respectively. PWPT= Pod weight per Plant (g); SKWPT= Sound kernel weight per plant (g); DI = aphid damage Index; AUDPC = area under disease progress curve.

Table 3. Variance component for GCA, SCA and their interactions with location, Bakers ratio, additive and dominance variances considering random effect model for 9 parents and 36 F_2 evaluated across Samaru and Lafia Locations in 2012.

Trait	Variance components of F_2					Bakers ratio		
	σ^2_E	σ^2_{GCA}	σ^2_{SCA}	σ^2_{GCA} x L	σ^2_{SCA} x L		(σ^2_A)	(σ^2_D)
Pod weight per plant (g)	22.41	241.72	585.84	467.775	447.845	0.45	483.43	585.84
Sound kernel weight per plant (g)	11.57	132.13	135.49	61.345	4.715	0.66	264.25	135.49
Aphid damage Index	55.26	329.90	261.86	503.05	211.96	0.72	659.80	261.86
AUDPC	5.87	68.13	323.08	437.105	64.64	0.30	136.27	323.08

obtained for AUDPC (0.30) (Table 3). The estimates of $\hat{\sigma}^2_A$ and $\hat{\sigma}^2_D$ showed that greater proportions of total genetic variance were attributed to non – additive (that is dominance and epistasis) for AUDPC (with $\hat{\sigma}^2_A$ = 136.27 and $\hat{\sigma}^2_D$ = 323.08).

Partitioning of genotypes into genetic effects indicated significant (p < 0.01) GCA and SCA effects for all the traits. The GCA effects for AUDPC ranged from – 10.61 for ICGX - SM 00020/5/9 to 5.44 in MANIPENTA (Table 5). Genotypes with the lowest desirable negative GCA effects were ICGX – SM 00020/5/9 (-10.61), SAMNUT14 (-2.04), ICGV IS 07890 (-1.58) and ICGX – SM 00017/5/P_{10}/P_1 (-1.41).The highest GCA effects for this trait were exhibited by KWANKWASO (5.44) and MANIPENTA (3.58) which were the most susceptible genotypes in this study. The GCA effect for sound kernel weight per plant (g) was highest in ICGV IS 07890 (5.74) and lowest in MANIPENTA (- 9.25). The parents, ICIAR-19BT (4.69), ICGX - SM 00020/5/9 (3.78) and ICGX - SM 00017/5/P_{10}/P_1 (3.10) depicted significantly high positive GCA effects.

The specific combining ability effects for AUDPC ranged from -15.65 to 35.96. Most crosses revealed positive SCA effect, 13 out of 36 crosses (36.11 %) had negative SCA effects (Table 5). The F_2 combinations, ICGX - SM 00017/5/P_{10}/P_1 X ICIAR-19BT (-15.65) had the best desirable negative SCA effects. Other crosses with desirable negative and significant SCA effects for this trait includes ICGX – SM 00020/5/P_4/P_1 X SAMNUT 14 (-9.66), ICGV IS 07899 X SAMNUT 14 (–8.73) and ICGX - SM 00017/5/P_{10}/P_1 X ICGV IS 07890 (–8.1). In contrast, ICGV IS 07890 X ICGV IS 07899 (-7.64), ICGX - SM 00020/5/9 X MANIPENTA (-7.22) and ICGX – SM 00020/5/9 X SAMNUT 14 (-5.52) had high negative but not significant SCA effects. The greatest SCA effect (35.96) was recorded for ICGX - SM 00020/5/P_4/P_1 X MANIPENTA.

Other crosses depicting significantly positive SCA effects includes ICGX – SM 00020/5/P_4/P_1XICGV IS 07890 (12.56), ICGV IS 07890XMANIPENTA (15.48), ICGX - SM 00017/5/P_{10}/P1XKWANKWASO (15.84) andICGX - SM 00017/5/P10/P1XSAMNUT 14(18.61) (Table 4).

The SCA effects for sound kernel weight per plant (g) was highest in ICGV IS 07890 X SAMNUT 14 (19.03) and lowest in ICGX – SM 00020/5/P_4/P_1 XICGV IS 07890 (- 16.07) (data not shown). The other parents that recorded the significantly high SCA effects were ICGV IS 07899XICIAR-19BT (15.17), SAMNUT14 X KWANKWASO (13.94), ICIAR-19BT X SAMNUT14 (11.55), ICGX - SM 00020/5/9 X ICGV IS 07899 (7.88), ICGX - SM 00020/5/9XICGX - SM 00020/5/P_4/P_1 (7.74), ICGX - SM 00020/5/9XICGV IS 07890 (7.22) and ICGX - SM 00017/5/P_{10}/P_1 X ICIAR-19BT (6.75). Whereas

Table 4. Estimates of general combining ability (GCA) effects of 9 parental lines for four important morphological characters of groundnut evaluated across Samaru and Lafia Locations in 2012.

Parents	PWPT	SKWPT	DI	AUDPC
ICGX – SM 00020/5/9	1.22	3.78**	-4.77**	-10.61**
ICGX – SM 00017/5/P_{10}/P_1	5.74**	3.10**	-15.33**	-1.41
ICGX – SM 00020/5/P_4/P_1	4.72**	2.77**	-4.66**	2.53**
ICGV IS 07890	12.64**	5.74**	3.50**	-1.58
ICGV IS 07899	3.96**	0.39	-2.55*	2.20
ICIAR-19BT	-0.45	4.69**	-1.69	1.89
SAMNUT 14	-6.69**	-4.68**	2.20	-2.04
KWANKWASO	-12.62**	-6.54**	11.73**	3.58**
MANIPENTA	-8.50**	-9.25**	11.57**	5.44**
SE±	1.13	0.59	1.12	1.41

*, **Significant at P < 0.05 and P < 0.01 levels of probability respectively.

ICGX – SM 00020/5/9 X ICGX – SM 00017/5/P_{10}/P_1 (-10.64), ICGV IS 07890 X ICGV IS 07899 (-13.84) and ICGX – SM 00020/5/P_4/P_1 X ICGV IS 07890 (-16.07) had the lowest SCA effects for sound kernel yield per plant (data not shown). However, the F_2s that combined significant and desirable SCA effects for SKWPT and AUDPC were ICGX – SM 00017/5/P_{10}/P_1XICIAR-19BT (6.75, –15.67), ICGX – SM 00020/5/9XICGX – SM 00020/5/P_4/P_1 (7.74, –2.77), ICGX – SM 00020/5/P_4/P_1XSAMNUT 14 (6.00, – 9.66) and ICGV IS 07899XKWANKWASO (3.75, –2.10).

DISCUSSION

Both GCA and SCA made significant and important contribution to progeny variation for DI and AUDPC. All the parents of the most GCRD-resistant crosses; ICGX – SM 00017/5/P_{10}/P_1, ICIAR-19BT, ICGX – SM 00020/5/9 and ICGX – SM 00020/5/P_4/P_1 had appreciable resistance and also favourable GCA resistance values. This suggests that, although resistance to GCRD tends to be at least partly dominant (Clements et al., 2004), optimal resistance in progenies will require crossing parental genotypes that are both GCRD resistant, supporting the findings of Loffler et al. (2011) and Hung and Holland (2012). Therefore, selection for resistance should not be confined to a single group but should be performed in parallel in all groups. The study showed that crosses between two susceptible genotypes resulted in progenies with susceptibility to GCRD. In contrast, Loffler et al. (2011) observed that hybrids often had more disease than their parental inbreds, perhaps because they used highly susceptible tester lines and higher inoculum pressure for hybrids than parents. SCA as well as GCA were important for evaluating resistance of the progeny. Significant SCA detected in 13 of 36 possible combinations indicated the presence of non-additive gene effect. Significant SCA effects were observed for the combinations ICGX – SM 00017/5/P_{10}/P_1 X ICIAR-19BT (aphid resistant//rosette resistant), ICGX – SM 00020/5/9 X ICGX – SM 00020/5/P_4/P_1 (aphid resistant//aphid resistant), ICGX – SM 00020/5/P_4/P_1 X SAMNUT 14 (aphid resistant//rosette susceptible) and ICGV IS 07899 X KWANKWASO ((aphid resistant//rosette susceptible). These results indicate resistance of these progenies was higher than would be expected from average of their expected parents based on AUDPC symptom rating. The largest positive SCA effects correspond to ICGX – SM 00020/5/P4/P_1 X MANIPENTA. This combination was more susceptible than predicted average parent performance indicating the importance of non-additive gene effect in this particular cross. Kenga et al. (2004) suggest that the difficulty in predicting the resistance level of the hybrid, on the basis of GCA alone should necessitate testing of specific male-female combinations. The SCA values provide important information about the performance of the hybrid relative to its parents. Arunga et al. (2010) found that the SCA effect alone has limited value for parental choice in breeding programs. The authors suggested that *per se* performance of the lines, SCA and GCA effects should be considered in selecting desirable parents in a breeding programme which is desired by any breeder. Furthermore, it was observed that crosses involving one good combiner and one average or poor combiner showed negative SCA effects. For example, MANIPENTA and KWANKWASO had poor GCA values for GRD resistance, while their crosses with ICGV IS 07899 and ICGX – SM 00020/5/9, respectively, had significant and desirable SCA effects. This is in agreement with Habarurema et al. (2012) which made similar conclusion in a study on bacterial blight ((*Xanthomonas oryzae* pv.*oryzae*) in rice.

The combining ability ratio, also known as Baker's ratio, for resistance to GCRD observed in this study was less than unity. According to Baker (1978), when combining ability ratio approaches unity, GCA alone cannot predict

Table 5. Estimates of specific combining ability (SCA) effects measured in the 36 F2 progenies evaluated across Samaru and Lafia Locations in 2012.

Cross	PWPT	SKWPT	DI	AUDPC
ICGX – SM 00020/5/9 X ICGX – SM 00017/5/P10/P1	-5.66	-10.64**	2.59	-4.82
ICGX – SM 00020/5/9 X ICGX – SM 00020/5/P4/P1	12.30**	7.74**	2.84	-2.77
ICGX – SM 00020/5/9 X ICGV IS 07890	15.50**	7.22**	-8.39**	9.53**
ICGX – SM 00020/5/9 X ICGV IS 07899	-6.85**	7.88**	-5.86	1.74
ICGX – SM 00020/5/9 X ICIAR-19BT	-2.97	4.62**	12.98**	2.79
ICGX – SM 00020/5/9 X SAMNUT 14	0.70	-7.29**	1.09	-5.52
ICGX – SM 00020/5/9 X KWANKWASO	-0.93	-4.74**	1.50	-1.57
ICGX – SM 00020/5/9 X MANIPENTA	1.43	2.17	7.62	-7.22
ICGX – SM 00017/5/P10/P1 X ICGX – SM 00020/5/P4/P1	-7.85	3.69	-0.73	2.81
ICGX – SM 00017/5/P10/P1 X ICGV IS 07890	2.94	-4.75**	10.06**	-8.10
ICGX – SM 00017/5/P10/P1 X ICGV IS 07899	-0.31	-3.35	1.44	5.87
ICGX – SM 00017/5/P10/P1 X ICIAR-19BT	-1.86	6.75**	-1.97	-15.65**
ICGX – SM 00017/5/P10/P1 X SAMNUT 14	0.44	2.37	-4.02	18.61**
ICGX – SM 00017/5/P10/P1 X KWANKWASO	-4.17	-2.78	-12.33**	15.84**
ICGX – SM 00017/5/P10/P1 X MANIPENTA	-10.79**	-7.11**	-30.59**	7.97
ICGX – SM 00020/5/P4/P1 X ICGV IS 07890	-14.78**	-16.07**	-10.16**	12.56**
ICGX – SM 00020/5/P4/P1 X ICGV IS 07899	-17.51**	-1.22	0.97	9.57**
ICGX – SM 00020/5/P4/P1 X ICIAR-19BT	-20.29**	-5.21**	11.17**	0.71
ICGX – SM 00020/5/P4/P1 X SAMNUT 14	2.02	6.00**	-11.61**	-9.66**
ICGX – SM 00020/5/P4/P1 X KWANKWASO	-1.05	-8.59**	6.03	-4.18
ICGX – SM 00020/5/P4/P1 X MANIPENTA	-0.14	-0.24	2.57	35.96**
ICGV IS 07890 X ICGV IS 07899	-3.03	-13.84**	-3.86	-7.64
ICGV IS 07890 X ICIAR-19BT	52.39**	-1.52	16.45**	3.14
ICGV IS 07890 X SAMNUT 14	-11.99**	19.03**	6.65*	4.33
ICGV IS 07890 X KWANKWASO	-10.71**	0.98	4.75	-1.39
ICGV IS 07890 X MANIPENTA	-10.31**	-3.35	24.53**	15.48*
ICGV IS 07899 X ICIAR-19BT	2.07	15.17**	-8.96**	3.34
ICGV IS 07899 X SAMNUT 14	-5.79	-6.39**	6.99*	-8.73*
ICGV IS 07899 X KWANKWASO	-4.47	3.75*	6.37**	-2.10
ICGV IS 07899 X MANIPENTA	-2.79	5.69**	-10.45**	29.13**
ICIAR-19BT X SAMNUT 14	3.52	11.55**	-13.20**	3.43
ICIAR-19BT X KWANKWASO	1.91	-6.17**	-10.31**	9.46**
ICIAR-19BT X MANIPENTA	13.25**	1.43	9.46	4.05
SAMNUT 14 X KWANKWASO	11.79*	13.94**	7.99**	1.82
SAMNUT 14 X MANIPENTA	6.54*	3.30	8.45	0.81
KWANKWASO X MANIPENTA	8.40	5.10	14.00**	9.61
SE±	3.21	1.68	3.19	4.03

*, **Significant at P < 0.05 and P < 0.01 levels of probability respectively.

the performance of the parents. Thus, the GCA scores could not be used to predict the performance of the parents in the present study, because the value of Baker's ratio is much lower than the theoretical maximum of unity. Low Baker's ratio observed for AUDPC in this study highlighted the importance of SCA variance, and hence the importance of dominance and/or epistatic gene effects for increasing resistance to GRD. This implies that selection should be done on latter generations, based on better hybrid combinations, rather than the performance of the parents involved in crossing programs. Partitioning G x L into variance to GCA x L and SCA x L interaction effects indicated significant variances of both GCA x L and SCA x L effects. The significant of GCA x L variance

implied that GCRD symptom rating (AUDPC) was sensitive to environmental conditions and data from additional environments or seasons will lead to precise GCA .

Conclusions and Recommendations

The estimates of low values of ratio of combining ability variance indicated that nonadditive gene effects were more important than additive gene effects in determining GCRD resistance in groundnut germplasm evaluated in this study. The nature of genetic variation for aphid damage index and AUDPC and its relationships with sound kernel yield implies that planning for successful breeding program for GRD resistance will be possible. ICGX – SM 00017/5/P_{10}/P_1, ICIAR-19BT, ICGX – SM 00020/5/9 and ICGX – SM 00020/5/P_4/P_1 were found to have good GCA for GCRD resistance and could be deployed in groundnut breeding programmes to improve the level resistance to the GCRD. The study recommends multi-location evaluations of advanced breeding lines in a range of environments and also to identify environments that effectively discriminate genotypes based on reaction to GCRD.

Conflict of interest

The authors have not declared any conflict of interest.

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Short Communication

Acclimatization results of micro-propagated plantlets

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This work is an analysis of the structured-functional particularities of regeneration of introduced varieties of *Vaccinium corymbosum* L. and *Vaccinium vitis-idaea* L. under *in vitro* and *ex vitro* conditions. The anatomical structures of the leaves of *V. corymbosum* and *V. vitis-idaea* cultivated under aseptical culture, greenhouse and open ground were studied. It is shown that conditioned cultivation superimposes the imprint on structure and function regeneration, structured and functional organization regeneration: a mobile system and can reform in accordance with changed condition surrounding ambiences. The differences in construction and functions of sheet plants, growing in aseptic culture, green houses or open ground are indicative of plastic sheet; an organ, capable of reconstructing its structure and function adequately under conditioned cultivation. This is theoretically a guarantor to successful adaptation of the plants under *in vitro* (the cultural container) and *ex vitro* (the greenhouse and open ground) conditions.

Key words: Aseptic culture, greenhouse, open ground, anatomical structure, blueberry, cowberry.

INTRODUCTION

In the foundation of clonal micropropagation of plants, there are two completely different stages; *in vitro* and *ex vitro*. In the first (*in vitro*), vital functions of the material being propagated occur in a closed sterile space, on the nutrient medium under strictly controlled conditions. After the regenerants are transferred from *in vitro* conditions, the second stage begins (*ex vitro* system), which is quite different from *in vitro* conditions.

In *ex vitro* conditions, the plants have to pass from heterotrophic nutrition to autotrophic nutrition conjugated with structural and functional transformation of organism under new conditions. They must adjust themselves to changeable environmental factors inherent in them.

The transition of plants from *in vitro* to *ex vitro* conditions is critical in most cases and entails death of plants. From our point of view, the comparative analysis of structural and functional peculiarities of regenerants under *ex vitro* and *in vitro* conditions will help to understand and prevent the cause of death of plants during adaptation period.

Research conducted by Brainerd et al. (1981) on leaf anatomy and water stress with plump plants under *in vitro* and *ex vitro* conditions showed that loss of water occurred three times faster in plants obtained from *in*

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vitro culture compared to those obtained from greenhouse. The thickness of palisade cells was much lower in regenerants raised under aseptical conditions than those from greenhouse and open ground.

According to researches by Grout (1975), Sutter and Langhans (1979), leaves are deprived of wax bloom in plants cultivated *in vitro* and stoma function is imperfect because of failure of open-closed mechanism. Similar conclusions about stoma functioning were obtained by Lee et al. (1988), Brainerd et al. (1982) and Wardle and Short (1983).

According to data by Bunning and Sagromsky (1948), O'Leary and Knecht (1981), Penfound (1931), stoma development is influenced by factors such as CO_2 concentration in the retort, water regime and hormone level.

The stomata of plants under *in vitro* conditions are usually open which is not true in respect with stomata *ex vitro* conditions (Brainerd and Fuchigami, 1981; Wetzstein and Sommer, 1982, 1983). In our opinion, such behaviour of stomata under *ex vitro* conditions is quite justified because in cultural retorts a very high constant relative humidity rate is kept (over 90%) and temperature and illumination degrees are not responsible to over falls because they are being controlled. Should any condition in cultural container occur, stomata reaction will follow in response to the changes of the given conditions.

From this point of view, the failure is clear overtaking some researchers seeking to interfere with efficient performance of stomata responding to conditions under which they are. For instance, the use of antitranspirants during transfer of plants from *in vitro* to *ex vitro* conditions promoted decreasing of photosynthesis caused by worsening of plant growth (Danies and Kozlowski 1974).

According to researches done by Fabbri and Sutter (1986), the leaf structure of wild strawberry formed *in vitro* culture, was characterized by a relatively thin leaf plate, under developed palisade cells, big air cavities, weakly developed cuticular integument. At the same time the leaf of wild strawberry formed under *ex vitro* conditions was differentiated into palisade and spongy tissues with a well-developed cuticular integument.

Similar results were obtained by Donnelly and Vidaver (1984) when studying raspberry leaves regenerated *in vitro*. Waldenmaier and Schmidt (1990) observed histological differences of Rhododendron leaves *in vitro* and *ex vitro* when tempering them. The differences included absence of breathing pores, weakly-structured mesophyll with leaves *in vitro*. In the *ex vitro* leaves, their anatomical structure changed: their thickness grew, the number of layers of epidermis and palisade tissue increased, and cuticle appeared. The acclimatization by low humidity rate led to a clear differentiation of the tissue into palisade and spongy mesophyll.

The objective of this study was to analyze the introduced varieties of *Vaccinium corymbosum* L. (Dixi. Bluecrop) and *Vaccinium vitis-idaea* L. (Koralle).

MATERIALS AND METHODS

The leaves of *V. corymbosum* L. (Dixi. Bluecrop) and *V. vitis-idaea* L. (Koralle) were preserved in alcohol-acetic acid (3:1). The cross sections were made in the middle part of the leaf, at microtome by histological technique and razor. The sections were cleared with chloral hydrate and then stained with Genevez and Sudan III reagents (Braune et al., 1979; Toma and Rugină, 1998; Verzar-Petri, 1979). The thickness of leaf was measured by micrometer.

The analysis of anatomical structure was realized according to previous method described by Brainerd et al. (1981), Grout (1975), Sutter et al. (1979) and Lee et al. (1988).

RESULTS AND DISCUSSION

The research on dependence of internal leaf structure under cultivating conditions showed that regenerants of *V. corymbosum (*Dixi, Bluecrop*)* and *V. vitis-idaea (*Koralle*)* cultivated under *in vitro* conditions had no clear differentiation of mesophyll into palisade and spongy tissues, had a thin leaf plate, weakly developed cuticular integument and under developed stomata apparatus entailing continuous opening of stomata and over transpiration.

The leaves developed in the greenhouse had a clear mesophyll differentiation into palisade and spongy mesophyll, had cuticular integument, and well-developed stoma apparatus enabling normal transpiration.

The leaves of plants transplanted into open ground did not differ from greenhouse leaves in general structure. They had a leaf structure clearly differentiated into palisade and spongy mesophyll, a well-developed cuticular integument and a stoma apparatus. However, it should be pointed out that the difference was observed in the change of quantitative indices of the leaf structure. Thus, leaves of plants from open ground had a thicker leaf plate, more layers of palisade tissue, longer cells, reduced volume of ductus intercellularis compared to the greenhouse and *in vitro* leaves (Table 1).

It should be pointed out that the differences in leaf structure are conjugated with their functional differences. An example is a thorough research on comparative anatomy and physiology of Asian birch (*Betula platyphylla*) cultivated in the greenhouse on aseptic culture (Smith et al., 1986). The authors concluded about the weak development of vascular system under *in vitro* conditions followed by an increased sensitivity of such plants to water stress inherent in *ex vitro* conditions.

A low intensity of photosynthesis was discovered by a very low illumination degree conjugated with the absence of clear differentiation of the leaf into palisade and spongy tissues *in vitro* culture. After the transfer of plants into *ex vitro* conditions (greenhouse) the researchers observed the increase in photosynthesis intensity and changes in leaf anatomy. In their opinion, the plants

Table 1. Quantitative indices of anatomical leaves structure of *Vaccinium corymbosum* and *Vaccinium vitis-idaea* cultivated in the aseptical culture, greenhouse and open ground*.

Grade	Aseptic culture (*in vitro*) 4000 Lx			Greenhouse >15000 Lx					Open Ground > 50000 Lx				
	Leaf thickness (µm)	The number of stomata per 1 mm²	Stoma size length x width (µm)	Leaf thickness (µm)	Palisade cofficient	Length:width of cells of palisade tissue ratio	The number of stomata per 1 mm²	Stoma size length x width (µm)	Leaf thickness (µm)	Palisade cofficient	Length:width of cells of palisade tissue ratio	The number of stomata per 1 mm²	Stoma size length x width (µm)
Vaccinium corymbosum													
Bluecrop	76±2	16±1	15x11	154±16	0.75	1.8:1	251±11	25x17	210±11	0.87	2.5:1	260±12	23x16
Dixi	85±3	16±1	15x12	173±13	0.71	1.9:1	250±9	26x16	221±12	0.9	2.7:1	265±10	24x15
Vaccinium vitis-idaea													
Koralle	91±4	19±1	16x10	286±9	0.63	2.61:1	410±20	24x15	450±19	0.86	3.31:1	430±23	21x14

*In the table no indices are shown of palisade coefficient and of palisade tissue cells with the leaves of plants from aseptic culture, since the mesophill of the leaf was not differentiated into palisade and spongy mesophills.

grown in aseptic conditions change considerably in their anatomical and physiological features compared to their double cultivated *ex vitro* conditions. The changes are accounted for by the influence of a specific environment in aseptic culture and transfer of plants into *ex vitro* conditions due to a quick recovery of metabolism resulting from normal development of plants.

According to researches by Donnely et al. (1984), Grout and Millam (1985), photosynthetical activity is lower with *in vitro* shoots compared to that of *ex vitro* shoots. In the minimum photosynthetical activity during 14 days after transfer of leaves from *in vitro* culture, it was observed that plants survived during acclimatization using the stock of metabolites. The normal recovery of structure and function occurs with the regenerants within a month after placing them under *ex vitro* conditions. To increase the survival rate of plants during adaptation it is necessary to gradually decrease the relative air humidity and increase irradiation. This increases the space occupied by palisade cells which in turn causes increase in intensity of photosynthesis.

Interesting researches were conducted by Solarova (1989) on study of round-o'clock variability of CO_2 concentration in cultivating retorts, where regenerants plants cultivated were obtained from leaf pieces. It turned out that CO_2 concentration in retorts increased in dark period and was connected to the regenerant size and sucrose content in the medium. The concentration in retorts decreased in light period and the illumination reached the compensation point in 3-4 h despite the low illumination degree (100 µmol.m^{-2}.s^{-1}). The author made a conclusion that the low CO_2 concentration in closed retorts for cultivation of regenerant plants induces different growth.

Therefore, the decreased CO_2 concentration is one of the low photosynthetical intensity observed with regenerants plants *in vitro* culture. The CO_2 concentration is increased by transfer of plants to *ex vitro* conditions, causing an increase of intensity of photosynthesis followed by growth acceleration.

On the foundation of comparative analysis of structural and functional features of the regenerants under *in vitro* and *ex vitro* conditions based on written sources and results of our own researches, we came to a conclusion: 1) that *in vitro* and *ex vitro* cultivating conditions leave imprint on structure and functions of regenerants; 2) structural and functional organization of regenerants is a mobile system able to be transformed in accordance with the changed environmental conditions. That means that the differences in structure and function of plant leave growth under aseptic culture, greenhouse or open ground testify to the flexibility of the leaf – the organ able to transform its structure and function according to the cultivating conditions. This is theoretically the guarantor of a successful adaptation of plants when transferring them from *in vitro* to *ex vitro* conditions.

In practice, we managed to avoid losses of plant material at the critical point due to using techniques based on conclusions confirmed by the results of experimental researches. This was proved by our observations over adaptation process of introduced species of *V. corymbosum* (Dixi, Bluecrop, Herbert, Rancocas, Covill, Early blue*)* and *V. vitis-idaea (*Koralle, Masovia,

Erntedank, Erntecrone, Erntezegen*)* when transferring them from *in vitro* to *ex vitro* conditions.

To prevent death of material from over transpiration (refers not only to *V. corymbosum* and *V. vitis-idaea*) caused by the reasons known to us: 1) the humidity drop *ex vitro* conditions, 2) imperfect structural and functional organization of the leaf in terms of *ex vitro* conditions, there is need firstly to increase the turgor of regenerants to its maximum value. It is achieved by plunging of the material into retort containing distillated water for 5-6 h.

The second essential condition is to keep high humidity rate in the greenhouse (not under 90%) and removal of strong air flows that is elimination of any wind since it dries up leaves because of quick evaporation. Absence of wind and high humidity rate will cause steam pressure gradient between leaves and air. It is essential to create *in vitro* identical conditions in the greenhouse in the first two to three weeks of regenerant cultivation (before root for-mation). It means to strictly control humidity rate, keep temperature similar to that when cultivating plants *in vitro* conditions and relatively low illumination degree (500 lx). Thus, the high air humidity will not cause intensive transpiration preventing the plant from fading. High temperature (25°C) and low illumination degree (500 lx) favour low intensity of photosynthesis and stop regenerant growth. The stock of metabolites with the regenerant will be utilized for root formation. After root formation, it is necessary to gradually decrease the air humidity around the regenerant and increase the illumination degree. This will complete the structural transformation of the leaf: the cuticular layer will appear, the cells of epidermis will change their shape, the mesophyll of the leaf will change its texture. The leaves will acquire features of xeromorphic structure and the plant will not be frightened by the low air humidity and even by strong wind characteristic under open ground conditions.

The procedures mentioned strictly and implemented by us when transferring the introduced species of *V. corymsosum* and *V. vitis-idaea* from *in vitro* to *ex vitro* conditions allowed us to preserve the viability of the plants and to secure their 100% survival and adaptation.

To sum it up, it can be concluded that the successful adaptation of regenerant plants when transferring them from *in vitro* to *ex vitro* conditions depends, on one hand, on our theoretical knowledge, results of experimental researches and, on the other hand, on the strict observance of simple techniques. The confirmation is a case of 100% adaptation of regenerant plants of introduced species of *V. corymbosum* and *V. vitis-idaea* not only under greenhouse conditions but also under open ground conditions.

Conflict of interests

The authors have not declared any conflict of interest.

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Floristic diversity under anthropogenic activities in the protected forests of Duekoué and Scio in southwestern Côte d'Ivoire

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This study analyses the effects of anthropogenic disturbance on trees and shrubs floristic α-diversity in two protected rain forests in southwestern Côte d'Ivoire. These forests have been under timber harvesting since their protection in 1929. The forestry service had developed plantations of indigenous timber species and teak since 1996 to increase their productivities for timbers. Additionally, they host many plantations of cash crop among which coffee, cocoa and rubber are the most important. To understand how these plantations affect the local flora, the diversity of shrubs and trees with DBH ≥ 10 cm was analyzed through the species number and diversity indices. Plots were of 20 m x 50 m size and a total of 10 per vegetation type. Highest species numbers, Shannon-Wiener's index, Hill's index and Pielou's index, in both plots and vegetation types were found in natural forest and undergrowth cleared forest which had similar values of these parameters. Plot richness was ranked between 1 and 7 species whilst vegetation type richness varied from 4 to 12 species for all plantations. Yet Simpson's diversity index showed highest values in plantations. Richness in plantation was influenced by the location of plantation site and the nature of crop but no influence was found with the combination site and crop nature.

Key words: Forest protection, cash crops, agroforestry, flora and diversity, South-West Côte d'Ivoire.

INTRODUCTION

The tropical humid forests host higher vascular plant richness and diversity compared to European and North American forests (Richards, 1996; Myers et al., 2000; Blanc, 2002; Parmentier et al., 2007; Parmentier et al., 2011). Mixed mesophytic forests of China and Southeast America that are the richest among the non-tropical forests (Richards, 1996) harbor 20 to 30 species. These numbers are smaller than the richness of trees bigger than 10 cm DBH in a hectare of primary Tropical humid forest plot that is often estimated between 40 and 100

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species (ORSTOM and UNESCO, 1983; Kouamé, 1998) and can reach 251 species (Ghazoul and Sheil, 2010). These forests also harbor high abundance and diversity of lianas which constitute other fundamental characteristics of this vegetation type (Richards, 1996; Kouamé et al., 2007).

Agriculture has played an important role in the transformation of lowland tropical forest landscapes worldwide over the past centuries and continues to do so today (Lass, 2004; Schroth and Harvey, 2007). In many regions, cash crops have been a driver of deforestation, with plantations or agroforestry systems replacing the original forest ecosystems (Ruf and Schroth, 2004). In comparison to other land uses that replace intact forest, traditional Cocoa (*Theobroma cacao* L., Sterculiaceae) and Coffee (*Coffea canephora* Froenh., Rubiaceae) agroforests, with diverse and structurally complex shade canopies, are among the agricultural land uses that are most likely to conserve a significant portion of the original forest biodiversity (Perfecto et al., 1996; Moguel and Toledo, 1999; Rice and Greenberg, 2000; Schroth et al., 2004; Faria et al., 2006; Harvey et al., 2006). Although cocoa and coffee cultivation may represent a serious threat to biodiversity in certain countries such as Côte d'Ivoire, Ghana, and the Dominican Republic, where their agroforests make up a significant proportion of all woodland (Donald, 2004), there are a number of reasons for regarding their shaded cultivation as environmentally preferable to many other forms of agriculture in Tropical forest regions (Greenberg, 1998; Power and Flecker, 1998). Since economic prospects for Rubber (*Hevea brasiliensis* Müll.Arg., Euphorbiaceae) on the world market are positive (Smit and Vogelvang, 1997; Burger and Smit, 1998, 2000) and the production by smallholders is still profitable (Levang et al., 1999; Suyanto et al., 2001), large tropical forest areas have been converted into Rubber plantations responsible for drastic erosion of local trees richness (Beukema et al., 2007). For rubber cultivation, forest is fully cleared and crops are established as monoculture plantations on average replanted after about 40 years, but some plantations are maintained to an age of 70-80 years (Gouyon et al., 1993). In many Tropical countries, this loss of the natural forests has been counteracted by the rapid increase in degraded forestland allocated to plantation establishment and other policies (CTFT, 1989). Like many other tropical countries, the loss of Ivoirian's natural forests has been counteracted by comprehensive reform programmes in the forestry sector among which a key reform was the Government's initiative in plantation establishment in the country, not only to halt forest degradation but also to catalyze important native forest flora restoration after long period of anthropogenic and non-anthropogenic disturbances (Lemenih and Teketay, 2004; Baatuuwie et al., 2011). These programmes have increased plantations since 1992 of both native and exotic timber tree species amongst which Teak (*Tectona grandis* L.f., Verbenaceae) is predominant. Teak cultivation involves full local vegetation removal sometimes with mechanics.

In Côte d'Ivoire, there are two main categories of protected areas; the national parks exclude any human activities except management and research, and the classified (protected) forests whose purpose is management for sustainable logging (Kouamé, 1998). The definition and delimitation of these protected areas began in 1924 by their static conservation (de Koning, 1983; Ahimin, 2006). After the Ivorian independence in 1960, their legal status was created together with a national forest research institute (IDEFOR) and a national society for forest development (SODEFOR). Forty years later, anthropogenic activities in national parks, protected forests and biological reserves result in their degradation despite the promulgation of legal instruments/laws (Dao, 1999; Chatelain et al., 2004; Ahimin, 2006). Due to rarefaction of wastelands in the rainforest area, the farmers crossed the limits of protected forests within which they establish their crops. The politico-military crisis in Côte d'Ivoire since 2002 led to increase in the illegal occupation of its South-western protected areas, especially Duekoué and Scio forests. In areas undergoing rapid land use change such as the rainforest of Côte d'Ivoire, where undisturbed lowland forest has almost completely disappeared (Chatelain et al., 2004; BNETD, 2010), the question whether at least some of the native rainforest species can survive in disturbed forest types has become important. The potential significance of such agroforestry systems for biodiversity conservation is stressed by nature conservation agencies and the international research community (Siebert, 2002; Garrity, 2004; Schroth et al., 2004).

To understand the effects of Teak plantations created by the Forestry Service and the cash crops production by small farmers in the protected forests of Duekoué and Scio on the diversity of trees, shrubs and lianas, eighty 20 m x 50 m plots were investigated for their woody plant richness. We sampled woody plant individuals that had 10 cm DBH and above at the species level, with the aim of analyzing woody plant species composition and diversity in relation with the anthropogenic activities. Given that both the agroforestry systems of creating forestry plantations and farming cash crops aim to promote few targeted species at the expense of the local flora, we hypothesized to find higher plant species richness and diversity in natural vegetation than in plantations.

MATERIALS AND METHODS

Research site and data collection

Research was carried out in the classified forests of Duekoué (6° 30'- 6° 45' N, 7° 00'- 7° 15' W) and Scio (6° 30'-7° 00' N, 7° 30'- 8° 05'W) South-west of Côte d'Ivoire (Figure 1). Climate in both areas is sub-equatorial with a long wet season from February to November and a short dry season from November to January. Annual rainfall varies from 1600-1700 mm in Duekoué forest to 1700-1800 mm in Scio forest. The average monthly temperature is

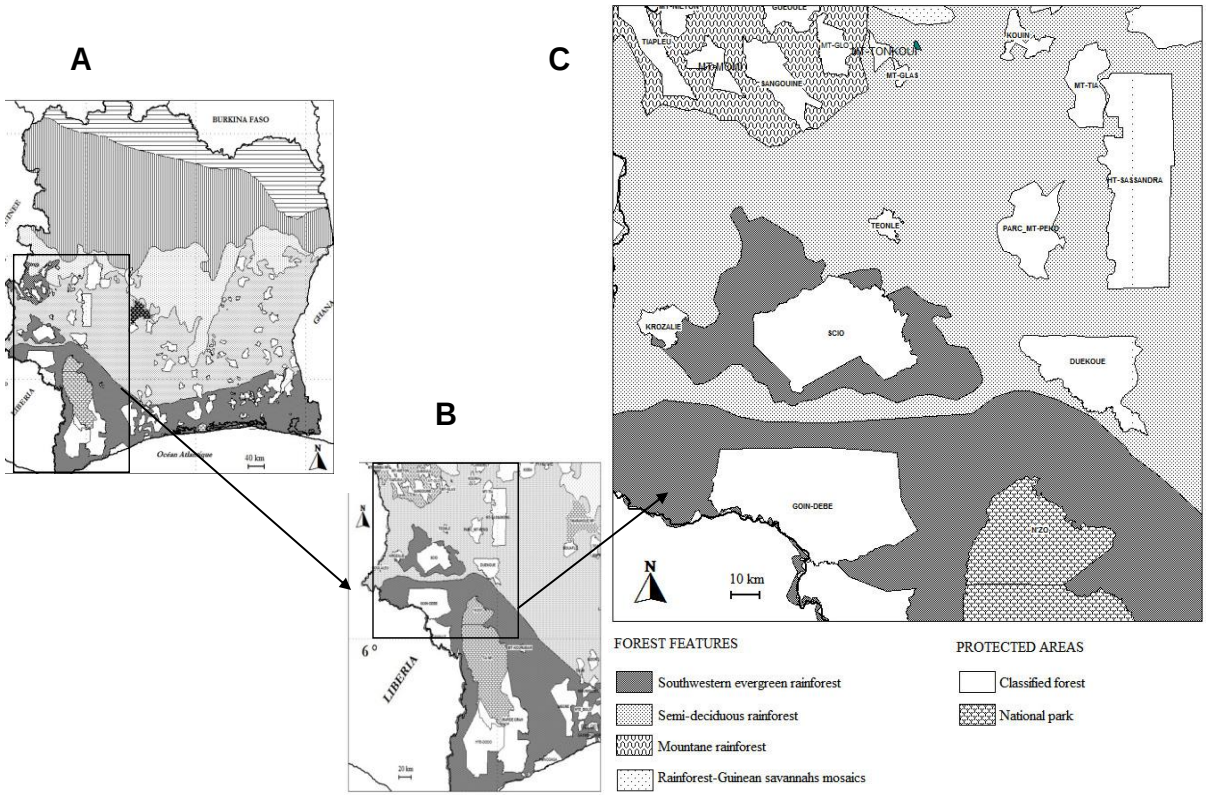

Figure 1. Localization with MapInfo 7.8 software of research sites on the map of protected areas and main floristic features distribution in Western Côte d'Ivoire rainforest zone (From Kouamé and Zoro Bi, 2010). A: General vegetation and protected areas map of Côte d'Ivoire, B: South-west region, C: research sites location.

25ºC while monthly and annual potential evapotranspiration of Duekoué and Scio are 123.5 and 1482 mm, respectively (Eldin, 1971). The soils of both forests belong to the remould ferrallitic group (Perraud and De La Souchère, 1970). The Duekoué forest, with an area of 53,600 ha (SODEFOR, 1994), consists of a moist semi-deciduous forest defined as a Tropical rainforest type in which part of the higher trees shed their leaves during the 3-4 months dry season in a region of 1350-1600 mm annual rainfall (ORSTOM and UNESCO, 1983) and interrupted by savannas areas and inselbergs (Monnier, 1983). The original vegetation of Scio forest, covering 88,200 ha (SODEFOR, 1996), belongs to South-western evergreen forest type of Côte d'Ivoire that spreads in the wettest forest area. (Kouamé, 2010; Kouamé and Zoro Bi, 2010)

Field data collection was carried out in eighty 1000 m² (20 m x 50 m) plots, as suggested by Thiombiano et al. (2010), established per 10 in four different vegetation types (biotopes) for each forest (Table 1). Homogeneity, local area, repetition, presence of plant individuals with DBH≥10 cm and availability were the criteria of these biotopes' selection. Thus, the biotopes plotted were the natural forest patches, the undergrowth cleared forests, the coffee plantations, the cocoa plantations, the rubber plantations and the teak plantations (Table 1). Each plot was sub-divided into ten 100 m² sub-plots where all plants with DBH≥10 cm were assessed for their scientific names and DBH.

Data analysis

Taxa identification followed Aubréville (1936), Lebrun and Stork (1991-1997), Aké Assi (2001, 2002) and Hawthorne and Jongkind (2006). Family and authors names have been updated with Mabberley (1997).

Floristic diversity was analyzed using the species number considered as the first diversity parameter (Gaston, 1996; Tuomisto, 2011) and the three commonest diversity indices (Shannon-Wiener, 1949; Simpson, 1949; Pielou, 1966). Simpson's diversity index (*D'*) checks the probability for 2 random individuals in a community to belong to the same species (Simpson, 1949).

$$D' = 1 - \sum Pi^2$$

Where, $Pi = ni/\sum ni$ with *ni* as average cover of a species *i* and $\sum ni$ the total cover of all species. *D'* varies from 0 (maximum diversity) to 1 (minimum diversity). This index is sensitive to the variation of importance for most abundant species (Peet, 1974; Grall and Coïc, 2006).

Shannon-Wiener's index (*H'*) which is the most recommended index to check richness diversity (Grall and Coïc, 2006) is below formulated:

$$H' = -\sum_{i=1}^{s} PiLnPi$$

with *Pi* as relative average cover of species *I* in a community (Shannon and Wiener, 1949). *H'* varies from 0 (monospecific settlement) to *LnS* (maximum diversity). This index is sensitive to the variation of importance for most rare species (Peet, 1974; Grall and Coïc, 2006).

Pielou's index (*J'*) measures the degree of a settlement diversity and corresponds to the average between the affective diversity

Table 1. Description and localization of plots.

Biotopes	Duekoué forest	Latitude N	Longitude W	Biotopes	Scio forest	Latitude N	Longitude W
Coffee plantations	PCAFD1	6° 42	7° 06	Coffee plantations	PCAFS1	6° 38	7° 52
	PCAFD2	6° 40	7° 06		PCAFS2	6° 38	7° 51
	PCAFD3	6° 41	7° 14		PCAFS3	6° 31	7° 48
	PCAFD4	6° 43	7° 12		PCAFS4	6° 39	7° 50
	PCAFD5	6° 41	7° 14		PCAFS5	6° 38	7° 52
	PCAFD6	6° 40	7° 06		PCAFS6	6° 38	7° 51
	PCAFD7	6° 42	7° 06		PCAFS7	6° 38	7° 53
	PCAFD8	6° 43	7° 02		PCAFS8	6° 38	7° 53
	PCAFD9	6° 40	7° 06		PCAFS9	6° 31	7° 48
	PCAFD10	6° 43	7° 12		PCAFS10	6° 39	7° 47
Cocoa plantations	PCAOD1	6° 42	7° 06	Cocoa plantations	PCAOS1	6° 31	7° 48
	PCAOD2	6° 42	7° 06		PCAOS2	6° 38	7° 51
	PCAOD3	6° 42	7° 12		PCAOS3	6° 39	7° 46
	PCAOD4	6° 41	7° 14		PCAOS4	6° 38	7° 51
	PCAOD5	6° 42	7° 12		PCAOS5	6° 38	7° 52
	PCAOD6	6° 43	7° 12		PCAOS6	6° 39	7° 46
	PCAOD7	6° 42	7° 12		PCAOS7	6° 38	7° 51
	PCAOD8	6° 42	7° 12		PCAOS8	6° 38	7° 51
	PCAOD9	6° 43	7° 12		PCAOS9	6° 39	7° 47
	PCAOD10	6° 42	7° 12		PCAOS10	6° 39	7° 47
Rubber plantations	PHEVD1	6° 42	7° 06	cleared	FDEFS1	6° 39	7° 46
	PHEVD2	6° 42	7° 06		FDEFS2	6° 38	7° 51
	PHEVD3	6° 42	7° 06		FDEFS3	6° 38	7° 51
	PHEVD4	6° 43	7° 06		FDEFS4	6° 39	7° 46
	PHEVD5	6° 43	7° 06		FDEFS5	6° 39	7° 51
	PHEVD6	6° 42	7° 14	Undergrowth forests	FDEFS6	6° 39	7° 50
	PHEVD7	6° 42	7° 14		FDEFS7	6° 39	7° 50
	PHEVD8	6° 42	7° 12		FDEFS8	6° 38	7° 48
	PHEVD9	6° 43	7° 06		FDEFS9	6° 38	7° 52
	PHEVD10	6° 42	7° 06		FDEFS10	6° 38	7° 53
Teak plantations	PTECD1	6° 42	7° 12	Natural forests	FNBAS1	6° 39	7° 46
	PTECD2	6° 42	7° 12		FNBAS2	6° 38	7° 51
	PTECD3	6° 42	7° 12		FNBAS3	6° 39	7° 48
	PTECD4	6° 42	7° 13		FNBAS4	6° 38	7° 53
	PTECD5	6° 42	7° 01		FNBAS5	6° 39	7° 49
	PTECD6	6° 41	7° 14		FNBAS6	6° 31	7° 48
	PTECD7	6° 41	7° 14		FNBAS7	6° 34	7° 51
	PTECD8	6° 42	7° 12		FNBAS8	6° 39	7° 49
	PTECD9	6° 42	7° 13		FNBAS9	6° 30	7° 51
	PTECD10	6° 42	7° 12		FNBAS10	6° 39	7° 50

H' and the maximum theoretical diversity *H'max* (Pielou, 1966).

$$J' = H'/H'max$$

with *H'* as Shannon-Wiener index. *J'* varies from 0 (monospecific settlement) to 1 (similar distribution of all species).

Additionally to these commonest indices, Hill's index which is a combination of Simpson's diversity index and Shannon-Wiener's index (Hill, 1973; Grall and Coïc, 2006) was used to analyze the diversity in biotopes as recommended by Peet (1974) and

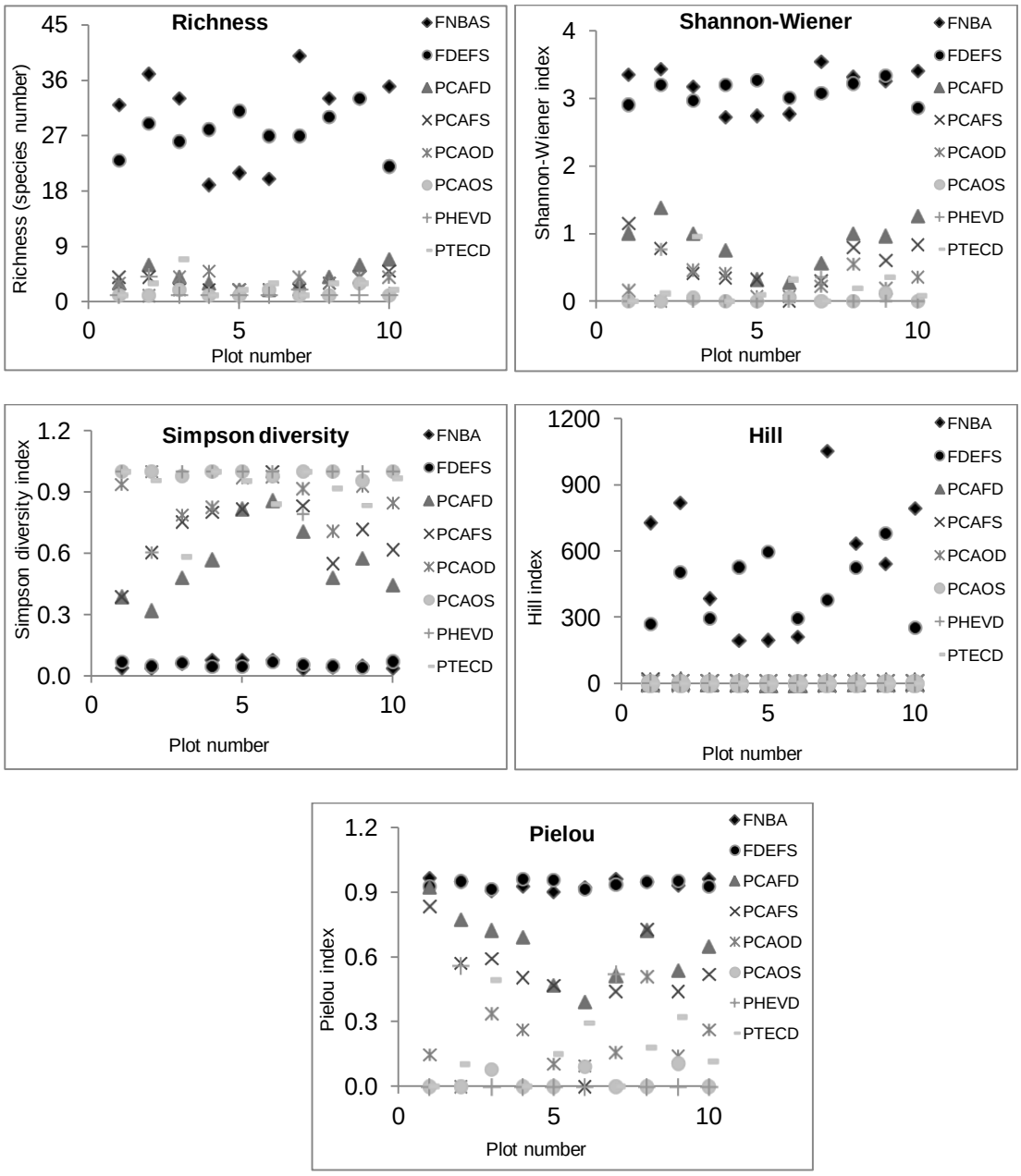

Figure 2. Richness and diversity indices in plots.

Routledge (1979).

$$Hill = (\sum Pi^2)^{-1} 1/exp[H']$$

Hill varies from 1 (monospecific settlement) to α (similar distribution of all species).

Such as data in plots showed normal distribution (Mead et al., 1993; Bar-Hen, 1998; Young and Young, 1998; Fowler et al., 1999), their statistical analyses were performed with parametric tests as recommended by Mead et al. (1993) and Fowler et al. (1999). Plot richness and diversity indices were compared using paired samples *t test* of Student (Student, 1908; Greig-Smith, 1983) with SPSS 18.0 software. Richness of coffee plantations and cocoa plantations that was assessed in both research sites (Table 1) was analyzed with ANOVA (Scherrer, 1984; Mead et al., 1993; Fowler et al., 1999) using Statistica 7.1 software for checking prospective impacts of site and/or crop nature on plot richness. Bonferroni's Post-Hoc test with Statistica 7.1 software led to segregate impacts of site and crop nature as the ANOVA showed their effects on plot richness.

RESULTS

The natural forest patches (FNBAS) in Scio site showed the highest α-diversity in plots and biotopes whereas the undergrowth cleared forests (FDEFS) in Scio showed the second highest α-diversity (Figure 2, Table 2). Both

Table 2. Richness and diversity indices in biotopes.

Parameters		FNBAS	FDEFS	PCAFD	PCAFS	PCAOD	PCAOS	PHEVD	PTECD
Richness	Minimum	19.00	22.00	2.00	2.00	1.00	1.00	1.00	1.00
	Maximum	40.00	33.00	7.00	5.00	5.00	3.00	4.00	7.00
	General	85.00	58.00	12.00	12.00	7.00	12.00	4.00	11.00
	Mean	30.30	27.60	4.00	4.00	3.20	4.00	1.60	2.60
	Std. dev.	7.50	3.41	1.76	1.76	1.23	1.76	1.07	1.78
Simpson diversity index	Minimum	0.03	0.04	0.32	0.39	0.71	0.96	0.60	0.58
	Maximum	0.08	0.07	0.86	1.00	1.00	1.00	1.00	1.00
	General	0.02	0.03	0.50	0.69	0.89	0.99	0.93	0.91
	Mean	0.05	0.06	0.56	0.71	0.89	0.99	0.94	0.91
	Std. dev.	0.02	0.01	0.18	0.17	0.09	0.01	0.13	0.13
Shannon-Wiener index	Minimum	2.73	2.87	0.27	0.00	0.00	0.00	0.00	0.00
	Maximum	3.55	3.34	1.39	1.15	0.56	0.12	0.77	0.97
	General	4.03	3.70	1.26	0.85	0.30	0.03	0.19	0.28
	Mean	3.18	3.11	0.86	0.56	0.25	0.02	0.11	0.21
	Std. dev.	0.31	0.16	0.37	0.34	0.19	0.04	0.26	0.29
Hill index	Minimum	195.19	252.40	1.53	1.00	1.00	1.00	1.00	1.00
	Maximum	1056.34	679.56	12.47	8.18	2.47	1.18	3.59	4.50
	General	2285.43	1191.98	7.10	3.38	1.51	1.04	1.31	1.45
	Mean	557.10	432.91	5.28	3.02	1.51	1.03	1.34	1.56
	Std. dev.	301.74	152.86	3.33	2.08	0.48	0.06	0.83	1.06
Pielou index	Minimum	0.90	0.91	0.39	0.00	0.00	0.00	0.00	0.00
	Maximum	0.97	0.96	0.92	0.83	0.51	0.11	0.56	0.50
	General	0.91	0.91	0.51	0.33	0.15	0.02	0.14	0.11
	Mean	0.94	0.94	0.64	0.51	0.20	0.03	0.11	0.17
	Std. dev.	0.02	0.02	0.16	0.22	0.15	0.05	0.23	0.16

The total area of each biotope is a hectare (10 x 1000 m²). Thus for all parameters in table 2, general values correspond to hectare data while the others are research plot area (20m x 50 m)data.

biotopes had similar plot richness which was very significantly higher than all plantations (Table 3) and showed also higher richness variability (Figures 2 and 3). Among plantations, the Rubber (PHEVD) cultivation led to the lowest plot richness (Figure 2, Tables 2 and 3) and variability (Figures 2 and 3). Distribution of trees in plantations was determined prior to the openness in vegetation and later by the nature of crop (Figure 4). Thus, plots were segregated into five groups amongst which the biggest (group I) gathered the natural forest patches and the undergrowth cleared forests from Scio site, and the Teak plantations (PTECD) and the Rubber plantations (Figure 4, Appendix 1) from Duekoué site. This group that was represented by 48.75% of plots appeared in low vegetation openness conditions. The second important group, in term of plots number (group II) that gathered 80% of Coffee plantations from both sites and a Rubber plantation (PHEVD2) from Duekoué, is found in medium vegetation openness conditions (Figure 4, Appendix 1). In slight higher vegetation openness, there were the smallest group (group III) made of four coffee plantations from Duekoué site and the group IV which gathered 90% of cocoa plantations from Duekoué site and 30% of cocoa plantations from Scio site (Figure 4, Appendix 1). The last group (group V) made of 70% of cocoa plantations from Scio site and a Cocoa plantation (PCAOD2) from Duekoué site appeared in highest vegetation openness conditions. Highly significant impacts of the site and of the nature of crop were found on the richness in coffee and Cocoa plantations but no impact was found with the combination site and crop nature (Table 4). Bonferroni's Post-Hoc test showed a very highly significant difference between Coffee plantations of Duekoué and Cocoa plantations of Scio, and a significant difference between Cocoa plantations of both sites while Coffee plantations from both sites were similar (Table 4).

Table 3. Matrix of biotopes mean richness comparison with SPSS 18.0 software.

	FNBAS	FDEFS	PCAFD	PCAFS	PCAOD	PCAOS	PHEVD	PTECD
FNBAS		0.97	12.63	12.35	11.35	12.08	12.86	11.70
FDEFS	ns		18.90	19.98	20.12	25.97	24.19	21.47
PCAFD	***	***		3.00	1.24	4.63	4.80	1.95
PCAFS	***	***	*		0.36	3.75	3.75	0.56
PCAOD	***	***	ns	ns		4.32	2.95	0.85
PCAOS	***	***	**	**	**		0.00	2.45
PHEVD	***	***	**	**	*	ns		1.86
PTECD	***	***	ns	ns	ns	*	ns	

Student *t* test values are above while significances are below. ns : test non-significant (P≥0.05); * : test significant (P<0.05) ; ** : test very significant (P<0.01) ; *** : test very highly significant (P<0.001). Degree of freedom of the test is 9.

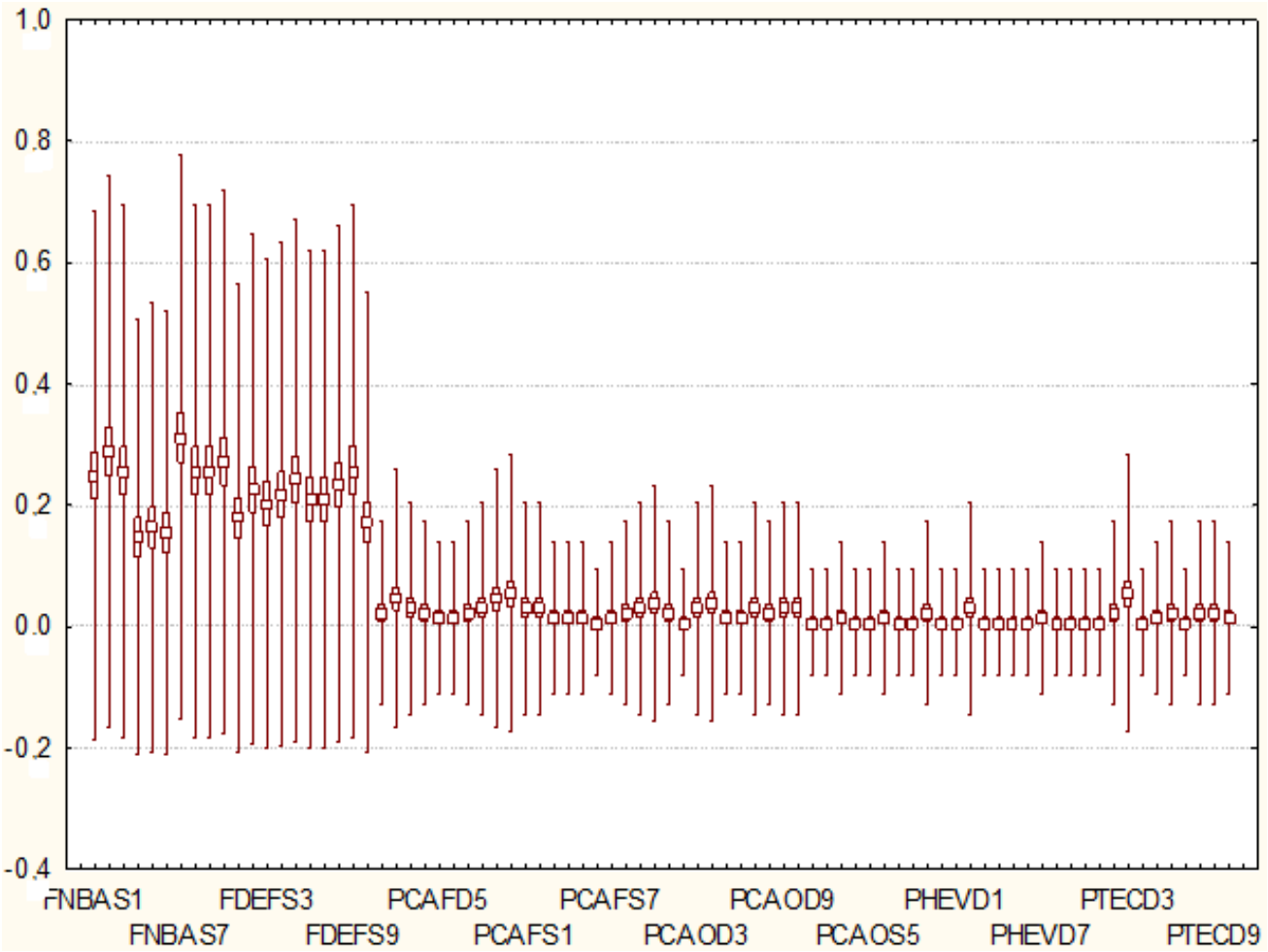

Figure 3. Boxplots of plot richness using factorial analysis with Statistica 7.1 software. Mean richness are in small central squares, error types are in small framing rectangles and standard deviation types are in vertical lines.

Shannon-Wiener's index showed highest (Figure 2) and similar (Tables 2 and 5) values in both natural forest patches and undergrowth cleared forests of Scio site. Despite the very significantly higher index value in coffee plantations of Duekoué site compared to value in coffee plantations in Scio site (Table 5), both biotopes showed

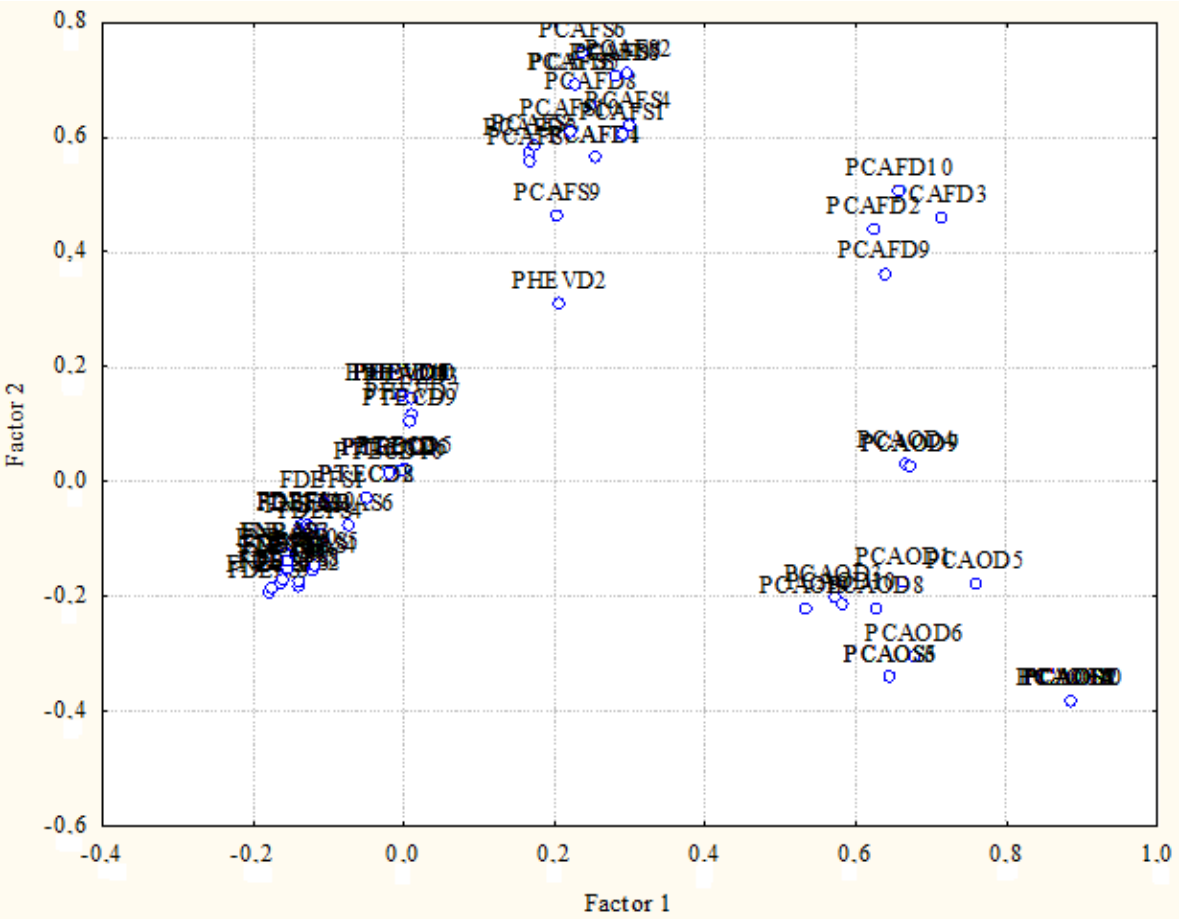

Figure 4. Projection of plots on the two first axes of the factorial analysis with Statistica 7.1 software according to their richness. Factor 1 on abscises explains 18.03% while factor 2 on ordinates is responsible for 12.06% of total variation of the analysis. The factor 1 that segregates natural forests and plantations expresses the openness in vegetation. The factor 2 distinguishes groups according the target species in plantations. Group I: Natural forest, undergrowth cleared forest, and Rubber and Teak plantations. Group II: Coffee plantations in both sites and one Rubber plantation (PHEVD2) except four Coffee plantations in Duekoué. Group III: the four remnant Coffee plantations in Duekoué site. Group IV: part of Cocoa plantations of both sites. Group V: remnant Cocoa plantations of both sites. Plots coordinates are given in Appendix 1.

Table 4. ANOVA and Bonferroni's Post Hoc test of effects of the site and the cash crop nature on richness in Coffee and Cocoa plantations.

	Parameter	SC	df	MF	F	P
ANOVA	Ord.of origin	336.4	1	336.4	208.8	***
	Site	19.6	1	19.6	12.2	**
	Crop	14.4	1	14.4	8.9	**
	Site*Crop	1.6	1	1.6	1.0	ns

	Site andCulture	Duekoué Cocoa	Duekoué Coffee	Scio Cocoa	Scio Coffee
Bonferroni	Duekoué Cocoa		ns	*	ns
	Duekoué Coffee	ns		***	ns
	Scio Cocoa	*	***		*
	Scio Coffee	ns	ns	*	

Error: MC Inter = 1.6111, df = 36 for Bonferroni Post Hoc test. ns, test non-significant (P≥0.05) ; *, test significant (P<0.05) ; *** : test very highly significant (P<0.001).

Table 5. Matrix of biotopes Shannon-Wiener's index comparison with SPSS 18.0 software.

	FNBAS	FDEFS	PCAFD	PCAFS	PCAOD	PCAOS	PHEVD	PTECD
FNBAS		0.55	25.83	29.30	26.49	31.22	32.57	21.02
FDEFS	ns		16.56	19.87	34.04	61.12	33.63	26.05
PCAFD	***	***		3.99	5.14	6.95	6.36	4.41
PCAFS	***	***	**		2.63	4.76	3.49	2.16
PCAOD	***	***	**	*		3.65	1.14	0.40
PCAOS	***	***	***	**	**		1.04	2.23
PHEVD	***	***	***	**	ns	ns		0.74
PTECD	***	***	**	ns	ns	ns	ns	

Student *t* test values are above while significances are below. ns : test non-significant (P≥0.05) ; * : test significant (P<0.05) ; ** : test very significant (P<0.01) ; *** : test very highly significant (P<0.001). Degree of freedom of the test is 9.

Table 6. Matrix of biotopes Simpson's diversity index comparison with SPSS 18.0 software.

	FNBAS	FDEFS	PCAFD	PCAFS	PCAOD	PCAOS	PHEVD	PTECD
FNBAS		0.33	9.58	12.78	27.95	115.56	22.08	20.10
FDEFS	ns		8.91	11.84	27.82	156.38	21.04	20.45
PCAFD	***	***		4.49	5.83	7.35	6.30	4.87
PCAFS	***	***	**		3.24	4.99	3.45	2.60
PCAOD	***	***	***	*		3.28	0.79	0.37
PCAOS	***	***	***	**	*		1.17	2.23
PHEVD	***	***	***	**	ns	ns		0.50
PTECD	***	***	**	*	ns	ns	ns	

Student *t* test values are above while significances are below. ns, test non-significant (P≥0.05) ; * , test significant (P<0.05) ; **, test very significant (P<0.01) ; ***, test very highly significant (P<0.001). Degree of freedom of the test is 9.

the highest values amongst plantations (Figure 2, Tables 2 and 5). Cocoa plantations of Scio site (PCAOS) expressed very significantly lower index value in comparison to those of Duekoué site but similar to values of rubber and teak plantations in Duekoué forest (Figure 2, Tables 2 and 5).

Simpson's diversity index showed its highest and similar values in Cocoa plantations of Scio site, Rubber and Teak plantations of Duekoué site (Figure 2, Tables 2 and 6). Cocoa plantations at Duekoué and Coffee plantations of both sites had medium index values, despite their variability, whereas natural forest patches and under-growth cleared forests expressed the lowest and similar Simpson's diversity index values (Figure 2, Tables 2 and 6).

Highest and similar Hill's index values were found in both natural forest patches and undergrowth cleared forests of Scio site (Figure 2, Tables 2 and 7). Cocoa plantations at Duekoué and Coffee plantations of both sites had slight medium index values, despite their variability, when cocoa plantations of Scio site, rubber and teak plantations of Duekoué site showed lowest and similar values (Figure 2, Tables 2 and 7).

Pielou's index expressed highest and similar values in both natural forest patches and undergrowth cleared forests of Scio site and medium values in coffee plantations of both sites (Figure 2, Tables 2 and 8). Cocoa plantations of Duekoué showed similar Pielou's index values with both rubber and teak plantations (Table 8) while Cocoa plantations of Scio expressed similar values with the rubber plantations and lower value compared to teak plantations. Rubber and teak plantations shared the same Pielou's index values (Figure 2, Tables 2 and 8).

DISCUSSION

The decreasing of α-diversity in both biotopes and plots documented by this study in all plantations, in comparison to the natural forest patches (Tables 2 and 3), and reveals that the farmers' cash crop production systems and forestry service teak production system

Table 7. Matrix of biotopes Hill's index comparison with SPSS 18.0 software.

	FNBAS	FDEFS	PCAFD	PCAFS	PCAOD	PCAOS	PHEVD	PTECD
FNBAS		0.55	25.83	29.30	26.49	31.22	32.57	21.02
FDEFS	ns		16.59	19.87	34.03	61.12	33.63	26.05
PCAFD	***	***		3.99	5.14	6.95	6.36	4.41
PCAFS	***	***	**		2.63	4.76	3.49	2.16
PCAOD	***	***	**	*		3.65	1.14	0.40
PCAOS	***	***	***	**	**		1.04	2.23
PHEVD	***	***	***	**	ns	ns		0.74
PTECD	***	***	**	ns	ns	ns	ns	

Student *t* test values are above while significances are below. ns, test non-significant (P≥0.05) ; *, test significant (P<0.05) ; **, test very significant (P<0.01) ; ***, test very highly significant (P<0.001). Degree of freedom of the test is 9.

Table 8. Matrix of biotopes Pielou's index comparison with SPSS 18.0 software.

	FNBAS	FDEFS	PCAFD	PCAFS	PCAOD	PCAOS	PHEVD	PTECD
FNBAS		0.01	6.28	6.43	15.83	48.09	12.03	13.66
FDEFS	ns		5.90	6.31	15.80	52.83	11.66	14.25
PCAFD	***	***		3.61	7.39	10.54	6.15	5.83
PCAFS	***	***	**		4.63	6.18	4.01	3.51
PCAOD	***	***	***	**		3.51	0.93	0.55
PCAOS	***	***	***	***	**		1.03	3.43
PHEVD	***	***	***	**	ns	ns		0.58
PTECD	***	***	***	**	ns	**	ns	

Student *t* test values are above while significances are below. ns, test non-significant (P≥0.05); ** , test very significant (P<0.01) ; ***, test very highly significant (P<0.001). Degree of freedom of the test is 9.

affect local rainforest flora and diversity. Indeed, the establishment of all these plantations involves prior clearance of forest undergrowth and lianas (FDEFD), as well as shrubs and trees, followed by burning (Donald, 2004; Beukema et al., 2007; Bisseleua et al., 2008; Baatuuwie et al., 2011). For coffee and cocoa, farms are mostly established following a similar model referred to as short-term boom-and-bust cycles: primary or secondary forests are selectively cleared, burned and the crop is planted along with understory food crops (Isaac et al., 2005). Moguel and Toledo (1999) distinguished five main systems of coffee production in Mexico according to management level, and vegetational and structural complexity (Donald, 2004; Schroth and Harvey, 2007). In our study area like the most part in Côte d'Ivoire South forest region, cocoa planting can take place under thinned primary-forest canopy, regenerating forest after clear felling, or under the canopy of artificially planted trees as documented Greenberg (1998) and N'goran (1998). The shade trees are vital for cocoa saplings survival and growth but provide also farmers with a variety of products, including firewood, construction materials, pharmaceutical products and food (Herzog, 1994).

According to Rice and Greenberg (2000), cocoa production in West Africa follows both the rustic system and the planted shade polyculture system (Moguel and Toledo, 1999) but Steffan-Dewenter et al. (2007) advocated planting cocoa at low tree density and thinning for economic viability. In Cameroon where the impacts of cocoa cultivation on the local biodiversity still being the most assessed in Africa (Schroth and Harvey, 2007), agroforests such as traditional cocoa plantations are gradually receiving increasing interest since several years (Guyer, 1984; Ruf and Schroth, 2004; Laird et al., 2007; Sonwa et al., 2007). Bisseleua et al. (2008) reported that their management practices were influenced by their relationship to the other components of the land-use system and were oriented at using a combination of multiple forest resources (Sonwa et al., 2001; Schroth et al., 2004; Perfecto et al., 2005). And their adaptive nature offers options for combining biodiversity conservation and cocoa production for human benefits (Greenberg et al., 2000; Reitsma et al., 2001; Perfecto et al., 2004; MCNeely and Schroth, 2006; Gordon et al., 2007; Steffan-Dewenter et al., 2007). Zapfack et al. (2002) set the richness of vascular plants in the cocoa fields between of the natural forest areas and, of the fallows and non-Cocoa

farms. They reported that many of the primary forest species were left standing in the course of burning, fruit trees were planted and other species (seedlings) were protected for further multiple uses (Zapfack et al., 2002). Schroth and Harvey (2007) reported that although both native and migrant farmers retain and plant useful species within their Cocoa farms, the native households retain and plant a higher density and diversity of non-Cocoa trees and use a wider range of non-tree species from their farms. In addition, the native farmers tend to have a greater number of local and wild species in their farms (Schroth and Harvey, 2007).

The lower richness in both cocoa and rubber plantations in Duekoué site documented by this study results from the near complete elimination of native trees species for their establishment than it is obtained in the cultivation of coffee plantations. Thus, medium values of diversity indices in Coffee plantations of both sites (Figure 2, Table 2-8) can be explained by the capacity of Coffee trees to grow and produce as well under the shade of many native or exotic tree species. Due to this capacity, famers preserve many useful tree species in their Coffee plantations for edible fruits and leaves, medicines, woods etc. on both sites of our study areas (Appendix 2). The crop effect shown by the ANOVA (Table 3) was due to this difference in intensity of tree species removed during Coffee and Cocoa plantations creating. Thus, the Coffee plantations in our study area correspond to the traditional polyculture system of Moguel and Toledo (1999) where several native and/or exotic species coexist with the crop. Hence, in both sites Legume tree species like *Albizia adianthifolia* (Schum.) WF.Wight, *A. glaberrima* (Schum. and Thonn.) Benth. *A. zygia* (DC.) JF.Macbr. and *Distemonanthus benthamianus* Baill. are especially preserved in both Coffee and Cocoa plantations (Appendix 2) in view of producing a mulch to supply organic matter for soil while exotic tree species as *Elaeis guineensis* Jacq. (Palm oil), *Mangifera indica* L. (Mango), *Musa paradisiaca* L. (Plantain Bananas) and *Persea americana* Mill. (Avocado) are introduced by farmers for their fruits (Appendix 2). Some natural and pioneer tree species like *Cordia guineensis* Schum. and Thonn. C. *platythyrsa* Bak., *Harungana madagascariensis* Lam. ex Poir., *Milicia excelsa* (Welw.) Berg and *Ricinodendron heudelotii* (Baill.) Pierre ex Heckel often survive as well in coffee and cocoa plantations for their products to the populations (Appendix 2) and their ability to promote quick shading of the Coffee and Cocoa trees, and to build refuges for Birds (Greenberg et al., 2000) which are benefic for Insects (Philpott and Armbrecht, 2006) and Mammals (Rolim and Chiarello, 2004). Thus, they lead to increase woody plant richness in such agrosystems. Rice and Greenberg (2000) suggested that the impact of cash crop production on biodiversity would be minimized if production was focused on already cleared lands, ensuring greater long-term stability of farms, and supporting greater levels of biodiversity. It is supported that the long-term incentives for promoting the management of a diverse shade canopy can be found in the ecological and agronomic services provided by the shade itself (Beer, 1987).

The similarity of the α-diversity and all diversity indices, between Rubber and Teak plantations shown by this study stems from the common practices of forest clearance, prior to the establishment of both types of plantations. Given that these sites are protected and managed for sustainable logging (SODEFOR, 1994, 1996; Kouamé, 1998), remnant timber species in these plantations increased their richness and diversity, especially in Teak plantations created by the SODEFOR. Higher and similar values of Shannon-Wiener, Hill and Pielou's indices and lower value of Simpson's diversity index in biotopes and plots of natural forest patches and undergrowth cleared forests compared to those in plantations (Tables 1-8, Figure 3) confirm the negative impacts of cash crop cultivation and Teak plantations on the flora of the study areas. Similar negative impact of Rubber cultivation on local natural plant species was also shown in Indonesia where plant richness decreased drastically from natural forests to Rubber plantations (Beukema et al., 2007). Except *E. guineensis* Jacq. and *M. indica* L. introduced in a young Rubber plantation at Duekoué site (PHEVD2), additional tree species in Rubber and Teak plantations were spontaneous and belonged to their undergrowth resproutings and remnant individuals (Appendix 2). And such introduction of exotic tree species explains the membership of PHEVD2 to the

Coffee plantation group II (Figure 4). The Teak's undergrowth self-regenerating capacity is shown by Baatuuwie et al. (2011) who pointed out no significant difference between the diversity of the socio-economic native tree saplings regenerating naturally under a Ghanaian's natural degraded forest, and a Teak mono-culture plantation and a mixed Teak-native tree species plantation.

The similarity of richness and all diversity indices found between the undergrowth cleared forests (FDEFS) and the natural forest patches (FNBAS) (Tables 1-8, Figure 2) shows that few big lianas and shrubs species were removed in our study area during this first step of cocoa plantations creating. The turnover of such biotope should be faster and very short if abandoned. Extinction of local tree species at the expense of cocoa starts with their destruction by felling or burning when cocoa trees become adults and need full sun for well fruiting. As the main features of the natural vegetation at Scio site, where such vegetation remains in our study areas, we assessed the same richness in the natural forest patches (Table 2) than Nusbaumer et al. (2005) despite differences in data collecting methods, plots' locations and ten years interval time between both studies. Scio's α-diversity is similar to those of Korup forest in Cameroon and, higher than the average 74±9 species per hectare documented by Kouamé (1998) in Haut-Sassandra protected semi-

deciduous forest (Figure 1) and those 64 species of the Ituri forest in DR Congo (Ghazoul and Sheil, 2010). But it is as far poorer than Yasuni forest in Ecuador and Pasoh forest in Peninsular Malaysia where the richness of a hectare plot is set at 251 and 206 species respectively (Ghazoul and Sheil, 2010). A larger interval of 46-180 species of trees with DBH ≥ 10 cm in 3 ha plots was documented by Sambuichi and Haridasan (2007) in Southern region of Brazil. Parmentier et al. (2007) attributed the lower tree α-diversity of African rainforests in comparison to Amazonian forests to climate variation in both regions. Shannon-Wiener's index values in Scio natural forest patches and undergrowth cleared forest plots (Table 2) fall within the 3.73-4.36 values of surrounding forest patches (Bakayoko, 2005) and the 3.31-4.22 values of forest in Southern region of Brazil (Sambuichi and Haridasan, 2007) whereas they are higher than the 1.6-3.0 values of forest in Yapo region, Eastern Côte d'Ivoire (Vroh Bi, 2013). The site effect documented by the ANOVA (Table 3) could be explained by the difference in original flora as both protected areas belong to two types of Ivorian rainforest (Kouamé and Zoro Bi, 2010).

Conclusion

Teak plantations created by forestry service and cash crop plantations in both Duekoué and Scio classified forests led to the decreasing of richness and diversity of woody plant individuals with DBH≥10 cm in accordance with the hypothesis of this paper. These impacts vary with the cash crop nature and the site separately but they are invariably together. When the forest undergrowth is just cleared for shrubs and lianas, the richness and diversity of individuals with DBH≥10 cm of Scio forest still being similar to those of the natural forest and its turnover should be faster if abandoned. In both Duekoué and Scio forest areas, coffee plantations where some natural trees survive and other exotic trees are introduced had higher richness and diversity of woody plant individuals with DBH≥10 cm among plantations. The rubber and teak plantations where few natural trees survive had the lowest richness and diversity of such category of plants.

Due to these results, we suggest to the Forestry Service 1) to remove all the cash crop plantations from Ivorian classified forests, 2) to circumscribe Teak and other wood plantations into some areas of these forests and 3) to promote the turnover of the less degraded areas in view to increase both richness and diversity of the Ivorian classified forests.

Conflict of Interest

The authors have declared that there is no conflict of interest.

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Appendix 1. Plots coordinates on the two first axes of the factorial analysis with Statistica 7.1 software.

Group	Plots	Factor 1	Factor 2	Group	Plots	Factor 1	Factor 2
	FDEFS1	-0. 107	-0. 039		PCAFS9	0. 206	0. 462
	FDEFS2	-0. 139	-0. 181		PCAFD1	0. 256	0. 563
	FDEFS3	-0. 158	-0. 151		PCAFD4	0. 256	0. 563
	FDEFS4	-0. 108	-0. 093		PCAFD5	0. 227	0. 689
	FDEFS5	-0. 161	-0. 179		PCAFD6	0. 168	0. 574
	FDEFS6	-0. 156	-0. 148		PCAFD7	0. 283	0. 707
	FDEFS7	-0. 136	-0. 077		PCAFD8	0. 250	0. 654
	FDEFS8	-0. 140	-0. 175		PCAFS1	0. 291	0. 603
	FDEFS9	-0. 176	-0. 196		PCAFS2	0. 298	0. 712
	FDEFS10	-0. 127	-0. 075		PCAFS3	0. 227	0. 689
	FNBAS1	-0. 123	-0. 081		PCAFS4	0. 301	0. 621
	FNBAS2	-0. 175	-0. 185		PCAFS5	0. 175	0. 583
	FNBAS3	-0. 155	-0. 130		PCAFS6	0. 238	0. 744
	FNBAS4	-0. 120	-0. 153		PCAFS7	0. 168	0. 557
	FNBAS5	-0. 118	-0. 146	Group II	PCAFS8	0. 283	0. 707
	FNBAS6	-0. 072	-0. 078		PCAFS10	0. 222	0. 607
	FNBAS7	-0. 157	-0. 128		PHEVD2	0. 208	0. 310
	FNBAS8	-0. 154	-0. 151		PCAFD2	0. 626	0. 440
	FNBAS9	-0. 159	-0. 170	Group III	PCAFD3	0. 714	0. 458
	FNBAS10	-0. 154	-0. 139		PCAFD9	0. 639	0. 361
	PHEVD1	-0. 002	0. 149		PCAFD10	0. 659	0. 506
	PHEVD3	-0. 002	0. 149		PCAOD1	0. 665	-0. 173
	PHEVD4	-0. 002	0. 149		PCAOD3	0. 572	-0. 202
	PHEVD5	-0. 002	0. 149		PCAOD4	0. 668	0. 031
	PHEVD6	-0. 002	0. 149		PCAOD5	0. 760	-0. 179
	PHEVD7	0. 013	0. 115		PCAOD6	0. 679	-0. 304
	PHEVD8	-0. 002	0. 149		PCAOD7	0. 673	0. 024
	PHEVD9	-0. 002	0. 149		PCAOD8	0. 627	-0. 222
	PHEVD10	-0. 002	0. 149		PCAOD9	0. 673	0. 024
	PTECD1	-0. 017	0. 018		PCAOD10	0. 582	-0. 216
	PTECD2	-0. 048	-0. 028	Group IV	PCAOS3	0. 647	-0. 341
	PTECD3	0. 012	0. 143		PCAOS6	0. 647	-0. 341
	PTECD4	-0. 017	0. 018		PCAOS9	0. 534	-0. 224
	PTECD5	0. 003	0. 022		PCAOD2	0. 885	-0. 384
	PTECD6	-0. 003	0. 019		PCAOS1	0. 885	-0. 384
	PTECD7	-0. 017	0. 018		PCAOS2	0. 885	-0. 384
	PTECD8	-0. 048	-0. 028		PCAOS4	0. 885	-0. 384
	PTECD9	0. 010	0. 106		PCAOS5	0. 885	-0. 384
Group I	PTECD10	-0. 019	0. 013	Group V	PCAOS7	0. 885	-0. 384
					PCAOS8	0. 885	-0. 384
					PCAOS10	0. 885	-0. 384

Appendix 2. Frequencies, origins and uses of shrubs and trees with DBH≥10 cm assessed in biotopes of Duekoué and Scio forests.

Taxa	FDEFS	FNBAS	PCAFD	PCAFS	PCAOD	PCAOS	PHEVD	PTECD	Origin	Uses
Aidia genipiflora (DC.) Dandy	3	1							Natural	
Albizia adianthifolia (Schum.) W.F.Wight		4		1					Natural	
Albizia glaberrima (Schum. and Thonn.) Benth.			3	1				2	Natural	
Albizia zygia (DC.) J.F.Macbr.		3	2	1					Natural	
Amphimas pterocarpoides Harms	3	5							Natural	Wood
Anthonotha fragrans (Bak.f.) Exell and Hillcoat		2							Natural	
Anthonotha macrophylla P.Beauv.	5								Natural	
Antiaris toxicaria Loes. var. *africana* C.C.Berg	3								Natural	Wood
Antrocaryon micraster A.Chev. and Guill.		2							Natural	
Baphia nitida Lodd.	6	4							Natural	
Baphia pubescens Hook.f.	9	8							Natural	
Belonophora hypoglauca (Welw. ex Hiern) A.Chev.		2							Natural	
Blighia unijugata Bak.		3							Natural	
Blighia welwitschii (Hiern) Radlk.		2							Natural	
Bombax brevicuspe Sprague		4							Natural	Wood
Bombax buonopozense P.Beauv.		4							Natural	Edible
Bussea occidentalis Hutch.		2							Natural	
Caloncoba gilgiana (Sprague) Gilg		4							Natural	
Calpocalyx aubrevillei Pellegr.	5								Natural	
Calpocalyx brevibracteatus Harms		4							Natural	
Ceiba pentandra (L.) Gaertn.				1					Natural	Wood, edible
Celtis adolfi-fridericii Engl.		8							Natural	
Celtis mildbraedii Engl.	9	9							Natural	
Celtis zenkeri Engl.	3	8							Natural	
Christiana africana DC.		1							Natural	
Chrysophyllum perpulchrum Hutch. and Dalz.	8	5							Natural	
Chrysophyllum taiense Aubrév. and Pellegr.	3	7							Natural	
Cleistopholis patens (Benth.) Engl. and Diels		4							Natural	
Coffea canephora Froenh. (= Coffee)			1	1					Exotic	Crop
Cola caricaefolia (G.Don) Schumann		2							Natural	
Cola lateritia Schumann	8	5							Natural	Edible
Cola nitida (Vent.) Schott and Endl.	5	5							Natural	
Cordia guineensis Schum. and Thonn.			2						Natural	Medicine

Appendix 2. Contd.

Cordia platythyrsa Bak.	3				3					Natural	craft
Corynanthe pachyceras Schumann	3	7								Natural	
Dacryodes klaineana (Pierre) Lam	1									Natural	
Desplatsia dewevrei (De Wild. and Th.Dur.) Burret	3	4								Natural	
Diospyros canaliculata De Wild.		3								Natural	
Diospyros ferrea (Willd.) Bakh.									1	Natural	
Diospyros vignei F.White									1	Natural	
Diospyros viridicans Hiern		1								Natural	
Discoglypremna caloneura (Pax) Prain		2								Natural	
Distemonanthus benthamianus Baill.	4		1	1						Natural	Wood
Drypetes chevalieri Beille		1								Natural	
Elaeis guineensis Jacq.			3	1	5		1			Exotic	Edible, cosmetic
Entandrophragma angolense (Welw.) C. DC.		1								Natural	Wood
Entandrophragma cylindricum (Sprague) Sragues		7								Natural	Wood
Entandrophragma utile (Dawe and Sprague) Sprague	3									Natural	Wood
Eribroma oblongum (Mast.) Pierre ex Germain		6								Natural	Wood
Erythrophleum ivorense A.Chev.		2								Natural	craft
Erythroxylum mannii Oliv.		1								Natural	
Euadenia trifoliolata (Schum. and Thonn.) Oliv.		1								Natural	
Ficus exasperata Vahl						1	2		2	Natural	
Funtumia africana (Benth.) Stapf	5	2								Natural	craft
Funtumia elastica (Preuss) Stapf	9	7		1						Natural	craft
Glyphaea brevis (Spreng.) Monachino	6									Natural	Medicine
Greenwayodendron oliveri (Engl.) Verdc.	4	2								Natural	Medicine
Guarea cedrata (A.Chev.) Pellegr.	3									Natural	
Guibourtia ehie (A.Chev.) J.Léonard	3	1								Natural	Wood
Gymnostemon zaizou Aubrév. and Pellegr.		1								Natural	
Harungana madagascariensis Lam. ex Poir.			3	2						Natural	Medicine
Hevea brasiliensis Müll.Arg. (= Rubber)							1			Exotic	Crop
Holoptelea grandis (Hutch.) Mildbr.		2								Natural	
Irvingia gabonensis (O' Rorke) Baill.	3	2								Natural	Edible
Keayodendron bridelioides (Hutch. and Dalz.) Léandri		3								Natural	

Appendix 2. Contd.

Klainedoxa gabonensis Pierre	4								Natural	
Lannea welwitschii (Hiern) Engl.	3								Natural	craft
Lecaniodiscus cupanioides Planch.	6								Natural	
Maesobotrya barteri (Baill.) Hutch.	6								Natural	
Mangifera indica L.			6	4	3		1		Exotic	Edible
Mansonia altissima (A.Chev.) A.Chev.		2							Natural	Wood
Maranthes aubrevillei (Pellegr.) Prance		2							Natural	
Mareya micrantha (Benth.) Müll.Arg.		6						1	Natural	Medicine
Microdesmis keayana J.Léonard		4							Natural	
Milicia excelsa (Welw.) Berg	4	2	4	3				1	Natural	Wood
Millettia zechiana Harms	8	3						1	Natural	
Monodora tenuifolia Benth.		2							Natural	
Musa paradisiaca L.					3				Exotic	Food
Musanga cecropioides R.Br.		2							Natural	
Myrianthus arboreus P.Beauv.	8								Natural	Edible
Myrianthus libericus Rendle	3	4							Natural	Edible
Napoleonaea vogelii Hook. and Planch.	3								Natural	Edible, craft
Nauclea diderrichii (De Wild. and Th.Dur.) Merrill								2	Natural	Wood
Nesogordonia papaverifera (A.Chev.) Cap.	6	9							Natural	Wood
Newbouldia laevis (P.Beauv.) Seem. ex Bureau	3	5							Natural	Edible, craft
Ochthocosmus africanus Hook.f.	3								Natural	
Ongokea gore (Hua) Pierre		2							Natural	Medicine
Ophiobotrys zenkeri Gilg	3								Natural	
Panda oleosa Pierre	5	5							Natural	
Parkia bicolor A.Chev.	3	2							Natural	
Persea americana Mill.			1	2	4				Exotic	Edible
Petersianthus macrocarpus (P.Beauv.) Liben	9	8						2	Natural	Wood
Piptadeniastrum africanum (Hook.f.) Brenan	8	1							Natural	Wood
Placodiscus attenuatus J.B.Hall		1							Natural	
Pteleopsis hylodendron Mildbr.		2							Natural	
Pterygota macrocarpa Schumann		3							Natural	Wood
Pycnanthus angolensis (Welw.) Warb.		2						2	Natural	Wood
Raphia hookeri Mann and Wendl.					4				Natural	Edible
Ricinodendron heudelotii (Baill.) Pierre ex Heckel			1	1					Natural	Edible

Appendix 2. Contd.

Rinorea convallarioides (Bak.f.) Eyles		1							Natural	
Rothmannia hispida (Schumann) Fagerl.	4								Natural	
Rothmannia urcelliformis (Hiern) Robyns	3								Natural	
Samanea dinklagei (Harms) Keay	3	2							Natural	
Scottellia klaineana Pierre var. *mimfiensis* Pellegr.	4	4							Natural	Wood
Sterculia rhinopetala Schumann	5	9				2			Natural	Wood
Sterculia tragacantha Lindl.						1			Natural	
Stereospermum acuminatissimum Schumann		3							Natural	
Strombosia pustulata Oliv. var. *pustulata*	7	6							Natural	
Synsepalum afzelii (Engl.) Pennington	3								Natural	
Tectona grandis L.f. (= Teak)								1	Exotic	Wood
Terminalia ivorensis A.Chev.	3								Natural	Wood
Terminalia superba Engl. and Diels		3						1	Natural	Wood
Tetrapleura tetraptera (Schum. and Thonn.) Taub.		3							Natural	craft
Theobroma cacao L. (= Cocoa)			4		1	1			Crop	Crop
Tricalysia macrophylla Schumann	7								Natural	
Trichilia martineaui Aubrév. and Pellegr.		2							Natural	
Trichilia megalantha Harms	3	3							Natural	
Trichilia monadelpha (Thonn.) J.J.De Wild.		3							Natural	
Trichilia prieureana A.Juss.		7							Natural	
Triplochiton scleroxylon Schumann	3	4							Natural	Wood
Uapaca guineensis Müll.Arg.	5								Natural	
Vitex ferruginea Schum. and Thonn.		2							Natural	
Vitex micrantha Gürke	3								Natural	
Xylia evansii Hutch.		5							Natural	Wood
Xylopia quintasii Engl. and Diels		3							Natural	
Xylopia villosa Chipp	6	2							Natural	
Zanthoxylum gilettii (Engl.) Waterman	3	5							Natural	Wood
Zanthoxylum leprieurii Guill. and Perr.		5							Natural	
Total	58	85	12	13	7	4	4	12		

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Full Length Research Paper

NaCl tolerance studies at seedling stage among different genotypes of *Helianthus annus* L.

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A greenhouse research was conducted at the research area of Department of Plant Breeding and Genetics, University of Agriculture, Faisalabad to evaluate 20 accessions for the identification of salt tolerant genotypes of sunflower (Helianthus *annuus* L.) as well as their characteristics. The experiment was conducted in completely randomized design with three repeats. Salinity was developed with NaCl to achieve the final levels of 3, 6 and 9 dsm^{-1} salinity, whereas control contained tap water. After 60 days of planting, 10 seedlings of each accession from each treatment and replication were uprooted and data was recorded. Sunflower genotypes G-36, G-61, A-23, A-61 and A-185 performed better in both controlled and saline conditions. These genotypes showed better shoot and root growth and biomass by least concentration of Na^+ and higher concentration of Cl^- in leaf sap.

Key words: Tap water, NaCl, replication, leaf sap.

INTRODUCTION

Sunflower (*Helianthus annuus* L.) is the second most important oilseed crop after soybean worldwide (Paniego et al., 2002). Sunflower is high yielding and non-conventional oilseed crop. It had desirable traits that is high oil contents (40-47%), protein (23%), high linoleic acid, toxic free elements and contain vitamins A, D, E, K. Salinity in soil or water is one of the major stresses and especially in arid and semi arid regions, can severely limit crop production. Salinity impairs seed germination, reduces nodule formation, retards plant development and reduces crop yield. High levels of soil salinity negatively affect productivity of most field crops (Munns, 1993). Saline soils remarkably reduce oil production potential and oil yield of sunflower (Szabolcs, 1994).The plants that grow in saline soils have diverse ionic compositions and a range in concentrations of dissolved salts. These concentrations fluctuate because of changes in water source, drainage, evapotranspiration, and solute availability. About 7% of arable lands of the world are under salinity pressure (Jumsoon et al., 1996). Soil

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Table 1. Soil analysis.

Determination	Value
Electrical conductivity (E.C.)	1.23 (d Sm^{-1})
Saturation percentage (S.P)	25.70 (%)
Total soluble salt (TSS)	17.7 (me L^{-1})

Table 2. Water analysis.

Parameter	Value
EC	1.036 (me L^{-1})
Na^{+}	3.83 (me L^{-1})
$Ca^{+} + Mg^{++}$	6.53 (me L^{-1})
TSS	10.36 (me L^{-1})

salinity reduces water availability of plant roots via negative (low) osmosis potential, as well as decrease of germination dynamics of plant seeds by ionic toxicity of behavior and response of different accessions of sunflower to tolerate salt stress at seedling stage. The Na+ and Cl- (Munns et al., 1988).

The study was conducted to understand the genetic information so obtained will be useful in formulating criteria for salt stress tolerance and high yield. The objective was also the development of selection criteria through correlation and path analysis studies. The selected types could be used in hybridization programme aimed at breeding for sunflower yielding high under salt stress conditions. The availability of high yielding salt stress tolerant sunflower is perceived to attract farmer to use the land resources otherwise left fallow due to salt stress.

MATERIALS AND METHODS

The present study was carried out under the glass house of the department of Plant Breeding and Genetics, University of Agriculture, Faisalabad. The research material was comprised of 20 accessions of sunflower developed by the Oilseed Research Programme of the Department. These accessions (G-16, G-30, G-32, G-36, G-44, G-45, G-61, G-64, G-66, G-68, G-86, A-2, A-14, A-23, A-56, A-60, A-61, A-79, A-133 and A-185) were planted and evaluated for various traits in the salinity experiments.

Experimental layout

Experiment was conducted in a glass house with no control of humidity, temperature and light. The experiment was laid out following factorial complete randomized design in three replications. The sunflower seed were planted in iron trays. Each tray was filled with soil and sand in the ratio of 1:1. The seeds were sown at the depth of 1.5 cm by maintaining distance of 2.5 cm each for row to row and seed to seed.

Planting medium

Normal soil free from any salinity and sodicity hazards was collected from the research area of department of Plant Breeding and Genetics, University of Agriculture Faisalabad. The mixture of sand and soil was air dried, ground and passed through 2 mm sieve and analyzed for chemical characters (Table 1).

Treatments

Tap water was applied for irrigation for 15 days according to requirement. After germination, four salt (NaCl) levels of irrigation water were maintained: treatment 1 = normal water (tap water); treatment 2 = 3 dsm^{-1}; treatment 3 = 6 dsm^{-1}; Treatment 4 = 9 dsm^{-1}. Composition of tap water is given in Table 2.

Tissue sap analysis for ion uptake

Sample collections

The 10 randomly selected plants per replication and per treatment for each genotype were uprooted. Two lower leaves (form the basal node) and two upper leaves (from the top node) were collected, washed with tap water to remove the soil residues and then dipped instantly in distilled water for a short period of time. The samples were blotted dry with the help of a sheet of blotting paper, placed in polyethylene bags, marked with the spirit marker and stored in the deep freezer for tissue sap extraction.

Extraction of leaf sap

Frozen leaf samples were thawed, after washing with distilled water, the tissue sap was extracted by using metal rod .The tissue sap oozing out from the samples was collected in epindroph tubes and immediately stored back in the deep freezer.

Centrifugation of tissue sap

The epindroph tubes were taken out from the freezer and placed at the room temperature to thaw. Then the thawed tissue sap was centrifuged at the 6500 rpm for 5 min. The supernatant tissue sap samples from epindroph tubes were analyzed for chloride, sodium and potassium ions.

Determination of chloride ions

Chloride ions in the tissue sap was determined by chloride analyzer (Sherwood chloride analyzer 926).

Determination of sodium ions

The tissue sap was diluted as required with distilled water. Sodium ions were determined using Flame Photometer (Sherwood flame photometer 410).

Data recording

After 60 days of planting, ten seedlings of each accession from each treatment and replication were uprooted. Data were recorded from the experiment on following parameters viz. Germination percentage (G %), emergence index (EI), emergence rate index (ERI), chlorophyll content, root: shoot ratio, mortality (M %).

Table 3. Comparisons of mean square values of analysis of variance of different salt stress levels on different traits.

Character	Control (0 d sm^{-1})		Salt stress level 1 (3 d sm^{-1})		Salt stress level 2 (6 d sm^{-1})		Salt stress level 3 (9 d sm^{-1})	
	Genotype	Error	Genotype	Error	Genotype	Error	Genotype	Error
Shoot length	24.820**	1.323	40.789**	7.374	50.263**	10.248	21.455**	2.115
Root length	0.432**	0.007	0.892**	0.106	0.563**	0.061	0.361**	0.042
Na^+ content	34.856**	1.317	139.807**	0.950	285.863**	0.933	767.530**	1.517
Cl^- content	1317.631**	1.717	2608.351**	1.183	1752.754**	1.667	3275.074**	5.800
Chlorophyll	26.799**	1.937	27.316**	4.588	34.025**	2.897	37.409**	0.434
Mortality %	10.877**	0.283	33.010**	4.908	57.202**	5.343	190.789**	9.207
Root / shoot ratio	0.014**	0.005	0.001**	4×10^{-3}	0.001**	2.5×10^{-4}	0.001**	1.75×10^{-3}

*Significant, **highly significant; ^{NS}Non significant.

Table 4. Mean Squares from the analysis of variance for germination percentage (G%), emergence index (EI), emergence rate index (ERI) of different sunflower genotypes.

SOV	DF	G %	E.I.	E.R.I.
Genotype	19	245.263^{NS}	71.835*	0.005^{NS}
Error	40	140.000	30.773	0.004
Total	59			
C.V.		12.20%	6.65%	7.00%

LSD value of emergence index = 9.154.

RESULTS AND DISUCSSION

The experimental results were obtained, presented and discussed separately.

Analysis of variance under four treatments

Under salt stress condition level 2 (6 dS m^{-1}) and level 3 (9 dS m^{-1}), significant differences and marked variation among accessions for all the traits were detected under normal and all salt stress levels, furthermore, the genotypes behaved differently to the stress. The comparison of treatment expression of various plant traits under normal and salt stress level 3 (9 dS m^{-1}) conditions suggested that salt stress adversely affected for the characters (Table 3).

Germination percentage (G%)

The analysis of variance of germination percentage of sunflower population under the study (Table 3). Non significant differences existed among of genotypes of the sunflower.

Emergence index (EI)

The analysis of variance of sunflower genotypes for emergence index is shown in Table 4. The results indicate that sunflower genotypes had significant differences and ranged from 91.80 to 75.00 (Table 5). The maximum value 91.80 was observed in the genotype A-85 followed by the genotype A-14 (90.40) and genotype A-133 (89.13).

Emergence rate index (ERI)

Table 4 reveals that the differences for emergence rate index among genotypes were non-significant.

Effect of salt stress (NaCl) on shoot length (cm)

Table 6 indicates that the shoot length was decreased significantly with increasing salinity levels. Interaction of all the genotypes and treatments was found significant.

Table 6 shows that accession G-36 closely followed by G-66 and A-23 had maximum shoot length and accession A-60 followed by A-2 and G-30 had minimum shoot length under normal condition. Accession A-23 closely followed by G-36 and G-64 had maximum shoot length under salt stress level 1 (3 dsm^{-1}). Accessions G-36 closely followed by G-45 and G-44 had maximum shoot length under salt stress level 2 (6 dsm^{-1}). Accession G-86 closely followed by G-30 and A-14 had maximum shoot length under salt stress level 3 (9 dsm^{-1}). Ramoliya and Panday (2003); Mer et al. (2000) and Ramdiya and Panday (2003) also demonstrated that salinity in nutrient

Table 5. Statistical Mean values of emergence index among various genotype of sunflower.

Genotype	Mean
A-185	91.80 A
A-14	90.40 A B
A-133	89.13 ABC
G-61	88.47 ABCD
G-45	87.80 ABCD
A-79	87.00 ABCD
G-64	86.93 ABCD
G-32	84.93 ABCDE
A-2	84.53 ABCDE
A-60	84.40 ABCDE
A-61	84.40 ABCDE
A-56	82.40 ABCDE
G-68	80.80 BCDE
G-66	80.40 BCDE
G-86	79.27 CDE
G-16	78.52 CDE
G-44	77.87 DE
G-30	77.67 DE
G-36	77.50 DE
A-23	75.00 E

The lines sharing common letters do not differ significantly from each other at 5% probability level.

solution reduced the growth of black spot (*Diospyros digvna* Jacq).

Effect of salt stress (NaCl) on root length (cm)

Table 7 shows that accession A-133 closely followed by A-60 and A-185 had maximum root length under normal condition. Accession G-66 closely followed by A-2 and A-79 had maximum root length under salt stress level 1 (3 dsm^{-1}). Accessions G-36 closely followed by A-2 and A-23 had maximum root length under salt stress level 2 (6 dsm^{-1}). Accession G-44 closely followed by G-36 and A-185 had maximum root length under salt stress level 3 (9 dsm^{-1}). Qureshi et al. (1998), Hussain and Rehman (1995) and Ghumman (2000) also conducted experiments on sunflower and found that root length and relative root length decreased with increase in salinity.

Effect of salt stress (NaCl) on chlorophyll content

Table 8 shows that accession A-79 closely followed by G-68 and G-32 had maximum chlorophyll under normal condition. Accession G-86 closely followed by G-68 and A-23 had maximum chlorophyll under salt stress level 1 (3 dsm^{-1}). Accessions A-61 closely followed by A-185 and A-60 had maximum chlorophyll under salt stress level 2 (6 dsm^{-1}). Genotypes G-68 closely followed by A-185 and G-66 had maximum chlorophyll under salt stress level 3 (9dsm^{-1}).

Effect of salt stress (NaCl) on sodium concentration (mol m^{-3}) in extracted leaf sap

Table 9 shows that accession A-23 closely followed by A-14 and A-61 had maximum Na+ content under normal condition. Accession A-56 closely followed by A-133 and A-61 had maximum Na^+ content under salt stress level 1 (3 dsm^{-1}). Genotype A-133 closely followed by A-56 and A-60 had maximum Na^+ content under salt stress level 2 (6 dsm^{-1}). The line A-133 closely followed by A-56 and A-60 had maximum Na^+ content under salt stress level 3 (9 dsm^{-1}). Nawaz et al. (2002) in sunflower also reported that the increase in sodium contents in leaves with increasing salinity was attributed to the increased amount of sodium ion in rooting medium, passive Na^+ diffusion through damaged membranes, decreased efficiency of exclusion mechanism.

Effect of salt stress (NaCl) on potassium concentration (mol m^{-3}) in extracted leaf sap of sunflower genotypes

Table 7 shows that accession A-60 closely followed by G-68 and G-32 had maximum K^+ content under normal condition. Accession G-32 closely followed by G-44 and G-45 had maximum K^+ content under salt stress level 1 (3 dsm^{-1}). The line A-56 closely followed by G-61 and G-44 had maximum K^+ content under salt stress level 2 (6 dsm^{-1}). Accession G-68 closely followed by G-66 and A-56 had maximum K^+ content under salt stress level 3 (9 dsm^{-1}). Decrease in K^+ concentration with increasing salinity was also reported a significant reduction of potassium in sorghum with increasing salinity. There is a debate that K^+ influx could be used as an index to salinity tolerance Shainberg and Levy (1992).

Effect of salt stress (NaCl) on chloride concentration (mol m^{-3}) in extracted leaf sap

Table 10 shows that genotype G-30 closely followed by G-185 and G-68 had maximum Cl^- content under normal condition. Accession G-68 closely followed by A-2 and A-56 had maximum Cl^- content under salt stress level 1 (3 d Sm^{-1}). The line G-32 closely followed by G-36 and A-61 had maximum Cl^- content content under salt stress level 2 (6 d Sm^{-1}). Accession A-185 closely followed by G-36 and G-61 had maximum Cl^- content under salt stress level 3 (9 dsm^{-1}).

Table 6. Statistical comparison of varietal means for shoot length for various salt stress levels.

Genotype	Normal (0 dsm^{-1})	Salt stress Level 1 (3 dsm^{-1})	Salt stress Level 2 (6 dsm^{-1})	Salt stress Level 3 (9 dsm^{-1})
G-16	43.44De	39.19a-f	30.97d-g	24.52ef
G-30	40.78Gh	40.70a-e	34.57b-e	29.81ab
G-32	43.54De	37.68b-f	30.23d-g	27.60b-d
G-36	51.14A	42.76ab	42.21a	26.77c-e
G-44	43.16d-f	41.46a-c	37.85a-c	26.97c-e
G-45	45.86C	41.65a-c	38.66ab	24.22ef
G-61	44.30Cd	41.34a-c	32.24c-g	27.40b-d
G-64	46.22C	42.11a-c	35.89b-d	24.89d-f
G-66	48.87B	33.95fg	31.85c-g	24.54ef
G-68	44.32Cd	35.52d-g	28.93e-g	23.15fg
G-86	44.40Cd	41.82a-c	36.46b-d	30.34a
A-2	40.29Gh	30.82g	28.37e-g	21.00gh
A-14	43.22d-f	39.29a-f	34.37b-e	27.87a-c
A-23	46.41C	43.45a	33.28b-f	23.02fg
A-56	43.44De	40.89a-d	30.94d-g	26.48c-e
A-60	39.57H	31.76g	26.18g	20.14h
A-61	41.81e-g	36.98c-f	30.32d-g	26.81c-e
A-79	43.64De	35.90d-g	29.11e-g	26.27c-e
A-133	40.84Gh	35.53e-g	27.54fg	22.75fg
A-185	41.23f-h	37.74b-f	31.54d-g	24.47ef

Means sharing common letters do not differ significantly at 5% probability level using LSD. LSD for genotypes under Normal condition =1.898; LSD for genotypes at salt stress level 1 (3 dsm^{-1}) = 4.589; LSD for genotypes at salt stress level 2 (6 dsm^{-1}) = 5.283; LSD for genotypes at salt

Table 7. Statistical comparison of varietal means for root length for various salt stress levels.

Genotype	Normal (0 dsm^{-1})	Salt stress Level 1 (3 dsm^{-1})	Salt stress Level 2 (6 dsm^{-1})	Salt stress Level 3 (9 dsm^{-1})
G-16	6.730J	6.540c-e	6.349c-f	5.93b-d
G-30	6.730j	6.650c-e	6.117ef	5.47f-h
G-32	7.097g-i	5.533f	6.553b-e	5.20h
G-36	7.357De	7.117a-c	7.717a	6.25ab
G-44	7.130gh	6.233de	6.473b-f	6.54a
G-45	7.407d	7.147a-c	6.067f	5.92b-d
G-61	7.283d-f	6.850b-d	6.207d-f	5.90b-d
G-64	7.117g-i	6.623c-e	6.097ef	5.66d-g
G-66	7.080g-i	7.690a	6.260d-f	5.45f-h
G-68	6.973I	7.160a-c	6.273d-f	5.44f-h
G-86	7.187f-h	6.790c-e	6.343c-f	5.89b-e
A-2	6.593Jk	7.630a	7.513a	5.51e-h
A-14	7.217e-g	6.210e	6.570b-e	5.50f-h
A-23	7.620C	7.417ab	6.870b	5.78c-f
A-56	6.497K	6.250de	6.443b-f	5.57d-h
A-60	7.797ab	6.597c-e	6.773bc	5.29gh

Table 7. Contd.

A-61	7.227e-g	6.917bc	6.327c-f	5.45f-h
A-79	7.053Hi	7.537a	6.580b-e	5.42f-h
A-133	7.883A	6.530c-e	6.627b-d	5.33gh
A-185	7.703Bc	7.080a-c	6.797bc	6.07bc

Means sharing common letters do not differ significantly at 5% probability level using LSD. LSD for genotypes under Normal condition =0.138; LSD for genotypes at salt stress level 1 (3 dsm^{-1}) = 0.537; LSD for genotypes at salt stress level 2 (6 dsm^{-1}) = 0.408; LSD for genotypes at salt stress level 3 (9 dsm^{-1}) = 0.338.

Table 8. Statistical comparison of varietal means for chlorophyll for various salt stress levels.

Genotype	Normal (0 dsm^{-1})	Salt stress Level 1 (3 dsm^{-1})	Salt stress Level 2 (6 dsm^{-1})	Salt stress Level 3 (9 dsm^{-1})
G-16	31.73e-g	31.39e-g	35.79de	30.81l
G-30	35.44a-c	28.24g	33.06ef	36.07hi
G-32	36.14ab	30.24fg	34.65de	35.18ij
G-36	24.96i	34.19c-f	40.38ab	34.63j
G-44	31.22f-h	33.38def	35.35de	36.42gh
G-45	29.02h	32.84ef	41.28ab	37.48fg
G-61	34.77a-d	35.03b-e	40.69ab	37.52fg
G-64	29.66gh	37.57a-d	35.85de	36.18hi
G-66	32.76d-f	35.32b-e	41.91ab	39.59c
G-68	37.02a	39.11ab	39.57bc	46.46a
G-86	31.10f-h	40.58a	36.41d	39.44cd
A-2	31.74e-g	37.29a-d	35.36de	38.26d-f
A-14	34.13b-e	37.11a-d	36.93cd	36.42gh
A-23	35.56a-c	37.86a-c	35.72de	37.91ef
A-56	32.02e-g	33.96c-f	37.47cd	38.76c-e
A-60	33.37c-f	34.28c-f	42.21ab	38.98c-e
A-61	31.45f-h	34.57c-e	42.95a	32.86k
A-79	37.15a	37.81a-c	37.02cd	38.84c-e
A-133	32.62d-f	33.88c-f	31.75f	34.81j
A-185	35.14a-d	34.71c-e	42.90a	44.50b

Means sharing common letters do not differ significantly at 5% probability level using LSD. LSD for genotypes under Normal condition= 2.297; LSD for genotypes at salt stress level 1 (3 dsm^{-1})= 3.535; LSD for genotypes at salt stress level 2 (6 dsm^{-1}) = 2.440; LSD for genotypes at salt stress level 3 (9 dsm^{-1})= 1.087.

Table 9. Statistical comparison of varietal means for na^{+} concentration (mol m^{-3}) for various salt stress levels.

Genotype	Normal (0 dsm^{-1})	Salt stress Level 1 (3 dsm^{-1})	Salt stress Level 2 (6 dsm^{-1})	Salt stress Level 3 (9 dsm^{-1})
G-16	17.67e-g	23.00j	55.00e	72.00g
G-30	16.00G	31.00fg	50.00gh	62.67j
G-32	19.00c-f	40.00c	44.67i	49.67k
G-36	17.33e-g	24.33ij	51.67fg	71.33g
G-44	17.00Fg	29.00h	38.67k	43.33l
G-45	12.67h	36.00de	69.00b	68.33h
G-61	16.00g	23.00j	50.00gh	73.33fg
G-64	20.00b-d	25.33i	52.00f	65.00i
G-66	19.00c-f	38.67c	59.67c	73.33fg

Table 9. Contd.

G-68	17.00fg	29.67gh	45.33i	90.00c
G-86	16.00g	26.00i	60.67c	91.00c
A-2	17.00fg	36.67d	59.00c	83.00d
A-14	21.33b	34.33e	51.00fg	77.00e
A-23	30.33a	35.00de	49.00h	75.00ef
A-56	19.00c-f	46.33a	70.00b	100.00a
A-60	18.00d-g	31.67f	69.67b	95.00b
A-61	20.67bc	41.67b	59.00c	65.33i
A-79	17.00fg	31.00fg	41.00j	51.67k
A-133	19.33b-e	43.00b	72.33a	101.30a
A-185	19.00c-f	31.00fg	57.00d	73.00fg

Means sharing common letters do not differ significantly at 5% probability level using LSD. LSD for genotypes under normal condition = 1.894; LSD for genotypes at salt stress level 1 (3 dsm^{-1}) = 1.608; LSD for genotypes at salt stress level 2 (6 dsm^{-1})=1.594; LSD for genotypes at salt stress level 3 (9 dsm^{-1})= 2.032.

Table 10. Statistical comparison of varietal means for cl^- concentration (mol m^{-3}) for various salt stress levels.

Genotype	Normal (0 dsm^{-1})	Salt stress Level 1 (3 dsm^{-1})	Salt stress Level 2 (6 dsm^{-1})	Salt stress Level 3 (9 dsm^{-1})
G-16	46.00j	80.67k	152.30f	128.00hi
G-30	98.00a	71.00m	136.30h	126.70i
G-32	61.00g	41.00q	169.70a	173.70c
G-36	78.67e	71.33m	166.00b	181.70b
G-44	66.00f	67.67no	76.67o	82.67l
G-45	82.67d	74.00l	150.00f	83.00l
G-61	78.33e	117.00f	126.30k	175.00c
G-64	47.00j	87.33j	141.70g	152.30e
G-66	39.00l	119.00e	150.30f	155.70e
G-68	86.00c	145.70a	135.00h	165.00d
G-86	33.00m	123.70d	128.70j	147.30f
A-2	38.00l	137.70b	152.00f	131.00h
A-14	42.00k	56.67p	100.30n	144.70fg
A-23	57.00h	73.33l	132.00i	147.00f
A-56	39.67l	134.00c	158.00d	93.67k
A-60	54.67i	66.00o	152.30f	124.70i
A-61	55.00hi	68.33n	160.30c	141.00g
A-79	32.33m	101.00g	114.70l	111.30j
A-133	34.00m	91.00i	103.00m	91.67k
A-185	88.67b	93.67h	154.70e	196.00a

Means sharing common letters do not differ significantly at 5% probability level using LSD. LSD for genotypes under Normal condition= 2.162; LSD for genotypes at Salt Stress Level 1 (3 dsm^{-1})= 1.795; LSD for genotypes at Salt Stress Level 2 (6 dsm^{-1}) = 2.131; LSD for genotypes at Salt Stress Level 3 (9 dsm^{-1})= 3.974

Effect of salt stress (NaCl) on mortality % in sunflower genotypes

Table 11 shows that accession A-185 followed by A-66 and A-79 had minimum mortality % under normal condition. The genotype G -14 followed by G-61 and G-45 had minimum mortality % under salt stress level 1 (3 dsm^{-1}). The line G-44 followed by A-79 and G-16 had minimum mortality % under salt stress level 2 (6 dsm^{-1}). Accession G-30 followed by A-79 and G-68 had minimum

Table 11. Statistical comparison of varietal means for mortality (%) for various salt stress levels.

Genotype	Normal (0 dsm^{-1})	Salt stress Level 1 (3 dsm^{-1})	Salt stress Level 2 (6 dsm^{-1})	Salt stress Level 3 (9 dsm^{-1})
G-16	0.00c	7.41bc	14.81d	36.70de
G-30	3.33b	6.33bc	20.00c	16.67l
G-32	6.67a	13.33a	23.33a-c	50.00A
G-36	0.00c	10.37ab	21.48bc	36.30de
G-44	0.00c	6.67bc	10.00e	40.00cd
G-45	3.33b	3.33c	23.33a-c	36.67de
G-61	0.00c	3.33c	23.33a-c	30.00fg
G-64	0.00c	6.67bc	23.33a-c	33.33ef
G-66	0.00c	13.33a	26.67a	33.33ef
G-68	3.33b	13.33a	23.33a-c	26.67gh
G-86	0.00c	6.70bc	24.81ab	42.59bc
A-2	0.00c	3.33c	26.67a	26.67gh
A-14	0.00c	3.33c	20.00c	40.00cd
A-23	0.00c	7.04bc	21.48bc	28.89fg
A-56	0.00c	10.00ab	20.00c	33.33ef
A-60	0.00c	10.00ab	20.00c	26.67gh
A-61	0.00c	10.00ab	20.00c	36.67de
A-79	0.00c	6.67bc	13.33de	23.33H
A-133	3.33b	10.00ab	20.00c	46.67ab
A-185	0.00c	10.00ab	26.67a	33.33ef

Means sharing common letters do not differ significantly at 5% probability level using LSD. LSD for genotypes under Normal condition= 0.878; LSD for genotypes at salt stress level 1 (3 dsm^{-1}) = 3.656; LSD for genotypes at salt stress level 2 (6 dsm^{-1}) = 3955; LSD for genotypes at salt stress Level 3 (9 dsm^{-1})= 5.007.

mortality % under salt stress level 3 (9 dsm^{-1}). Several researchers have also documented that higher concentration of salt in the rooting medium cause mortality of many plant species (Donahave et al., 1983).

Conclusion

The research concluded that the accession G-86 closely followed by G-30 and A-14 had maximum shoot length under salt stress level 3 (9 dsm^{-1}) whereas genotype G-44 closely followed by G-36 and A-185 had maximum root length under salt stress level 3 (9 dsm^{-1}). Genotypes G-68 closely followed by A-185 and G-66 had maximum chlorophyll under salt stress level 3 (9 dsm^{-1}) whereas line G-30 followed by A-79 and G-68 had minimum mortality % under salt stress level 3 (9 dsm^{-1}).

Conflict of interest

The authors have not declared any conflict of interest.

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Review

Maize: Panacea for hunger in Nigeria

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Maize (*Zea mays*) is always preferred to other crops, and it is fast becoming an industrial crop in Sub-Saharan African countries. Nigeria has been divided into low, medium, medium to high and high maize production potential groups. Traditionally, maize was mostly grown in forest ecology in Nigeria but large scale production has moved to the savanna zone, especially the Northern Guinea savanna, where yield potential is much higher. Maize yields in Nigeria is still very low due to biotic, abiotic agronomic factors like soil infertility, pests and diseases, drought, unavailability of improved germplasms, weeds, unremunerative prices, uncertain access to markets etc. Maize pests and diseases in Nigeria include downey mildew, rust, leaf blight, stalk and ear rots, leaf spots and maize streak virus, Striga attack, stem borers, termites, storage insects, beetle etc. Collaborative research efforts in Nigeria led to development of agronomic package for maize production for different farming systems. There are different readily-available ethnic maize dishes in Nigeria and due to lower cost and high starch contents, maize is commonly used as roughage feed for livestock, and also included in poultry feeds. Importance of maize as an easily harvested crop food with potential to mitigate food insecurity and alleviate poverty cannot be over-emphasized in the developing world.

Key words: Agronomy, ethnic foods, food insecurity, fertilizer, maize, sub-sahara Africa.

INTRODUCTION

Agbado (corn) sweet *agbado* (corn)
Loveliest food of the raining season
Strictly loved by old and young
The joy of the raining season
Oh come, how often do I see you come
The green oblong leaves.

Maize has always been preferred to any other crop, including cassava because most of the world's civilizations developed around grains rather than tuber crops (Fakorede, 2001). Maize is an astonishing plant, with an astonishing capacity to surprise humans. For instance, one seed planted can produce over 500 kernels in return. It is a plant that utilizes sunlight effectively and outstrips yield/hectare of other grains. Indeed, maize, which is fast becoming an industrial thereby, making it the first crop to be harvested for food during hunger period.

Although, maize did not originate from Africa, it was introduced to the continent in the 16th century, and by the 19th century, it had spread all over the continent. It is, perhaps, the most important cereal crop of significant economic importance in African countries that has replaced sorghum and millet. It is a staple food crop

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grown in diverse environments and consumed by people with varying food preferences and socio-economic background in Africa. Its taste has been easily accepted by the local population and therefore, it has been rapidly replacing traditional starchy foods like cassava. It is also an important crop when food items need to be transported to not self-sufficient population countries.

Other reasons for rapid adoption and expansion of maize include the facts that, it gives one of the highest yields per man-hour of labour spent on it; it provides nutrients in a compact form and it is easily transportable; the husks give protection against birds and rain; it is easy to harvest and does not shatter; it stores well if properly dried; it can be harvested over a long period first as immature cobs, but can be left standing in the field to maturity before harvesting; cultivars with different maturing periods are available. Also, in terms of taste, many people prefer maize to their local cereals (Purseglove, 1972; Pingali and Heisey, 1999; Verheye, 2010).

Importance of maize cannot be over-emphasized in the developing world, including the potential to mitigate the present food insecurity and alleviate poverty. Maize is a preferred staple food for over 900 million poor consumers, 120-140 million poor farm families and about one third of malnourished children (CIMMYT and IITA, 2010). In sub-Saharan Africa, absence or shortage of maize invariably leads to famine and starvation. It is estimated that by 2025, maize would have become the crop with the greatest production in developing countries and the world, and by 2050, the demand for maize in developing countries will double (CIMMYT and IITA, 2010). Due to the fact that maize is highly responsive to production inputs; its food and industrial uses are many, and its production potential can hardly be matched by any of the other major cereals. It is therefore, definitely a solution to hunger, which can salvage the famine population. As the popular Yoruba saying in Nigeria goes: *Igba gbogbo ni agbado ngba ni* (maize saves at all times).

The origin of maize is controversial (Galinat, 1988) due to the fact that modern maize is so far removed from ancestral form, which has its original form of seed dispersal through self-propagation. In addition, no wild race of maize currently exists, hence prototypes of maize have been difficult to reconstruct. Until recently, there were two major schools of thought regarding the origin of maize. The first one posited that Teosinte was the ancestor of maize, while the second one expressed that the cultigen was derived from hypothetical wild maize (Norman et al., 1995). Moreover, over the years, the former theory has become generally accepted (Doebly, 1990).

IMPORTANCE OF MAIZE

Maize is the world's most widely grown cereal, as it is grown in a range of agro-ecological environments, and more maize is produced annually than any other grain. It is the most important cereal crop in the economy of African countries, and is one of the most important commodities used for food aid. Owing to the fact that it is cheaper than other cereals (such as rice and wheat), it is more affordable to the vast majority of the population, and therefore, occupies a prominent position in the agricultural development agencies of several countries in Africa. It is an important staple food for more than 1.2 billion people in Sub-Saharan Africa (SSA) and Latin America. All parts of the crop can be used as food and non-food products (IITA, 2009) and as a versatile crop; maize has been put to a wider range of uses than any other cereal. Maize is widely consumed as food in many parts of the world, and it is a staple food in developing countries, particularly in continents of Latin America, Asia and Africa. It is also a basic ingredient for some indigenous drinks and food products. In the developed world, maize is largely used as livestock feed and raw material for industrial products, while in developing countries it is mainly used as food.

Maize is a staple food for about 50% of Sub-Sahara African population (IITA, 2009). It is an important source of carbohydrate, protein, iron, vitamin B and minerals. As food, the whole grain, freshly green or dried, may be used or may be processed traditionally by wet and dry milling methods to give a variety of food products. Preparation and uses of maize alone or in combination with other food material as staple food or snacks in Nigeria include the followings: *ogi* (in hot and cold forms), *tuwo*, *donkunnu*, *maasa*, Couscous, *akple*, *gwate*, *nakia*, *egbo*, *abari*, *donkwa*, *ajepasi*, *aadun*, *kokoro*, *elekute* etc. (Abdulrahaman and Kolawole, 2006) (Figure 1).

Maize as livestock feed

The bulk of the concentrated feed to farm animals consist of grains, and maize is the most important and preferred one due to its low cost (compared to other cereals), low fiber content and high starch content which consist of concentrated energy food that gives highest conversion of dry substance to meat, milk and eggs. Maize stover, which is the plant residue after the ear has been removed (contains 30 to 40% of the plants total nitrogen, 75% of the potassium, sulphur and magnesium and almost all the calcium) is used by many farmers in developing countries as roughage feed for livestock (Dowswell et al., 1996).

Silage maize is important feed in temperate areas (United States, Canada and Europe), and consists of entire plant, which is cut, chopped and placed in a structure for anaerobic fermentation so as to allow for storage. In 2005, of the 42% of the world maize produced in United States, 58% served as feed; while 17% went into industrial uses and ethanol production (FAO, 2006).

Maize can be processed into different products for

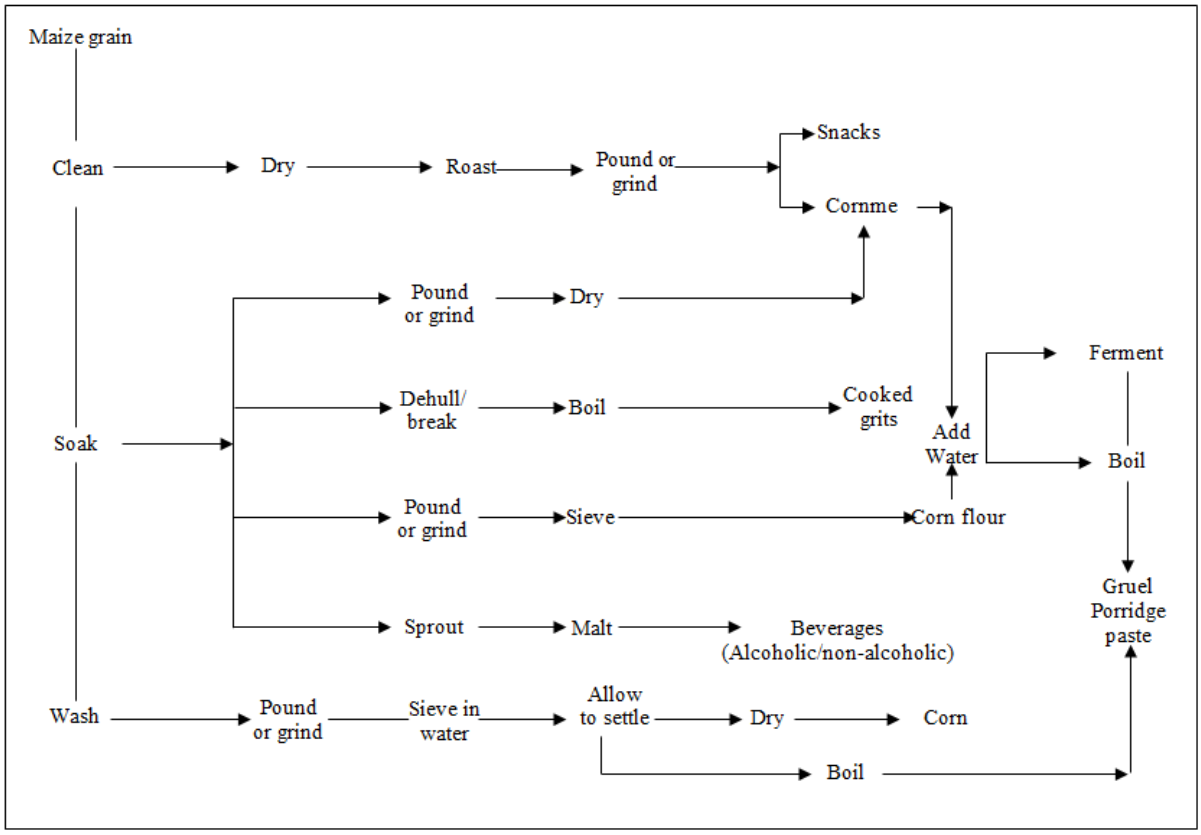

Figure 1. Utilization of dry maize grains. Source: Okoruwa, 1997.

Table 1. Annual world cereal harvest (3 year average 2002-2004).

Crop	Cultivated area ('000 ha)
Wheat	212,193
Rice	149,292
Maize	**143,355**
Barley	56,569
Sorghum	43,812
Millet	35,601
Oat	12,082
Rye	7,639

Source: FAOSTAT, 2005.

various end users at the traditional level and on industrial scale. A large proportions of products utilized in developing countries are obtained from traditional processing, while industrial processing meets the bulk of the demand in developed countries. Traditional commercial products obtained from maize are based on certain endosperm and some quality parameters, which influenced the choice and suitability of maize varieties for various uses. The properties include chemical, physical, biochemical, physico-chemical, organoleptic and rheological properties that can be influenced or altered favourably through breeding and other agronomic practices (Okoruwa, 1997).

GLOBAL MAIZE PRODUCTION TRENDS

Globally, maize ranked third after wheat and rice in terms of area harvested (Table 1) but in terms of annual production maize ranked first (Table 2). There is continuous increase in maize production all over the world and this is attributed to both increase in area of production and increase in yield per hectares. Maize grain production in the world in 1970 was about 266 million metric tons (MMT), while in year 2000, it was over 592 MMT and exceeded 844 MMT in 2010. Of the total global production, United States is far the biggest producer, contributing between 37-43% of the total world production (Table 3). In 2010 world ranking, United States was followed by China (177MMT), Brazil (56MMT), Mexico (23MMT) and Argentina (21MMT) (FAOSTAT, 2012). Maize production in United States and other developed countries of the world is highly mechanized on large scale production, where hybrids

Table 2. Annual Cereal Production (3 year average 2002-2004).

Crop	Average production ('000 metric tonnes)
Maize	**654,907**
Rice	588,947
Wheat	587,186
Barley	143,901
Sorghum	57,511
Millet	28,896
Oat	25,941
Rye	17,814

Source: FAOSTAT, 2005.

Table 3. World maize production and fraction produced in USA (metric tonnes).

Year	World production	USA	% by USA
1970	265831023	105471000	39.7
1980	396623417	168647000	42.5
1990	483343614	201832000	41.8
2000	592479279	251852000	42.5
2010	844405181	316165000	37.4

Source: FAOSTAT, 2012.

Table 4. Maize production, yield and area harvested in Africa and the World, 1986-2006.

Production (m tons)	1986[a]	1996[a]	2006[a]
World	472.3	564.1	736.1
Africa	31.7	40.3	48.9
Africa as % of World	6.7	7.1	6.6
Yield (tons/ha)	1986	1996	2006
World	3.6	4.1	4.9
Africa	1.4	1.6	1.7
Africa as % of World	39.6	38.8	35.7
Area harvested (ha)	1986	1996	2006
World	130.8	139.1	151.6
Africa	22.2	25.6	28.2
Africa as % of World	17.0	18.4	18.6

[a]Three-year averages for 1985-87; 1995-97 and 2005-07. Source: FARA, 2009.

maize varieties are commonly grown with high inputs in terms of agrochemicals.

Africa is a minor producer of maize by world standard (Table 4), accounting for only 7% of global production, while the average annual productions were estimated at 32MMT during 1985 - 1987, which increased to 49MMT during 2005-2007. Area planted with maize in West and Central Africa increased from 3.2 million in 1961 to 8.9 million in 2005, leading to increased production from 2.4MMT in 1961 to 10.6MMT in 2005 (IITA, 2009). Until the middle of the 20th century, maize production in the Sub-Sahara Africa grew mainly through expansion in the area planted. However, shortage of the land mass has eliminated area expansion as a potential source of production growth and there is an increased attention on increasing productivity. Basic facts about maize production and trade in Africa as highlighted by FARA (2009) stated that:

1) Africa accounts for 7% (49MMT) of global maize production.
2) Maize yield in Africa (1.7 tons/ha) account for 36% of global maize yield (4.9 tons/ha) (Table 4).
3) Maize production in Africa is increasing faster (2.8% per annum) than global production (2.5% per annum); however; global yields are increasing faster (1.6% per annum) than yields in Africa (1.3% per annum).
4) Africa is a net importer of maize. Average annual net imports have more than doubled from 4.57 million tons in 1995-1997 to 10.64 million tons in 2005-2007.
5) Maize imports have risen by 76% between 1995-1997 (6.82 million tons) and 2005-2007 (12 million tons), while exports have declined by 40% from 2.25 million tons to 1.35 million tons during the same period.
6) Africa accounts for 12% of the global maize imports.
7) Africa spends more than US$2.0billion on net imports of maize in 2005-2007.

MAIZE IN NIGERIA

Increase in maize production in Nigeria has been achieved greatly by expansion in area harvested rather than increase in yield. The area harvested increased from 2.8 million hectares in 1986 to over 3 million hectares in 2000 and over 6 million hectares in 2011. Of the total world production (844M tons) in 2010, Nigeria, the largest producer in Sub-Sahara Africa produced 7.7 million tons representing 0.9% of the world production.

Based on production potentials, Nigeria has been divided into four groups namely low, medium, medium to high and high maize production potential (ATA, 2011) (Figure 2). The average yield of maize in Nigeria as in other Sub-Sahara Africa countries is generally low 1.68 tons/hectare, which is very low compared to average yield in United States 9.3 tons/hectare over the same period (Table 5).

Maize production constraints in Nigeria

In view of the importance of maize in Nigeria, efforts are continuously made to increase maize yield per unit area of land and to extend areas where it can be grown,

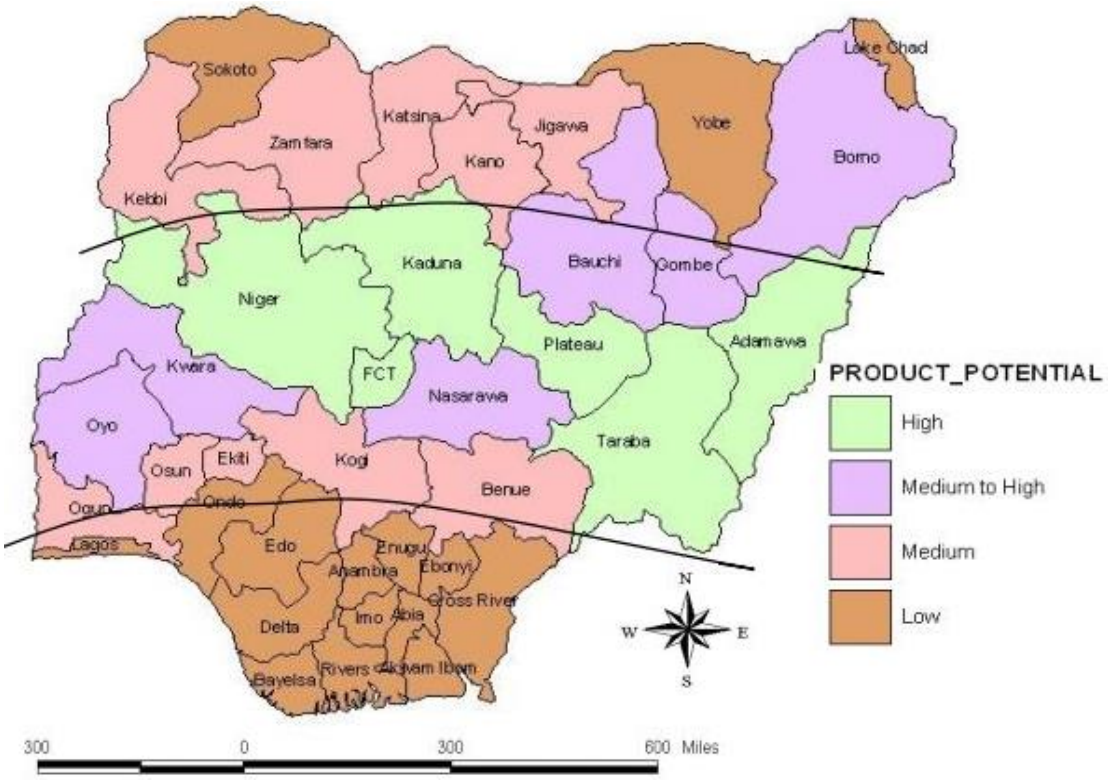

Figure 2. Maize production map of Nigeria. Source: ATA, 2011.

Table 5. Maize production in Nigeria and USA.

Year	Area harvested ('000ha)		Total production ('000t)		Yield (t/ha)	
	Nigeria	USA	Nigeria	USA	Nigeria	USA
1986	2,800,000	27,885,000	3550000	208,943,000	1.268	7.493
1987	3,408,000	24,080,000	4612000	181,142,000	1.353	7.523
1988	3,212,000	23,573,000	5268000	125,914,000	1.640	5.311
1999	3,423,000	28,525,000	5476000	239,549,000	1.600	8.398
2000	3,159,000	29,315,700	4107000	251,852,000	1.300	8.591
2001	3,283,000	27,829,700	4596000	241,375,000	1.400	8.673
2002	3,282,000	28,057,200	4898000	227,765,000	1.490	8.118
2003	3,469,000	28,710,300	5203000	256,227,000	1.500	8.925
2004	3,479,000	29,797,700	5567000	299,874,000	1.600	10.064
2005	3,589,000	30,399,000	5957000	282,261,000	1.660	9.285
2006	3,905,000	28,586,500	7100000	267,501,000	1.818	9.358
2007	3,944,000	35,013,800	6724000	331,175,000	1.705	9.458
2008	3,845,000	31,796,500	7525000	307,142,000	1.957	9.660
2009	3,350,560	32,168,800	7358260	332,549,000	2.196	10.338
2010	4,149,310	32,960,400	7676850	316,165,000	1.850	9.592
2011	6,008,470	33,986,300	9180270	313,918,000	1.528	9.237

Source: FAOSTAT 2013.

especially the cultivation of dry areas as improved through irrigation. Traditionally, maize has been mostly grown in forest ecology in Nigeria but large scale production has moved to the savanna zone, especially the Northern Guinea savanna, where yield potential is much higher than in the forest. The environmental condi-

Table 6. Grain yield (kg/ha) of maize genotypes grown at varying densities in South-western Nigeria.

Parameter	Density (plants/ha)			
	Ibadan		Ilora	
Maize genotype	53,333	80,000	53,333	80,000
Open-pollinated	3598.7	3913.7	3346.7	4899.0
Single cross hybrid	3542.3	5284.9	N.A.	N.A.
Double cross hybrid	3879.5	5162.3	3818.8	4889.7

N.A. = Not available; Source: Olaniyan and Lucas, 2004.

tions required for maize cultivation are therefore, superior in the savanna zone with high solar radiation, less incidence of biotic stresses and natural dryness at time of harvest (Kim et al., 1993). However, in spite of all efforts, maize yields in Nigeria, like in many other Sub-Sahara countries, is still very low compared to developed countries due to many constraints, which may be biotic, abiotic agronomic or others like low soil fertility, pests and diseases, drought, unavailability of improved germplasm, weeds, unremunerative prices, uncertain access to markets etc.

Agronomy of maize

Density of planting

Density of planting has significant effect on the yield of crop and specifically on maize. Studies on the impact of plant density on maize had shown that yield response to plant density varied substantially with management practices, maize genotypes, location, year and soil fertility level (Carolene and Russell, 1987).

Application of optimum plant density in maize production helps the proper utilization of solar radiation, which influences leaf area, interception and utilization of solar radiation and consequently maize dry matter accumulation and yield (Pepper, 1987). Generally, the dry matter per unit area of land of hybrid maize increased significantly with plant density and the highest density of planting produced the highest dry matter yield per unit area (Olaniyan and Lucas, 2001). However, in temperate regions, the relationship between total dry matter and density of planting in maize is asymptotic (Adelana and Milbourn, 1972).

Yield increases with increase in plant density up to a maximum for a maize genotype grown under a set of particular environmental and management conditions and decline when plant density is increased further. In other words, the relationship between grain yield and density in maize is parabolic (Cardwell, 1982).

Decline in grain yield per plant when plant density increased can be attributed predominantly to a decline in kernel number and kernel weight. Maize yield is low with low plant density because of little plasticity in leaf area per plant (Cox, 1996) and if plant density is too high, the reduction in the availability of resources per plant in the period surrounding silking generates a marked fall in yield per plant that is not offset by the increase in the plants number (Vega et al., 2001).

Maize as a sole crop is grown at population densities ranging from 18,000 to 55,000 plants/ha in Nigeria and the general recommendation is inter-row spacing of 75 and 25 cm as intra-row spacing to give 53,333 plants/ha. This recommendation is likely to be based on earlier studies where optimum density of 53,000-66,000 plants/ha gave optimum grain yield (Lucas, 1981). However, using high yielding FARZ 27 genotype, optimum yield at population density of 88,000 plant/ha was reported (Lucas, 1986). The density of 80,000 plants/ha was found optimal for hybrid maize varieties grown in Kaduna that is, Northern Guinea savanna zone of Nigeria and 53,333 plants/ha for Ikenne and Mokwa representing the forest and southern Guinea savanna zones respectively (Akintoye et al., 1997). Results of studies carried out in Ibadan and Ilora representing the Southwestern part of Nigeria, confirmed 80,000 plant/ha as the optimum density as against recommended density of 53,333 plants/ha for the areas (Table 6). Hybrid maize planted at the higher density produced 23 and 27% higher yields at Ibadan and Ilora respectively.

Efforts to push the density of planting to 100,000 plants/ha with high nitrogen level reduced grain yield by 15% (Olaniyan and Lucas, 2002). Maize grain yield is associated with the number of kernels per area, numbers of ear per plant and number of kernels per ear (Sangoi and Salvador, 1998). Echarte et al. (2000) found grain yield response to plant density to be positively and strongly related to number of kernel/m^2 and negatively and weakly related to weight/kernel.

Maize improvement in Nigeria

Maize researchers' efforts in Nigeria have no doubt improved maize production. The collaborative efforts of research institutes in Nigeria and advance maize breeding research programmes has led to many achievements including: (i) Development of agronomic package for maize production for different farming systems. (ii) Development and release of many maize genotypes based on the needs, requirements, prevailing pest and diseases in different agro ecological zones. (iii) Development of new varieties that yield up to twice the yield of the traditional varieties. (iv) Release of different maturing varieties: extra early, early, intermediate and late maturing which enable expansion of maize production to different areas, including areas with short rainy season. (v) Improvement in nutrient composition: quality protein maize were developed which provides better quality protein than normal maize in terms of

Table 7. Some improved open pollinated maize varieties in Nigeria.

Name of variety	Original source	Developing institution	Outstanding characteristics	Year of release	Year of Registration
Western yellow-1	Mixed germplasm from Caribbean and Mexico	I.A.R. &T Ibadan	High carotene content good for ogi. Also good for poultry feeds	1971	1991
096-EP6	Nigeria	FDAR Ibadan	High carotene content, good for pap and poultry feeds	1975	1991
TZPB	IITA, Ibadan	IITA, Ibadan	Big cobs, high yielding rust blight resistant	1975	1991
TZB	IITA, Ibadan	IITA, Ibadan	High yielding, good for pap	1975	1991
TZSR-W/Y	CIMMYT/ IITA, Ibadan	IITA, Ibadan	High yielding and widely adapted streak resistant	1981	1991
DMR-LSR (W&Y)	IITA, Ibadan	IITA, Ibadan	Resistant to downy mildew, sturdy and vigorous plants	1984	1991
KEWESOKE	I.A.R. and T Ibadan	I.A.R. and T Ibadan	Good for mixed cropping	1980	1991
TZESR – W	I.A.R. and T/IITA, Ibadan	I.A.R. and T/IITA Ibadan	Resistant to downy mildew, rust and blight	1982	1991
TZESR – Y	I.A.R.andT/IITA, Ibadan	I.A.R. and T/IITA Ibadan	Resistant to streak rust and blight	1982	1991
95 TZEE – W1 SAMMAZ 12	IITA, Ibadan	IITA, Ibadan, IAR, Samaru, Zaria	Extra earliness, high yield potential, and suitable in area with ≥ 600 mm rainfall distribution with 80days	2001	2001
95 TZEE–Y1 SAMMAZ 13	IITA, Ibadan	IITA, Ibadan, IAR, Samaru, Zaria	Extra earliness, high yield potential, and suitable in area with ≥ 600 mm rainfall distribution with 80days	2001	2001
PH 2 (OBA-FEMI)	Premier Seed Nig. Ltd. Zaria	Premier Seed Nig. Ltd. Zaria	Short plant type, high yield potential, resistant to lodging, good for mechanized harvesting	2001	2001

Table 7. Contd.

PH 5 (OBA - 99)	Premier Seed Nig. Ltd. Zaria	Premier Seed Nig. Ltd. Zaria	High yielding potential, quality protein maize	2001	2001
PH 6 (OBA - 98)	Premier Seed Nig. Ltd. Zaria	Premier Seed Nig. Ltd. Zaria	High yielding potential, quality protein maize	2001	2001
OBATANPA SAMMAZ 14	CRI, Kumasi Ghana	IAR, Samaru	High lysine and tryptophan contain, medium maturing, good seed quality, high yield, tolerance to Striga.	2005	2005
IWDC2SynF2 SAMMAZ 15	IITA, Ibadan	IITA, Ibadan, IAR, Samaru, Zaria	Medium maturing, good seed quality, high yield potential, tolerance to Striga hermonthica	2008	2008
TZLComp 1SynW-1	IITA, Ibadan	IITA, Ibadan, IAR, Samaru, Zaria	Late maturing, good seed quality, high yield, resistance to Striga hermonthica	2008	2008

lysine, tryptophan and micronutrients to combat diseases caused by macro and micronutrient deficiencies (Ado et al., 2007; IITA, 2009).

A list of some of the improved varieties of maize (open pollinated and hybrids) developed in Nigeria are presented in Tables 7 and 8.

Pests and diseases of maize

Maize in Nigeria is attacked by an array of diseases that can cause significant damages. These include the downey mildew, rust, leaf blight, stalk and ear rots, leaf spots and maize streak virus (IITA, 2009). However, Striga is one of the most dangerous weeds in maize production especially in savanna area of Nigeria. Yield losses from Striga range from 10 to 100% depending on time of parasite infection (Lagoke et al*.,* 1991). Studies on time and rate of application of nitrogen were carried out at Mokwa, Southern Guinea savanna zone of Nigeria using four hybrid cultivars (Kim and Adetimirin, 1997). Timing and nitrogen application rates significantly affected Striga emergence, host plant damage scores, agronomic traits and grain yield. Nitrogen application at two weeks after planting (WAP) (compared with 0, 4 and 6 WAP) at 60kg/ha gave the best result in terms of maize performance and reduction of Striga emergence. The tolerant cultivar produced 188% higher grain yield than susceptible cultivars across all treatments. Grain yield of tolerant cultivar at 60kgN/ha was 88% higher than that of susceptible cultivar at 120kgN/ha. In a similar study, Striga infestation reduced grain yield of two susceptible hybrids by 49% and of two tolerant hybrids by 24%. The two tolerant hybrids produced on average 87% higher grain yield than the two susceptible hybrids under low N rates (0-60 kg/ha) and 51% higher yields under high N (90-150 kg/ha) (Kim et al., 1997). Efforts are continuously made to reduce or control these diseases; however, the efforts were complicated by several factors that are making it difficult to achieve an impact in a relatively short time, due to the following reasons:

i. Important diseases change with time
ii. Some diseases are specific to particular ecologies, whereas others are present in all ecologies iii. Difficulty in infecting maize with some of the diseases for effective screening of the germplasm for resistance for example downey mildew.
iv. Difficulty of artificially inoculating maize plants to screen for resistance; therefore, plant breeders could not study the genetics of resistance of

Table 8. Some improved hybrid maize varieties in Nigeria.

Name of variety /original	Original source	Developing institution	Outstanding characteristics	Years of release	Yeas of regist.
8321-18	IITA	IITA	Resistant to streak, Striga/ weevil, semi-flint grain, high yield – 6.5t/ha	1984	1991
8321-21	-do-	-do-	High yield 6.0 ton/ha, resistant to streak, rust and blight dent grain texture	1984	1991
8522-3	-do-	-do-	-do-	1984	1991
8322- 13	-do-	-do-	High yield 6.5t/ha, resistant to streak, downy mildew, and drought, dent grain type.	1984	1991
8341-5	-do-	-do-	-do-	1984	1991
8425-8	-do-	-do-	-do-	1985	1991
8428-19	-do-	-do-	High yield 6.0 ton/ha, resistant to streak, Striga, semi-dent	19885	1991
8434-11	-do-	-do-	High yielding, resistant to streak and storage weevil semi flint grain, high yielding 5t/ha.	1985	1991
8505-2	-do-	-do-	High yield 6.0ton/ha, resistant to streak and Striga, semi-dent grain	1986	1991
8505-3	-do-	-do-	Same as above but semi flint grain	1986	1991
8505-4	-do-	-do-	High yield 6.0ton/ha, tolerant to streak and Striga, semi-flint grain texture.	1986	1991
8505-5	-do-	-do-	-do-	1986	1991
8505-13	-do-	-do-	-do-	1986	1991
8341-5	-do-	-do-	High yielding, resistant to streak and weevil, flint grain texture	1984	1991

Source: National Centre for Genetic Resources.

some of the diseases (Fakorede, 2001).

The most debilitating pests in maize production include stem borers, termites, storage insects, beetle and host of others. Various species of stem borer rank as the most devastating, causing maize pest loss of 20 - 40% during cultivation and 30 - 90% post-harvest storage (IITA, 2009). As a result of significant yield loss due to pest and diseases, extensive research has been done along this area. Many national maize programs with the aim of developing maize resistant varieties to prevailing diseases in different agro ecological zones of the country had been organized. On account of these national programs, germplasms both of pollinated and hybrids resistant to prevailing diseases have been developed. Several agronomic practices have also been investigated to minimize the effect of many of these pests and diseases.

Although many insects, pests and diseases can be controlled with chemicals, these chemicals, often times, are not available, are dangerous and often expensive for small-holders who lack access to credit facilities. Moreover, chemicals are increasingly viewed as environmentally hazardous, threat to human life and safety, such that their use are been discouraged by many policy makers. A variety of chemical and cultural practices can help control many of the diseases; however, using resistant germplasms, which is the least harmful to the environment, is the most effective method for controlling them.

Low soil fertility and maize

Low soil fertility ranks among the most serious constraints of maize productions which are brought mainly by reduction in the fallow period because of ever increasing population pressures. Nigeria as in many other tropical climates is characterized by high rainfall and insolation, the attendant problem of nutrient leaching and low level of soil organic matter which has made nitrogen the most nutrient limiting maize production in Nigeria (Azeez et al., 2006). Increase of fertility level has played a key role in the increase of maize yields and the dominant plant nutrient causing this increase has been nitrogen.

One important characteristic of maize is its high nutrient requirement especially N, P and K. On the average, maize grain contains up to 2% N and 2.6% for all above

ground parts. 20 kg of nitrogen is removed from the soil for every ton of maize grain harvested (Fakorede, 2001). Farmers understand the importance of nitrogen in maize production and employ different strategies to minimize the adverse effects of low soil fertility such as using low population density, applying mineral fertilizer or animal manure, leaving the land fallow and switching to less demanding crops (Carsky et al., 1998). However, even with all these efforts farmers are not able to supply adequate quantity of fertilizer and most often do not apply fertilizer mostly due to high cost or unavailability. Therefore, maize yield is generally low.

Fertilizer requirements of maize

Increase in fertility level has played a key role in the increase of maize yields in Nigeria, and the dominant plant nutrient responsible for this increase has been nitrogen, which is a major nutrient needed in large quantity for high yields in maize production. Many field experiments have shown responses in grain yield of maize to application of nitrogen fertilizer. But the magnitude of response to applied nitrogen varies across experiments due to confounding influences of soil nitrogen supply from non-fertilizer sources, weather variation, variety and cropping practices. Consequently, recommendations of nitrogen management are site and season specific.

Nitrogen is a component of protein and nucleic acids but when it is sub-optimal, growth is reduced (Haque et al., 2001). Nitrogen is also a characteristic constituent element of protein and also integral components of many other compounds essential for plant growth processes including chlorophyll and many enzymatic processes. Nitrogen plays a significant role in protein synthesis and thus strongly influences grain production and grain protein content (Gallais and Hirel, 2004). Its supply also affects both leaf area development and leaf senescence, and consequently crop radiation interception (Lemcoff and Loomis, 1986). Meanwhile, photosynthetic activity of the leaf canopy, and thus radiation use efficiency, varies with leaf nitrogen (expressed as the amount of nitrogen per unit leaf area (Muchow and Sinclair, 1994).

During reproductive development, nitrogen is mobilized from the leaves and stem to the grain (Ta and Weiland, 1992) with consequent reduction on leaf photosynthetic capacity and the consequences for grain yield depend on the relative contribution of crop nitrogen uptake and mobilization of leaf nitrogen to grains as moderated by the grain concentration. Common nitrogen recommendation for old NS series in Nigeria is 75 kgN/ha (Jones, 1973). Lucas (1986) recommended 150 kgN/ha for FARZ series based on profitability, although the yield of maize at 75 kgN/ha was not significantly different from the yield at 150 kgN/ha, while Akintoye et al. (1999) reported responses up to 70, 140 and 210 kg N/ha for maize grown in Ikenne, Mokwa and Kaduna respectively, using different maize genotypes.

With regard to dry matter distribution in maize as influenced by nitrogen, studies on nitrogen levels at Ilora, Southwestern Nigeria using three genotypes of maize (open pollinated, single cross and double cross hybrids) indicated that at final harvest, among all genotypes and fertilizer levels, the least dry matter was partitioned to tassels while the highest was found in stem and grains. The proportion of dry matter partitioned to grains are 27% for single and double cross and 25% for open pollinated while the proportion partitioned to grain was similar (30%) for 75,100, 150 and 200 kgN/ha (Olaniyan,1999) (Figures 3 to 7).

The biggest problem to fertilizer use in Nigeria is the cost because the government has reduced drastically the subsidy on fertilizer. In view of this a bag of nitrogenous fertilizer costing only ₦3.00 a bag (50 kg) to the farmer in 1980s, ₦ 1,500 in 1990s is now N6,000. Fertilizer is relatively expensive in Nigeria than countries in Asia or South America, probably because fertilizer imports have to be financed with foreign exchange which is often in short supply. Another obstacle to fertilizer use in Nigeria is the unavailability at the time when the farmer needs it or in the formulations they desire. Many factors contribute to fertilizer supply problem. Planning and administering a national fertilizer program require skills that are not always available in the government agency that oversee input supply, and the private sector also may experience problems in distribution of fertilizer (Shepherd, 1989).

Furthermore, deep-seated corruption in Nigeria is a major obstacle to fertilizer supply. For instance, medical practitioners, engineers, politicians and others who have no business in farming are the major players in fertilizer and other inputs distribution of fertilizer; thereby, benefiting from government support towards fertilizer supply, while the farmers who are supposed to be major beneficiaries are relegated to the background.

Data from sites throughout Africa suggested that modest doses of fertilizer, especially nitrogen in maize often generate significant yield increases. Whether or not crop responses are sufficient to justify the increased cost of purchasing and applying fertilizer depends on a number of factors, including the price of fertilizer, the price of maize grain and cost of additional inputs required.

Applying chemical fertilizer is a major strategy for maintaining soil fertility. Other technology such as crop rotation, crop residue management, use of live mulches, use of organic fertilizer (compost, animal manure, organo-mineral fertilizer) and other techniques have been researched into. Moreover, nitrogen use efficient genotypes have been researched and developed. Research into many of these soil fertility technologies has shown that they could be viable, and trials on various farmers need to test whether they are economically viable and sustainable from the farmers' point of view.

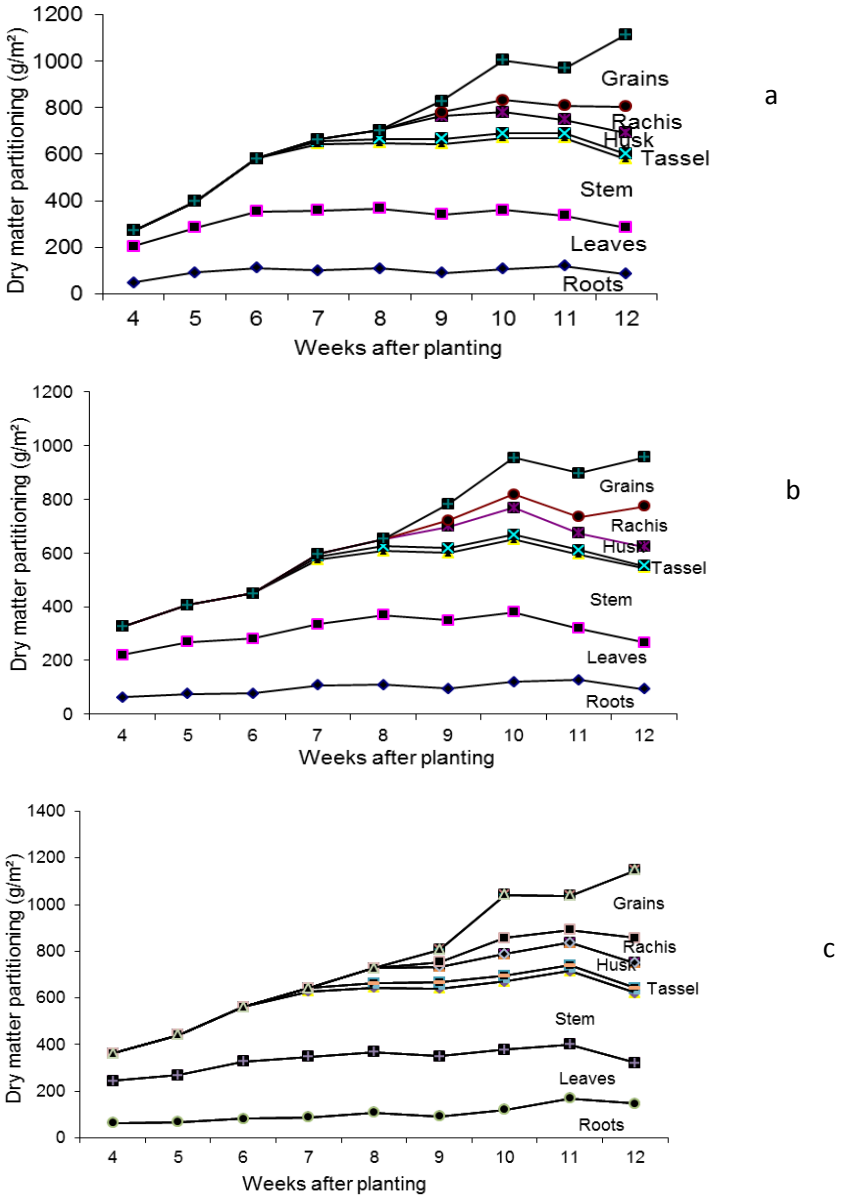

Figure 3. Dry matter partitioning in (a) Open pollinated (b) Single cross (c) Double cross maize genotypes grown at 80,000 plants/ha at zero N level.

Weed infestation and maize

Maize require minimum weed invasion to exhibit its yield potential. Heavy weed interference results in competition for essential resources and, consequently, yields loss. Weeds seriously limit maize productivity in farms in Nigeria. Indeed, it is estimated that weed control takes 50 to 60% of the total cost of maize production (Fakorede, 2001). Weeding takes between 21 to 32% of the total time devoted to maize production in Nigeria. Maize is susceptible to competitions from many annual weeds in the first six to eight weeks after planting and the extent of weed infestation varies from one ecology to the other. It has been reported that weeding during a critical period of 10 to 30 days after crop emergence greatly enhances grain production, while uncontrolled weed growth during this period could reduce maize yield by 40 to 60% (Olaniyan and Lucas, 2004).

One of the most dangerous weeds in maize production is Striga, which is also known as *witch weed*. It is indigenous parasitic weeds that attack maize crop, especially in the savanna areas of Nigeria. Striga is becoming a detrimental pest of maize as a result of the intensification and expansion of maize in the savanna, where Striga is endemic. Apart from savanna region, the weed has been reported in other parts of Nigeria,

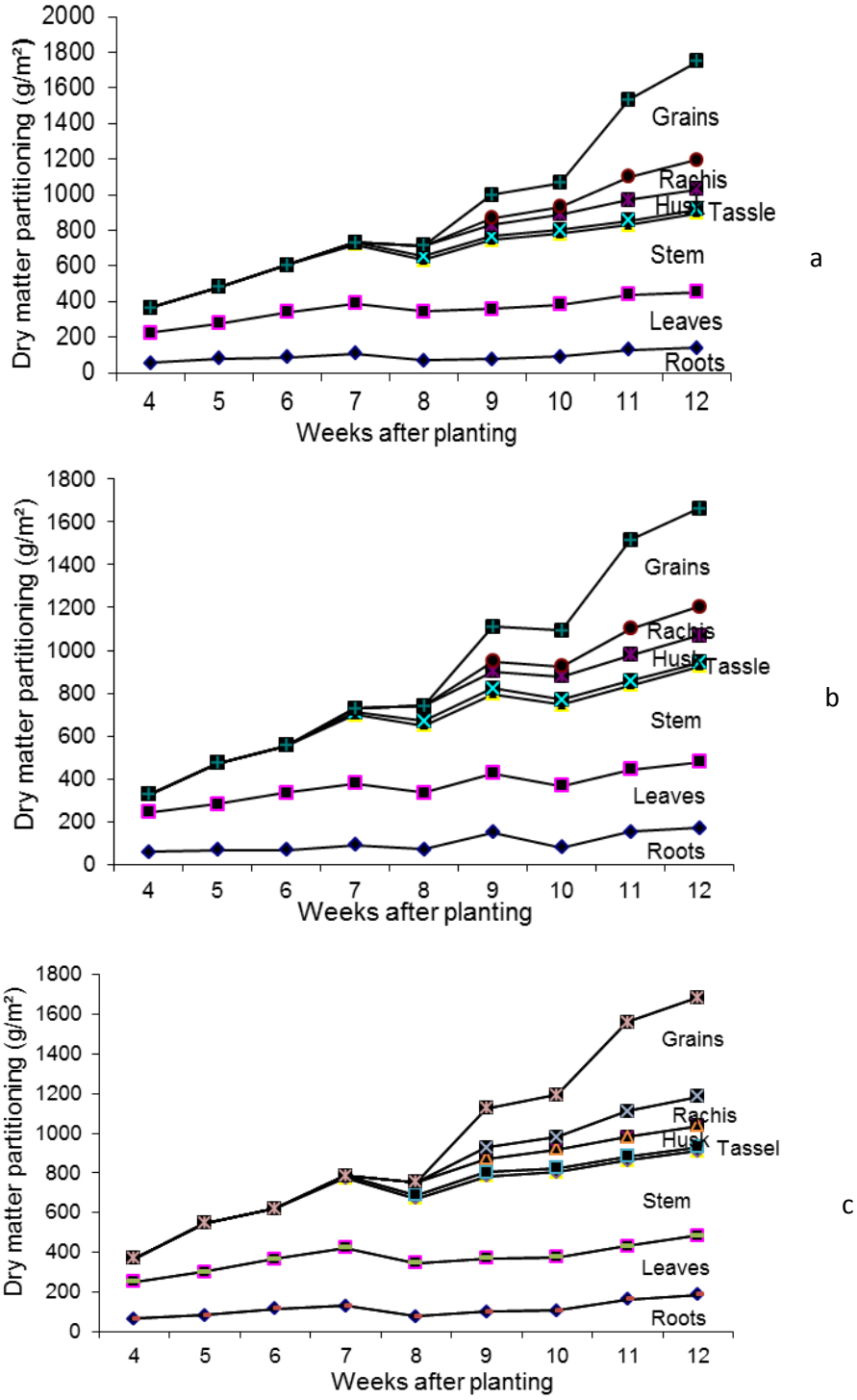

Figure 4. Dry matter partitioning in (a) Open pollinated (b) Single cross (c) Double cross maize genotypes grown at 80,000 plants/ha with 75kg N/ha.

including the derived savanna of southern Nigeria. The species observed in the southern part of Nigeria are *Striga asiatica, Striga aspera, Striga gesnerioides* and *Striga forbesii*. However, *Striga hermonthica* which is prevalent in the Northern part of Nigeria is the most damaging and widespread among Striga species. Yield losses from Striga range from 10 – 100% depending on time of parasite infection (Lagoke et al., 1991). Although major technologies exist to control Striga weeds but since most of the effective control practices require expensive

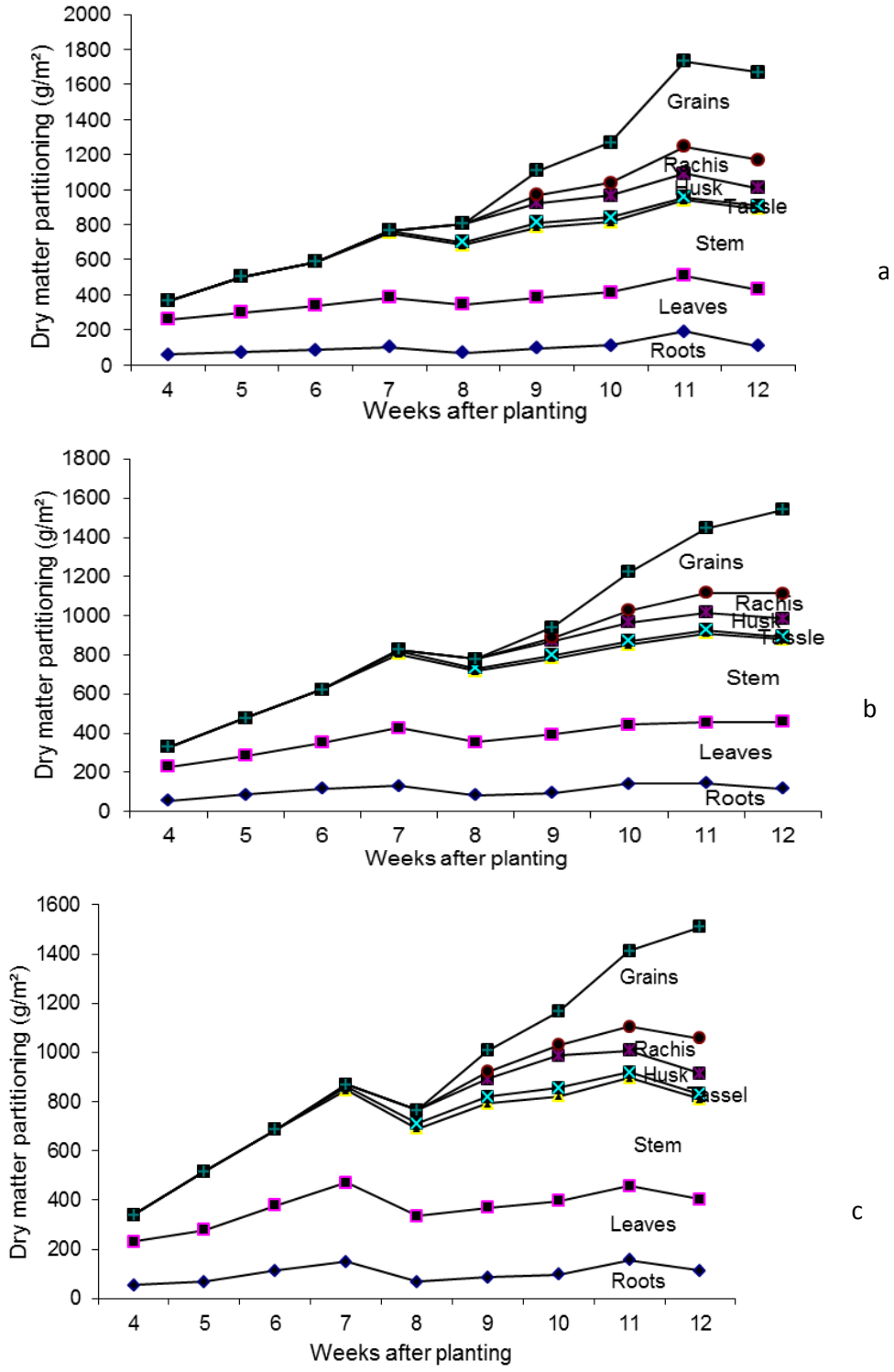

Figure 5. Dry matter partitioning in (a) Open pollinated (b) Single cross (c) Double cross maize genotypes grown at 80,000 plants/ha with 100kg N/ha.

inputs or special equipment, they are not suitable for small scale farmers who constitute the large family population in Nigeria (Ogborn, 1987). However, it has been recommended that the most practical approach of controlling Striga species is the use of cultivars that are resistant to or tolerant to the weed infestation.

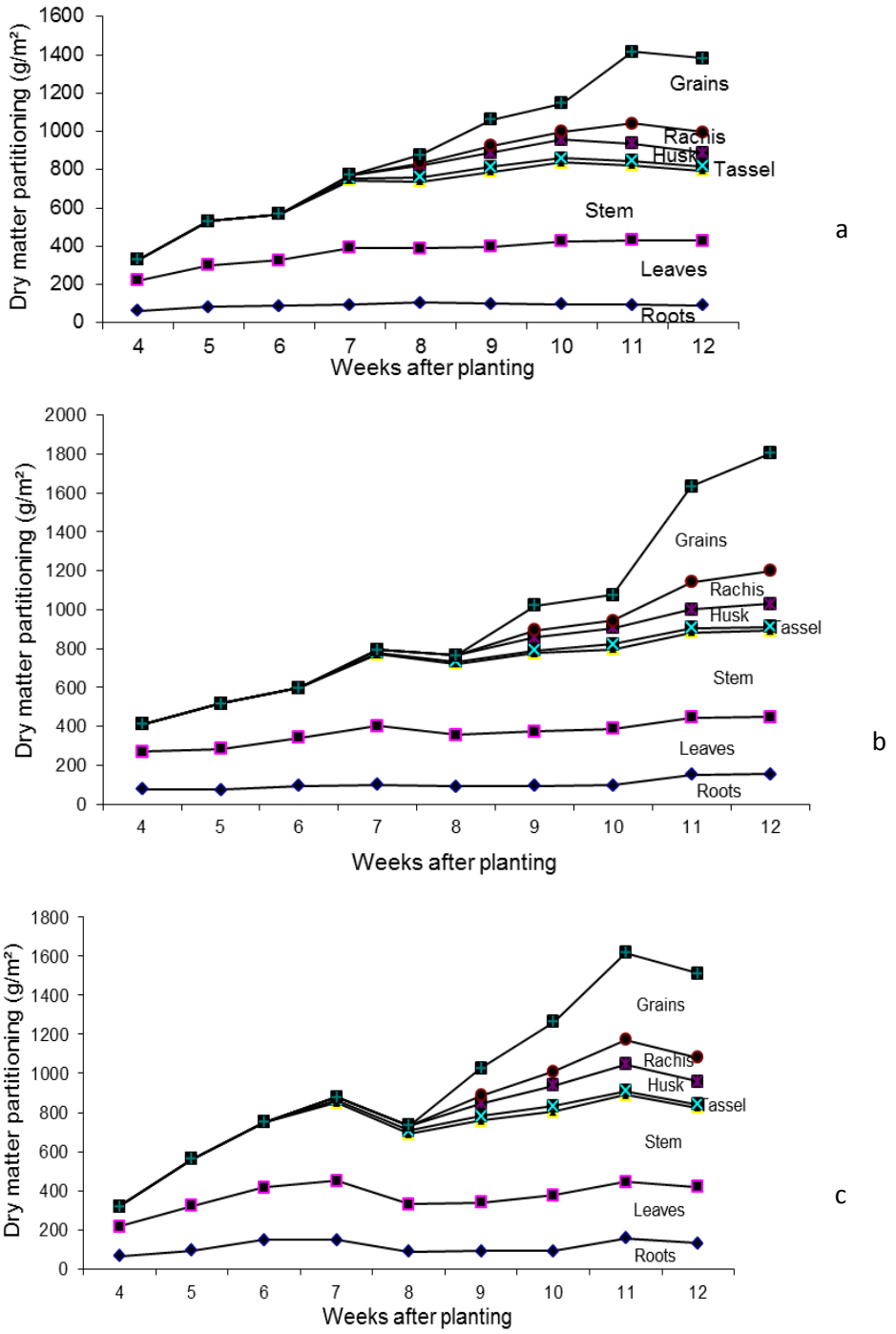

Figure 6. Dry matter partitioning in (a) Open pollinated (b) Single cross (c) Double cross maize genotypes grown at 80,000 plants/ha with 150 kg N/ha.

Weeding requirements of maize

Weed control in most agricultural system in Nigeria involves the use of hoes and cutlasses which are quite tedious, time consuming and often ineffective. Recommendation is usually hand weeding 14 to 21 days, and 57 days after planting, while third weeding may be necessary, depending on the varieties and severity of weeds. Weed may also be controlled by various other methods, including tillage practices, planting of weed - free seeds, cultivation using animal or mechanical power, cultural methods such as the use of cover crops, planting

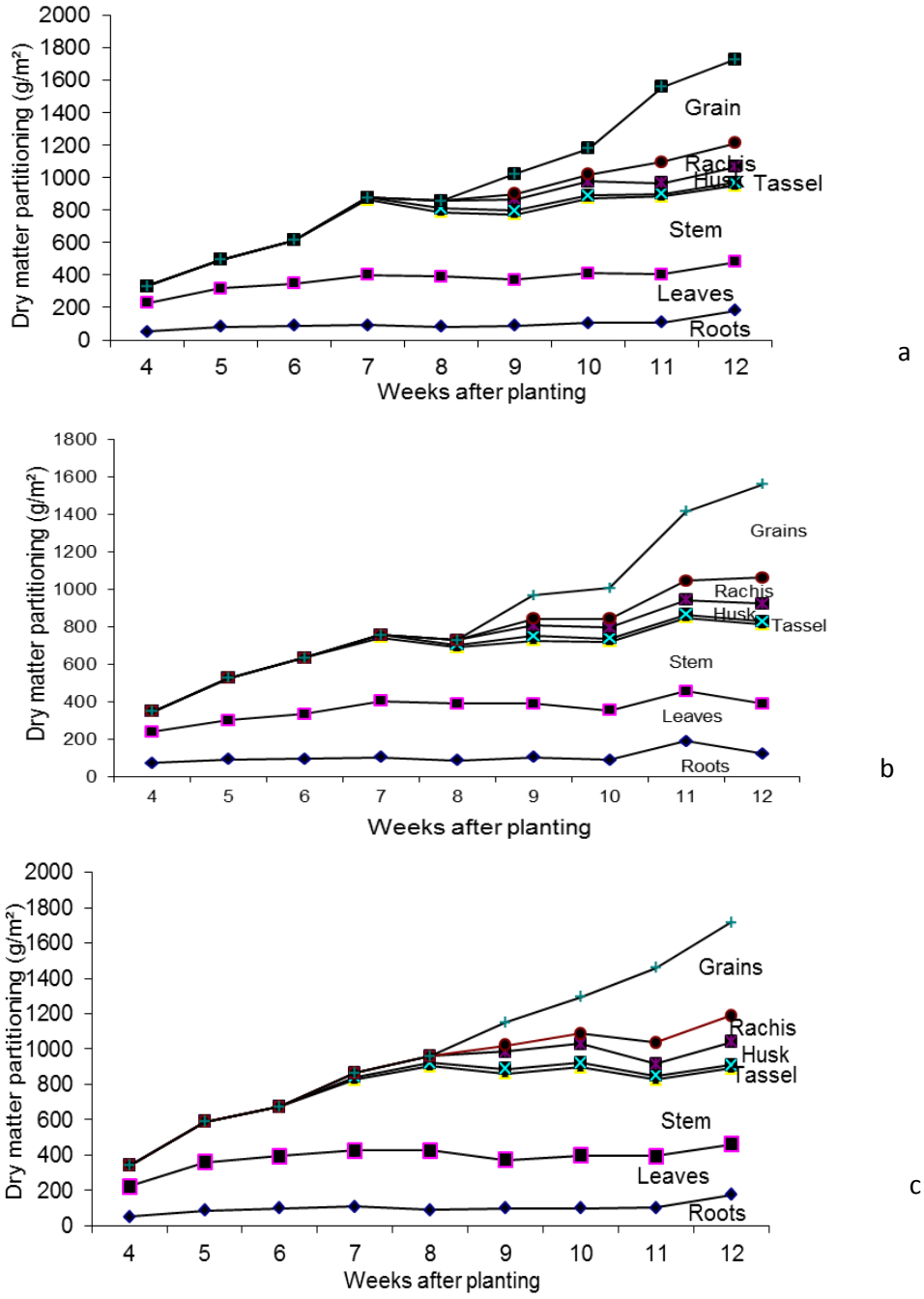

Figure 7. Dry matter partitioning in (a) open pollinated, (b) single cross, (c) double cross maize genotypes grown at 80,000 plants/ha with 200 kg N/ha.

immediately after land clearing and use of herbicides. Maize is affected by many weed species but weeds such as *Cyperus rotundus, Rottboellia Cochinchinensis* and *Imperata cylindrical* are quite problematic because they are very competitive and difficult to control when routine crop husbandry practices are employed. Others such as *Euphorbia heterophylla* occur in large number (high density) and grow as rapidly relative to crop that the young maize are shaded out. Other weeds common in maize plots included *Talinum triangulare, Amaranthus spinosus, Chromolaena odorata, Agerantum conyzoides* (Akobundu and Agyakwa, 1987).

Weeding takes between 21 to 32% of the total time devoted to maize production in Nigeria (Table 9) (Lucas, 2007). Moreover, different herbicides are recommended and for maize and maize based crop mixtures, however,

Table 9. Mean man-hours used for different cultural operations by maize farmers in Nigeria.

Operation	% time used
Land Preparation (including slashing and burning	24.6
Planting	5.1
Weeding	25.6
Fertilizer application	7.7
Harvesting	18.5
Shelling and storage	18.5
Total	100

Source: Lucas, 2007.

unavailability, cost and knowledge of handling and environmental effects are drawbacks to its use.

Drought and maize

Maize grown in Nigeria and many other countries in Sub-Sahara Africa are usually rain-fed. Rainfall, and to a lesser extent, temperature are the most important climatic factors that determine crops' growth and timing of agronomic practices in different ecological zones of Nigeria. Although, agricultural drought occurs when the levels of precipitation are sufficiently low to cause serious decrease in crop yield through its effects on the physiological process whereas, maize is essentially sensitive to moisture stress around the time of tasselling and cob formation. It also needs optimum moisture condition at the time of planting. Drought may occur at any stage of maize growth but when it coincides with flowering and grain filling periods yield loss could be between 40 to 90% (Menkir and Akintunde, 2001). Drought stress at flowering disrupts the synchrony between pollen shed and silking, which is the major cause of yield reduction (Banziger et al., 1999).

In spite of great potential of Nigeria in maize production, frequent occurrence of drought occasioned by erratic rainfall distribution and/or cessation of rain during the growing season is the greatest hindrance to increased production, and this is more serious in the northern parts of Nigeria. In order to reduce yield loss due to drought, drought resistant varieties are being developed and the Nigerian Meteorological Agency (NIMET) are taking responsibilities for weather forecast into periods of planting for different cropping zones.

MAIZE IN THE FARMING SYSTEM

Small scale farmers in Nigeria as well as other Sub-Sahara African countries prefer intercropping to sole cropping because they found the latter to be costly, risky and characterised by inadequate production of wide range of food crops needed by an average household. Maize is often intercropped with legumes, cassava, melon etc.

Cereal legume intercropping is often employed as a basis for enhancing resource use and the sustainability of tropical smallholder farming systems (Tsubo et al., 2005) In Africa, maize does well when intercropped with beans or other legumes. Productivity of mixture of maize and legumes often exceeds that of sole crops due to synergistic effects that favour the growth and yield components of both crops (Agegnehu et al., 2010). The productivity of intercropping system of maize and soybean in guinea savanna agro ecosystem of Nigeria indicated yield advantage of 2-63% as depicted by the LER of 1.02 to 1.63, indicating efficient utilization of land resources by growing intercropping maize with soybean (Muoneke et al., 2007).

In another study in Nigeria on the effect of maize/cassava intercropping on the micro-environment, intercropping cassava with maize was found to lead to better light interception than growing cassava alone (Lucas,2007). Unfortunately, small scale farmers could not maximize the production from their intercropping system because most of the agronomic practices introduced to the farmers are usually for sole cropping.

Growth analysis of maize

Growth is a vital function of plants and is an indication of a gradual increase in number and size of cells. Growth analysis is commonly used in ecological studies to determine success of species in various habitats, competition among species, genetic differences in yield and agricultural treatment on crop growth (Hayness et al., 1967). Growth analysis is still the most simple and precise method to evaluate the contribution of different physiological indices in plant development despite its drawback. In growth analysis two basic measurements are made from which large numbers of parameter are derived. These are measure of plant material present (dry weight) and leaf area which is the measure of the assimilatory system of plant material. Generally, maize growth analysis is carried out so ascertain the formation and accumulation of plant biomass as determined by the environment or internal factors or both (Nevado and Cross, 1990). Some important parameters of growth in maize studies are as follows:

Dry matter

Total crop dry matter is the spatial and temporal integration of all plant processes and, therefore, crop dry matter is the most relevant parameters in the study of crop canopies. Rate of dry matter accumulation varies across the life cycle of a crop and leaf area sampled at

intervals ranging from days to weeks to quantify the effect of environmental influences or to analyze genotypic differences between maize cultivars.

The pattern of rate of dry matter accumulation is typically characterized by a sigmoid curve and always in three phases (i) a period of exponential growth during early development followed by (ii) period of more or less constant rate dry matter accumulation and (iii) a period of declining crop growth rate during the final phase of development when green leaf decline due to leaf senescence and leaf photosynthesis declines due to leaf aging (Echarte et al., 2008).

Rate of dry matter accumulation is the product of total incident solar radiation, the absorptance of incident solar radiation by the crop canopy and the efficiency of conversion of absorbed solar radiation into plant dry matter. Dry matter accumulation increases for maize hybrids at high than at low density due to light interception. With increase in plant population per hectare dry matter yield per plant decreases steadily, but yield per hectre increases up to a maximum and then falls (Tollenaar, 1991). Differences in dry matter accumulation arise mainly in variation in leaf area.

Crop growth rate

Crop growth rate (CGR) is defined as the increase in plant material per unit time. It is a measure of rate of dry matter production per unit area of land (Watson, 1952). CGR can be expressed in energy units as a percent of daily mean total of incident radiation or transformed into a coefficient of solar energy utilization. This coefficient indicates how economically a crop uses the solar radiation available during and interval between two harvests. CGR is generally higher in C4 plant than C3 plants (Tsubo et al., 2005). CGR patterns can be defined accurately by taking plant samples at different time intervals during the growth season. Values of CGR are normally low during early growth stages and increase with time, reaching maximum values at time of flowering.

Analysis of CGR is important for evaluating treatment differences among maize crop or maize genotypes in relation to yield. CGR of tropical maize genotypes are generally higher than the temperate genotypes, but they could not out yield the temperate genotypes. The peak CGR rate observed for most studies in Nigeria ranged between 20.7 to 46.3 g/m^2/day (Lucas 1981) and peak value for CGR in temperate region ranged from between 12.0 to 24 g/m^2/day (Iremiren and Milbourn, 1978). Higher values in the tropics have been associated to higher production of dry matter per unit leaf area when compared with values reported in temperate regions.

Leaf area index

Leaf area index (LAI) describes the size of assimilatory apparatus of the plant stand and serves as a primary value for calculating other growth characteristics. LAI is defined as the leaf area of a plant over a certain area of ground (Watson, 1952). Leaf area index is the primary factor that determines crop growth rate on crop communities. Olson and Sanders (1988) concluded that one simplest ways of increasing LAI is increased plant density.

LAI increases linearly as the plant density increases but the leaf area per plant decreases as the plant density increases. Hence dry matter produced decreases with decreasing of LAI. The differences in values reported for optimum LAI in maize in different parts of the world may be due to differences in environmental factors and leaf arrangement. These two factors have been shown to affect net assimilation rate (NAR) and consequently, CGR (Pendleton, 1968). Maize varieties with erect leaf arrangement which are better suited for light interception have higher NAR at dense canopies and therefore higher optimum LAI for CGR.

Net assimilation rate

NAR is defined as increase in plant dry weight per unit time. It provides an estimate of photosynthetic production. NAR is describes as a measure of the efficiency of the unit leaf surface whose value depends on light interception characteristics of plant (plant angle and leaf inclination); gas exchange properties of leaf, characteristics for drought resistance, water use and several other factors (Williams, 1946). Warren-Wilson (1966) reported that NAR is more dependent on intercepted light than any other environmental factor while Jolliffe et al. (1990) reported that NAR is more dependent on plant density. It was reported that NAR value decreases with crop growth due to mutual shading of leaves and reduced photosynthetic efficiency of older leaves.

NAR would decrease with increased LAI; NAR per plant would decrease with increased LAI at high than at low density but increase than in per area and this trend decrease will continue from the beginning till the end of growth season (Brogeham, 2000). Thus, increasing plant density accelerated leaf senescence, increased shading of leaves, and reduced the net assimilation of an individual plant but increase NAR per unit area (Boyat et al., 1990).

Leaf area ratio

Leaf Area Ratio (LAR) is defined as ratio between leaf area and the total dry weight of the plant. It characterises the relative sizes of assimilatory apparatus as a useful measure of the growth differences that occur between experimental crops. LAR is environment specific and higher LAR was reported in the forest zone than in derived zone in an experiment conducted in two ecological

zones using two varieties of maize in Nigeria (Lucas, 1981).

Harvest index

Harvest Index (HI) is an estimate of economic yield of a crop over its total dry matter represented (Donald, 1951) as:

$$HI = \frac{\text{Maximum dry matter of economic yield}}{\text{Total dry matter}} \times 100$$

In a comparison of two groups of hybrid maize, approximately 15% of the yield difference between the two groups is attributed to harvest index hence 85% of the difference is due to increased dry matter accumulation. Plant density produced an increase in total dry matter production and a decrease in harvest index and optimum plant density was a tradeoff of both effects (Tollenaar, 1989).

CONCLUSION

Maize, an efficient crop in utilizing solar energy by converting it to food, has an important role to play in providing food for the current and future needs of Nigeria and the world at large. As species, maize contains enormous genetic variation which, under good management and favorable conditions will give very good yield. In Nigeria, maize has high yield potential but yields are substantially below their potential. However, sustainable maize production is attainable in Nigeria. At present, the cost of production of 1 hectare of maize ranged between ₦ 100,000 to ₦ 120,000 while farmer's sell maize between ₦ 45,000 to ₦ 50,000 / ton. With the average maize yield, farmers are running at loss. Therefore, maize productivity should be enhanced. A wide gap between yield in researchers and farmers field needs to be addressed through efficient linkage between researcher, extension, market and input. Other factors militating against maize production should be identified and properly addressed. Storage facilities need to be improved upon, although the silo for storing grain has increased to 500,000mts capacity. However, there is need to create grain aggregation and collation centers in the rural maize growing areas for temporary storage and linkage to bigger silos or end-users.

In maize, two inputs, improved seeds and fertilizer are of overriding importance in maize intensifications system. In recent years, the private sector has become increasingly involved in the supply of improved maize seed, fertilizer and other agricultural chemicals and equipment. Development of efficient seed industries for providing and distribution of improved maize seed variety to farmers at affordable price should be prioritized in transformation agenda of government. Agriculture should be regarded as a social service as it is done in developed countries like United States and European Union. At present, the mechanisms for the distribution of subsidized fertilizer are very inefficient, and in this regard, regulatory system should be streamlined and made more transparent. The planned appropriation of ₦ 60 billion for purchase of 10 million cell phones for farmers to buy inputs and access information (people who live far from the cities and where network connection is poor) may not be appropriate.

Conflict of Interest

The authors have not declared any conflict of interest.

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Full Length Research Paper

Assessment of nodulation of *Mucuna pruriens* by promiscuous native rhizobia population, Southeast Nigeria

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The potency and competitive ability of indigenous rhizobia population in soil to nodulate a non-native legume host has been contentious especially in tropical Africa. This study examined the symbiotic compatibility between the indigenous rhizobia population and a non-native legume species. Soil samples were randomly collected with soil auger at 0 - 30 cm depth from agricultural fields southeast, Nigeria. The control soil samples were obtained from the Badagry beach which had no previous history of legume cultivation at the same depth. *Mucuna pruriens* seeds were aseptically sown in plastic pots containing these soils kept in the greenhouse for 10 weeks. The test pots were watered with sterile distilled water while the control pots received inorganic nitrogen (N) as source of N nutrient. The agronomic features of the host legume were evaluated after 10 weeks and the presence of appropriate Rhizobium inoculant contributed positively to biomass production in *M. pruriens*. The *Mucuna* microsymbiont was found to thrive under favorable climatic and edaphic factors for effective nodulation of the host legume. The microsymbiont was identified on yeast-extract mannitol salt agar (YEMA) containing bromothymol blue (BTB) as *Bradyrhizobium* and *Rhizobium* species. This study convincingly showed the importance of rhizobia to soil fertility maintenance in sustainable agricultural practice as well as the adaptability of the legume microsymbiont to different ecological zones particularly the tropical humid environment.

Key words: Inoculation, *Mucuna pruriens,* nodulation, organic agriculture, *Rhizobium,* soil fertility.

INTRODUCTION

The challenge facing agriculture in sub-Saharan Africa is decreasing soil fertility and decreasing productivity. This is due to excessive cultivation of farmlands which results in low fertility in most of the soils in sub-Saharan Africa

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and the limited use of fertilizers which often are not readily available (Anonymous, 1999).

Leguminous cover crops such as in alley cropping have the potential to contribute to soil nitrogen and increase the yields of subsequent or associated cereal crops through symbiotic N_2-fixation. Although, it has often been wrongfully assumed that *Mucuna pruriens* will freely nodulate, fix N_2 and therefore contribute to soil N. Recent findings suggested *M. pruriens* failure to nodulate in some farmer's field in the derived Savanna in Republic of Benin. Although, *Mucuna* could play an important role as a source of N, it may also depend solely on soil N and therefore may deplete soil N, hence behaving as non-fixers when either not effectively nodulated or there is too low nutrient availability particularly phosphorous (P) (Hougnandan et al., 2000; Jemo et al., 2007).

M. pruriens has been reported to be adequately established and well nodulated in soil in the presence of its microsymbiont, favorable climatic and edaphic factors which enhances its establishment in soil within the humid and moist savanna zone of Nigeria (Ojo, 2001; Jemo et al., 2007).

The severity of the spear grass (*Imperata cylindrical)* problem, the decline of the traditional fallow system as a means of soil fertility management and lack of inorganic fertilizer, necessitated the adoption of *M. pruriens* technology by small holders in some areas in the derived Savanna of West Africa (Akobundu and Okigbo, 1984; Versteeg and Koudokpon, 1993; Sanginga et al., 1996). In West Africa, crop productivity declines rapidly when the fallow period is shortened below a minimal period of time needed to restore soil fertility to a level accepted for sustainable agricultural practice. Hence, soils are degrading rapidly and nutrient balances are negative (Van der Pol et al., 1993). In order to tackle this problem in West Africa, the development of cropping systems that enhances sustainable crop production at an economically accepted level with minimal external inputs has long been proposed. Alley cropping systems (Kang et al., 1981) and other soil fertility regenerating techniques such as intercropping of maize and Pigeon pea (*Cajanus cajan)*, relay planting of *M. pruriens* one month after planting maize or planting tree fallows of *Acacia auriculiformis* on severely degraded soils have since been tested in the Mono province of Benin Republic by Versteeg and Koudokpon (1993). In alley cropping systems, trees are intercropped with crops and cut back regularly to avoid tree-crop competition for water, light or nutrients. The tree pruning is subsequently applied to soil surface thereby supplying nutrients. Although, alley cropping systems have proven to sustain crop productivity on soils of high base saturation with minimal supplies of mineral fertilizer (Kang et al., 1995), the use of *M. pruriens* as green manure for intensive maize (*Zea mays L*.) production in West Africa has given much positive yield effect to suggest its integration into sustainable agriculture practice within the region (Hauser and Nolte, 2002). The socio-economic conditions of the peasant farmers have prevented the absolute adoption of this system as well as reports that N_2 released from decomposing residues is poorly recovered by food crops (Vanlauwe et al., 1996b, 1998).

The selection of adequately adapted inoculants strains and understanding of changes in rhizobia population as well as the factors precipitating such changes would enhance the application of appropriate indigenous rhizobial strains in the desired low-input farming system and subsequently enhance both *M. pruriens* biomass and N uptake (Sanginga et al., 1994; Jemo et al., 2007). The challenge of decreasing agricultural productivity in tropical Africa would be eradicated by synchronization of low-input systems such as *Rhizobium*/legume association into farming systems under favorable climatic and edaphic factors, which are not only sustainable but also economically viable (Anonymous, 1991). The potency and competitive ability of introduced rhizobia inoculants in soil for over 10 years have been reported (Ojo and Fagade, 2002). However, in many soils adequate nodulation and N_2-fixation by legumes require inoculation with the appropriate and specific rhizobia inoculants particularly where such legumes has not been previously cultivated (Sanginga et al., 1988; Jemo et al., 2007).

The objectives of the current study were to assess the ability of promiscuous native rhizobia population in a humid tropical environment to adequately nodulate *M. pruriens* as well as their potential to produce biomass in spite of the prevailing climatic and edaphic factors in the environment under investigation.

MATERIALS AND METHODS

Soils

Soil samples were randomly collected from agricultural field in Aba, Calabar, Enugu, Onitsha, Lokoja, Owerri and Benin within the tropical humid zone, Southeast of Nigeria at a depth of 0-30 cm using a soil auger (5 cm diameter). Ten core soil samples were randomly collected with the soil auger from each of the agricultural fields. Soil samples with no previous history of being inoculated with *Mucuna* microsymbiont as well as not having been cultivated with legumes were collected at same depth from another location (Badagry beach) to serve as the control. The composite soil samples from each of the experimental fields sampled in each of the ecological zones were then used for the pot experiment in the green house using the randomized complete block design.

Seeds

Legume seeds were obtained from the Genetic Resources Unit (GRU), International Institute of Tropical Agriculture (IITA) Ibadan, Nigeria.

Media and plant nutrients

Yeast-extract mannitol agar (YEMA), Jensen's Nutrient solution and potassium nitrate (KNO_3) solution at 0.05% N were used (Vincent,

1970).

Soil analysis procedures

Preparation of the soil samples

The composite soil sample from each location was passed through a 6-mm mesh sieve and mix thoroughly (Ocio and Brookes, 1990b). Then, three portions of the soil were weighed out, 15-50 g, one portion into a weighing container for water content determination and two portions into 100 ml glass bottles, one sample to be fumigated for 24 h and then extracted as well as one control sample to be extracted immediately.

Extraction of microbial biomass C and N

The fumigation was carried out using $CHCl_3$. The bottles containing the unfumigated control and fumigated subsamples had 0.5 M K_2SO_4 dispensed into them using the equivalent oven-dry soil weigh (g): extractant volume (ml) ratio 1:2 to 1:5. The jars with their caps tightened were placed on a rotatory shaker for 1 h. After shaking, the soil suspension was pass through the Whatman No.5 filter paper; excessive evaporation was avoided during the filtration process. Thereafter, measurements of organic C and total N were done using 20 ml of extracts in each case. Then, the filtrate was capped and stored at 4°C for not more than 2 -3 days (Voroney et al., 1993).

Determination of organic C, organic N and exchangeable NH_4^+ in the extract

Organic C, organic N and exchangeable NH_4^+ dissolved in the K_2SO_4 extracts were determined colorimetrically using automated equipment as described by Voroney et al. (1993).

Nodulation experiment

The composite soil samples were tested with scarified *M. pruriens* seeds to evaluate their potential at nodulating with promiscuous native rhizobia population in the soils. Seven plastic pots, 20 mm diameter were used to cultivate the legume and replicated twice. Two plastic pots of the same diameter were used for the control experiment and this was replicated twice. A total of sixteen plastic pots were used for the experiment, randomly arranged on a platform in the greenhouse. Four scarified *M. pruriens* seeds were sown aseptically into each pot and later thinned to two plants per pot with sterile forceps 2 weeks after planting (WAP). Each of the pot for the test experiment was watered with sterile distilled water while one pair of the control pot received Jensen's nutrient solution and the other pair 0.05% KNO_3 solution (Vincent, 1970).

The experiment was monitored for 10 weeks. The above-ground plant materials were harvested with sterile surgical blades and then oven-dried at 65°C for 48 h. Roots were carefully removed and examined for nodulation. Fresh nodules were counted, cleaned of soil particles and then used for strain identification (Sanginga et al., 1994).

Nodule-typing on YEMA

Aseptic procedures were practiced in typing the harvested nodules on YEMA containing bromothymol blue (BTB). The nodules were crushed with sterile glass rods in sterile Petri dishes and then inoculated on YEMA containing BTB (0.0005%) incubated at 28°C for 48 h. Pure cultures were prepared from the isolates and used for plant re-infection experiment as well as for biochemical characterization (Somasegaran and Hoben, 1994; Ojo, 2001).

Morphological and biochemical tests

The morphological and biochemical characterization of rhizobia from harvested nodules of *M. pruriens* were executed following the methods described by Somasegaran and Hoben (1994) and Gerhardt et al. (1981).

Gram reaction

A thin film of each of the isolate was prepared on a grease-free slide and it was air-dried as well as heat-fixed by passing it horizontally over the Bunsen flame. The smear was stained with crystal violet for 60 s and rinsed off under slow running tap water. The resulting smear was thereafter stained with Gram's iodine and left for 30 s. This was rinsed off with tap water. The decolorizing agent (75% Ethanol) was used to flush the smear on the slide and was immediately rinsed with water. Thereafter, few drops of the counter stain Safranine was applied on the smear for 30 s and then rinsed with tap water. The slide was left to dry and later examined under oil immersion lens (100x) of the microscope. Gram positive organisms appear purple in color while Gram negative organisms appear pink or red under the microscope (Gerhardt et al., 1981).

Catalase test

This was perfected by adding 3% hydrogen peroxide to 18-24 h culture on agar slant or glass slide. The culture was observed for immediate appearance of bubbles (Alexander and Strete, 2001). Presence of bubbles is considered a positive test.

Oxidase test

This was performed by adding oxidase reagent (tetramethyl–p–phenylenediamine dihydrochloride) to a bacterial growth on agar plate. Color change was observed for up to 60 s (Alexander and Strete, 2001). A dark purple color represents a positive test.

Indole test

Suspected isolates were inoculated into test-tubes containing peptone water with a sterile transfer needle. Test-tubes were incubated at 28±2°C for 24 - 48 h. Thereafter, five drops of Kovac's reagent (p-dimethylaminobenzaldehyde) was added to the medium (Alexander and Strete, 2001). A red color on medium represents a positive test for indole production.

Growth on YEMA supplemented with BTB

The growth reactions in the standard yeast-extract medium (YM) containing bromthymol blue (BTB) acted as pH indicator. Fast-growing rhizobia produce an acid reaction in the YM medium (yellow color) containing BTB (pH 6.8) while slow-growing rhizobia produce an alkaline reaction (blue or purple color) (Somasegaran and Hoben, 1994).

Motility test

This was used to detect the presence of flagella. Suspected

Table 1. Selected physico-chemical properties of the soils.

Location	pH (H_20)	NO_3–N (%)	NH_4– N (%)	Organic C (%)	CEC	PO_4^{-3} (%)
Enugu	5.9	72	17	4.0	1.0	47.7
Owerri	6.28	25.3	12.4	1.00	1.0	49.8
Onitsha	6.20	39.00	12.36	2.15	1.0	52.52
Benin	6.30	10.32	8.60	0.50	1.0	9.40
Lokoja	7.70	36.38	24.00	0.27	1.0	21.78
Aba	8.60	23.65	17.00	1.71	1.0	16.48
Calabar	7.30	23.63	14.00	0.67	1.0	23.30
Control	7.80	23.60	12.00	0.34	1.0	4.89

isolates were inoculated into nutrient agar slant with a sterile transfer needle inserted and withdrawn in a straight line in the centre. The inoculated slants were incubated at 28±2°C for 24 - 48 h (Alexander and Strete, 2001). Growth away from line of inoculation indicates the organism is motile.

Statistics

Statistical Package for Social Sciences (SPSS) software model (Version 16) was used for the statistical analysis and analysis of variance (ANOVA).

RESULTS

The mean physico-chemical properties of the soil samples showed that all the locations had some level of fertility that could support sustainable agricultural practice. The soil samples from Benin, Enugu, Owerri and Onitsha were slightly acidic while other locations were slightly alkaline (Table 1). The general presentation of soil pH from the southeast ecological zone was neutral (7.01) (P> 0.05) as shown in Table 4. The mean nitrogen (N) content showed that the soil had more NO_3–N than NH_4–N (Tables 1 and 4). Generally, with the quantity of soil N in the soil, it is expected that N_2- fixation should complement the soil N to facilitate the establishment of the legume host.

The mean PO_4^{3-} nutrient was low (28.2%) (P>0.05) but this is adequate for *M. pruriens* establishment in the soil (Tables 1 and 4). Phosphorus (P) and nitrogen are essential mineral nutrient for the survival of rhizobia population in soil which had been provided by the soil samples. The mean organic carbon (C) content (1.33%) though extremely low, the CEC property of the soils were expected to support the proliferation of *M. pruriens* microsymbiont as well as cation exchange in the soil (Tables 1 and 4). The control soil samples had mineral nutrient in them but they were sterile soils hence no *M. pruriens* microsymbiont in them. The rhizobia count using yeast-extract mannitol salt agar (YEMA) was above 300 cells/g of soil at the commencement of this study from each location. The mean agronomic features of *M. pruriens var cochinensis* 10 weeks after planting (WAP) showed that the legume was established in all soil samples. The moisture accumulation was relatively high in all pots, this was as a result of quantity of watering process applied in the greenhouse and the experiment was conducted during rainy season. It was obvious that nodulation failed with soil samples from Onitsha despite the firm establishment of the legume host and its biomass production. The nodule number (#) was highest with Enugu, Calabar, Benin, Owerri, Lokoja and Aba in a descending order.

Root and shoot dry weights which are pointers to the quality of biomass production were highest in Calabar and Lokoja, respectively (Table 2). The mean root and shoot dry weight were 1.27 and 3.61 g, respectively while mean nodule number (6) suggested poor nodulation 10 WAP (P> 0.05) as shown in Table 5.

Generally, nodulation of *M. pruriens* was relatively poor in all locations 10 WAP because only few of the indigenous rhizobia strains were specific for this *M. pruriens* species which is of Asian origin.

The mean agronomic features of *M. pruriens var utilis* showed firm establishment of the legume host but it failed to nodulate with native rhizobia species specifically in Onitsha soil samples (Table 3). Biomass production 10 WAP in terms of root and shoot dry weight was highest in Calabar and Onitsha, respectively. The mean root and shoot dry weight were 1.62 and 3.80 g, respectively with mean nodule number 5.88 (P> 0.05) as shown in Table 6. In both varieties of legume used, the uninoculated controls were not comparable to the inoculated pots in terms of biomass production. There was no nodulation in the control pots since they were uninoculated. The presence of *Rhizobium* species in the legume roots could only have been the factor that enhanced the biomass production in most of the inoculated pots. Furthermore, the contents of the nodules aseptically inoculated on YEMA containing BTB showed yellow (acidic) and blue (alkaline) coloration which suggested the presence of *Rhizobium* and *Bradyrhizobium* species, respectively. The results of this experiment validates the null hypothesis (H_0); *M. pruriens* microsymbiont are quite

Table 2. Mean agronomic features of *M. pruriens* var. *cochinensis* at 10 weeks after planting (WAP).

Sources	Root fresh wt (g)	Root dry wt (g)	Root moisture content (%)	Shoot fresh wt (g)	Shoot dry wt (g)	Shoot moisture content(%)	Nodule freshwt (g)	Nodule number
Enugu	4.99	0.80	84.0	19.20	3.01	84.3	1.50	16
Calabar	12.41	2.33	81.2	11.54	3.42	70.4	1.15	12
Aba	7.91	1.36	82.8	11.13	2.92	73.8	1.03	5
Onitisha	4.70	1.46	90.2	17.29	4.42	74.4	0	0
Lokoja	7.56	1.39	81.6	16.15	4.55	71.8	0.96	5
Benin	9.30	1.84	80.2	12.76	3.98	61.5	1.18	8
Owerri	4.69	0.41	91.3	19.31	2.87	84.2	0.91	
Control KNO_3	4.78	1.36	71.5	71.5	3.04	75.5	0	0
Control Jensen's	12.04	0.50	95.8	95.8	4.29	72.6	0	0

Wt = Weight.

Table 3. Mean agronomic features of *M. pruriens* var. *utilis* 10 WAP.

Sources	Root fresh wt (g)	Root dry wt (g)	Root mixture content (%)	Shoot fresh wt (g)	Shoot dry wt (g)	Shoot moisture content (%)	Nodule fresh wt (g)	Nodule number
Enugu	6.80	0.96	85.9	20.40	4.00	80.39	2.07	18
Calabar	7.06	2.98	57.8	16.54	3.46	79.08	1.08	12
Aba	8.01	2.00	75.03	11.13	2.92	73.77	0.76	5
Onitisha	6.06	1.16	82.51	17.29	4.72	76.17	-	-
Lokoja	8.06	2.05	74.57	18.15	4.65	74.93	0.99	5
Benin	9.80	1.46	80.0	12.77	3.19	75.02	0.90	8
Owerri	7.69	1.41	81.66	10.98	3.96	63.93	0.86	5
Control KNO_3	5.01	1.01	79.8	12.41	3.04	75.50	-	-
Control Jensen's	10.98	1.56	85.79	15.65	4.29	72.59	-	-

Wt = Weight.

scanty in population southeast, Nigeria while the native rhizobia population are incompatible and non-specific for *M. pruriens* varieties cultivated. The alternative hypothesis (H_1); the native rhizobia population in soils southeast, Nigeria is compatible and specific for the introduced *M. pruriens* varieties.

Therefore, statistical analysis results showed that native rhizobia species specific for nodulation of *M. pruriens* in southeast Nigeria are scanty, however, the legume host was well established.

Table 4. ANOVA: Two-factor with replication.

Summary	pH (H_20)	NO_3–N (%)	NH_4– N (%)	Organic C (%)	CEC	PO_4^{3-} (%)	Total
Enugu							
Count	8	8	8	8	8	8	48
Sum	56.08	253.88	117.36	10.64	8	225.87	835.86
Average	7.01	31.735	14.67	1.33	1	28.23375	13.9965
Variance	0.948228571	341.8392	21.8312	1.611828571	0	362.5205982	204.276
Total							
Count	8	8	8	8	8	8	
Sum	56.08	253.88	117.36	10.64	8	225.87	
Average	7.01	31.735	14.67	1.33	1	28.23375	
Variance	0.948228571	341.8392	21.8312	1.611828571	0	362.5205982	
ANOVA							
Source of Variation	SS	Df	MS	F	P-value	F crit	
Sample	0	0	65535	65535			
Columns	2015.525038	5	403.1050076	3.331292325	1.35508E-03	2.178155558	
Interaction	0	0	65535	65535			
Within	5082.234963	42	121.0055943				
Total	7097.76	47					

DISCUSSION

The root-nodule bacteria have been studied for long but little of its potential contribution to organic farming as well as sustainable agriculture has been explored. The detection of nodules showed presence of indigenous promiscuous *M. pruriens* microsymbiont with the competitive edge over other rhizobia strains in the soil hence its ability to nodulate *M. pruriens*. The absence of nodulation of *M. pruriens* in control pots showed the effectiveness of sterilization procedures applied to control soils as well as the fact that rhizobia species specific for *M. pruriens* were absent in the control pots.

M. pruriens varieties planted were well established in all the ecological zones. The two varieties (*M. pruriens* var. *utilis* and *M. pruriens* var. *cochinensis*) failed to nodulate with native rhizobia population in soil samples from Onitsha, Southeast Nigeria. Soils from Onitsha, Owerri, Benin and Enugu were acidic (Table 1). Comparatively, Onitsha soil was the best in terms of root moisture retention while in terms of shoot biomass production, Lokoja soil was the best. The percentage (%) moisture in the root/shoot showed that more water absorption occurred in the soils from the core east (Enugu, Aba, Onitsha and Owerri) due to the soil texture (Table 2). The presence of rhizobia species as biological N fertilizer contributed to the improvement of soil fertility thus enhancing the establishment of *M. pruriens* in the soil environment southeast, Nigeria; although, soil acidity has been reported to adversely affect agricultural production and limits legume productivity (Bordeleau and Prevost, 1994; Correa and Barneix, 1997). This observation suggested that certain environmental stress may be responsible for the absence of nodulation in Onitsha soil. In this case, indigenous strains were unable to adapt to acid stress and probably some other environmental stress not investigated. While this same soil samples had relatively adequate N and P nutrient supply as well as organic C, these findings corroborated earlier report by Walsh (1995) and Zahran (1999).

Although, at the commencement of this study, the mean indigenous rhizobia population was found to be above 300 cells/g of soil for these locations, the presence of these rhizobia species was confirmed using standard and conventional methods as reported by Vincent (1970).

Typical environmental stress earlier discovered by previous researcher include photosynthetic deprivation, water stress, poor water-holding capacity (Bottomley, 1991), salinity, soil nitrate, temperature, presence of heavy metals, acidic soil and biocides to militate against the legume-microsymbiont systems in soil which further corroborated the deductions of Walsh (1995). More so, the population of *Rhizobium* and *Bradyrhizobium* species vary in their level of tolerance to adverse environmental changes. However, Enugu soil which is the most acidic (Table 1) had the highest nodulation. One physico-chemical factor that was outstanding with this location was the relatively high NO_3–N and PO_4^{-3} nutrient in this location. The tolerance of the *M. pruriens* microsymbiont (*Rhizobium* sp.) to the prevailing climatic and edaphic factors might have enhanced the success of the symbiotic association in Enugu soil as well as its water-

Table 5. ANOVA: Two-factor with replication.

Summary	Root fresh wt (g)	Root dry wt (g)	Root moisture content (%)	Shoot fresh wt (g)	Shoot dry wt (g)	Shoot moisture content (%)	Nodule fresh wt (g)	Nodule number	Total
Enugu									
Count	9	9	9	9	9	9	9	9	72
Sum	68.38	11.45	758.6	274.68	32.5	668.5	6.73	54	1874.84
Average	7.597777778	1.272222222	84.28888889	30.52	3.611111111	74.27777778	0.747777778	6	26.03944
Variance	9.684994444	0.383569444	51.99361111	953.47565	0.485461111	48.48694444	0.342869444	31.75	1167.789
Total									
Count	9	9	9	9	9	9	9	9	
Sum	68.38	11.45	758.6	274.68	32.5	668.5	6.73	54	
Average	7.59777	1.272222	84.28888	30.52	3.61111	74.27777	0.74777	6	
Variance	9.68499	0.38356	51.99361	953.47565	0.48546	48.48694	0.34286	31.75	
ANOVA									
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*			
Sample	0	0	65535	65535					
Columns	74140.18378	7	10591.4548	77.26737103	9.24509	2.156423973			
Interaction	1.81899	0	65535	65535					
Within	8772.8248	64	137.075387						
Total	82913.008	71							

Wt = Weight.

Table 6. ANOVA: Two-factor with replication.

Summary	Root fresh wt (g)	Root dry wt (g)	Root mixture content (%)	Shoot fresh wt(g)	Shoot dry wt (g)	Shoot moisture content%	Nodule fresh wt (g)	Nodule number	Total
Enugu									
Count	9	9	9	9	9	9	9	9	72
Sum	69.47	14.59	703.06	135.32	34.23	671.38	6.66	53	1687.71
Average	7.7188888	1.62111	78.1177777	15.0355555	3.8033333	74.5977777	0.74	5.88888	23.44042
Variance	3.3170361	0.40893	74.0713444	11.2409527	0.464275	21.966569	0.453775	36.8611	981.3565

Table 6. Contd.

Total								
Count	9	9	9	9	9	9	9	9
Sum	69.47	14.59	703.06	135.32	34.23	671.38	6.66	53
Average	7.7188888	1.62111	78.1177777	15.0355555	3.8033333	74.597777	0.74	5.88888
Variance	3.3170361	0.40893	74.071344	11.240952	0.464275	21.966569	0.453775	36.8611
ANOVA								
Source of VARIATION	SS	df	MS	F	P-value	F crit		
Sample	0	0	65535	65535				
Columns	68486.0	7	9783.71964	526.06299	5.28103	2.156423		
Interaction	-3.18323	0	65535	65535				
Within	1190.272	64	18.598					
Total	69676.309	71						

Table 7. Micromorphology and biochemical characterization of *M. pruriens*.

Isolate code	Gram reaction	Cellular morphology	Catalase	Oxidase	Indole test	YM-BTB	Probable isolate
X_1	-	R	+		-	Acid (yellow)	*Rhizobium* sp.
X_2	-	R	+		-	Acid (yellow)	*Rhizobium* sp.
X_3	-	R	+		-	Alkaline (blue)	*Bradyrhizobium sp.*
X_4	-	R	+		-	Alkaline (blue)	*Bradyrhizbium* sp.

YM = Yeast extract mannitol medium; BTB = bromothymol blue; R = rod; - = negative; + = positive.

holding capacity (Table 2). Screening for the tolerant strains of *M. pruriens* microsymbiont indigenous to Onitsha and well adapted to its climatic and edaphic conditions failed because nodulation did not take place at this location. Poor nodulation is also possible in soils where a viable rhizobia population can be demonstrated (Graham et al., 1994). However, *M. pruriens* microsymbiont (*Bradyrhizobium* sp.) isolated from Lokoja, Aba and Calabar soils were influenced by the alkaline soil pH. The pH of the rhizosphere thus determines the type microsymbiont that occupies the nodules of the host legume.

High soil temperature in tropical and sub-tropical areas has been identified to be inhibitory or delay nodulation (Graham, 1992), particularly in creeping legumes nodulation which is affected by high tropical temperatures which may be as high as between 28 and 35°C in the January – April period. The optimum temperature for effective nodulation is between 25 and 30°C (Michiels et al., 1994; Arayankoon et al., 1990). Furthermore, the presence of heavy metals contaminants on cultivated plots as a result of the use of sewage sludge as organic fertilizer which often contains materials potentially toxic to soil microorganisms have been reported to have adverse effects on nodulation and N_2-fixation in legumes (McGrath et al., 1988). Other chemical contaminants such as polycyclic aromatic hydrocarbons (PAHs) which occur as ubiquitous environmental contaminants due to the combustion of fossil fuels can affect nodulation and N_2–fixation in legumes (Wetzel and Werner, 1995). The presence of rhizobia as N fertilizer contributed to the improvement of soil fertility thus enhancing the establishment of *M. pruriens* in the soil environment southeast, Nigeria.

The nodule number profile (Table 2) suggested

the relative presence of more *M. pruriens* microsymbiont in the soil from Enugu than in all other locations. Considering both the root and shoot dry weight (Table 2) which suggested the degree of soil fertility available to the legume particularly in collaboration with the microsymbiont, the agronomic parameters of *M. pruriens var cochinensis* showed that soils from Benin, Onitsha and Lokoja relatively supported biomass production more than in other locations. Although, nodulation failure was observed on Onitsha soil, this calls for further investigation of probable causes of this apart from climatic and edaphic reasons. Whereas *M. pruriens var utilis* (Table 3) showed excellent agronomic features with soils from Calabar, Lokoja and Aba in terms of root dry weight. However, considering shoot dry weight, soil from Onitsha, Lokoja, Enugu and Owerri performed excellently (Table 3). This performance can be attributed to the level of fertility of these soils. These findings are in agreement with the conclusion of Alhou et al. (1999) and Jemo et al. (2007).

The statistical analysis was critical with ANOVA using two-factor replication. Some ecological zones in this study have compatible and specific *M. pruriens* microsymbiont which have adapted to the introduced *Mucuna* varieties.

Conclusion

Biological nitrogen fixation (BNF) is an efficient source of N for increasing agricultural productivity. However, its success is inhibited by various adverse environmental conditions which subsequently affect crop yields. The *M. pruriens* seeds used in this study were of Asian origin but were perfectly adapted to the tropical humid climate in this study. The use of rhizobia as inoculants could be a successful biological process capable of improving crop yield while simultaneously providing biomass for other industry use.

The *Rhizobium*-legume symbiosis is hereby suggested as an ideal solution to the improvement of soil fertility as well as the rehabilitation of adversely degraded soil and this is worthy of priority for future research.

Natural selection of stress-tolerant rhizobia strains would be a non-polluting and cost-effective way to improve soil fertility as compared to other common ways such as inorganic NPK fertilizer and sewage sludge application. This will improve international trade volume because the crop yield from this type of agricultural practice would have conformed to the specifications of the federation of international organic agriculture standards.

Conflict of interest

The authors did not declare any conflict of interest.

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Survey of ethno-veterinary medicinal plants at selected Horro Gudurru Districts, Western Ethiopia

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Plant remedies are still the most important in therapeutics of treat livestock diseases, though large knowledge of ethno medicinal plants is declining to deterioration due to the oral passage of herbal heritage verbally. The objective of the study was to identify and document ethno-veterinary medicinal plants. The study was carried out from January to July 2014 at selected Horro Gudurru districts of western Ethiopia. The study sites were selected purposefully based on the recommendations of elders and local authorities. Ethno-botanical data were collected using semi-structured interviews, field observations and group discussion. About 51 study participants were involved in this study during the study period. A total of 25 ethno-veterinary medicinal plant species belonging to 19 families were documented with details on their local name, family, habitat, their traditional preparation and mode of application. *Solanaceae* families constituted the highest proportion (16%) followed by *Euphorbiaceae* (12%). The informants reported that there were 14 known livestock diseases which are treated by traditional healers. Herbs (44%) were the most widely used followed by shrubs (32%). Oral route of administration (76%) was the most commonly used followed by topical (24%). About 78.4% of the plant taxa were available every time. Agricultural expansion (43.3%) has been found to be the first main threat followed by deforestation (21.2%). The study revealed that the traditional healers and some livestock owners had knowledge of medicinal plants used to treat livestock diseases. Hence, further research should be conducted to evaluate the efficacy and possible toxicity of the plants in the study area.

Key words: Ethno-veterinary, disease, Horro Guduru Wollega, medicinal plants, livestock.

INTRODUCTION

In Ethiopia, livestock production remains crucial and represents a major asset among resource-poor smallholder farmers by providing milk, meat, skin, manure and traction. However, the economic benefits of livestock populations remain marginal due to prevailing livestock diseases which are among the principal bottle necks of livestock performance and cause of high economic losses of the resource poor farmers (Mesfin and Lemma, 2001).

Plant remedies are still the most important and sometimes the only sources of therapeutics for nearly 80% of human and more than 90% in livestock population

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in Ethiopia. Estimated floras of 6500 to 7000 species of higher plants are medically important and out of these medicinal plants, 12% are endemic to Ethiopia (Mengistu, 2004). Despite their vital role in catering for the health of human and livestock population, large part of the knowledge of ethno medicinal plants is on the verge of irreversible loss and declining to deterioration due to oral passage of herbal heritage from generation to generation verbally rather than in writings (Pankhurst et al., 2001).

Traditional healers and local farmer's (traditional health practitioners) have made remedies from plants that play an important role in treating both animals and human diseases. Ethno-veterinary medicine studies traditional knowledge, folk beliefs, skills, methods and practices used for the treatment of livestock ailments (Tabuti et al., 2003). It offers medicines which are cheap and locally available than pharmacotherapy. The traditional practitioners can prepare and use homemade remedies without any expenditure (Yirga et al., 2012). The use of ethno-veterinary practices to treat and control livestock diseases is an old practice in a large part of the world, particularly developing countries where animal health service facilities are still very poor or/and are found scarcely located at urban areas (Kokwaro, 1976; McCorkle, 1995; Sinha et al., 2002). Even still, those in close proximity to conventional drugs also use traditional medicinal drugs to treat their animals (Gemechu et al., 1997) due to shortage of modern drug of choice, cultural acceptability, efficacy against certain diseases and economic affordability (Teklehaymanot and Giday, 2007).

However, the traditional knowledge of ethno-veterinary medicinal plant is not compiled in Ethiopia (Giday et al., 2009; Sori et al., 2004). Traditional medical knowledge of medicinal plants and their use by indigenous cultures are not only useful for conservation of cultural traditions and biodiversity, but also for livestock healthcare and drug development in the present and future (Tamiru et al., 2013).

The studies conducted on the traditional ethno-veterinary medicinal plants in Ethiopia are very limited when compared with the multiethnic cultural diversity and the diverse flora of Ethiopia (Giday et al., 2009). Even though traditional ethnoveterinary knowledge of medicinal plants is very crucial to treat different animal diseases, there is no study conducted in Horro Guduru Wollega Zone of western Ethiopia on this regard. Hence, the present study was designed to identify and document ethno-veterinary medicinal plant species and traditional medicinal knowledge of the traditional health practitioners in study area.

MATERIALS AND METHODS

Study area

The study was conducted from January to July 2014 at selected Horro Guduru Districts/Woredas in Oromia regional state of Western Ethiopia. The zone is found about 251 Km from West of Addis Ababa. The study area is located at an elevation of 2,088 m above sea level. The annual minimum and maximum temperature is 15-27°C and the annual average rainfall is 1800 mm (NAMSA, 2013). The rural and peri-urban areas of the zone are featured by mixed agricultural system where livestock play an important role. There are no adequate veterinary services in case of drug availability, only some broad spectrum drugs were offered.

Study population

The target populations used in this study were elderly livestock owners and traditional animal healers. The survey was conducted through questioner surveys designed for farmer particularly knowledgeable elders and traditional animal healers.

Data collection

The study sites were selected based on the availability of practice of traditional medicine and on the recommendations of local authorities and agricultural developing agents. Moreover, the agro-climatic zones was considered to select the study districts' and kebeles purposely. An ethno-veterinary botanical survey was conducted to gather information on the traditional usage of plants in livestock health care system using a semi-structured interview, observations and field guided walks (Martin, 1995) with the local farmers and traditional healers who were willing to share their indigenous knowledge. A total of 51 individuals were purposively selected and interviewed based on their knowledge on traditional medicine in the study area. The market survey was made to distinguish and record the type of herbal drugs sold in the market, and the multipurpose role of some medicinal plants was observed during the study period. Furthermore, herbal drug sellers and others who brought plant species were interviewed. The group discussions were conducted to elaborate the methods of preparation, administration and conservation of the medicinal plants. Interviews were conducted in "Afan Oromo" language. During the study period, each informant was visited two to three times in order to confirm the reliability of the ethnobotanical information. The responses that were not in harmony with each other were rejected.

Plant specimen collection and identification

The medicinal plants were collected from the wild and home gardens based on the report of the informants. The collected plants and the necessary recorded information were taken. Preliminary identification was done at the site (field) and the collected voucher specimens were taken to the Natural Herbarium of Wollega University and identified by botanist. After the specimens were taken to the department, plant species were identified using taxonomic keys; the volumes of the Flora of Ethiopia and Eritrea (Edwards et al., 2000; Hedberg et al., 2006) and by making a comparison with the already identified specimens that were deposited at the herbarium.

Data analysis

Descriptive statistical methods: proportions and tables were used to summarize the collected ethno-veterinary medicinal data.

RESULTS

General characteristic of the informants

In the present study, traditional healers (72.5%) and

Table 1. Summary of group interviewed, source of plants and availability of the medicinal plants in the study area.

Variables		Total no. of respondents	No. of identified major risk factors
Sources of Plants	Wild	39	76.5%
	Domestic	7	13.7%
	Both	5	9.80%
Total		51	100
Group of interviewed	Livestock owners	14	27.5%
	Traditional healers	37	72.5%
Total		51	100
Age of respondents	Elderly	44	96%
	Young	7	4%
Total		51	100
Availability of the medicinal plants	Every time	40	78.4%
	Seasonally	8	15.7%
	Difficult to get	3	5.90%
Total		51	100

livestock owners (27.5%) participated during the study period. Almost all of the respondents were elderly, age group: 96.0% and younger age group: 4%, respectively (Table 1).

Sources and habitat of ethno veterinary medicinal plants

The ethno-veterinary medicinal plant data collected from the study site revealed that most of the medicinal plants were collected from the wild (76.5%) followed by from home gardens (13.7%) and both (9.8%), respectively (Table 2). In addition, the most indicated habit of the medicinal plants was herbs (44%) and then shrubs (32%) (Table 3).

Medicinal plant parts used for the preparation of the remedies

The study showed that the widely used plant part for the preparation of the remedies in the study area was leaves (48%) and followed by roots (36%) and the rest were seed and fruits (20%) (Figure 1 and Table 3).

Mode of preparation, route and ingredients added

The study revealed that the highest mode of preparation was in the form of grinding (49.3%); followed by crushing (27.7%) and others like chopping, decoction, roasting, consumption of whole plant part, streaking (23.0%). The majority of the plant remedies were prepared from fresh material of the plants which accounted for 87% followed by fresh/dry (13%). The most widely used route of administration was oral which accounted for 76% followed by topical (24%) and inhalation (4%) (Table 3).

Indications

The informants reported that there were 15 known livestock diseases and disease conditions in the study area which are treated by traditional healers. Of the diseases, rabies (29.4%) was the most common one. Traditional healers (key informants) ranked these plant taxa based on their perception of the degree of effectiveness (Table 5).

Availability of medicinal plants based on the season

Availability of some ethno-veterinary medicinal plants were affected by season; many of the plants were available all the time (78.4%), some are available seasonally (15.7%), and the rest, difficult to get (5.90%) as described in the study area (Table 1).

Medicinal plant families frequently used at study area

Solanaceae and Euphorbiaceae families constituted the highest proportion (16%) followed by Cuccurbitaceae (12%), Cuccurbitaceae (8%) and all other plant families were constituted proportionally (4%) (Table 4).

Inheriting knowledge of medicinal plants

According to the survey, transfer of ethno-veterinary knowledge of medicinal plants follows vertical transfer to the most selected family member orally with great secrecy. The highest number of transfer of knowledge on the plant is to trust eldest son that accounted for 45.0% followed by trusted sons (23.5%), and others are all members of the family (19%), relatives (9%) and friends (4%). The findings of the study showed that as people

Table 2. Summary of ethno veterinary medicinal plants, disease treated, habit, part, route and uses of the plants.

Scientific name	Family name	Local name	Habit	Parts	Other uses	Routes	Indications
Achyranthes aspera	*Amaranthaceae*	Maxxannee	Herb	Root	Fence	Oral	Wound, mastitis
Allium sativum	*Alliaceae*	Qullubbii adii	Herb	Leaf	Food	Oral	Abdominal pain, Pastuerellosis, relief bloat
Brassica carinata	*Brassicaceae*	Gomenzeera	Herb	Seed	Food	Oral	Stop bloat, wound
Brucea-anti dysentrica	*Simaroubaceae*	Qomonyoo	Herb	Fruit		Oral	Rabies, ring worms
Calpurnia aurea	*Fabaceae*	Ceekkataa	Shrub	Leaf	Fence	Topical	Lice infestation, leech
Capsicum annum	*Solanaceceae*	Mimmixa	Herb	Seed	Food	Oral	Abdominal pain, relief bloat, Pastuerellosis, leech, Tapeworm
Carrissa- spinarum	*Apocynaceae*	Hagamsa	Shrub	Root	Fence, Food	Oral	Ring worm, wound
Clucia lanceolata	*Euphorbiaceae*	Ulee foonii	Shrub	Root	Fence	Oral	Rabies
Coffea Arabica	*Rubiaceae*	Buna	Shrub	Seed	Food	Topical	Wound, abdominal pain
Croton macrostachyus	*Euphorbiaceae*	Bakkanniisa	Tree	Leaf	Shed	Oral, Topical	Ring worm, relief bloat, wound
Grewia bicolar	*Tliaceae*	Harooressa	Tree	Leaf	Fence	Oral	Retained fetal membrane
Justicia- schimperiana	*Acanthaceae*	Dhummugaa	Shrub	Root, Leaf	Fence	Oral	Rabies, coccidiosis
Nicotiana- tabacum	*Solanaceae*	Tamboo	Shrub	Leaf	stimulant	Oral	Leech, Tape worm
Prunus africana	*Rosaceae*	Hoomii	Tree	Leaf	Shed, furniture	Topical	Wound
Phytolacea- dodecandra	*Phytolacaceae*	Handoodee	Herb	Leaf	Detergent	Oral	GIT parasites, rabies
Ricinus- communis	*Euphorbiaceae*	Qobboo	Shrub	Root	Shed, Food	Oral	Retained fetal membrane, rabies
Solanium incanum	*Solanaceae*	Hiddii	Herb	Root	-	Oral	Pastuerellosis
Vernonia anygdalina	Asteraceae	Eebicha	Tree	Leaf	Fence	Oral	Retained placental membrane
Zehneria scara	Cuccurbitaceae	Hidda reeffaa	Herb	Leaf	Fence	Oral	Rabies, Pastuerellosis
Zingiber officinale	Zingebiraceae	Jinjibila	Herb	Root	Food	Oral	Rabies, Pastuerellosis, abdominal pain, leech
Olea europaea	*Oleaceae*	Ejersa	Tree	Leaf	Charcoal	Inhalation	Rabies, snake bite
Cucumis ficifolius	*Cucurbitaceae*	Hiddi hooloto	Herb	Root	-	Oral	Rabies
Solanum gigantum	*Solanaceae*	Hiddii saree	Herb	Root	-	Oral	Rabies
Dodonaea angustifolia	*Sapindaceae*	Itacha	Shrub	Leaf	-	Topical	Lice infestation
Citrus aurantifolia	*Rutaceae*	Lommi	Tree	Fruit	Food	Topical	Tick infestation, wound

become older and older, their knowledge of traditional medicine becomes better and better. Most of the informants were elders which indicated the trend of transferring (inheriting) the knowledge is usually at old age. The study also indicated that there is no widely observed trade of medicinal plants in the study area though some practitioners and women sell some medicinal plants in the market and in their homes. Some of the plant medicines that are grown in home gardens and sold were *Olea europaea*, *Nicotiana tabacum*, *Capsicum annum*, *Coffea arabica*, *Justicia schimperiana* and *Ricinus communis.*

Conservation and threats of medicinal plants

The study indicated that many of the informants who have knowledge on traditional medicine usage give priority to the immediate use of the medicinal plants than to its sustainable future uses, as a result their harvesting style is destructive. However, some plants are protected for spiritual and cultural purposes. Thus, these places are good sites for the protection of the medicinal plants since cutting and harvesting are not allowed in such particular areas. This indicated that a good practice for the conservation of medicinal plants is through cultivation. The study revealed that there were a number of threats that affect the medicinal plants in the study area. The factors include agricultural expansion (43.3%) followed by deforestation (27.9.2%) and overgrazing (16.3%) (Table 6).

DISCUSSION

In this study, a total of 25 ethno-veterinary medicinal plant species belonging to 19 families were identified and documented with details on their local name, family,

Table 3. Summary of ethno-veterinary medicinal plant habit, parts and routes of administration.

Variables		Frequency	Percent (%)
Habit	Herb	11	44
	Shrub	8	32
	Tree	6	24
Parts	Leaf	12	48
	Seed and fruit	5	20
	Root	9	36
Routes	Oral	19	76
	Topical	6	24
	Inhalation	1	4

Table 4. Summary of ethno-veterinary medicinal plants' frequencies in the study area.

Family name	Frequency	Percent (%)
Amaranthaceae	1	4
Alliaceae	1	4
Brassicaceae	1	4
Simaroubaceae	1	4
Fabaceae	1	4
Solanaceceae	4	16
Apocynaceae	1	4
Euphorbiaceae	3	12
Rubiaceae	1	4
Tliaceae	1	4
Acanthaceae	1	4
Rosaceae	1	4
Phytolacaceae	1	4
Asteraceae	1	4
Cuccurbitaceae	2	8
Zingebiraceae	1	4
Oleaceae	1	4
Sapindaceae	1	4
Rutaceae	1	4

habitat and their traditional preparation, and mode of application. *Solanaceae and Euphorbiaceae* families constituted the highest proportion (10.7%) followed by Fabaceae and Cuccurbitaceae with the same proportion (7.14%). The finding was in line with other findings that were conducted in different parts of Ethiopia (Gebre, 2005; Tolesa, 2007; Teklehaymanot et al., 2009; Gebrezgabiher et al., 2013; Lulekal et al., 2014).

Most of the respondents were older age groups, however, very few youths were involved in traditional livestock treatment in the study area. This was in line with report of Yirga et al. (2012a, b) from Jimma. The findings also agree with reports of Tamiru et al. (2013) from Dabo Hana District, West Ethiopia and Gebrezgabiher et al. (2013) from Tigray region. The less medicinal knowledge in relation to young age might be attributed to the fact that traditional knowledge is built with years of experience (Awas, 2007). This might be also be due to the transfer of ethno-veterinary knowledge of medicinal plants which follows vertical transfers to the most selected family member orally with great secrete usually at old age.

The finding revealed that the majority of the traditional healers and some livestock owners in the villages rely on traditional veterinary knowledge, practices and locally available materials (Giday and Ameni, 2003), primarily medicinal plants to cure and prevent livestock diseases such as wound, rabies, retained fetal membrane, abdominal pain, bleeding, pastuerellosis, mastitis, leech infestation, bloating, dermatophilosis, skin diseases, lice infestation, ring worm and gastro intestinal parasites. This indicated that herbaceous medicinal plants were the widely used for the treatment of various diseases. The finding agrees with studies in other parts of Ethiopia (Tessema et al., 2001; Giday and Ameni, 2003; Sori et al., 2004; Teklehymanot and Giday, 2007; Birhanu et al., 2014).

In this study, most of the medicinal plants were collected from the wild (76.5%) and others were from home gardens (13.5%) and both (9.80%). This was in line with studies in other parts of Ethiopia (Giday et al., 2009), Pakistan (Farooq et al., 2008) and Brazil (Barboza et al., 2007; Monteiro et al., 2011). This indicated that the practice of cultivation of plants for their medicinal purpose in home gardens in most of the country is low though many plants are cultivated for other purposes, mainly for food. In a similar way, people in the study area have less effort to cultivate medicinal plants in their home gardens rather, they go to nearby or far places and harvest the plants. In addition, the habit of the medicinal plants indicated that most of them were herbs (44%) followed by shrubs (33.6%) and tree (24%). The knowledge on the type, part used, dosage, administration of the medicinal plants is circulating only among traditional practitioners of traditional medicine based on the severity of diseases (Tadesse, 1986). All plant growth forms were not equally used as remedies, because of the difference in distribution among the growth forms. This leads to the wide use of herbs and shrub for their medicine. In the present study, the part of the medicinal plant which is highly used for the preparation of the remedies were leaves (48%) followed by roots (36%). This agrees with the reports of Giday et al. (2003) and Mesfin (2007). Based on the information gathered from the key informants especially from those who are highly accepted by the society for their ability in healing different health problems, the condition of preparation of remedies was not the same. The highest condition of preparation was fresh (87%) followed by fresh/dry (13%). In contrast to

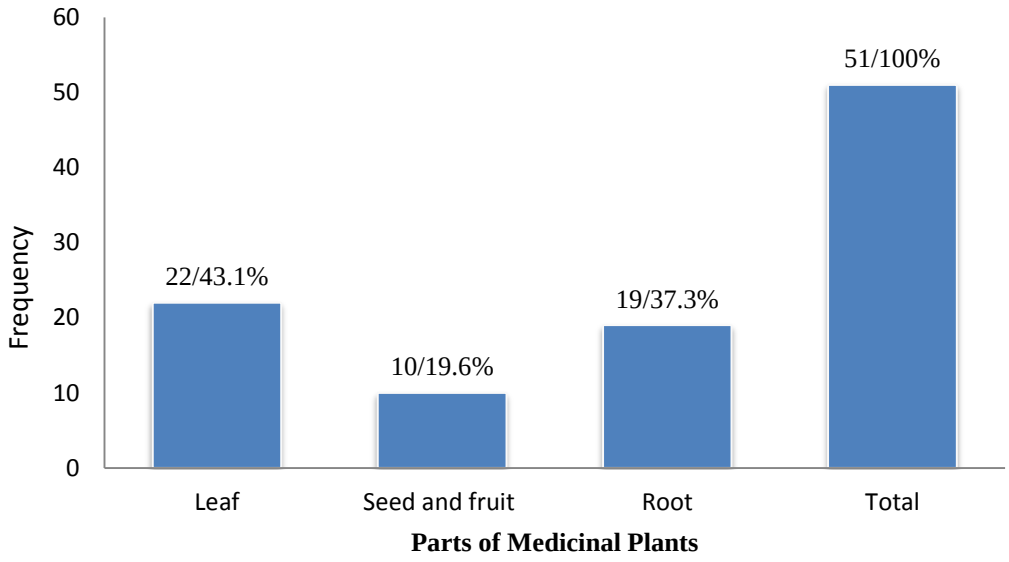

Figure 1. Parts of ethno-veterinary medicinal plants used for treating livestock diseases in the study area.

Table 5. Summary of ethno-veterinary medicinal plant with respect to indications in the study area.

Livestock diseases (indications)	Frequency and Percent
Rabies	15 (29.4%)
Wound	5 (9.80%)
Retained fetal membrane	2(3.92%)
Abdominal Pain	4 (7.84%)
Pastuerellosis	5(9.80%)
Mastitis	1 (1.96%)
Leech infestation	3(5.90%)
Relief bloating	5 (9.80%)
Dermatophilosis	3 (5.90%)
Ring worm	3 (5.90%)
Lice infestation	1 (1.96%)
GIT parasites	1 (1.96%)
Tape worm	1 (1.96%)
Snake bite	1 (1.96%)

this, some professional traditional healers sell their plant medicines in dried form in the market and also store the dried plant medicines in different containers in their homes. This agrees with another finding at Tigray region (Gebrezgabiher et al., 2013).

The study also showed that the information gathered from the key informants especially from those who are highly accepted by the society, most of the plant remedies were administered orally (76%) followed by topical (24%) and inhalation (4%). The result agrees with similar studies elsewhere in Ethiopia (Abebe and Ayehu, 1993; Teklehaymanot and Giday, 2007). But, the dosage determination was the big problem in the study area because there is no standardized known unit of measurements of the plant remedies. However, the dose was determined by using homemade remedies using cup, glass, plant parts like number of bulbs and number of seeds and their own hand as handful were the identified means to treat animals in the study area. The dosage regime is generally dependent on the degree and duration of the diseases, age and body condition of the animal. This agrees with report of ethno-medicinal plant knowledge and practice by Abera (2014) from Gimbi district, Southwestern Ethiopia.

In this study, the information gathered from the key informants indicated that the treats of medicinal plants

Table 6. Priority ranking of factors perceived as threat to ethno-veterinary medicinal plants on the level of destructive effects in Horro Guduru based on interviews (n=51).

Factors	Frequency	Percentage (%)	Rank
Agricultural expansion	45	43.3	1
Deforestation	29	27.9	2
Overgrazing	17	16.3	3
Drought	13	12 .5	4

increase from time to time in the study area. The agricultural expansion was the major medicinal plant treats (43.3%) followed by deforestation (27.9%). This might be due to continuous agricultural expansions, deforestation and draught in addition to lack of attention towards the medicinal plants. The plants disappeared because of rapid socioeconomic, environmental and technological changes and as a result of the loss of cultural heritage under the guise of civilization (Lulekal et al., 2008; Khan et al., 2012).

The traditional medicinal use of some surveyed medicinal plants is recorded in other parts of the country. *Hagenia abyssinica* is used to treat taneasis in human in Bale, Debark and Kofle rural communities of Ethiopia (Assefa et al., 2010). Similar, its use for treatment of livestock ailments have been also documented (Mesfin and Obsa, 1994; Abebe et al., 2000; Wondimu et al., 2007; Yineger et al., 2008). The therapeutic value of *Achyranthes aspera* is known for skin diseases (Goyal et al., 2007) and various gastrointestinal and respiratory problems (Bhandari, 1990). The medicinal use of *Azadirachta indica* to treat endoparasites and ectoparasites is also documented by Sori et al. (2004) in the Borena pastoralists, southern Ethiopia. Moreover, the efficacy of leaves of *A. indica* to reduce the parasitic load (Khan, 2009) and that of the Aloe species in treating *Trychostogylus* in sheep (Ibrahim, 1986) has also been confirmed. The study depicted that the traditional healers and local farmers have rich knowledge on ethno-veterinary medicines to treat and manage their livestock through indigenous knowledge, to protect the health and increase productivity.

Conclusion

In this study, 25 species and 19 families of ethno-veterinary medicinal plants were identified and documented during the study period in the study area. There were a large number of valuable resources, practices and knowledge of ethno-veterinary medicine which can solve problems of shortage of drugs at rural areas as well as drug resistance in different diseases. The plants were mainly collected from the wild by consultation of traditional health practitioners. Agricultural expansion and deforestation were found to be the main threats to the medicinal plants. Thus, attention should be given to the medicinal plants and research is needed to determine safety, toxicity and determine the dose.

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Conflict of interest

The authors declare that there is no conflict of interest.

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Short Communication

The effective method of potato cultivation

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Potatoes occupy a considerable place in food ration of Uzbekistan's population. That is why over the past few years, there are various researches on traditional ways of increasing the amount of potatoes produced as well as the generally accepted methods of their cultivation. One of such methods is generative propagation of potatoes.

Key words: True potato seed, greenhouse, seedlings, minitubers, potato seed tubers.

INTRODUCTION

As it is well known, the cultivation of potatoes from botanical seeds has a number of advantages over vegetative propagation; they include economical seed tuber material of 3.0-3.5 t from every hectare and the low expenditures connected with storage and transportation of potatoes (Ergashev, 2006; Budin, 1987).

When taking into consideration, that the expenditure for the seed materials comprises 55-60% of the total expenditures for the crop cultivation, it becomes evident that it is the prime cost of the product produced (Ergashev, 2006; Ross, 1989).

Research shows that in generative propagation of crop area of 1 ha, 70-100 g of botanical seeds are used; and virus, viroidal, micoplasmous and bacterial diseases affecting potatoes are not spread to the following generations, both during the period of vegetation and storage of tubers. That is why this method is the best for seed-growing of potatoes because it is virusless (Ergashev, 2006).

Taking into consideration the above circumstances, in 2012-2014 we conducted a research on the selection of sorts, hybrids, hybrid populations and self-pollinated lines of potatoes, suitable for generative propagation and also for elaboration of effective technology for its cultivation, leading to obtaining high yields of marketable and seed potatoes with the least prime cost for product produced.

MATERIALS AND METHODS

The research was carried out on the experimental plot of Samarkand Strong Point of Scientific-research Institute of vegetable-gourd Crops and Potatoes of the Republic of Uzbekistan. The botanical seeds of 42 sorts, hybrids, hybrid populations and self-pollinated lines, obtained from the Scientific Production Association on Potatoe-growing (Moscow), all-union Institute of plant-growing and in the firm "Sedek" (Moscow) and collected units from the sorts cultivated under local condition of Uzbekistan, served as initial materials.

The seeds were sown in the film hot-houses early in March to get seedlings. The seedlings were planted on open ground on 2nd of April according to the scheme 70 x 20 cm. During the research, the germinating power of seeds and acclimatization of seedlings was determined. Phonological observations, biometrical measurements,

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Table 1. The attack of plants by viruses with regards to potato production from true potato seed (2012 – 2014 years).

S/N	The origin of seeds	In overt form (%)	In latent form (%)	The attack of plants by viruses (%)			
				X	S	M	Y
1	Surkhan-1	0	4.0	-	1.0	2.0	1.0
2	Curado	0	2.3	-	-	-	2.3
3	Triumph	0	1.5	1.5	-	-	-
4	Ballada	0	4.5	2.5	-	-	2.0
5	Deva	0	3.6	0.6	2.0	1.0	-
6	Assol	0	3.8	3.5	0.3	-	-
7	Vilona	0	2.9	0.7	1.2	-	1.0
8	Sante (at planting with tubers of the first reproduction	9.2	35.2	8.2	11.2	6.3	9.1

Table 2. The peculiarities of the growth, development and tuber formation of potatoes at generative propagation.

S/N	The origin of seeds	Germinating power of seeds (%)	Acclimatization of seedlings (%)	The productivity of plants g/bush	Yield per ha in center	Profitableness of the production
Generative propagation						
1	Surkhan-1	92	97	520	160,0	201,7
2	Curado	89	93	210	149.0	147.2
3	Triumph	91	96	580	150.0	152.7
4	Ballada	85	90	286	130.0	166.0
5	Deva	88	92	450	185.0	212.2
6	Assol	86	91	320	145.0	140.8
7	Vilona	90	94	380	135.0	128.2
8	Sante (at planting with tubers of the first reproduction	-	-	465	232.0	126.7

serological and immunophermental analysis, visual method of determining viral infection of the plants, the productivity of plants and yield-capacity of samples were also conducted (Okhrimenko, 2013). The Methodology of the Research on the Potato Crop (1967) was followed.

The economic effectiveness of potatoes cultivation with true potato seeds was determined according to generally accepted methods (Tables 1, 2).

RESULTS AND DISCUSSION

The research ascertained that the samples had different indexes according to germinating power of seeds, and acclimatization of seedlings. Thus, they were much higher intersort hybrids as compared to intertype hybrids. There was positive correlation between the germinating power of seeds and acclimatization of seedlings. The highest indexes in this respect were obtained from hybrid populations.

It is necessary to note, that the vegetation period of plants in average comprises 120-136 days depending on genetic origin. Serological and immunophermental analysis showed that the total viral infection of plants at the generative propagation had the least index as compared to plants of tuber origin and 1.5-4.5%, depending on the sample and year of cultivation.

The insignificant infection of plants, grown from botanical seeds can be the result of their infection during vegetation period. 35.2% of plants of the first reproduction positively reacted to the content of viruses in latent form under productive conditions at the tuber propagation. The productivity of plants at generative propagation comprised 210-580 gr/bush depending on their genetic origin. During cultivation of potatoes with tubers, the average productivity of plants comprised 465 g from every bush.

The determination of economic effectiveness of

different methods of cultivation has shown a great advantage of generative propagation in comparison with generally accepted method. Thus, by the saving of planting material and by obtaining healthy, free from viruses plants and owing to high productivity, the profitableness of the production is up to 212.2%

Conclusions

This research has shown that with generative propagation, 1-2 tuber potatoes can be used as seeds. In spite of the genetic diversity of qualities, they give high and qualitative yield. This affirms that the new method can serve as an effective method for creating the initial material for true potato seed-growing.

The obtained data show that the generative propagation of potatoes can serve as an effective way of getting qualitative seed and marketable potatoes.

Conflict of interest

The authors did not declare any conflict of interest.

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Full Length Research Paper

Spatiotemporal variations of *Tricolia affinis* (C.B. Adams, 1850) associated with the macroalgal community on reef stretches at Piedade beach, southern coast of Pernambuco State, Brazil

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The aim of the present study was to assess the correlation between the spatiotemporal variation of the gastropod *Tricolia affinis* and the macroalgal community on the sandstone reef at Piedade beach on the southern coast of Pernambuco State (PE) during the rainy and dry seasons in 2009 and 2010. Five square plots were randomly sampled on each date on the reef flat and pool to sample the gastropod population and measure the biomass of the dominant algae. Abiotic parameters were measured near the sampling sites. Three macroalgal genera, the Rhodophyta *Gracilaria* and *Hypnea* and Chlorophyta *Bryopsis*, showed representative biomasses at the sampling sites and were found throughout the sampling period. The abundance of *T. affinis* on the reef flat was significantly lower during the rainy season; however, no significant difference in this gastropod's abundance was found between the reef and tide pool. The density of *T. affinis* was significantly correlated with the biomass of *Gracilaria*, which showed the highest biomass and structural complexity in the studied area.

Key words: Sandstone reefs, benthic algae, *Gracilaria*, mollusca, seasonality.

INTRODUCTION

Coastal regions and their associated reefs are among the zones that are most susceptible to environmental impacts because of the high human population densities and economic activities in these areas, including the existence

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of port complexes and tourism and leisure activities. These factors may cause changes in algal communities and the associated fauna, which are sensitive to human impacts (Sousa and Cocentino, 2004), such as trampling in the intertidal zone. Organic pollution is currently one of the main threats to coastal environments. This type of pollution can affect the development of benthic communities, causing a decrease in species diversity and potentially promoting the proliferation of opportunistic species, including ephemeral macroalgae (Breves-Ramos et al., 2005; Simões et al., 2009).

The sandstone reefs located on the southern coast of Pernambuco state are marine outcrops forming ridges parallel to the coast that often emerge during low tide. These reefs consist mainly of quartz and bioclasts, primarily originating from shellfish and algae (Sousa and Cocentino, 2004). Many of these reefs represent a key consolidated substratum for the attachment of benthic algae and a diverse associated fauna, therefore representing an ecosystem with significant biological interactions (Simões et al., 2009) that forms the base of the local food webs.

The *Tricolia* genus has a wide geographical distribution and is found in North Carolina (USA) to the coast of Brazil. The species *Tricolia affinis* is a prosobranchiate gastropod of the family Tricoliidae found at coastal locations in Florida, the Caribbean, Colombia, Venezuela and Brazil, exhibiting sizes varying between 0.2 and 4.0 mm (Rios, 1994). On the Brazilian coast, this genus is found from Santa Catarina State to the northeastern region, including the Fernando de Noronha archipelago (Nogueira et al., 2003). According to Braga (1983), *T. affinis* is an abundant species in the studied area (Piedade beach). This gastropod is commonly found in estuaries and on rocky shores, coral reefs, unconsolidated substrata and seagrass banks, often associated with algae.

T. affinis is an herbivorous species that is most frequently found in the subtidal zone, where there are higher nutrient concentrations. This species feeds on microscopic algae and bacteria (periphyton) on the surface of debris-covered macroalgae (Marcus and Marcus, 1960). According to Ourives et al. (2011), *T. affinis* is also an abundant component of the estuarine fauna of Rio Cachoeira, Bahia State, Brazil, where its distribution was found to be positively correlated with salinity. The aim of the present study was to assess the temporal and spatial variation of *T. affinis*, associated with a *Gracilaria* bank, in the intertidal zone of the reef at Piedade beach since, this alga has been commercially used on the Brazilian coast, for agar production, and its abundance was found to be reduced in several locations along the northeastern coast. While in other areas, it is considered as an invasive species (Fukunaga et al., 2014; Zi-Min and Lopez-Bautista, 2014).

Two areas were sampled: a tidal pool where the algae were always immersed and more likely subjected to water transport, the reef flat algae where they are better attached to the substrate and subjected to less water transport. Therefore, the present study was based on the hypotheses that a correlation exists between the dominant algal biomass, abiotic factors and the abundance of *T. affinis* at the sampling sites and that spatial and temporal differences exists in the mollusk and algae abundance.

MATERIALS AND METHODS

Study area

Piedade beach in the Jaboatão dos Guararapes municipality is located on the southern coast of Pernambuco state, between the coordinates 08° 11´ 08.48´´ S, 34° 55´ 04.66´´ W and 08° 11´ 03.45´´ S, 34° 55´ 03.24´´ W. Its reef area is approximately 9.8 km in length and encompasses approximately 234 km², with a formation running parallel to the coast. The reef is immersed at high tides, forming a lagoon between the beach and the reef at low tides. The region exhibits a tropical Atlantic climate and average air temperature near 26°C, and the distribution of rainfall defines two seasons: a dry season (September-February), with rainfall below 100 mm, and a rainy season (March-August), with rainfall greater than 100 mm (Cavalcanti and Kempf, 1969). During the study period, the precipitation in Piedade was highest in April 2009 (268.9 mm) and lowest in October 2009 (6.0 mm) according to data from the local meteorological station. The coast of Piedade consists of a coastal plain formed by fluvial and marine deposits, where there was once a strip of typical coastal vegetation referred to as restinga that is now occupied by quickly expanding real estate developments and is still influenced by the Jaboatão River, which receives pollution from industrial hubs and urban centers (Pereira et al., 2002).

Field work

Sampling was conducted in the intertidal zone of the Piedade reef (PE) from April 2009 to March 2010. The first sampling site was a reef pool at the coordinates 8° 10´ 48.8´´ S and 34° 54´ 57.9´´ W. The second site was located on the reef flat in an area dominated by *Gracilaria caudata* (8° 10´ 49.4´´ S and 34° 54´ 58´´W) and a few meters away from the tide pool. Five 20 x 20 cm squares were randomly sampled using a spatula at each study site on each sampling date. Samples were collected in April, June, August, October and December 2009 and January and March 2010.

The collected material was packed in plastic bags, labeled with the date, sampling site and square number and then immediately transported to the University of Pernambuco Marine Biology laboratory for freezing and subsequent triage. The water temperature and soluble oxygen were measured at the sampling sites near the square plots with an oximeter. The soluble oxygen values ranged between 9.2 and 18.1 mg/L at the sampling sites, and thus, oxygen was not a limiting factor for the *T. affinis* populations. Water samples were collected in an area near the sampling sites where there is water influx to the reef flat, through an opening on the edge of the reef, for subsequent analysis of salinity, pH and nitrogen concentrations (ammonia-N [NH^{3-}+NH^{4+}.N], nitrite-N [NO^{2-}.N] and nitrate-N [NO^{3-}.N]) at the Chemical Oceanography Laboratory of the Federal University of Pernambuco (Table 1). The salinity and nutrient analyses were conducted according to Strickland and Parsons (1972).

Table 1. Temperature values, salinity, total nitrogen and pH of Piedade beach.

Months	Temperature	Salinity (‰)	Total N (µmol/L)	pH
April	33	20.18	2.34	8.64
June	28	15.26	4.60	8.69
August	28.8	20.45	2.85	8.94
October	33.6	24.29	2.11	8.63
December	33.8	27.53	0.45	8.45
January	32.3	26.73	3.70	8.41
March	34.2	31.54	1.56	8.64

Laboratory and statistical analyses

The mollusks were separated on a plastic tray using a stereomicroscope, metallic tweezers and Petri dishes. Then, they were fixed in 70% ethanol for subsequent counting. Only *T. affinis* samples in good condition and with an intact operculum were included in the counts, with empty shells being excluded. The dominant algae were identified taxonomically at the genus and species level. Algae were transferred to laminated paper bags and dried at 60°C for 72 h to assess their biomass. The samples were then weighed on an analytical scale, and the algal biomass values were expressed per m². Bivariate and univariate analyses of variance (ANOVAs), in addition to Tukey's tests, were used to assess the seasonal variation of algae at the reef flat and tide pool after logarithmic transformation of the biomass data.

Two-way ANOVAs were performed to assess the spatiotemporal variability of the *T. affinis* density in the samples, following confirmation of the normal distribution of the data and square root (x) + 0.5 transformation. Subsequently, one-way ANOVAs were performed for each sampling station individually, in addition to Tukey's test. Canonical correspodence analyses were performed with the mean algal biomass and the mean density of *T. affinis* with the abiotic variables (water temperature, salinity, pH and total nitrogen) separately for the two sampling sites. The Pearson correlation coefficients between the dominant algal biomass and abundance of *T. affinis* were assessed. The statistical programs R (R Core Team, 2014) and Biostat 5.8.4 were used for these analyses.

RESULTS AND DISCUSSION

A two-way ANOVA showed that there was significant temporal variation in the *T. affinis* density, but no significant differences were found in gastropod abundance between the sampling sites. The temporal variation of *Tricolia* was significant in the reef flat samples, significant differences between the sampling months were found ($p < 0.01$) (Figure 1). The mean gastropod density on the reef flat differed between April 2009 and June, August and October 2009. A significant difference was also found between October 2009 and March 2010, when the mean density of *Tricolia* was approximately 850 individuals per m^2, demonstrating a population recovery at the end of the dry season.

The Rhodophyta *G. caudata*, *Hypnea* (*Hypnea musciformis, Hypnea spinella* and *Hypnea cenomice*) and the Chlorophyta *Bryopsis* (*Bryopsis plumosa* and *Bryopsis pennata*) were the dominant algae in terms of biomass (Figures 2, 3 and 4). These algal genera were found on all of the sampling dates at both the tide pool and reef flat. Their mean dry weights were subjected to statistical analyses in combination with the density of *T. affinis*. *Ulva* spp. was also found during all of the sampling campaigns on Piedade reef, but at a lower biomass than *Gracilaria*, *Hypnea* and *Bryopsis* and was therefore not included in the statistical analyses. The macroalgae showed seasonal variation, reflected by a lower biomass of *G.caudata* in rainy season samples ($p < 0.05$). However, the biomass of *Hypnea* showed no significant differences between the sampling months at either the tide pool or reef flat ($p > 0.05$) but was different between sites. No significant differences in *Bryopsis* biomass were found on the reef flat between the sampling dates ($p > 0.05$). In turn, *Bryopsis* showed a peak biomass in December 2009, which was significantly higher ($p < 0.01$) than the mean biomasses detected in the other sampling months at the tide pool, although no differences in biomass were found between sampling sites. A two-way ANOVA demonstrated a higher abundance of *G. caudata* at the reef flat than in the pool.

The *T. affinis* density was positively correlated with the *Gracilaria* biomass for the samples collected at both the reef flat ($r = 0.91$, $p < 0.01$) and tide pool ($r = 0.88$, $p < 0.05$). The salinity in the study area was low (15.26 – 20.45‰) during the rainy season, which may have been a limitation for *T. affinis* populations. The canonical correspondence analysis for the reef pool showed the correlation between total nitrogen and *Hypnea* biomass and *Tricolia* density close to *Gracilaria* biomass (Figure 5) where the mollusk was less variable, in the same site *Gracilaria* is opposite to *Bryopsis*. In the reef flat, salinity is close to *Gracilari*a which is opposite of *Hypnea* and *Bryopsis* (Figure 6).

The opposite canonical correspondence analyses ordering of algal genera indicates competition between functional groups (Gao et al., 2014), evidenced also by

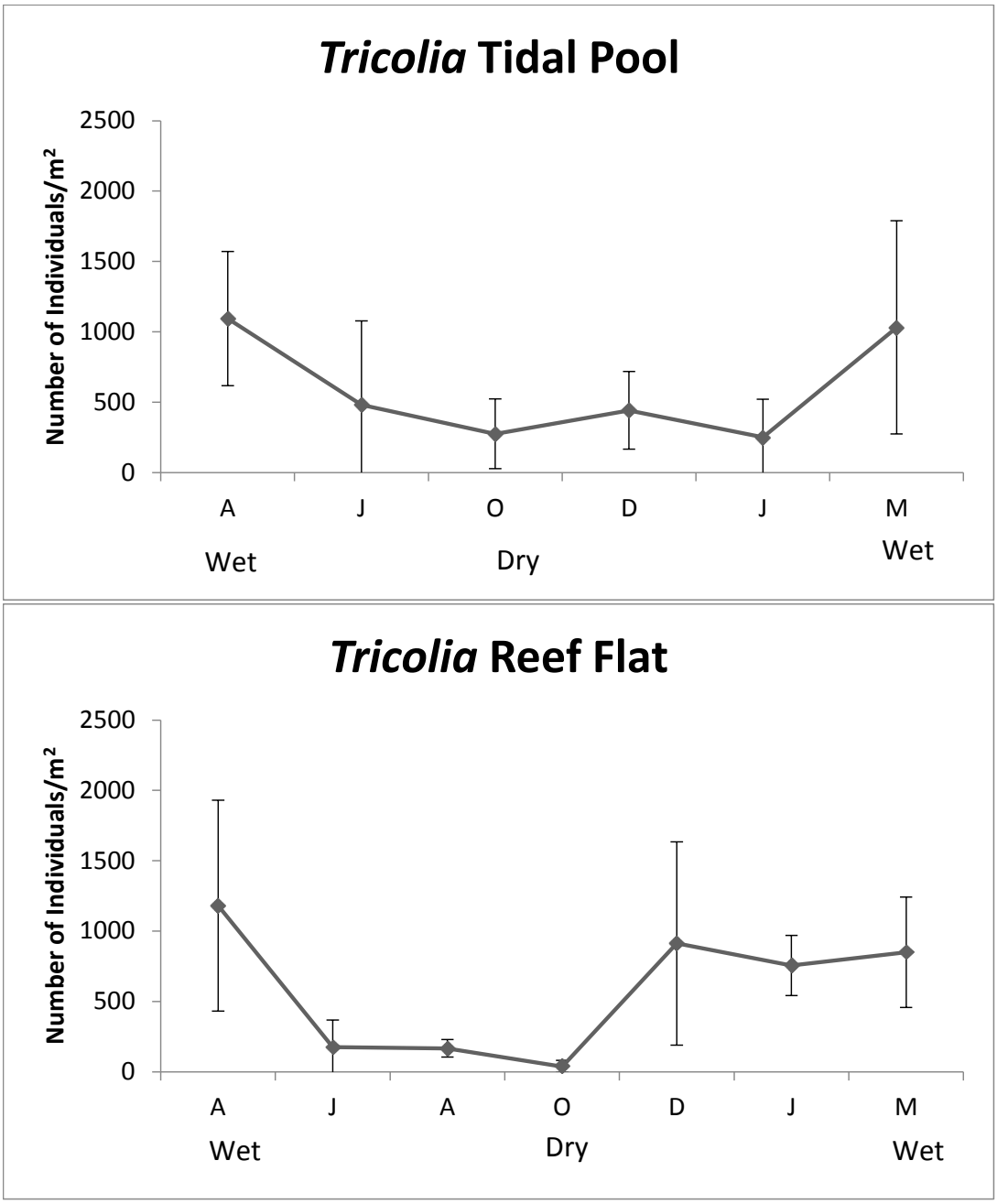

Figure 1. Seasonal variation of *Tricolia affinis* number of individual averages and standard deviations.

the analyses of variance since *Hypnea* spp. was more abundant at the tidal pool whereas *G.caudata* biomass was greater at the reef flat. A two-way ANOVA showed that there was temporal variation in the *T. affinis* density; however, there was no significant difference in the gastropod's abundance between the two sampling sites. We can therefore conclude that the spatial distribution of *T. affinis* was proportional at the two sampling sites. Therefore, a correlation between *T. affinis* density and *G. caudata* biomass was found, at the reef flat and pool, possibly related to the alga's higher structural complexity or the provision of greater protection for recruitment and from predation and ocean currents, in addition to being a reliable source of food. *Gracilaria* exhibited a lower biomass in the samples from the rainy period, directly affecting the number of sampled *Tricolia*. *Gracilaria*'s stem complexity confirms the correlation of *T. affinis* with its structural composition, which may be related to the

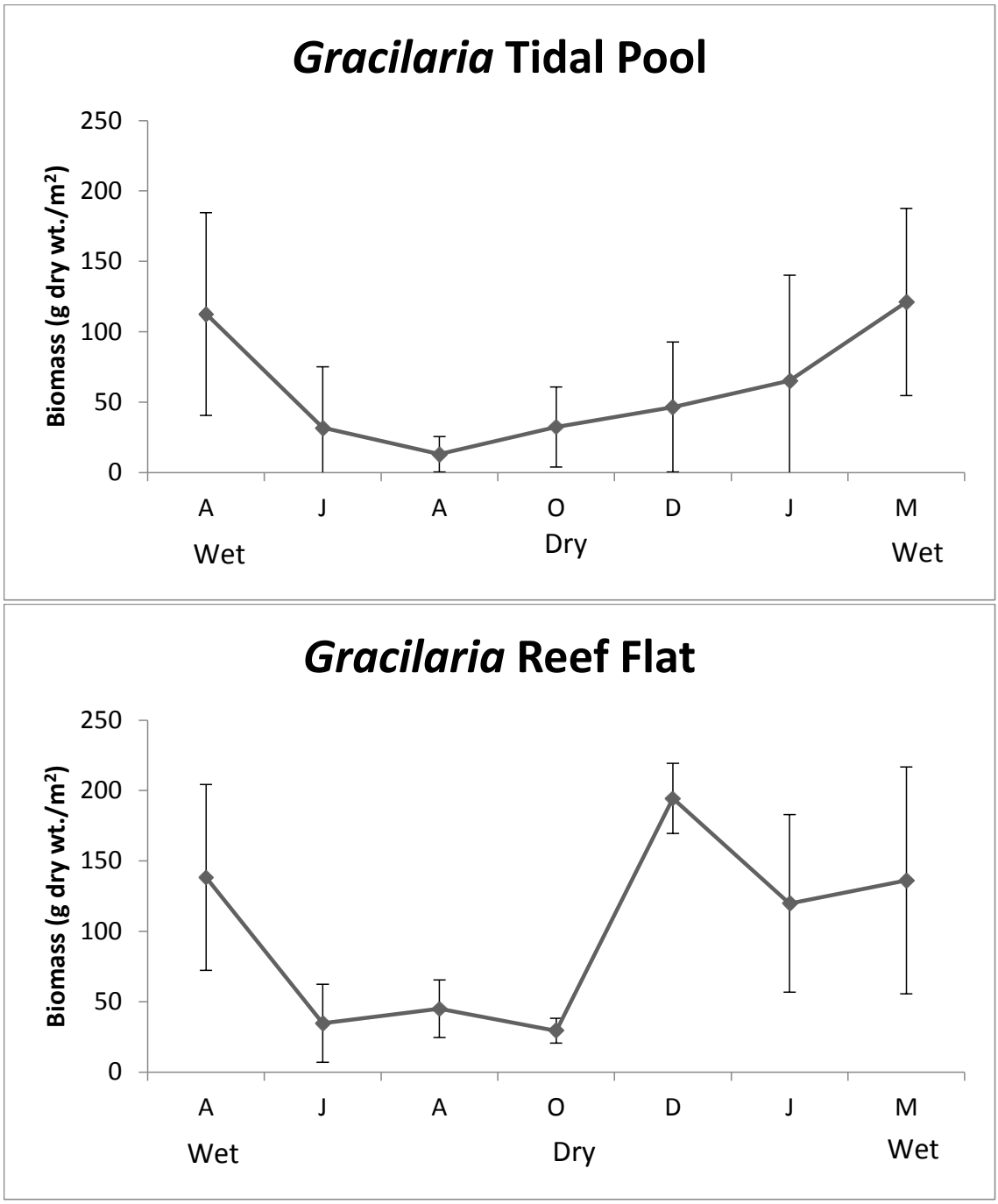

Figure 2. Seasonal variation of *Gracilaria* biomass averages and standard deviations.

retention of sediments that provide a greater supply of nutrients and protection against predation and hydro-dynamic counterflows for the mollusk (Chemello and Milazzo, 2002). Pereira and Jacobucci (2008) suggested that although *T.affinis* has been registered as a diet staple for fishes and crabs and an important link in the food chain of aquatic environments, it may not play a key role in the diets of marine species due to its low nutritional quality and calcareous shell (Dubiski-Silva and Setuko, 2008). The study area is a highly impacted region, constant trampling by bathers, which may be one reason for the success of the gastropod and relationship with *Gracilaria*. Invasive *Gracilaria salicornia* in the coast of Hawaii improved the density of epifauna and detritivores

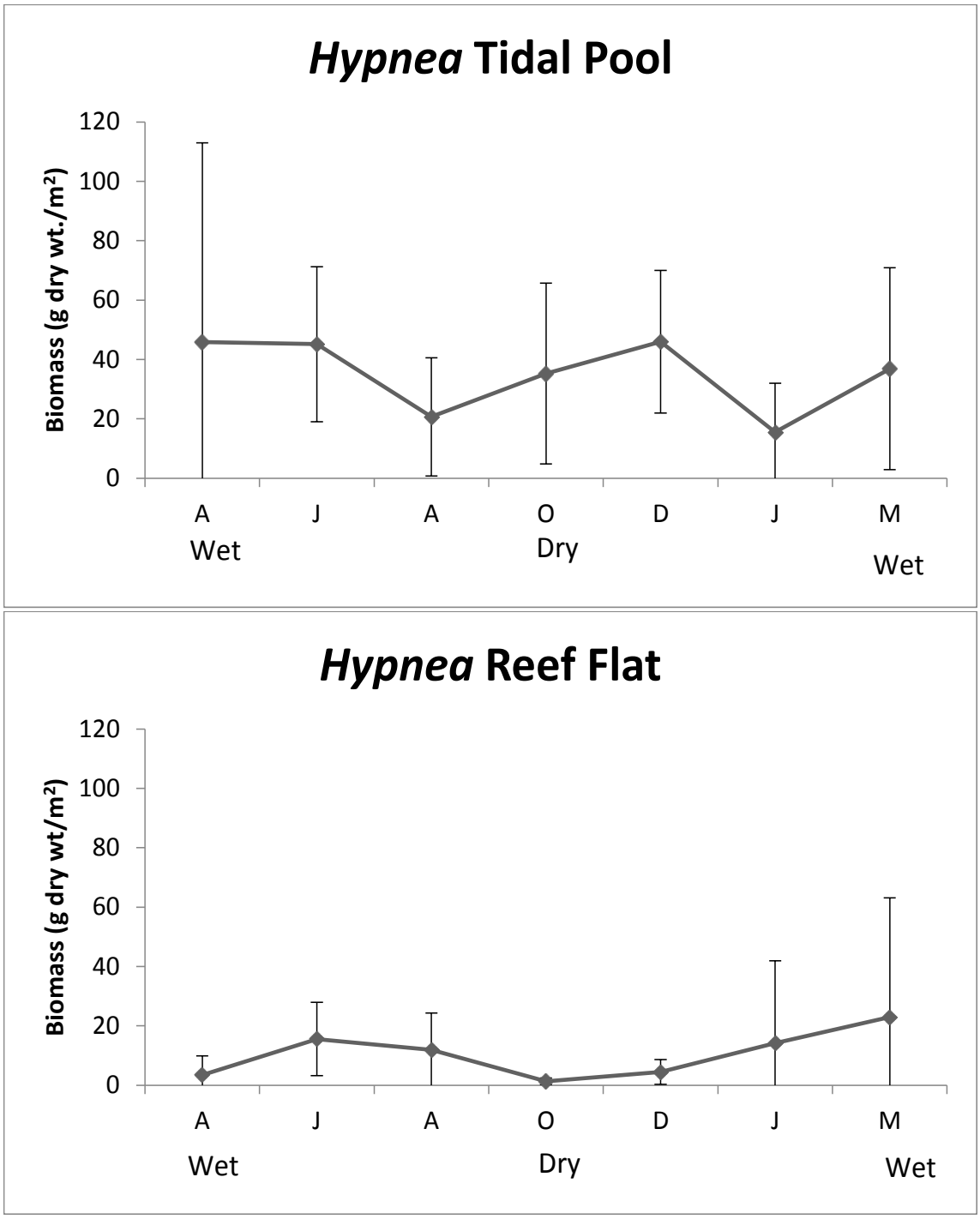

Figure 3. Seasonal variation of *Hypnea* biomass averages and standard deviations.

in comparison with *Padina thivyi*, while the invasive *Acanthophora spicifera* did not cause any epifauna improvement (Fukunaga et al., 2014).

The density of this gastropod was positively correlated with the biomass of *G. caudata* which showed the highest dry weight values at the sampling sites and presented a significantly higher biomass at the reef flat than the pool, where the temporal variation in *Tricolia* was less evident. Along with *Tricolia, G. caudata* showed seasonal variation between the dry and rainy periods, with a higher

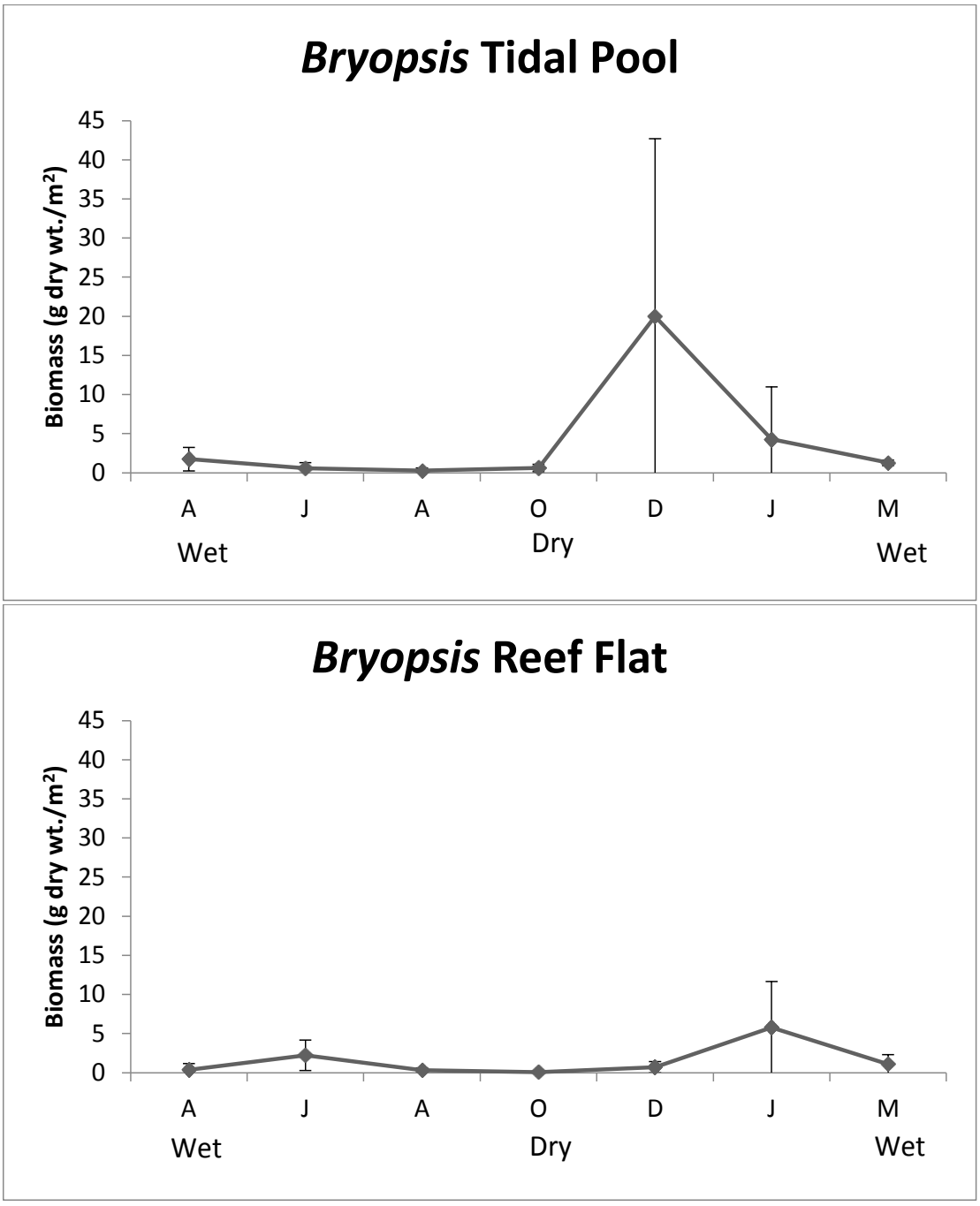

Figure 4. Seasonal variation of *Brypsis* biomass averages and standard deviations.

biomass found in dry periods, when there was also a higher temperature and salinity (Silva et al. 1987; Marinho-Soriano et al., 2001). Pereira et al. (2010) found no seasonal variations in *T. affinis* density or a positive correlation with *Sargassum* spp. biomass; however, these authors observed a constant presence of juvenile *T. affinis* throughout the year in the phytal fauna of *Sargassum*, indicating that factors such as habitat availability may affect this gastropod's abundance and recruitment. On the Piedade reef, climatic and hydrodynamic factors should be considered, because they may affect the dispersal of organisms in the reef pool, thereby causing a greater variability in the population distribution of *T. affinis* in the tide pool, in

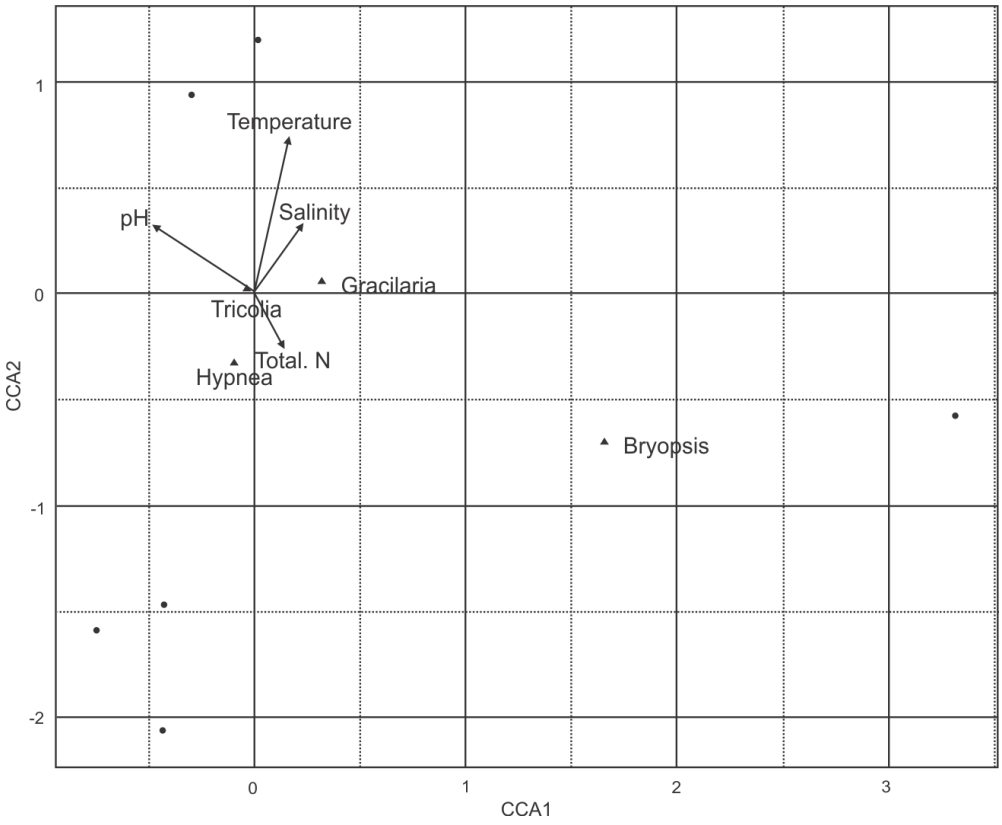

Figure 5. Canonical correspondence analysis for data from the tidal pool.

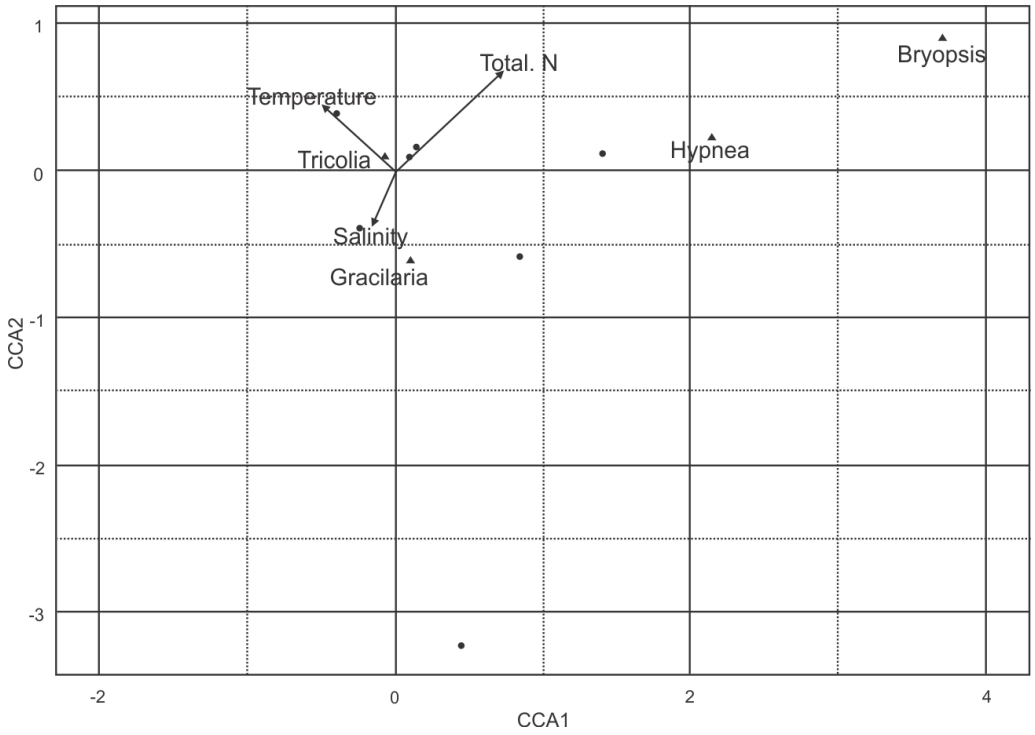

Figure 6. Canonical correspondence analysis for data from the reef flat.

addition to the trampling of the reef flat.

Conflict of interests

The authors declare that there is no conflict of interests.

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