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Pedality And Soil Moisture Retention Characteristics In Relation To Erodibility Of Selected Soils

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Abstract: Research on the pedality and soil moisture retention properties as they influence susceptiblity to soil erosion was conducted in the onset of 2006 rainy season in selected sites of Southeastern Nigeria. Minipedons were sampled for the in situ analyses. Soil data were subjected to analysis of variance, correlation and regression analyses. Results indicate that pedality is influenced by management type, with soils under bush fallow having higher pedality values. Variation in pedality attributes were significant ($p \leq 0.05$) in soils under bush fallow and conventional tillage. Some pedality attributes and soil moisture retention characteristics had significant correlation with erodibility factor: Available soil data showed that soil texture and organic carbon greatly influenced pedality and soil moisture retention characteristics in the site. However, more parameters and intensive soil sampling may be needed to improve the certainty of prediction. [Nature and Science. 2007;5(1):1-7].

Keywords: Degradation, hydraulic properties, morphology, tropical soils, soil structure.

Introduction

Variability in soil structure due to agricultural management practices has been studied using different techniques (Coughlan *et al.,* 1991; Boersma and Kooistra, 1994). However, description of some visible features is a rapid approach that can be used for an initial global evaluation of soil structure (Eynard *et al.,* 2004). Pedality is used to describe soil structure in terms of strength, size, shape and arrangement of aggregates Eynard *et al.* (2004) calculated pedality as a product of grade, size and type of structure.

The concept of pedality has been developed as it relates to hydraulic properties (Bouma, 1992; Lin *et al.,* 1999) especially as they influence soil moisture retention characteristics. Soil moisture content which is a variable amount of water contained in a unit mass or volume of soil and the energy state are important factors influencing plant growth (Igwe *et al.,* 1997). Soil moisture content influences wettability (Dekker *et al.,* 2001; Doerr *et al.,* 2002) and repellency (Hallet and Young, 1999). Measurement of stability of soil peds to water is used to estimate structural changes due to cultivation, since water is a major agent of aggregate breakdown but these attributes can be quantified using morphological features of a soil structure. These changes due to effects of human use and management were referred to as dynamic soil quality (Seybold *et al.,* 1998) and they influence vulnerability of soils to erosive forces.

Land use types cause differences in soil properties (Geeves *et al.,* 1995). In Southeastern Nigeria, Akamigbo (2001) reported marked differences in soil properties due to two different land uses, with intensive cropping of most productive soils leading to gully formation. Attempts have been made to quantitatively assess near surface soil quality using the soil quality morphological index (SQMI) (Grossman *et al.,* 2001). The SQMI combines information about soil texture, soil structure, moist rupture resistance, dry crust strength, thickness of surface horizon, surface connected macropores and cracks in determining soil quality. Soil texture influences plasticity, compatibility and consistency (Hillel 1998).

Changes in near-surface morphology due to land use and management are not substantially recognized in soil survey (Seybold *et al.,* 2004) and have not been considered in soil quality assessments (Grossman *et al.,* 2001).

Southeastern Nigerian soils have been subjected to intensive cropping with little or no additional soil fertility inputs. Soil fertility regeneration depends on natural bush fallow whose length has been shortened due to high population pressure (Onweremadu, 1994). Slash-and-burn system of clearing is still a farming practice. All these have heightened the spate of land degradation through soil erosion. However, there is current desire to acquire soil data, and such acquisition through soil survey might be too expensive considering the low socio-economic status of farmers in the area. Based on the foregoing, the study aimed at investigating the pedality and moisture retention characteristics of soils while relating them to erodibility and soil texture under three tillage practices.

Materials and Methods

Study area: Southeastern Nigeria lies between latitudes 4°30' and 7°30°N, and longitudes 6°45' and 9°00'. The major geological materials in the area include shale, cretaceous sandstones, upper coal measures, lower coal measures, coastal plain sands and basement complex rocks (Orajaka, 1975). The area is predominantly a lowland with highlands above 200 metres above sea level (Ofomata, 1975). It is characterized by a bimodal annual rainfall about 1700 to 2500 mm. Daily temperatures are generally high ranging from 28 to 35°C. The area has a rainforest vegetation. Farming is a major socio-economic activity in the area.

Field studies: Thirty soil samples from surface horizons were collected from selected parts of southeastern Nigeria for the study in the beginning of rainy season in 2006. The choice of sampling points was guided by the geological map of the area, such that all soil groups were represented. Soil samples were air-dried, crushed and sieved using 2-mm sieve.

Soils were morphologically described using the Natural Resources Conservation Services (Soil Survey Staff, 2003). The morphological description of soil structure was quantified in relation to hydraulic properties of soils, based on the scale of Lin *et al.* (1999) (Table 1). Pedality was calculated as a product of grade, size and type of soil structure. Ped size was rated independently of type (fine <10 mm, medium 10-50 mm, coarse > 50 mm). The scale for platy structures was one-tenth less than other structure types and only very thin plates received maximum points because the orientation of platy structures is unfavourable to vertical flow and root penetraction.

Laboratory study: Particle size distribution was determined hydrometer method (Gee and Or, 2002). Bulk density was evaluated using the core method (Grossman and Reinsch, 2002). Total carbon was determined by a Leco CS444 analyzer (Leco Corp., St. Joseph, M. I). Soil samples were placed in a furnace overnight at 475°C temperature to estimate inorganic carbon, and organic carbon was measured after subtraction from the total carbon content (Yang and Kay, 2001).

Soil moisture retention characteristics were determined by soaking disturbed soil samples for 48 hours to allow the samples get saturated. The saturated soil samples were put in the pressure plate extractor and pressure applied at 0.01, 0.05, 0.1 and 1.5 Mpa suction until water ceased to drain-out. The soil samples were weighed and oven-dried at 105°C for 24 hours. The volumetric moisture content (θ_v), values obtained were multiplied by their corresponding bulk density. Available Water Capacity (AWC) was calculated as the water retained between suction 0.01 and 1.5 Mpa. Atterberg limits were determined according to the procedure of Sowers (1965). The liquid limit was estimated with the aid of Casagrande apparatus while the plastic limit was obtained by kneading and rolling the soil on a glass plate. Plasticity index (PI) was calculated as the difference between liquid limit and plastic limit (Grieve, 1980).

The Wischmeier erodibility factor (K) was computed using the Nomograph (Wischmeier *et al.,* 1971) and the Slaking index (SI) was calculated as liquid limit divided by water retained at 0.01 MPa (De Boodt, 1967).

Statistical analysis: Soil data analyses were carried out using statgraphics (STSC Inc/Statistical Graphics Corporation, 1987). The software was also used to perform correlation and regression analyses on the data. Variability in soil properties was ranked according to the procedure of Aweto (1982).

Results

Soil properties: Value ranges in selected soil properties are shown in Table 2. High values of coefficient of variation were recorded in Atterberg limits, sand and organic carbon contents while moderate variations occurred in erodibility factor, silt and clay content. Bulk density showed slight variation (CV=15%).

Pedality: Pedality was better developed in a 5 – year old bush fallow fields on the surface soils when compared with both the conventional and minimum tillage management practices. But predality of minimum tillage was better than of conventional tillage. At depths greater than 20 cm, structural changes due to tillage management practice were less marked. There were non-significant pedality attributes in soils under minimum tillage. Granular aggregates and fine wedges predominated in the 0-20 depth of soils in bush fallow whereas blocky structures were common in conventionally tilled soils. Very large lumps or blocks dominated below 20 cm of conventionally tilled soils. In soils with high clay content, wedge

formation was observed and pedality rating tended to be greater, especially under bush fallow. In line with the findings of Sparrow *et al.* (1999), ped type was not primarily responsible for the differences in pedality.

Soil moisture retention characteristics: Values of volumetric moisture retention characteristics as shown in Table 4 show that soils do not retain much water at high suctions. Amount of available water varied and decreased as soil texture changed from finer materials to more coarse forms. Soils with more clayey textures retained highest level of water at 0.01 Mpa and this water influenced availability of soil moisture. Soils of Awgu with relatively high sand content had very high AWC. The more clayey soils had least slaking index values while sandier textures were more slakeable.

Relationship between pedality, soil moisture retention characteristics, slaking index and erodibility: Table 5 presents correlaton coefficient values between erodibility factor and pedality attributes in addition to slaking index. Grade had the greatest relationship with erodibility factor while it related weakly with slaking index. Unlike in pedality attributes, soil erodibility factor had a better relationship with soil moisture contents (Table 6). Highly significant negative correlation coefficients ($p=0.01$; 0.05) were obtained between erodibility factor and soil moisture content at different pressures, namely 0.01, 0.05, 0.1 and 1.5 Mpa.

Modelling: Results of stepwise regression analysis conducted in the study are shown on Table 7. Soil organic carbon and textural parameters contributed highly to variability in pedality and volumetric moisture retention characteristics.

Table 1: Scores for quantifying morphological features of soil structure in relation to water retention (Adapted from Lin *et al.*, 1999).

Morphological feature	Class	Score
Structure grade	Massive	0
	Weak	1
	Moderate	5
	Strong	25
	Single-grained	50
Aggregate size	>50 mm (>5 mm if platy)	1
	10-50 mm (1-5 mm if platy	3
	<10 mm (< 1 mm if platy)	10
Structure type (shape)	Massive	0
	Platy	1
	Prismatic, blocky, wedge >10 mm	10
	Granular, single grained wedge <10 mm	30

Table 2: Selected soil properties of studied soils

Soil property	Range	Mean	CV	Ranking
Liquid limit (%)	10-62.0	39.0	109	HV
Plastic limit (%)	10-45.0	26.0	67	HV
Plasticity index (%)	0.0-17.8	0.4	120	HV
Wischmeier Erodibility (K)	0.1-0.8	0.4	35	MV
Bulk Density (g cm^{-3})	1.2-1.7	2.0	15	LV
Clay (%)	7.0-57.0	25.0	48	MV
Silt (%)	2.0-30.0	19.0	31	MV
Sand (%)	43.0-90.0	54.0	52	HV
Organic carbon (%)	0.5-1.6	16.0	60	HV

HV=High variation, MV=moderate variation, LV=little variation

Table 3: Differences between soil samples (N=30) in means of pedality, and structural type, grade and size averaged over 0-20 cm in the study site.

Management type	Pedality property	Soils samples scores	Level of significance
Minimum Tillage	Pedality	880.0	NS
	Type	8.3	NS
	Grade	6.8	NS
	Size	5.0	NS
Conventional Tillage	Pedality	286.0	**
	Type	5.2	**
	Grade	4.8	**
	Size	5.3	**
5-year old bush fallow	Pedality	1960.0	**
	Type	11.0	**
	Grade	8.9	**
	Size	7.6	*

** significant at $p \leq 0.01$, * significant at $p < 0.05$, NS = not significant

Table 4: Volumetric moisture retention characteristics and slaking index values of studied soil (%)

	MPa					
Location	0.01	0.05	0.1	1.5	AWC	SI (%)
Agulu	18.3	13.8	11.9	11.6	6.7	1.0
Ihiala	12.4	9.7	6.6	6.5	5.9	2.2
Opi	20.1	14.0	15.3	13.9	6.2	0.1
Izzi	38.0	18.1	15.1	14.0	24.0	0.2
Ezzamgbo	44.1	30.2	28.0	20.3	23.8	0.4
Ezzikwo	39.1	18.1	15.2	14.3	24.8	0.8
Mgbidi	13.5	9.4	6.7	6.4	7.1	2.4
Agbani	33.7	21.0	17.9	18.1	15.6	0.8
Afikpo	36.5	17.2	14.8	13.1	23.4	0.7
Owerri	10.0	8.1	6.1	5.9	4.1	2.3
Mbaise	9.8	7.6	7.0	5.3	4.5	2.2
Ukehe	24.2	15.6	11.6	11.4	12.8	0.2
Ehamufu	27.1	16.4	13.1	12.8	14.3	0.1
awkuzu	16.2	8.9	8.0	7.3	8.5	1.8
Obosi	12.2	10.1	8.8	6.8	5.4	2.1
Obolloafo	21.3	15.1	14.1	14.9	7.2	0.1
Oguta	10.6	9.2	7.8	4.7	5.9	2.0
Egbema	11.2	9.5	8.2	5.7	5.3	1.9
Okigwe	19.6	13.4	12.6	10.2	9.4	1.2
Ibeku	60.2	50.1	47.2	29.1	21.1	0.6
Uturu	25.6	19.6	16.7	11.9	13.7	0.9
Amakama	29.2	17.1	14.6	10.2	19.0	1.8
Anuro	64.8	46.7	43.8	30.0	34.8	0.7
Lokpanta	44.1	29.5	25.4	9.9	34.2	0.7
Awgu	33.2	22.8	21.8	8.3	24.9	0.8
Aguata	15.9	11.7	11.8	9.1	6.8	1.3
Awka	17.0	9.0	8.0	7.2	9.8	1.2
Nenwe	31.6	19.0	11.3	10.4	21.2	0.7
Aninri	32.7	22.7	22.0	19.0	13.7	0.8
Akidi	64.0	54.0	48.1	39.1	24.9	0.6

Table 5: Correlation coefficients between erodibility factor (K), pedality, type, grade size and slaking index of soils studied (n=30)

Soil property	Correlation coefficient (r)	Level of significance
Pedality	0.516	*
Type	0.166	NS
Grade	0.774	*
Size	0.623	*
Slaking index	0.296	NS

Table 6: Correlation coefficient between erodibility factor (K) and moisture retention characteristics of soil (n=30)

Moisture content (MPa)	Correlation coefficient	Level of significance
0.01	-0.612	*
0.05	-0.635	**
0.10	-0.648	**
1.50	-0.608	**
AWC	-0.498	*

AWC=available water capacity
** significant at p = 0.01, * significant at P=0.05

Table 7: Stepwise regression models of pedality and soil moisture retention characteristics with selected soil properties (n=30)

Department variable	Regression equation	r2
Padality	Y=17.79+0.62(clay)-0.76(sand)+6.1(OC)	0.77
Size	Y=40.25+0.31(clay)-0.43(sand)+5.5.(OC)	0.81
Grade	Y=30.15+0.36(clay)+4.55(OC)	0.85
0.01 Mpa	Y=19.21+0.29(clay)44(sand)+4.7(OC)	0.80
0.05 Mpa	Y=33.23+0.47(clay)-0.42(sand)+1.22(OC)	0.78
0.10 Mpa	Y=2.36-0.28(sand)	0.82
1.50 Mpa	Y=2.16+0.33(clay)+0.81(OC)	0.72
AWC	Y=47.21-0.33(sand)	0.58

OC=organic carbon, AWC=available water capacity
** significant at $p \leq 0.01$

Discussion

Differential pedality in soils of the study site is a result of variation in aggregation due to land use. Below 20 cm depth, there was almost a homogenous pedality in all soils under study. As granular aggregates dominated 0-20 cm in bush fallow, blocky soil structures were found at the same depth at Conventional and Minimum tillage soils, suggesting the influence of tillage and traffic on pedality. In a similar study, Akamigbo (1999) reported marked differences between virgin forest and cultivated soils of a humid tropics in morphological properties.

Pedality and pedality attributes of grade and size significantly ($p \leq 0.05$) influenced erodibility of soils (Table 5). Weaker soil structures are more vulnerable to erosive forces than stronger grades just as large ped sizes resist easy transportability by runoff water. However, these peds are modified by alternate wetting and drying (Caron *et al.,* 1992) and slaking occurs on these soils when wet moisture status reverses upon drying (Igwe, 2001) during the dry season.

Variation in soil moisture retention characteristics suggests differential transmissivity of soil water in these soils. Significant negative correlation coefficients between erodibility and soil moisture retention characteristics, implies that they could be used to detect the level of susceptibility to soil erosion. Earlier, Hardley *et al.* (1985) observed that detachment of soil peds by impacting rain drops responds to various

levels of soil wetness, suggesting that alternate drying and wetting promotes soil erosion. However, hydraulic properties of soils are influenced greatly by human management with Ley *et al.* (2002) affirming that soil immediately after tillage is highly unstable and undergoes dynamic changes in the interaggregate stability. However, this study did not assess the soil moisture characteristics under different management practices considering the report of Blanco-Canqui *et al.* (2004) that residual effects of tillage diminish within 21 to 28 days.

Modelling using the available soil data confirms the significance of particle size distribution and organic carbon (organic matter) in the aggregation of soils and in pedality, especially as they relate to the susceptibility of soils to erosion. But the relevance of organic carbon in soil structural formation is at macroagregation in tropic soils (Igwe *et al.,* 1999). Gantzer *et al.* (1990) observed that soil erodibility was related to soil texture, stating that long-term productivity of clayey soils increases their erodibility. High r^2 values in the study implies that the associating parameters are good predictors of pedality and soil moisture retention characteristics. Determination of soil texture and soil moisture rententivity is cheap when compared with highly expensive and sophisticated probing sensors, suggesting the use of these parameters in low-input agriculture of the study area.

Conclusions

Pedality varies due to management practice, with soils under bush fallow exhibiting higher values when compared with conventional and minimum tillage practices. Soil moisture retention characteristics varied due to management practice in the site. Both pedality and soil moisture retention characteristics influenced erodibility of soils.

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Prevalence and shedding of *Renibacterium salmoninarum* in Brook trout (*Salvelinus fontinalis*) in Michigan

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ABSTRACT: Bacterial Kidney Disease has been reported wherever susceptible salmonid populations are present. There has been a dispute about the status of the diseases among different salmonid species and their susceptibility to the infection over the past years. In this regard, a considerable number of indicated the susceptibility of brook trout to BKD infection. In order to determine the status of bacterial kidney disease (BKD) in hatchery and wild populations of brook trout (*Salvelinus fontinalis*) in Michigan, (*Renibacterium salmoninarum*) *R. salmoninarum* prevalence and intensity were determined in representative samples from adult hatchery raised and wild stocks as well as their offspring from 2001 through 2005. The hatchery raised adult Iron River brook trout presented higher BKD prevalence than the brook trout caught from Cherry Creek. Generally, the BKD prevalence and intensity in hatchery and wild brook trout strains gradually decreased throughout the period from 2001 to 2004. The critical role played by hatchery practices to control the spread and minimize the prevalence of BKD among Michigan brook trout populations was discussed. Although most of the previous studies reported unimportant role of the male in transmission of *R. salmoninarum*, yet our results clearly demonstrated that males shed more *R. salmoninarum* along with their gametes than females. [Nature and Science. 2007;5(1):8-17].

Keywords: BKD, *Renibacterium salmoninarum*, prevalence, Shedding, Brook trout, *Salvelinus fontinalis*

INTRODUCTION

Brook trout (*Salvelinus fontinalis*) is an indigenous salmonid species in the Great Lakes (Coon 1999) that has been artificially propagated and stocked in Michigan's public waters for years (Dexter and O'Neal, 2004). Two strains of brook trout, the Assinica and Iron River strain, are being reared by Michigan Department of Natural Resources (MDNR) for stocking resident stream populations where there is a deficiency of natural recruitment (Dexter and O'Neal, 2004). Iron River Brook trout strain is considered a pure native strain which was originally from the Iron River in Michigan's Upper Peninsula. Unlike the Assinica strain, Iron River brook trout are slow to reach maturity and are characterized by a very slow growth rate because of their wild characteristics (Driver 1995., Dexter and O'Neal 2004).

Fish health poses major challenges to development and progress of the brook trout restoration in the Great Lakes basin. Among these health challenges, bacterial kidney disease, caused by *R. salmoninarum*, is an eminent threat due to the enzootic nature of the pathogen within Great Lakes waters (Eissa 2005). Moreover, affinity of *R. salmoninarum* for the kidneys, which possesses an essential lymphoid function and its obligate intracellular nature, makes this pathogen and its soluble antigens a major threat to the host by suppressing the fish immune system (Ellis 1999; Fredriksen et al. 1997; Grayson et al. 2002).

A considerable number of studies have been performed on brook trout in the USA which indicated the susceptibility of brook trout to BKD (Belding and Merrill 1935; Snieszko and Griffin 1955). However, most of these studies involved the use of experimental infection, whilst few discussed natural BKD infection among brook trout (Allison (1958); Bullock et al. (1971); Mitchum et al. 1979; Mitchum and Sherman 1981).

Interestingly, the first report of BKD in the USA occurred in brook trout at a Massachusetts State fish hatchery (Belding and Merrill, 1935). During the late 1940s and early 1950s, *R. salmoninarum* infection caused mass mortalities in brook trout at the federal hatcheries in Berlin, New Hampshire, Cortland, and

New York (Snieszko and Griffin, 1955). Mitchum et al. (1979) found that the prevalence of BKD in brook trout during epizootics in southeastern Wyoming, USA were 100% and 83% among dead and live brook trout respectively.

In Michigan, the first case of BKD was discovered in 1955 in brook trout yearlings at the Oden and Marquette state hatcheries, where eggs were originally imported from a hatchery in New England in which BKD had been endemic for many years (Allison, 1958). Since the first report of the disease in 1955, none of the published studies have reported any data about the recent occurrence of BKD outbreaks, prevalence, or magnitude of the disease in brook trout in Michigan or in the Great Lakes basin.

Thus, the aim of the current research was to investigate the status and magnitude of BKD in brook trout in Michigan by assessing the *R. salmoninarum* prevalence in the hatchery raised brook trout populations in Michigan. The role-played by the male and the shedding of the bacteria along the gamete were also studied.

MATERIALS AND METHODS

Fish. To investigate the prevalence and intensity of *R. salmoninarum* infection among brook trout (BKT) populations in Michigan waters, a total of 628 adult brook trout were collected from the hatchery raceways of Marquette State fish hatchery (MSFH) and the Cherry Creek water stream outside the hatchery in Marquette, Michigan, from 2001-2004. MSFH is the primary facility for brook trout broodstock that are used for the production of fingerlings to be stocked in both inland and Great Lakes waters.

A total of 567 hatchery-raised brook trout broodstocks (529 Iron River strain (IR-BKT) sample, 38 Assinica strain (AS-BKT) sample) and 61 adult Cherry Creek wild brook trout (CC-BKT) (Table1) were used for BKD prevalence analysis. Following approximately 18 months of egg incubation and fish rearing in the Marquette State Fish Hatchery, MDNR releases fingerlings in the spring of each year. From 2002-2005, a total of 420 pre-stocking fingerlings were collected to determine the prevalence of *R. salmoninarum* prior to release into the basins of Lakes Michigan (Table 1).

The fish were euthanized by exposure to an overdose of MS-222 (tricaine methane sulfonate, Finquel-Argent Chemical Laboratories, Redmond, WA). During collection of the broodstock fish samples, following gamete collection, the abdominal cavity was cut open and all internal organs were examined for signs of BKD. About one gram of tissues were sampled from anterior, posterior and middle kidney sections using separate sterile dissecting tools.

Fingerlings were collected, euthanized and whole kidney tissue was collected individually using separate sterile dissecting tools.

To test the shedding of *R. salmoninarum* antigens with the gametes, a total of 200 coelomic/ovarian fluid samples were collected from females using sterile transfer pipette during egg collection, transferred to 5 ml sterile polypropylene tubes, and kept on ice until processed at the laboratory at Michigan State University, East Lansing, MI. Additionally, a total of 200 semen samples were collected from males by squirting the middle stream of semen directly into sterile 5 ml polypropylene tubes.

Clinical examination. Fish were euthanized using an overdose of MS 222 and examined externally for the presence of any lesions, parasites, or abnormal growths on the skin or gills. Fish were dissected and examined internally for any lesions, swelling, or color changes in the kidneys other internal organs and viscera.

Sample processing. Kidney samples representing the anterior, posterior and middle sections of the kidney were transferred in sterile 7.5 cm x 18.5 cm Whirl Pak® bags (Nasco, Forte Atkinson, and WI), kept on ice, and were softened as much as possible through multiple cycles of physical pressure. The homogenized kidney tissues were diluted in 1:4 (w/v) Hank's Balanced Salt Solution (HBSS, Sigma Chemical Co, St. Louis, MO) then stomached for 2 minutes at high-speed in a Biomaster Stomacher-80 (Wolf Laboratories Limited, Pocklington, York, UK). In the case of coelomic/ovarian fluid or milt samples, 1 ml from fluid sample was diluted 1:2 (v/v) in HBSS for Q-ELISA testing.

Measurements of *Renibacterium salmoninarum* antigen using the Quantitative Enzyme-linked Immunosorbent Assay (Q-ELISA). Aliquots of 250 µl volume of each processed samples were transferred into 1.5 ml safe-lock microfuge tube to which an equal volume of 0.01 M phosphate buffered saline-Tween 20 (0.05 %) (PBS-T20) (Sigma) with 5 % natural goat serum (Sigma) (Olea et al., 1993) and 50 µl CitriSolv solution (Fisher Chemicals, Fairlawn, New Jersey) (Gudmunsdottir et al., 1993) were added.

The mixture were thoroughly mixed by vortexing then incubated at 100 °C on heat blocks with rotary shaker for 15 minutes and followed by 2 hours of incubation at 4 ºC. After incubation, the mixture was centrifuged at 6000g for 15 minutes at 4 ºC. The aqueous supernatant of each sample was carefully collected and then transferred to a 1.5 ml microfuge tube for Q-ELISA testing. The Q-ELISA method used in this study is that described in details by Pascho and Mulcahy (1987) and Alcorn and Pascho (2000). The positive negative threshold absorbance is calculated according to the method described by Meyers et al. (1993) .The positive–negative cutoff absorbance for the kidney homogenate was 0.10. The tested positive samples were assigned the following antigen level categories: low (0.10 to 0.19), medium (0.20-0.99) and high (1.000 or more) (Pascho et al., 1998). Intensity of infection among certain group of fish is expressed by percent of fish with high titers of *R. salmoninarum* soluble antigens using Q-ELISA.

Nested PCR. A DNeasy tissue extraction kit (Qiagen-Valencia, CA, USA) was used for the extraction of DNA from 100µl aliquots of kidney tissue homogenates. The DNA was extracted according to the manufacturer's instructions, with a few minor modifications from the method described by Pascho et al. (1998). The tissue pellets were obtained by centrifugation at 6000 g for 20 minutes at 4 °C and the pellets were incubated with lysozyme buffer consisting of 180 µl of 20 mg lysozyme (Sigma), 20mM Tris-HCl, pH 8.0; 2 mM EDTA (Sigma) and 1.2 % (v/v) Triton X 100 (Sigma) at 37 °C for 1 hour. The nPCR method and primers recommended by Pascho et al. (1998) were employed with slight modifications to the volume of DNA (5 µl for first round and 2 µl for second round PCR reaction), water, and master mixes (45 µl for first round and 48 µl for second round nPCR reaction) .The controls were composed of a PCR mixture containing no DNA template reagent (negative control), positive *R. salmoninarum* and positive tissue control. A volume of 10 µl of the nPCR product and controls were mixed with 2 µl of 6X loading dye (Sigma) and used on a 2 % agarose gel (Invitrogen Life Technologies, Carlsbad, CA). Each electrophoresis gel included a 1kbp DNA ladder with 100 bp increments (Invitrogen). Gels were run in 1 X Tris Acetate Buffer (1 X TAE) gel buffer (Sigma). Gels were visualized under the KODAK EDAS Camera System and UV Trans-illuminator. Samples were considered positive when a 320 bp band was detected. Molecular confirmation of the purified bacterial isolates was also conducted using nPCR according to the method described by Chase and Pascho (1998).

Statistical Analysis. Because of the nature of data collected in this study required basic statistical description, data analysis was primarily relied on descriptive statistics. For year to year and brook trout strains comparisons, the data was tested for normality and then student t test (parametric) or Mann-Whitney Rank Sum Test was used (with an alpha level = 0.05).

RESULTS

A. Prevalence of *R. salmoninarum* infection in captive and wild brook trout stocks. *Renibacterium salmoninarum* antigens were detected in the kidneys of Marquette State Fish Hatchery captive broodstock and fingerlings as well as Cherry Creek brook trout. The prevalence of *R. salmoninarum* in the Iron River broodstock exhibited a steady decline from 87% in 2001, to 80% in 2002, to 60% in 2003, and to 43% in 2004. Similarly, the intensity of *R. salmoninarum* in the Iron River brook trout (expressed by the percent of fish showing high titer of *R. salmoninarum* antigen) exhibited a comparable decline. The percent of fish with high *R. salmoninarum* antigen levels decreased from 17% in 2001 to 7.5% in 2004 (Figure 1).

Assinica brook trout Broodstock (BS AS-BKT) were tested only in 2003 and 2004. The results demonstrated a decrease in prevalence from 80 % in 2003 to 25% in 2004 (Table 2). However, the intensity of the *R. salmoninarum* infection in the BS AS-BKT did not exhibit a similar decline.

In the case of Cherry Creek brook trout (CC-BKT), samples were collected in the falls of 2001-2004. Prevalence of *R. salmoninarum* in CC-BKT decreased from 80% in 2001 to 67% in 2003. There was no consistent pattern in the intensity of *R. salmoninarum* (Table 2).

The prevalence of *R. salmoninarum* in IR-BKT fingerlings between 2003 and 2005 were comparable to the parents and consistent with the high prevalence in the gamete donor broodstock, although 2004 showed a significantly low prevalence. However, the intensity of *R. salmoninarum* antigens in BKT fingerlings was higher than those detected in their parents during 2003 (48% vs 17% in their parents) before sharply declining in subsequent years (table 2).

The prevalence of *R. salmoninarum* in 2005-AS-BKT fingerlings was lower (28%) than those of their parent BS AS-BKT-2003. Similarly, the prevalence of *R. salmoninarum* in 2005-AS-BKT fingerlings was

much lower than that of the 2005-IR-BKT fingerlings (28% vs 45% in IR-BKT fingerlings). The intensity of the *R. salmoninarum* infection in the AS-BKT fingerlings showed a decline, which was similar to the prevalence through the time of testing.

To compare the prevalence and intensity of *R. salmoninarum* in males and females BKT, two hundred pairs of IR-BKT broodstock were tested in 2004. Results indicated that the prevalence of *R. salmoninarum* infection was higher in the kidney tissue of the females than males (48.5% in females vs 37.5% in males). Similarly, the intensity of *R. salmoninarum* infection was clearly higher in the females (11% in female vs. 4 % in male). The shedding of the bacterial antigen along with the gametes was tested in broodstock IR-BKT in 2004. Data shown in Figure (2) illustrated that *R. salmoninarum* antigen shed with the gametes in both males and females IR-BKT. The prevalence of individuals that shed the bacterial antigen along with the gametes is lower in males than in females (10% in milt vs 15% in ovarian fluid). Also, the intensity of samples with high *R. salmoninarum* antigen was much higher in females (2.5%) than in males (0.5%). The majority of the females (20 out of 30) that shed the antigen in their ovarian fluids exhibited similar levels of *R. salmoninarum* antigens in their kidneys. However, 9 females shed the *R. salmoninarum* antigen along with the ovarian fluid without detecting *R. salmoninarum* antigen in the kidneys and one female shed the antigen at a low level in the ovarian fluid and tested negative for the kidney tissue. Likewise, the majority of male shedders (13 out of 20) had similar levels of *R. salmoninarum* in their kidneys with only 7 fish shed the antigen without detected titer of *R. salmoninarum* antigen in the kidney.

Table 1. Details of samples collected from brook trout broodstocks throughout the period from 2001-2004 and pre-stocking 18-month-old fingerlings collected throughout the period from 2002-2005.

Brook trout strain and life stage	Date Collected	Number of fish tested
Iron River broodstocks	October, 2001	54
	October, 2002	45
	October, 2003	30
	October, 2004	400
Assinica broodstocks	October, 2003	30
	October, 2004	8
Iron River 18 month old pre-stocking fingerlings	March, 2002	60
	March, 2003	60
	March, 2004	60
	March, 2005	60
Assinica 18 month old pre-stocking fingerlings	March, 2003	60
	March, 2004	60
	March, 2005	60

Table 2. *Renibacterium salmoninarum* infection prevalence and intensity among brook trout broodstocks and their corresponding 18 months pre-stocking fingerlings throughout the period from 2001 - 2005. Data in this table was generated using a polyclonal antibody-based quantitative ELISA (Q-ELISA) performed on kidney tissues. Prevalence was determined by % of Q-ELISA positive samples of the total number of samples tested. Intensity was considered high if the antigen concentration ≥1; medium if the antigen concentration = 0.20-0.99; and Low if the antigen concentration = 0.10 to 0.19.

Strain	**Parent stocks (Gamete donors)**		**18 months old pre-stocking fingerlings**	
	Year	***Renibacterium salmoninarum* prevalence**	**Year**	***Renibacterium salmoninarum* prevalence**
Iron River	2001	87.0 % Total 16.7% high 50.0 % medium 20.4% low	2003	98.0 % Total 48.0 % high 35.4 % medium 14.6 % low
Iron River	2002	80.0 % Total 15.55 % high 13.3 % medium 51.1 % low	2004	20.0 % Total 0.0 % high 10.0 % medium 10.0 % low
Iron River	2003	60.0 % Total 10.0 % high 0.0 % medium 50.0 % low	2005	45.0 % Total 0.0 % high 12.5 % medium 32.5 % low
Assinica	2003	80.0 % Total 0.0 % high 10.0 % medium 70.0 % low	2005	27.5 % Total 0.0 % high 2.5 % medium 25.0 % low

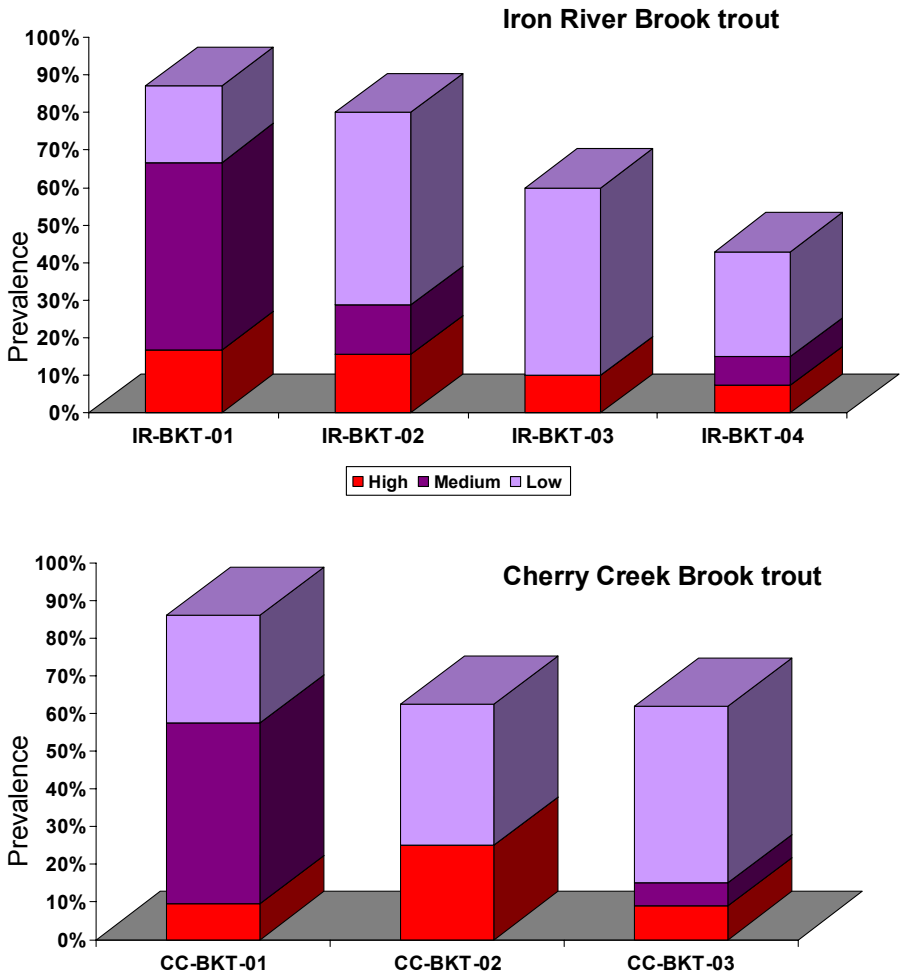

Figure 1. Prevalence and intensity of *Renibacterium salmoninarum* among the adult brook trout collected through 2001-2003。 Data in this Figure was generated using a polyclonal antibody-based quantitative ELISA (Q-ELISA) performed on kidney tissues. Prevalence was determined by % of Q-ELISA positive samples of the total number of samples tested. Intensity was considered high if the antigen concentration ≥1; medium if the antigen concentration = 0.20-0.99; and Low if the antigen concentration = 0.10 to 0.19. IR-BKT: Iron River Brook trout CC-BKT: Cherry Creek Brook trout

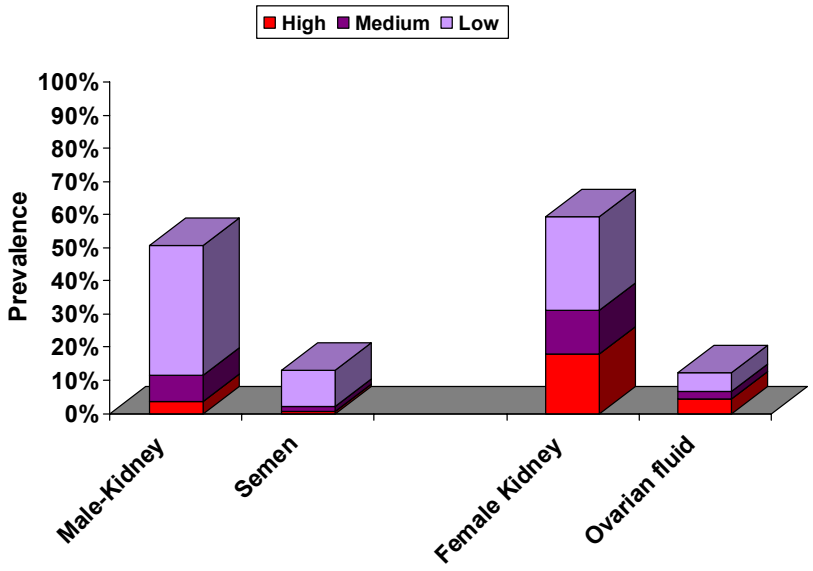

Figure 2. *Renibacterium salmoninarum* Prevalence and intensity in kidneys and gametes of the Iron River brook trout broodstock in from Marquette State Fish Hatchery. Samples were collected during the fall spawning season of 2004. Data in this Figure was generated using a polyclonal antibody-based quantitative ELISA (Q-ELISA) performed on kidney tissues. Prevalence was determined by % of Q-ELISA positive samples of the total number of samples tested. Intensity was considered high if the antigen concentration ≥1; medium if the antigen concentration = 0.20-0.99; and Low if the antigen concentration = 0.10 to 0.19.

DISCUSSION

For decades, brook trout has been known for its high susceptibility to *R. salmoninarum* infection (Snieszko and Griffin 1955; Mitchum and Sherman 1981). Brook trout infected with *R. salmoninarum* either naturally or experimentally, suffer from high mortalities (Belding and Merrill 1935; Snieszko and Griffin 1955; Mitchum et al. 1979).

Data obtained in this study demonstrated a high prevalence and intensity of *R. salmoninarum* infection in both hatchery raised and wild stocks. This concurs with previous reports. For example, Mitchum and Sherman, 1981, recorded a relatively high prevalence and severity of *R. salmoninarum* infection in wild and hatchery raised brook trout populations (58 %, 45 % respectively).

A general trend of declining prevalence and intensity of *R. salmoninarum* in IR-BKT broodstock was observed over the period of this study. This trend might be explained by the improvement of hatchery hygienic practices. Among these practices are the prophylactic erythromycin phosphate administration and hardening of eggs in erythromycin containing water. Evelyn et al. 1986 and Lee and Evelyn (1989) found that intramuscular injection of broodstock with erythromycin phosphate dramatically minimized the vertical transmission of *R. salmoninarum*. Also, Rinsing of broodstock in iodophores solution before collecting gametes could minimize the *R. salmoninarum* on the eggshell (Ross and Smith 1972). Maule et al. (1996) described similar observations that lead to remarkable decrease of *R. salmoninarum* prevalence among other salmonid species.

Although the Assinica strain demonstrated inconsistent *R. salmoninarum* infection prevalence when compared to the Iron River, yet Iron River strain showed higher intensity than Assinica strain. The Assinica strain is known for its superior survival and growth (Gowing 1986), and these characteristics could play a vital role in the general defense of the fish against severe infections with *R. salmoninarum*. In addition, variable susceptibility of fish strains to different diseases is not unusual. For example, some strains of steelhead showed variable susceptibility to *R. salmoninarum* (Winter et al., 1980).

Wild populations of BKT (CC-BKT) showed a comparable prevalence to that of the hatchery reared BKT. The fact that Cherry Creek supplies the hatchery with water and that BKT exists in this water may explain the similarity in infection levels.

Analysis of the data of *R. salmoninarum* prevalence and intensity of infection among the Iron River brook trout pre-stocking fingerlings indicated that fingerlings from 2003 exhibited the highest prevalence and infection intensity (approaching 100 %). The 2003 pre-stocking fingerlings are the offspring of the 2001 Iron River brook trout parent stocks that also exhibited high BKD prevalence (83 %). Vertical transmission have probably played a major role in this high incidence of infection, particularly that erythromycin prophylactic administration was not implemented in 2001. On the contrary, the 2004-2005 Iron River and Assinica brook trout offspring showed relatively lower *R. salmoninarum* prevalence and intensity, although they originally hatched from fertilized eggs collected from 2002-2003 parents with relatively high *R. salmoninarum* prevalence and intensity. This could only be explained by the strict hygienic measures adopted by the hatchery starting from 2002.

Data obtained from the Q-ELISA testing of gametes indicated that the *R. salmoninarum* was shed with the gametes in both males and females (10% in males versus 15% in females), with a higher intensity in females than males. These results suggest a contribution of the male in the vertical transmission of *R. salmoninarum* to the offspring, in addition to the role played by females. Allison (1958) was the first to report vertical transmission in the brook trout, albeit with circumstantial evidence that gametes from infected adults resulted in infected offspring. Our data agreed with Wiens and Kaattari (1989), which were able to detect the *R. salmoninarum* antigens in the milt of infected males. However, the studies of Klontz (1983) and Evelyn, et al. (1986) demonstrated that males play an insignificant role in the vertical transmission of *R. salmoninarum*. However, the data may be complicated by discrepancies between levels of *R. salmoninarum* in the ovarian fluid and their levels in the kidney of corresponding individual fish.

In conclusion, this study supports the previous reports, which emphasized that brook trout are highly susceptible to *R. salmoninarum* infection. In addition, this study shed the light on the possible contribution of a number of factors to development of BKD epizootics in Michigan hatcheries, such as the seasonal changes and the presence of external parasites. Further, the possible role of males and females in shedding the bacterium and its soluble antigens was fully discussed.

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Multi-variable Grey Model based on Genetic Algorithm and its Application in Urban Water Consumption

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ABSTRACT: Owing to the influence of economy, population, standard of living and so on, the urban water consumption possesses certain characteristics of grey. As the expansion and complement of grey system (GM(1,1)) model, the multi-variable grey model (MGM(1,n)) reveals the relationship of restriction and stimulation between variables. Genetic algorithm possesses the whole optimal and parallel characteristics. In this paper, through using the genetic algorithm, the parameter q of MGM(1,n) model has been optimized, and a multi-variable grey model (MGM(1,n,q)) based on genetic algorithm has been built. The model has been validated after examining the urban water consumption in Dalian city from 1990 to 2003. The result indicates that the multi-variable grey model (MGM(1,n,q)) based on genetic algorithm is better than MGM(1,n) model, and the MGM(1,n) model is better than GM(1,1). [Nature and Science. 2007;5(1):18-26].

KEYWORDS: Grey system; MGM(1,n,q); genetic algorithm; urban water consumption

1. INTRODUCTION

With the rapid development of economy, persistently increase of population and constantly improvement of standard living, the urban water demand has been constantly rising, but the amount of water supply is limited. The conflict between urban water demand and supply is gradually exacerbating, and the settlement of urban water shortage is the austere challenge of urbanization development. The forecast of urban water consumption is the premise and basic to plan and manage water resources. The results of forecast directly influence the reliability and practicability of assignment decision-making of water resources system, and also directly influence the sustainable consumption of urban water resources and sustainable development of social economy [1]. At present, there are many methods to predict the urban water consumption, such as the regress analytical method, the exponent smoothness method, the ration of water consumption method, the grey system forecast method, and the artificial neural network (ANN) method. ANN method needs long series of data, so it is difficult to predict due to the lack of historical data. The grey system theory takes uncertainty system as the study object, such as small sample and poor information. Through the creation and development of partial known information, the valuable information is picked out, so the operation behavior and evolvement rule are correctly described and effectively monitored and controlled. Practice has proved the grey system model needs less information but the precision of result is better, and can preferably reflect the practical condition of the system [2]. The grey system has been extensively applied in producing, engineering, science and technology. Owing to the influence of economy, population, living standard and so on, the urban water consumption has certain grey characteristics. Especially when the longer series of reliable data are unavailable, the grey system model is an available method to predict the urban water consumption [3].

2. MULTI-VARIABLE GREY MODEL MGM(1,n) [4]

Grey system model GM(1,1) is disposed through one accumulation of single variable time series $\{X_i\}$ $(i=1,2,\cdots,n)$. Through first-order differential equation, the intrinsic rule of generating sequence can be revealed. The GM(1,1) can only be applied to modeling and predicting of single time series data.

$$\frac{dX^{(1)}}{dt}+aX^{(1)}=b \qquad (1)$$

Only using the single time series data, the grey system model GM(1,1)can not reflect the influence and development between each other; however, the GM (1,n) model can mainly be applied to describing the correlation of variable between each other, not to predicting. The GM (1,1) model has been detailed described in reference [5]. In this paper, the Multi-variable Grey Model MGM(1,n) had been introduced. MGM(1,n) model is "n" variables first-order differential equations, it is the natural expansion of GM(1,1) model in "n" variables, not simple combination of GM(1,1) model. The first-order differential equation of MGM(1,n) model can be written as follows:

$$\begin{cases} \dfrac{dx_1^{(1)}}{dt}=a_{11}x_1^{(1)}+a_{12}x_2^{(1)}+\cdots+a_{1n}x_n^{(1)}+b_1 \\ \dfrac{dx_2^{(1)}}{dt}=a_{21}x_1^{(1)}+a_{22}x_2^{(1)}+\cdots+a_{2n}x_n^{(1)}+b_2 \\ \qquad\vdots \\ \dfrac{dx_n^{(1)}}{dt}=a_{n1}x_1^{(1)}+a_{n2}x_2^{(1)}+\cdots+a_{nn}x_n^{(1)}+b_n \end{cases} \qquad (2)$$

The first-order differential equation of MGM(1,n) model is as follows:

$$\frac{dX^{(1)}}{dt}=AX^{(1)}+B \text{ or } \frac{dX^{(1)}}{dt}-AX^{(1)}=B \qquad (3)$$

The sequence time response formula is

$$X_{(t)}^{(1)}=e^{A(t-1)}X_{(0)}^{(1)}+A^{-1}(e^{A(t-1)}-I)\bullet B \qquad (t=1,2,\cdots,n) \qquad (4)$$

Where $e^{At}=I+At+\dfrac{A^2}{2!}t^2+\cdots=I+\sum_{k=1}^{\infty}\dfrac{A^k}{k!}t^k$

The parameter A and B can be estimated by least-square method:

$$\hat{a}_i=\begin{bmatrix}\hat{a}_{i1}\\ \hat{a}_{i2}\\ \vdots\\ \hat{a}_{in}\\ \hat{b}_i\end{bmatrix}=(L^TL)^{-1}L^TY_i \quad (i=1,2,\cdots,n) \qquad (5)$$

$$\hat{A}=\begin{bmatrix}\hat{a}_{11} & \hat{a}_{12} & \cdots & \hat{a}_{1n}\\ \hat{a}_{21} & \hat{a}_{22} & \cdots & \hat{a}_{2n}\\ \vdots & \vdots & & \vdots\\ \hat{a}_{n1} & \hat{a}_{n2} & \cdots & \hat{a}_{nn}\end{bmatrix}, \text{ and } \quad \hat{B}=\begin{bmatrix}\hat{b}_1\\ \hat{b}_2\\ \vdots\\ \hat{b}_n\end{bmatrix}$$

where

$$L=\begin{bmatrix}\frac{1}{2}(x_1^{(1)}(2)+x_1^{(1)}(1)) & \frac{1}{2}(x_2^{(1)}(2)+x_2^{(1)}(1)) & \cdots & \frac{1}{2}(x_n^{(1)}(2)+x_n^{(1)}(1)) & 1\\ \frac{1}{2}(x_1^{(1)}(3)+x_1^{(1)}(2)) & \frac{1}{2}(x_2^{(1)}(3)+x_2^{(1)}(2)) & \cdots & \frac{1}{2}(x_n^{(1)}(3)+x_n^{(1)}(2)) & 1\\ \vdots & \vdots & & \vdots & \vdots\\ \frac{1}{2}(x_1^{(1)}(m)+x_1^{(1)}(m-1)) & \frac{1}{2}(x_2^{(1)}(m)+x_1^{(1)}(m-1)) & & \frac{1}{2}(x_n^{(1)}(m)+x_n^{(1)}(m-1)) & 1\end{bmatrix}$$

and $Y_i=(x_i^{(0)}(2),x_i^{(0)}(3),\cdots,x_i^{(0)}(m))^T$

3. THE ESTABLISHMENT OF MGM(1,n,q) MODEL

Since original data are mainly time-series data in practice, the derivative can be transformed into a forward difference equation [6], such as

$$\frac{X_{t_1}^{(1)}-X_{t_2}^{(1)}}{t_1-t_2}-AX_{t_2}^{(1)}=B$$

In fact, $t_1-t_2=1$, so we can get

$$X_{t+1}^{(1)}-X_t^{(1)}-AX_t^{(1)}=B \qquad (6)$$

in the same way, a backward difference equation is

$$X_t^{(1)}-X_{t-1}^{(1)}-AX_t^{(1)}=B \text{ or } X_{t+1}^{(1)}-X_t^{(1)}-AX_{t+1}^{(1)}=B \qquad (7)$$

Different sequence satisfies different equation. Some satisfy the Eq.(6), while others satisfy the Eq.(7). The common difference equation is

$$X_{t+1}^{(1)}-X_t^{(1)}=B+qAX_t^{(1)}+(1-q)AX_{t+1}^{(1)} \qquad (8)$$

The Eq.(8) is the common difference equation of MGM(1,n). The building model is MGM(1,n,q) by Eq.(8). When q=0.5, the MGM(1,n,q) model turns to the GM(1,1) model. For the any q_0 the background matrix is

$$L_0=\begin{bmatrix}(q_0x_1^{(1)}(2)+(1-q_0)x_1^{(1)}(1)) & (q_0x_2^{(1)}(2)+(1-q_0)x_2^{(1)}(1)) & \cdots\\ (q_0x_1^{(1)}(3)+(1-q_0)x_1^{(1)}(2)) & (q_0x_2^{(1)}(3)+(1-q_0)x_2^{(1)}(2)) & \cdots\\ \vdots & \vdots & \\ (q_0x_1^{(1)}(m)+(1-q_0)x_1^{(1)}(m-1)) & (q_0x_2^{(1)}(m)+(1-q_0)x_1^{(1)}(m-1)) & \cdots\end{bmatrix}$$

$$\begin{bmatrix} (q_0 x_n^{(1)}(2) + (1-q_0)x_n^{(1)}(1)) & 1 \\ (q_0 x_n^{(1)}(3) + (1-q_0)x_n^{(1)}(2)) & 1 \\ \vdots & 1 \\ (q_0 x_n^{(1)}(m) + (1-q_0)x_n^{(1)}(m-1)) & 1 \end{bmatrix} \quad (9)$$

$$\hat{a}_i = \begin{bmatrix} \hat{a}_{i1} \\ \hat{a}_{i2} \\ \vdots \\ \hat{a}_{in} \\ \hat{b}_i \end{bmatrix} = (L_0^T L_0)^{-1} L_0^T Y_i, \quad i = 1,2,\cdots,n \quad (10)$$

According to the above, if only given q_0, the $\hat{A}$ and $\hat{B}$ may be obtained by Eq.(9) and Eq.(10), then the $X_t^{(1)}$ may be obtained by Eq.(4). When the original series $X_i^{(0)}$ are given, the parameter q is the only factor that influences the precision of MGM(1,n) model. The relationship is very non-linear between q and errors. If the genetic algorithm is adopted, an ideal value about the parameter q may be obtained. In this paper, the MGM (1,n) model is combined with the genetic algorithm (GA), which is called MGM (1,n,q) model.

4. THE SOLUTION OF MGM(1,n,q) MODEL WITH GA

4.1 The genetic algorithm

The genetic algorithm(GA)[7,8] is an adaptive whole search and probability optimization arithmetic, which simulates the heredity and evolution of biology in environments. This arithmetic was initially presented by John Holland, professor of Michigan University U.S. in 1975. Through genetic operation of selection, and mutation to current population, the new generation is created and gradually evolves to optimal state. Only the evaluation function is used during seeking optimization and the differentiability of objective function is not required, can the genetic algorithm be whole, parallel, speed, adaptable and robust, so it has been extensively applied in the field of function optimization, production control, automation control, image disposal, artificial life and so on.

4.2 The basic steps of GA

1) Encoding. The $q \in [0,1]$ can be expressed by a binary cluster. The length of q (chromosome) can be determined by the precision of q.

2) Initialization of the population. N numbers selected from 0 to 1 at random are regarded as initial population. (N is the number of population).

3) The fitness evaluation of individual. The fitness function indicates the degree of adaptation capability to the environment, which is related with objective function. The fitness value of the No. i individual

$Fit(q(i,k))$ (k is the iterative times) may be calculated by using the following formula.

$$Fit(q(i,k)) = \begin{cases} C_{\max} - fit(q(i,k)) & , fit(q(i,k)) \prec C_{\max} \\ 0 & , fit(q(i,k)) \geq C_{\max} \end{cases}$$

The objective function can be expressed by the square sum of error; $fit(q(i,k)) = \sum_{i=1}^{N} (\hat{x}_i^{(0)} - x_i^{(0)})^2$ .where $C_{\max}$ may be the import parameter, the maximum of $fit(q(i,k))$ until now or the maximum of $fit(q(i,k))$ at current population or in latest several generation populations.

4) Selection. The survival probability of the i individual in the k generation is

$$p_i^{(k)} = \frac{Fit(q(i,k))}{\sum_{j=1}^{N} Fit(q(j,k))}$$

We can choose a strategy (such as roulette), so the selected probability of the i individual is $p_i^{(k)}$. The higher fitness of individual, the more chance of the individual can be selected as the new individual. The lower fitness of the individual, the fewer chance of individual can be selected as the new individual, and the more probability of being eliminated.

5) Crossover. A pair of individuals for crossover is selected stochastically. The simplest method of crossover is to select a truncation point stochastically, split each gene chain into two sections at this point, and then exchange their tails, for instance:

1100110011 | 10001 → 1100110011 01010
1011100101 | 01010 → 1011100101 10001

The crossover embodies the process of exchanging information in course of biology heredity.

6) Mutation. Several individuals are selected from the population with probability (p_m). For the selected individual, a bit is selected randomly for mutation, namely turns 1 to 0 (or 0 to 1). For example 10110[1]011 is changed to 101100011. The mutation embodies the accidental gene mutation in the course of biology heredity.

7) Evolutionary iteration. The filial generation from the previous is regarded as the new one, then repeat the 3) step to 6) step until an acceptable solution will be found or the reserved iteration times fulfilled.

Through using the genetic algorithm, the ideal q_0 can be obtained; the matrix L_0 can be obtained by

putting the q_0 into Eq.(9); then the value of $\hat{A}$ and $\hat{B}$ can also be obtained by the Eq.(10).

The calculation process of MGM(1,n,q) model is

$$\hat{X}^{(1)}(t) = e^{\hat{A}(t-1)}X^{(1)}(1) + \hat{A}^{-1}(e^{\hat{A}(t-1)} - I) \bullet \hat{B} \qquad (t = 1,2,\cdots,n)$$

$$\hat{X}^{(0)}(1) = X^{(0)}(1)$$

$$\hat{X}^{(0)}(k) = \hat{X}^{(1)}(k) - \hat{X}^{(1)}(k-1) \qquad (k = 2,3,\cdots n)$$

The relative error and the square sum of errors can be used to evaluate the MGM(1,n,q) model forecasting. The relative error is $\frac{\left|\hat{x}_i^{(0)} - x_i^{(0)}\right|}{x_i^{(0)}}$; the square sum of errors is $\sum_{i=1}^{n}(\hat{x}_i^{(0)} - x_i^{(0)})^2$.

5. CASE STUDY

Owing to the influence of economy, population, standard of living and so on, the urban water consumption possesses certain characteristics of grey. Due to the need of less information and higher precision, the grey system can preferably reflects system practical condition. The following is an analysis and calculation of urban water consumption for several years in Dalian city. Due to the available data of water consumption are scarce in Dalian city, only the data from 1990 to 2003 can be analyzed

Table 1. The Statistic of urban water consumption from 1990 to 2003 in Dalian

Year	Urban water consumption (10^4m^3)	Urban population (10^4 people)
1990	20826	239.64
1991	19305	241.57
1992	25107	244.94
1993	25416	248.67
1994	27485	252.35
1995	30354	254.74
1996	30910	257.23
1997	32784	259.71
1998	27105	262.40
1999	33142	264.17
2000	32249	267.78
2001	31868	270.68
2002	33262	273.23
2003	35460	274.78

The data from 1990 to 2000 year are regarded as the basic, and the data from 2001 to 2003 as the test. According to the above theory, we perform a simulation forecast. In course of genetic algorithm, the binary coding has been adopted, the number of population is 10, the length of coding is 20, the probability of crossover is 0.95, and the probability of mutation is 0.08. After optimization of genetic algorithm, the parameter q=0.456534. At last, we compare the simulation value and the errors of GM(1,1), MGM(1,n) and MGM(1,n,q). The result is shown in table 2.

Table 2. The analysis of simulation value and errors about three models

year	water consumption (10^4m^3)	GM(1,1)		MGM(1,n)		GM(1,n,q)	
		Simulation value (10^4m^3)	Relative errors (%)	Simulation value (10^4m^3)	Relative errors (%)	Simulation value (10^4m^3)	Relative errors (%)
1990	20826	20826.00	0	20826.00	0	20826.00	0
1991	19305	23538.98	21.93	19847.87	2.81	19741.59	2.26
1992	25107	24506.24	2.39	23939.15	4.65	23866.78	4.94
1993	25416	25513.24	0.38	26536.8	4.41	26495.03	4.24
1994	27485	26561.62	3.36	28228.06	2.70	28210.75	2.64
1995	30354	27653.08	8.90	29369.68	3.24	29370.61	3.24
1996	30910	28789.39	6.86	30178.46	2.37	30192.29	2.32
1997	32784	29972.40	8.58	30786.09	6.09	30808.66	6.02
1998	27105	31204.01	15.12	31272.61	15.38	31300.80	15.48
1999	33142	32486.24	1.98	31686.65	4.39	31718.21	4.29
2000	32249	33821.15	4.88	32057.78	0.59	32091.12	0.49
2001	31868	35210.92	10.49	32403.97	1.68	32437.97	1.79
2002	33262	36657.79	10.21	32736.13	1.58	32770.04	1.48
2003	35460	38164.12	7.63	33060.92	6.77	33094.21	6.67
Mean relative error(%)		7.33		4.05		3.99	

From the result in table 2, we can draw the conclusion that the precision of MGM (1,n) is much higher than the GM (1,1) due to the restriction and stimulation between variables. The MGM (1,n,q) is higher than the MGM (1,n) due to the parameter q being wholly optimized. The mean relative error is enhanced from 7.33% to 3.99%. The curves of modeling and forecast of three models are shown in Figure 1.

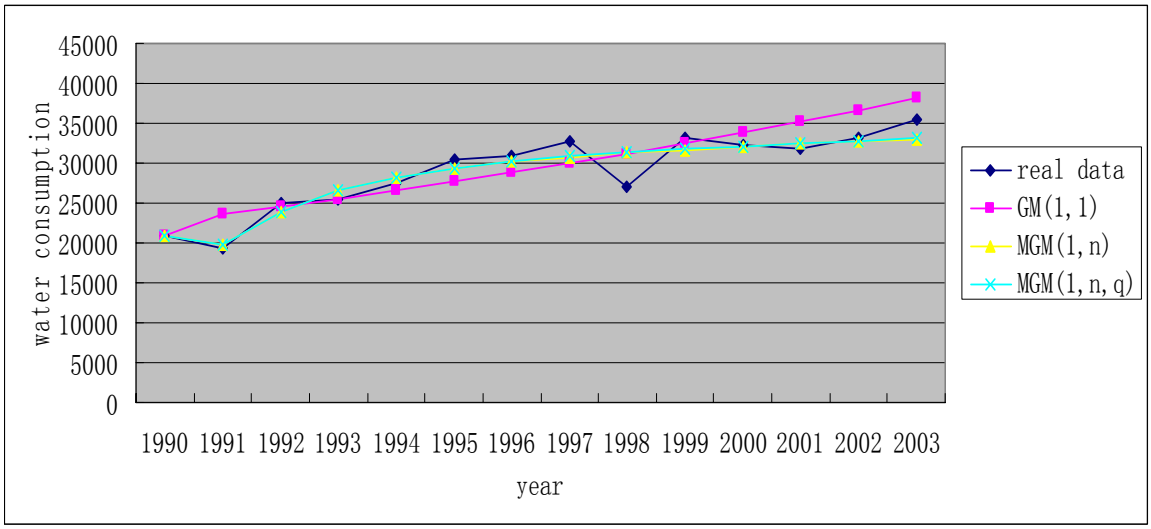

Figure 1. The curves of simulation and forecast of models

6. CONCLUSIONS

As the expansion and complement of GM (1,1) model, the MGM (1,n) can reflect the relationship of restriction and stimulation between variables. According to the characteristics of whole search and connotative parallel calculation, the genetic algorithm is adopted and combined with the multi-variable grey model (MGM (1,n)) well, and a Multi-variable model (MGM (1,n,q)) based on Genetic Algorithm has been established. Owing to the influence of economy, population, standard of living and so on, the urban water consumption possesses certain characteristics of grey. The grey system model is an available method to predict the urban water consumption. Taking the data of urban water consumption in Dalian city from 1990 to 2003 as example, the model is proved. The result indicates that the MGM(1,n,q) model is better than MGM(1,n) model, and the MGM(1,n) model is better than GM(1,1). At the same time, in the course of modeling of multi-variable grey model, if the choice of variable is improper, the morbidity of matrix or inverse matrix would occur. How to choose the proper variable is difficult in the course of applying, which needs to be further researched.

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Sorptivty Characteristics of Soil Phosphorus In Relation to Land Utilization Types

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Abstract: Phosphorus (p) adsorption characteristics of 45 soil samples from 3 land utilization types (LUTs) were studied in soils of Owerri, Southeastern Nigeria during 2005/2006 cropping season. Some soil properties as well as selected P- adoption characteristics were studied in these LUTs, namely secondary forest (SF), cassava-based farm (CF) and continuously cultivated arable farm (CCF). The experiment was laid out in a randomized complete block design (RCBD) and farms were owner–managed. Results show differences in P- adsorption was greatly influenced by soil organic carbon (SOC), soil pH, exchangeable calcium, exchangeable aluminium and aluminium. Regression analysis shows that SOC and pH were the highest predictors of P-adsorption in soils of the study site, using the investigated LUTs. There is need for inclusion of more soil chemical, physical and mineralogical properties in predicting soil P- adsorption to enhance reliability of information. [Nature and Science. 2007;5(1):27-38].

Keywords: Adsorption, agriculture, land use, pedogenesis, phosphorus, tropical soil.

Introduction

The release of phosphorus (P) through natural processes is very scanty, whereas the sinks for P, especially at the floor of oceans are huge. In most agro ecosystem, P – losses outweigh gains, and unless augmented by man, P-cycles lose momentum. This is particularly true for tropical upland soils where deficiency of P is the most prevalent initial constraint to plant growth (von Uexkull, 1989). The uncertainties about P-chemistry in soils are due to its strong interaction with many organic and inorganic solid phases, continual uptake by plants and micro-organisms, continual return from organic decay and slow reaction rates (Isirimah et al., 2003). Although P-adsorption capacities of soil are influenced by Fe and Al oxides (Hakim, 2002), exchangeable calcium and magnesium, soil texture, porosity, pH, ionic strength and hydraulic conductivity (Bubha *et al.,* 2003), it has been reported that land utilization type influences P-adsorption capacity (Amapu *et al.,* 2000).

Man's land use activities affect global P-cycle. If p is applied to soil in excess of crop requirement, P will generally build up in the soil (Zhang *et al.,* 2005) and this increases the chances of P loss in the soil system (Sharpley *et al.,* 1999). Often, farmers practice land application of animal manure in order to meet crop nitrogen (N) needs and this results in over application of P as N/P ratio of most crops and pastures is 8:1 (USDA, 2001 while that of animal manures is 4:1 (Zhang et al., 2004). In paddy soils, Isirimah *et al.,* (2003) attributed high and continued phosphate availability to cycling of P between iron and aluminium forms.

Nnaji et al. (2002) observed variation in available phosphorus when soils of five land utilization types were evaluated. They reported that soils under maize –pepper intercrop, cassava-maize-pepper intercrop, sole cassava and cassava-maize intercrop were 68%, 27%, 14% and 11%, respectively higher than forest soil in available P. On highly weathered soils of Kenya, maize (Zea mays L.) yield doubled by applications of 50kg P ha^{-1} yr^{-1} as triple super phosphate (Bunemann *et al.,* 2004b) while crops had significant yield when it followed one –season Crotatlaria grahamiana planted fallow (Niang *et al.,* 2002; Smestad *et al.,* 2002). Application of 2tha^{-1} ash was optimum for soil pepper production and resulted to relatively high asoil P after the treatment (Odedina *et al.*, 2003). Intensive donkey drawn tillage on a steep slope led to a decline of available P within the tilled layer of 0-15 cm in upper and middle portions of the slope (Li *et al.,* 2004).

Phosphorus is a critical element in natural and agricultural ecosystems throughout the world (Onweremdu, 2007) as its limited availability is often the main constraint for plant growth in highly weathered soils of the tropics (Bunemann *et al.,* 2004a). Phosphorus deficiency problems are common in well –weathered oxisols and ultisols because of strong acidic reactions and abundance of Al and Fe ions (Saleque *et al.,* 2004), and the situation can be worsened with inappropriate P management (Saleque *et al.,* 1998) Rice removed about 2 to 3 kg P for 1 mg of grain produced (Timsina and Connor, 2001). All these show that P-availability and use by plants vary among plants. We hypothesize that P- availability and uptake relate to sorption characteristics of soil. Pased on the above, the major objective of this study was to investigate P- sorption characteristics as influenced by land utilization type (LUT).

Materials and methods

Study area: The research was carried out during the 2005/2006 cropping season at Owerri, Southeastern Nigeria. The study site is located on latitude $5^{0}43^{1}$ 14^{11}.623 N and $7^{0}37^{1}34^{11}$.490 E., and with an elevation of 55 metres (handheld Global Positioning System – GPS) receiver (Garmin Ltd, Kansas, USA). The predominant parent material for underlying the area from which most soil formed is the Coastal Plain Sands (Benin formation) of the Miocene-Oligocene geological era. Soil are referred to as: "Acid sands", and are characteristically acidic, of low cation exchange capacity, low base saturation, low fertility status and suffer from multiple nutrient deficiencies (Oti, 2002). Earlier, soils were classified as Isohyperthemic Arenic Kandiudult (Onweremadu, 2006). The area has a humid tropical climate (Igwe and Stahr, 2004), with two distinct seasons, namely wet and dry seasons. Rainfall distribution is bimodal with

peaks during the months of July and September. Temperatures are high and change only slightly during the year. Vegetation is described as rainforest and has multiple plant species that shade their green leaves at different times, making the forests "evergreen". However, the density of the rainforest has drastically reduced due to anthropogenic activities. Subsistence farming is a prevalent socio-economic activity in the area.

Experimental design: The study identified 15 plots of farmland located within the same area and at close proximity. These plots have been consistently under cultivation for 15 years and under one of the following land utilization types (LUTs): secondary forest (SF), cassava- based arable farm (CF) and continuously cultivated arable farm (CCF). Adequate care was taken to ensure the fields selected for a particular LUT have been subjected to similar cultural practices, such as uniform tillage system and application of manures over years. Farms were owner –managed and farmers belong to the same social setting and great uniformity was found in their farming practices. The experiment was managed by farmers except for researchers' technical input and collection of data. The experiment was a randomized complete block design (RCBD) with three LUTs as treatments. Five farmers' plots were used as replicates for each LUT. Each plot measured 400 m^2 (20 x 20 m). All data were taken from the inner 10 x 10 m^2 of each plot.

Soil Sampling: Fifteen soil samples were collected form each LUT at 0-20, 20-40 and 40-60 cm depths. A total of 45 soil samples were used for the study. In each treatment were 5 replicates and soil samples were collected at 3 depths. These soil samples were air-dried, and sieved using 2 –mm sieve in readiness for laboratory analysis.

Laboratory analysis: Particle size distribution was determined by hydrometer method (Gee and Or, 2002). Soil pH was measured in 1:2.5 soil /liquid ratio in 0.1 N KCl (Hendershot *et al.,* 1993). Soil organic carbon (SOC) was estimated by combustion at 840^0C (Wang and Anderson, 1998). Exchangeable calcium and aluminium were measured using inductively coupled plasma atomic emission spectrometer (ICP – AES). Cation exchange capacity (CEC) was determined by percolating 2.5 g soil with 100 ml of I M ammonium chloride for about 4 hours. Before percolating soil samples, samples were soaked with extraction solution overnight. Aluminium saturation (Alsat) was computed as exchangeable aluminium (Al) divided by CEC multiplied by 100 percent.

Adsorption Isotherms: Phosphorus adsorption isotherms were determined according to the procedure of Graetz and Nair (2000). A gram of soil sample was equilibrated with 25 mL of varying concentrations of P in 0.01 M $CaCl_2$ solution in 50-ml centrifuge tubes. The concentrations of the solutions were 0.0, 0.5, 1.0, 5.0, 10.0, 15.0 and 20.0 mg P L^{-1}. The tubes were shaken for 24 hours on an end –to- end shaker at 150 oscillations per 60 seconds. The samples were then centrifuged for 10 minutes at 5211 x g and the supernatant decanted. The P in solution was then quantified calorimetrically using the ascorbic acid method (Kuo, 1996). The amount of P adsorbed was determined by the difference between initial and final amounts of P in solution. Duplicate analyses were conducted on all soil samples. Phosphorus adsorption isotherms were estimated with the linearized form of the Langmuir equation:

$$\frac{C}{S} = \frac{1}{KSmax} + \frac{C}{Smax} \quad \ldots I.$$

Where S = total of amount of P retained

C = Concentration of P after 24-hr equilibrium

Smax = P – sorption maximum

The Smax was calculated by regressing C/S versus C, where C is the equilibrium solution P concentration and S is adsorbed P. The reciprocal of the slope 'of mean regression is Smax (Zhang *et al.,* 2005).

Computations: The following calculations were made:

1. The amount of P remaining in solution was taken as equilibrium concentration and the difference between the initial concentration and equilibrium concentration was taken as adsorbed P.
2. The adsorption isotherm versus equilibrium concentration was plotted for each soil being investigated to obtain a straight line with slope of 1/b and intercept of 1/kb
3. The following form of Langmuir equation: C/C x1m = 1/kb+ c/b) was used to obtain adsorption capacities and constants related to bonding energy.

Where C= P concentration in equilibrium solution

x/m = P adsorbed by soil

b = adsorption maximum

k = constant related to bonding energy of soil for P (affinity constant)

Statistics: Correlation and regression analyse were performed to relate some soil properties and P- sorption characteristics using PC SAS version 8.2 (SAS Institute 2001).

Results

The properties of soils under LUTs are given in Table 2. Soils were generally sandy irrespective of LUT but SOC values were highest in soils of SF although values decreased with depth in all LUTs. Soil were strongly to moderately acidic and least pH value was recorded at 20-40 cm depth under SF. The same layer had the highest value of Alsat (aluminium saturation) and least exchangeable calcium.

Phosphorus adsorption characteristics are shown on Table 3, indicating variabilities in the P-adsorption characterstics of studied soil. Soils under SF had steeper slopes. Values of Langmuir adsorption constants, equilibrium P and buffering capacity for soils of the LUTs are indicated on Table 4. The standard P requirements (P adsorbed at a standard concentration of 0.2 mg P) were low for the LUTs and results were consistent with the findings of Osodeoke (1999). Also, the adsorption maxima at low equilibrium solution P concentrations are generally low with values obtained in Western Nigerian soils (Osodeoke, 1999) but moderate with results of P-studies conducted by Kwari and Batey, (1991) in

Northcastern soils of Nigeria. Adsorption maxima decreased in this order: CCF, CF and SF and the same trend was followed by k-values (affinity constant values). But buffering capacity value was highest in CCF and least in SF.

Simple correlation result between P-adsorption characteristics and some soil properties are shown (Table 5). Soil clay and Alsat influenced adsorption maxima of P in soils under SF. Similar findings were made by Zhang et al. (2005) when clay was correlated with Smax (r=0.7.9) while Borling et al. (2001) found significant relationships between oxides of aluminium and Smax. Under CF, soil pH and exchangeable calcium had highest relationship with P-adsorption capacity while in all LUTs, there were negative relationship between P adsorption capacity and SOC. Unlike results of other researchers (Dodor and Oya, 2006; Zhang *et al.,* 2005) soil pH had significant ($P < 0.01$) relationship with P-adsorption. Exchangeable Ca was significantly and negatively correlated ($P<0.05$) with P-adsorption and this contrasts the results of other researchers (Sims *et al.,* 2002). Higher correlation values were established between exchangeable Ca and P – adsorption in CCF. Phosphorus predictive capacities of individual soil properties are presented on Table 6. The P- predictive ability of soil pH decreased in the order of CCF CF and SF while SOC had higher prediction of P- adsorption in CCF and SF when compared with CF. Least coefficient of alienation ($1-R^2$- 0.23 was found in the relationship between exchangeable Ca and P-adsorption

Table 1 Cultural practices associated with each land utilization type (LUT) in the study site

LUT	Cultural Practices
SF	Land clearing by slash and burn, natural fertility regeneration
CF	Land clearing by slash and burn, soil amended with animal manures and inorganic fertilizers. Cassava + maize + okra + pepper intercropping
CCF	Land clearing included slashing, stumping and packing debris in heaps more applications of animal manure, compost, farm yard manure and inorganic fertilizers multiple cropping including groundnut (Arachis hypogea)

SF = Secondary forest, CF = Cassava =based farm, CCF = continuously cultivated arable farm

Table 2 Selected properties of the studies soil (mean values)

LUT	Depth (cm)	Clay (g kg^{-1})	Silt (g kg^{-1})	Sand (g kg^{-1})	SOC (g kg^{-1})	Ca (cmol kg^{-1})	Al (cmolkg^{-1})	CEC (cmol $kg^{-1)}$	Alsat (g $kg^{-1)}$	pH (KCl)
Secondary forest	0-20	330	70	600	14.0	6.6	4.0	12.2	42	4.4
(SF)	20-40	350	60	590	6.0	5.1	4.3	8.2	50	3.8
	40-60	360	60	580	2.0	15.6	3.9	11.6	38	4.5
Cassava based	0-20	300	50	650	18.0	16.8	4.6	11.0	44	4.4
Arable farm	20-40	360	70	570	6.0	11.2	4.9	9.2	46	4.6
(CF)	40-60	305	70	625	1.0	18.6	3.7	10.6	38	4.6
Continuously	0-20	290	40	670	14.8	15.3	5.0	10.8	43	4.8
Cultivated arable	20-40	340	60	600	3.0	11.0	5.2	9.6	44	4.6
Farm (CCF)	40-60	400	80	520	2.6	17.9	3.6	10.6	39	4.8

LUT – land utilization type SOC = soil organic carbon, Alsat = aluminium saturation, Ca = calcium, Al = aluminium CEC = cation exchange capacity.

Table 3. Phosphorus adsorption properties of studied soil (mean values)

LUT	Depth (cm)	EPC (mg Pg^{-1})	Ad. P (mg P ml^{-1})	EPC/Ad.P
SF	0-20	0.02	10.01	0.002
	20-40	0.73	9.25	0.079
	40-60	0.60	9.55	0.063
CF	0-20	0.30	17.66	0.017
	20-40	2.71	21.25	0.127
	40-60	0.70	16.65	0.042
CCF	0-20	1.21	30.25	0.040
	20-40	2.72	33.40	0.081
	40-60	0.90	41.26	0.022

LUT = land utilization type SF = Secondary forest, CF = Cassava = based arable farm

CCF = Continuously cultivated arable farm, EPC= equilibrium P concentration

Ad.P = adsorbed P .

Table 4. Values of Langmuir adsorption constants (adsorption maximum affinity constant), equilibrium P and buffering capacity (mean values)

LUT	ECR (mg P g^{-1})	AM (b)	AC (k)	0.2 mg P	BC (mg P g^{-1})
SF	0.0 -0.8	20.0	1.0	4.1	28.0
	0.8-6.0	76.0	0.1	-	-
CF	0.0 -0.8	26.0	1.2	2.9	26.1
	0.8 -5.8	72.0	0.3	-	-
CCF	0.0 -0.8	28.8	1.8	2.2	24.3
	0.8 -5.2	78.4	0.4	-	-

LUT = land utilization type, SF = Secondary forest, CF = cassava-based arable farm
CCF = continuously cultivated arable farm, ECR = equilibrium concentration range, AM = adsorption maximum, AC = affinity constant, BC = buffering capacity.

Table 5. Simple correlation (r) between Langmuir adsorption constants, 0.2 mg P and selected soil characteristics (n = 45).

LUT	Soil properties	b	k	0.2 mg P
SF	pH (KCl)	0.62**	0.68*	0.66*
	SOC (g kg^{-1})	- 0.60**	0.58*	0.65*
	Ca (cmol kg^{-1})	- 0.60 *	0.38 NS	0.51*
	Alsat (g kg^{-1})	0.79**	0.88**	0.90**
	Clay (g kg^{-1})	0.88*	0.91**	0.92**
CF	pH (KCl)	0.88**	0.81*	0.68*
	SOC (g kg^{-1})	- 0.74*	0.63*	0.52*
	Ca (cmol kg^{-1})	- 0.88**	0.56**	0.53*
	Alsat (g kg^{-1})	0.62**	0.77*	0.80**
	Clay (g kg^{-1})	0.72**	0.82**	-0.86**
CCF	pH (KCl)	0.96 **	0.83**	0.51*
	SOC (g kg^{-1})	- 0.98**	0.43 NS	0.25 NS
	Ca (cmol kg^{-1})	- 0.82**	0.48*	0.34 NS
	Alsat (g kg^{-1})	0.41 NS	0.87**	0.88**
	Clay (g kg^{-1})	0.37*	0.96**	- 0.91**

LUT = land utilization types, SF = Secondary forest, CF = cassava-based arable farm,
CCF = continuously cultivated arable farm SOC = Soil organic carbon, Ca = calcium, Alsat = aluminium saturation, b = adsorption maxima, k = affinity constant, ** significant at P = 0.01, * significant at P = 0.05, NS not significant.

Table 6. Pedotransfer functions relating adsorption of P (Y) to some soil properties (n=45)

LUT	Independent variable	Regression equation	R^2	$1-R^2$
SF	pH (KCl)	Y = 17.6 – 41.63 pH	0.62	0.38
	SOC (g kg $^{-1}$)	Y = 38.6 – 6.11 OC	0.74	0.26
	Ca (cmol kg $^{-1}$)	Y = 31.2 – 1.25 Ca	0.59	0.41
	Alsat (g kg $^{-1}$)	Y = 26.8 + 0.98 Alsat	0.62	0.38
	Clay (g kg $^{-1}$)	Y = 33.2 + 1.16 Clay	0.46	0.54
	0.2 mg P	Y = 28 . 6 + 0.22 mg P	0.55	0.45
CF	pH (KCl)	Y = 22.1 – 46. 18 pH	0.77	0.23
	SOC (g kg $^{-1}$)	Y = 31.2 – 0.92 OC	0.55	0.45
	Ca (cmol kg $^{-1}$)	Y = 29.1 – 0.06 Ca	0.77	0.23
	Alsat (g kg $^{-1}$)	Y = 30.3 + 0.07 Alsat	0.38	0.62
	Clay (g kg $^{-1}$)	Y = 25.6 + 0.27 clay	0.52	0.48
	0.2 mg P	Y = 30.3 + 0.02 mg P	0.59	9.41
CCF	pH (KCl)	Y = 19.0 – 52. 12 pH	0.92	0.08
	SOC (g kg $^{-1}$)	Y = 29.8 – 0.13 OC	0.96	0.04
	Ca (cmol kg $^{-1}$)	Y = 23.2 – 0.09 Ca	0.67	0.33
	Alsat (g kg $^{-1}$)	Y = 35.1 + 1.11 Alsat	0.17	0.83
	Clay (g kg $^{-1}$)	Y = 26.3 + 0.09 Clay	0.59	0.41
	0.2 mg P	Y = 29.2 + 0.12	0.61	0.39

Discussion

Soil were generally sandy in all the LUTs, indicating similarity in parent material source. Slight variations in soil texture and other properties could be attributed to land use history and differential impact of climatic factors on soils. Onweremadu et al. (2007a) noted slight temporal textural differences in arable soils resulting from continuous cultivation. Exchangeable Ca was high in surface layers of soils under CCF and CF while it increased with depth in soil of SF, suggesting leaching of exchangeable Ca to deeper soil layers. This is further confirmed by high Alsat (50%) and strong acidity (pH_{KCl} = 3.8) Values of P-adsorption attributes varied indicating ability of soils under different LUTs to adsorb P. Steeper slopes in soil of SF is suggesting higher buffering capacity of soils when compared with soils of other LUTs. However, relatively lower buffering capacities of soil under CF and CCF could be attributed to varying additions of inorganic fertilizers while higher values of CEC in soils under SF is suggestive of higher buffering capacities. Soils with largest cation exchange capacities offer greatest resistance to change in pH and are most strongly buffered (Foth, 1984).

The standard P requirement values, that is, P adsorbed at a standard concentration .of 0.2 mg kg^{-1} varied among LUTs and were low in the study areas. Least values were obtained in CCF, possibly due to multiple cropping and continuous cultivation of soils. There exists two distinct linear potions as calculated form the regression equations implying that these soil had two adsorption maxima (b) and affinity constants (k). It is argued that P-adsorption capacity as suggested by the population of sites in the low equilibrium P (first linear portion) is more important than that of the second linear portion as they represent P- levels for crop production when fertilized (Udo 1981). High values of P-adsorption maxima in CCF soils at high ECR could be due to more population of adsorption sites. High values of k (affinity constants), implying greater bonding energy in the first linear portions show that the tenacity of P-adsorption is higher at low P equilibrium concentration, and these attributes varied with LUT.

In all LUTs, P- adsorption characteristics were influenced by some soil properties, although at varying levels. Soil properties that correlated with P- adsorption were pH, SOC Alsat, exchangeable Ca and clay. Similar relationships were recorded by Burt et al. (2002). Studied soils are highly weathered and the presence of organic matter reduce P- sorption capacity (Gillman et al., 1989) due to direct result of competition for sorption sites between phosphate and organic ligands (Hakim, 2002). He also reported that the same competition exists between Al and Ca. it is also possible that organic matter reduces positive charge on variable charge surfaces by lowering pH, and this decreases the attraction of P to the soil surface. This effect was more in soils under SF than in other soils, indicating that anthropogenic activities do alter soil properties.

Soil organic carbon and pH had high values of coefficient of determination, having $r^2 = 0.96$ and $r^2 = 0.92$, respectively, indicating that these predictors can be used to predict P-sorption and P – availability with high degree of confidence in soils of these LUTs.

Conclusion

Sound knowledge about P-sorption properties in soils under different LUTs is necessary in sustained use of soil for crop production. Results of this study revealed differences in P- adsorption due to land use and identified soil pH and SOC as main predictors of P activity in the study areas. There is need for more intensive sampling and multiple regression of physical, chemical and mineralogical properties of soils for more reliable information on soil properties on prediction of P.

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Adoption Levels And Sources of Soil Management Practices in Low – Input Agriculture

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Abstract: Socioeconomic characteristics as they affect adoption level and sources of soil management information were investigated in Owerri Agricultural Zone, Southeastern Nigeria in 2005. Structured interview was used as instrument of data collection. Data were subjected to percentage, mean and multiple regression analysis. Results showed that farmers were relatively young, literate and profit-oriented. Agricultural extension agents were the chief sources of information to the arable farmers. Adoption level was highly related with education ($t = 2.82; P \leq 0.05$), age ($t= 2.56$; $P \leq 0.05$) and income ($t= 2.48$; $P \leq 0.05$) Greater information dissemination is suggested through integration of selected arable farmers into Agricultural Development programme (ADP) as "contact" farmers for multiplier effects. [Nature and Science. 2007;5(1):39-45].

Keyword: Adoption level, change agent, humid tropics, low-input farming soil management, soil survey information,

Introduction

Soil survey information is an essential input in the efficient use and sustainable management of soil and soil-related resources. It is required for use in development planning and in eliciting support for policies that favour sustainable natural resources use. Such information should be usable and seen in terms of its value, status, use, accessibility and applicability (Okedi, 1999). He noted that soil information should include description, classification, mapping and evaluation of soils, slope classes and physiographic units of topography , land units; land ownership records; land cover and land use; and environmental requirements of crops. Soil survey information helps soil managers to recommend appropriate management practices. Soil data are scanty (Onweremadu 2006) but fundamental in minimizing food insecurity (Smith et al., 2006). Its scantiness could be responsible for slow progress in combating rural poverty (Jansen et al., 2006).

Lal and Ragland (1993) observed that the available soil data are not translated into problem solving technology. In addition to this, the language of delivery of soil survey information is so complex that physical scientists, social scientists and other land users who need it find it difficult to avail themselves of the information (Akamigbo, 2002). Again, where farmers and other land users are aware of several constraints on-farm, their perception of urgent ones may be at variance with the researchers (Mutsaers *et al.,* 1997). Yet, indigenous knowledge of farming community is rarely considered and incorporated in modern packages for sustainable land use (Oweremadu *et al.,* 2007), hence farmers persist on traditional technologies (Tanko 2003). These and other reasons could be why soil data are rarely used (Smith *et al.,* 2004). In addition to the above Isife *et al.* (2006) reported that low participation and adoption of technologies by farmers is among other things caused by poor field contacts between extension agents and farmers. Where the agricultural extension agents are available poor technical knowledge may hinder communication necessary for effective delivery (Nwachukwu, 2003; Matthews – Njoku et al., 2006).

Efficacy of any agricultural extension is judged by the level of mass adoption and spread of modern and scientific practices among farmers in the rural neighbourhood. In his study of the factors affecting adoption of improved practices by goat farmers in Southeastern Nigeria, Ajala (1992) reported that age, sex, education, herd size, nature of farming, organizational participation, experience and management system were positively related to adoption. Apart form the above, information is relevant in adoption (Minot et al., 2006) in particularly designing geographically targeted programmes for addressing disparities. Information sources are stimulants for adoption (Rogers, 1995), implying hopes for greater adoption in this era of information and communication technology (Venkatesan, 1994; Spore, 2006).

Unfortunately, the results of applying Green Revolution technologies have been slow with yields significantly lower and less uniform (World Resources Institute, 1994; Nerlove et al., 1996). Based on the above and on the need to apply scientific information in sub-Saharan Africa (Wilson, 2001), the major objective of this study was to investigate levels of adoption and information sources available to farmer in Owerri agricultural zone, Southeastern Nigeria.

Materials and methods

Study area: The study area is Owerri agricultural zone of Imo State, Southeastern Nigeria, lying between, latitude $5^0 15^1$ and $5^0 45^1$ N, and longitudes $6^0 45^1$ and $7^0 30^1$E. It has a humid tropical climate. The land area is about 3000 Km^2 and comprises eleven local government areas namely Aboh Mbaise, Ahiazu Mbaise, Ezinihitte Mbaise, Ikeduru, Mbaitol Ngor Okpala, Oguta, Ohaji Egbema Owerri North, Owerri Municipal and Owerri West.

The population density of the agricultural zone is over 500 persons/ km^2 (Agulanna , 1998) and agriculture is the major socio-economic activity, and mainly for the production of staple food crops (Asiabaka; 2005).

Sampling: Field studies were conducted in 2005. Three local government area were purposively selected based on intensity of farming activities. From each of the three local government areas, two towns were randomly selected, as follows: Awara and Umuokanne from Ohaji –Egbema, Agwa and Izombe from Oguta; and Emeabiam and Oforola from Owerri West. Ten arable farmers were randomly selected from each town from a list of 'big' farmer in the local government area. A big farmer is one that has about one hectare or more of arable farmland and duly registered with the Agricultural Unit of the Local Government Area. Thus, 60 arable farmers constituted the sample size for the purpose of the study.

Structured interview schedule was used to elicit information from the farmer.

Validation of interview schedule was done, using content validity method, which is a way of determining the relevance and suitability of items included in the study (Chuta, 1992) .Following the jury method as used by Ajayi (1996), items contained in the draft interview schedule for the research work were subjected to thorough examination and criticism by three lecturers in the Department of Agricultural Extension, Federal University of Technology, Owerri, Nigeria. The relevance and suitability of items determined by lecturer experts formed the basis for the development of final interview schedule which was used collect data for the study. In this study, the following socioeconomic characteristics were investigated: age, educational status, membership of social organizations farm size and estimated income. Adoption levels were estimated using 7 stages of adoption, which include unaware (UA) aware (A), interest (1), evaluation (E), trial (T), adoption (AD) and discontinuance (D).

Data analyses: Frequency distribution, mean and percentages were used in analyzing data collected. Adoption level (dependent variable) was regressed to socioeconomic attributes (independent variables).

Model used is as follows:

$$Y = a+b_1X_1 + b_2X_2 + b_3X_3 +b_4X_4 + b_5X_5 +e\ldots 1$$

Where Y = adoption level of soil management practice
a = Intercept
b_1-b_5 = regression coefficients
X_1 = age
X_2 = education
X_3 = membership of social organization
X_4 =farm size
X_5 = estimated income
e = error term

Results

Table 1 reveals the socio-economic attributes of the arable farmers. Most of the respondents (50.0%), were within 31-40 years showing that farmers are relatively young although a good umber (30%) of the farmers were more than 41 years old.

Only 5% of the respondent had no formal education while a majority (50%) had secondary education, indicating that the enterprise had a good proportion of literate people. Farmers belong to 3 to 4 social organizations, showing high social participation, which serve as a forum through which farmers could exchange ideas about new farm practices (Onu 1991 2005). Eighty-three percent of farm size ranges from 1.1 to 3.0 hectrares and with an estimated average income of N72, 000.00.

The distribution of farmerson different stages of adoption of soil management practices information is shown in Table 2. Organic fertilization, mulching, inorganic fertilization and minimum tillage are highly adopted in the study site while biomass transfer, liming and use of planted fallow are yet to gain grounds in the rationality of farmers. Many farmers are aware of crop rotation practice and herbicide application but are unwilling to adopt them.

In Table 3 are sources of soil information with agricultural extension agents playing a substantial role in informing farmers about soil resource. The ranking shows that least information on soil come to farmers through their children (students) who are in schools (7.08%).

Adoption level related with socio-economic characteristics as given in Table 4. Education had the highest relationship with adoption level ($t = 2.82$), followed by age ($t=2.56$) and estimated income ($t=2.48$). These results are consistent with the findings of Onyebinama (2000) that personal characteristics, especially age and education influence adoption level.

Discussion

Only 2% of the respondents were above 50 years, implying that majority of them were still in their active years, thus vibrant in carrying out farm work. Similar findings were made by Agwu and Chukwu (2006) that only 19% of rice farmers in Aninri local government area of Enugu State, Nigeria were above 50 years old. This is an advantage for adoption and spreading of sustainable soil management practices.

A very good number of the farmers were literate, especially at primary (28 13%) and secondary (50.20%) levels and this enhances transfer of soil management practices and other soil survey information. Rapidly increasing unemployment in industries, ministries and government parastatals has caused many school leavers to opt for agriculture and other menial jobs to sustain life. The literacy level of these farmers is capable of promoting local innovation, particularly in the area of framer led research and extension (FRE) and this will certainly reduce food insecurity.

Majority of the respondents belonged to an average of 3 social organizations since such social organizations may provide a forum for exchange of idea. However, farmers in the study area are communalistic and traditionally would like belong to many social groups as that is indicative of social status. Nonetheless, farmers frequently suggest that other farmers are an important source of information about farming.

With an average of 2.5 hectares of farmland, farmers were able to make N72,000.00 (mean value) under rainfed agriculture. Larger farms may attract more adoption tendencies since no farmer would like entertain crop failure. Further adoption and diffusion of soil management practices and information is likely when high income expectations are projected. Total and near total adoption of organic fertilization, mulching, inorganic fertilization and minimum tillage is not surprising. With the exception of inorganic fertilization others originate from indigenous practices hence their adoption. Adoption of inorganic fertilizers (98%) could be due to decreasing yield resulting form shortened fallow length since fertility regeneration is by fallowing. Although biomass transfer, planted fallows (CTA, 2002) and liming are sound soil management technological packages, farmers are not confident hence low adoption (2%) in each of them. Low adoption of crop rotation despite awareness (40%) and trial (30%) is possibly due to the rural setting of the study site, having relatively large expanse of farmland.

Tremendous impact of agricultural extension agents as information sources compared with other is attributable to the great emphasis of the present government on agriculture at all levels and that may have influenced more literate people choosing farming for livelihood. Contact farmers and/or contact groups receive the technologies first hand from extension agents and other farmers copy from project farmer (Aaji, 2002). But mass media did not contribute much in formation delivery to farmers possibly due to commercialization of mass media stations, which according to Arokoyo (1998) compels extension services to pay exorbitantly for air time. Education ($t = 2.82$), age ($t = 2.56$) and estimated income ($t = 2.48$) were significantly ($P \leq 0.05$) found to be related to adoption level. Training is an added input which enhances good performance and adoption (Meenambigai and Seetharaman, 2003). An educated farmer understands an innovation that may appear complex to an illiterate farmer as the latter prefers to adopt simple technologies (Cary and Barr, 1992). Age had a significant negative relationship ($P < 0.05$) with adoption

level, indicating that older farmers adopt less soil management practices. Results of this study agree with the findings of Ajala (1992), and this suggests that older farmers still hold tenaciously to traditional practice. Farming subcultures influence adoption process (van der Ploeg, 1993). Significant relationship between adoption level and estimated income suggests that farming to the respondents is profit – oriented thus are likely to adopt more technologies so long as income increase. Under the classical model of adoption of commercial innovations the more an innovation will provide concrete economic benefits, the greater the rate of adoption although farmers under certain circumstances do not act in an economically rational way (Van clay, 1992), especially if it is environmentally unfriendly.

Table 1. Distribution of respondents according to socio-economic characteristics (n = 60)

Socio-economic characteristics	Percentage	Mean
Age (Years)		
21-30	15.00	
31-40	50.00	35.0
41-50	28.00	
51-60	2.00	
Educational status		
No formal education	5.33	
Primary education	28.13	
Secondary education	50.20	
Post –Secondary education	13.33	
Membership of Social Organizations	40.33	
1-2		
3-4	53.00	3.0
5-6	6.67	
Farm size (Hectares)		
1.0	10.00	
1.1-20	38.33	
2.1-3.0	45.00	2.5
> 3.1	6.67	
Estimated Income (N)		
60,000,00- 65,000,00	10,00	
65,001,00 -70,000,00	15.33	
70,001.00 – 75,000.00	34.67	72,000.00
75,001.00 – 80,000.00	13.00	
80,001.00 – 85,000.00	8.00	
85,001.00 – 90,000.00	19.00	

(Source: Field Survey, 2005)

Table 2 Distribution of respondents according to stages of adoption (n =60)

	Adoption	Stage						
Soil management practice	UA	A	I	E	T	AD	D	Total
Inorganic fertilization	-	-	-	-	-	98	-	100
Organic fertilization	-	-	-	-	-	100	-	100
Liming	83	-	6	6	3	2.0	-	100
Mulching	-	-	-	-	-	100	-	100
Herbicide application	20	42	30	5	1	2	-	100
Pesticide application	-	2	-	-	10	88	-	100
Minimum tillage	-	5	2	-	3	90	-	100
Use of planted fallow	82	-	10	-	6	2	-	100
Crop rotation	-	40	10	10	30	10	-	100
Biomass transfer	83	7	-	5	3	2	-	100

UA = unaware , A = aware, I = interest , E = evaluation, T – trial AD = adoption,
D= discontinuance
(Source: Field survey, 2005)

Table 3. Sources of soil information for soil management by rank (n= 60)

Source of soil information	Percentage
Agricultural Extension Agents	30.05
Dealers on agrochemical	18.70
Farmers organizations	17.45
Mass media	16.49
Agricultural Exhibition/shows	10.25
Students	10.25
	7.08

Source: Field Survey, 2005).

Table 4. Multiple regression analysis on the relationship between adoption level and socio-economic attributes (n =60)

Independent variable	Coefficient	SE	T-value	F-ratio	R^2
Constant	4.83	0.51	10.09*	2.98	0.4
Age	-0.08	0.02	-2.56*		
Education	0.09	0.01	2.82*		
Membership of soil organization	0.43	0.16	0.88 NS		
Farm size	-0.06	0.01	-0.68 NS		
Estimated income	0.32	0.02	2.48*		

SE = Standard error, * Significant at $P < 0.05$
NS = not significant

Conclusion

This study revealed that arable farming was dominated by relatively young and educated people who can taken enhance adoption and soil management technological transfer. Results also indicated that farmers are exposed to a wide range of impersonal sources of soil information and have potentials of disseminating such soil information to neighbouring farmers. Again, age, education and income dictate adoption status in the study area.

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Intercropping - A Food Production Strategy for the Resource Poor farmers

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Abstract: Intercropping is practiced by majority of farmers in the tropical and subtropical regions of the world. This group of farmers produce majority of the food in our markets. This system of cropping suppresses weeds, reduces pest disease infestation, gives yield advantage and there is stable yield over time. Intercropping encourages high nutrient uptake than in sole cropping and water use efficiency is high because of intercoopertive interaction between the intercrops. It encourages high soil fertility maintenance especially where legumes are used as component crop they provide continuous soil cover, which prevents direct impact of raindrops, which causes erosion. By intercropping, a simple combination of maize/cassava can increase CE and pH as well as increase Mn content in the soil. It is a cheap way of food production as one input such as manure can be applied once and used by all the crop components in the farm thereby saving time for the farmer. It reduces risk of crop failure and ensures the farmer's stable income over time. Intercropping helps the farmer to spread his harvest over the season and so ensures a regular supply of food. The farmer makes optimal and maximal use of the land at any cropping season. Intercropping is done with crop rotation to break weed, diseases and pests' cycles and also provides complementary fertilization to crops in sequence with each other. [Nature and Science. 2007;5(1):46-59].

Introduction

The farmer is the central person in crop production. He is the planner and executor of any farm enterprise, and performs all functions involved in food crop production including clearing, planting, weeding, staking and training of vines, fertilizer application, harvesting, processing, storage and marketing. With the large variety of crops, the number of risks involved in crop production is numerous. These risks are centered on farm economics and farm management in connection with the duration of the growing period of the plant, the destination of the crop produce and the scale of production (ILACO 1985). Most crops are annual crops. In the smallholder farmers practice, the most important factors determining the date of planting and harvesting and the crop varieties selected for planting are the annual rainfall and temperature. The farmer starts early, utilizing the early rains before the soil temperature gets cold where he suspects that germination or sprouting as the case may be will not be realized again. Rain fed agriculture has been practiced in crop production for many years before the advent of irrigation agriculture and most smallholder farmers practice all sorts of irrigation farming especially in the dryer regions of the world. Farmers are great people because of their superb initiative to adapt to varied conditions and situations in order to feed their family and world population. Intercropping is practiced to reduce labour at planting time and harvesting .In fact, intercropping is indigenous to the wetter zones of West Africa and Southeastern Nigeria in particular. In home gardens intercropping is practiced at advanced and sophisticated level

The major annual crops which are of great importance to most categories of farmers include the grain cereals – maize, sorghum, millet and rice; tubers – cassava, yam, sweet potatoes and Irish potatoes; others include beans, cow pea, Soya bean and many varieties and species of vegetables. The world food revolve around these crops and the farmers makes use of factors of production - land labour, capital to ensure the feeding of the human race. There are some other crops whose growing period is over 12 months and they are referred to as perennial crops. Some of them become productive within their first year, such crops include sugar cane and others only become productive after a number of years example is oil palm, rubber, cocoa etc.

Throughout the world, farmers are classified into large-scale, medium scale and small-scale farmers. The focus of this paper is on the last category the small scale of which the subgroup in this category the resource poor farmers is drawn attention to. This group plant below one hectare of land and they are in majority mostly in the tropical environment. At least 55% of world farmers are resource poor found mainly in Africa, Asia and Latin American. The smallholder farmer produces crops for his family consumption and sales the surplus to the market. By so doing, the surpluses from these farmers when pulled together is very great that they contribute numerously in feeding majority of our people especially in the tropical a subtropical world.

Intercropping

A cropping system is an aspect of farming system or agricultural production system which consists of one or more enterprises, or business activities in which sets of resources and inputs are uniquely managed by the farmer in the production of one or more commodities to satisfy human needs for food, fibre, various products, monetary income and other objectives (Okigbo, 1982). This however differs from one region or zone to the other to confirm with the culture of the people.

Intercropping is the growing of two or more crops in proximity to promote interaction between them. In line with this definition, Wahua (1982), Ikeorgu (1983), Okigbo (1978) explained that intercropping is the growing of two or more crops simultaneously on the same field such that the period of overlap is long enough to include their vegetative stage. Further to this definition, Gomez and Gomez (1986), stated that where the overlap in time is too small for example only four weeks out of a growing season of 3-4 months, the term relay crop is used.

Sequential cropping, which is the growing of individual crop in sequence during one growing season on the same piece of land and intercropping, are the two basic principles of multiple cropping (Ruthenberg, 1971, Andrew and Kassam, 1975). They noted that, Agro-silviculture i.e. the growing of arable crop mixtures involving the intercropping of arable crops mainly is among the three broad areas of intercropping.

Intercropping is a common feature of agriculture in the tropical Africa as well as in the Asian and American tropics (Papendick et al. 1976, Okigbo 1978, Kurt 1984 and Dalrymple 1971). Specific intercropping systems have developed over the centuries in the different regions and they are closely adapted to the prevailing ecological and socio-economic conditions. Kurt (1984) explained that intercropping system differs frequently from one area to another with changes in soil and local climate while social and cultural conditions may superimpose on the ecological and economical zones. Thus, as regions and ethnic groups differ in their food preferences, so also do they differ in their cropping system Lagemann (1977) observed that the increasing demand for cassava in the densely populated area of southern Nigeria combined with migration of the active male population to urban areas, has caused a decline in yam cultivation in favour of cassava. He stressed that .the population pressure in southeastern Nigeria has also led to an intensification of intercropping in order to increase the production per unit area. In general, there is a high indication in the importance of intercropping since it has for sometime now become government policy to increase production by improving intercropping systems (Kurt, 1984).

Yam and Yam Based Cropping Systems

Yams rank second to cassava as the most important tuber crop in Africa. In turn, Africa accounts for nearly 98% of world yam production. A total of about 26 million tones of yams are produced on the continent annually (Onwueme, 1989) but from more recent report IITA (2005) explained that according to FAO statistics 37.5 million tones of yam were produced worldwide in the year 2000, 96% of this in Africa and Nigeria is leading producer with 26 million tones. According to a popular Igbo saying, "yam was given to man by God." Hence it is closely linked to the origin of mankind. Yam is part of the religious, social and cultural heritage of many Nigerian tribes and up to date often plays a key role in religious ceremonies (Arinze, 1970). The new yam festival marking the onset of the harvest period is still an outstanding social event almost everywhere in the yam-growing belt of West Africa (Coursey and Coursey 1971). It has been shown that several yam species originated from West Africa (Coursey, 1975, Ustimenko-Bakumovsky, 1983, Howard and Warren 1988, Onwueme and Sinha 1991, Degras, 1993) Thus, yam is truly an indigenous crop in the cultural and biological sense (Lothar 1982). Above all, yam has remained widespread among West African farmers up to date. The center of production however, lies in Nigeria (Lothar 1982, Onwueme 1978, Coursey 1975, Onwueme and Sinha 1991).

Yam, Dioscorea is a monocot (Onwueme and Sinha 1991). It is a large genus of over 600 species with subterranean tubers or rhizomes and it belong to the family Dioscoreaceae (Ustemenko-Bakumovsky 1983, Daisy 1987, Onwueme and Sinha 1991, Degras, 1993). The gender Dioscorea which include many species predominantly are spread in the tropical and partially in the subtropical countries of the world (Ustimenko-Bakumovsky 1983, Daisy 1987, Onwueme and Sinha 1991, Howard and Warren 1988). The tubers are storage organs and often grown to a considerable size. They produce short fibrous roots and annual shoot, which are twining except in the dwarf species and the direction of twining is specific (Daisy 1987, Onwueme 1978, Onwueme and Sinha 1991, Degras 1993). Some of the yams species produce bulbils in the axils of the leaves, which have the morphology and appearance of a condensed stem, and in a few instances are relatively large and tuberous. Yams usually flower and the flowers are small, and borne on long racemes, with male and female flowers separate and mainly borne on different plants (Ustimenko-Bakumovsky 1983, Daisy 1987, Onwueme 1978, Degras 1993). According to Degras, (1993), Onwueme

and Sinha (1991) all cultivated species of yam are large leafed with a thin coiling or lodging stem and juicy tubers. The most widely distributed species of yams are the Dioscorea alata, Dioscorea rotundata, Dioscorea esculenta Bourk and the Guinea yam Dioscorea cayenensis which are wide spread in West Africa. Yam is a perennial plant and the tubers mature in 6-10 months and remain dormant for 3-6 months when stored, depending on species and cultivars.

Yams grow best in deep, well-drained soils with a rainfall of 1000-3000mm in the absence of frost. Dioscorea alata for instance may be grown up to an altitude of about 2000m (Onwueme 1978, Bourke 1982 Daisy 1987, Onwueme and Sinha 1991, Degras 1993). The freshly harvested yam tuber consists of 70% water, 25% starch, 1-2% protein and only traces of sugar and vitamin (Onwueme and Sinha 1991, Howard and Warren 1988). The yam tuber can be boiled and eaten with oil, can be roaster or processed into yam flour. Some other processed yam forms include yam chips, which are used as snacks and yam flakes (Onwueme 1978, Onwueme and Sinha 1991, Degras 1993, Daisy 1987).

Many researchers reported that the average yield of yam is at 10-12 tonnes per hectare but may range from less than 10 to more than 50t/ha (Onwueme 1978, Bourke 1982, Ustimenko-Bakumovsky 1983, Quin 1984, Daisy 1987, Onwueme and Sinha (1991), Degras 1993).

Yams are usually intercropped with maize, and vegetables such as cucurbits, pumpkins, peppers and Okra (Daisy 1987). Monoculture is increasing in certain areas of West Africa and Caribbean. However, in yam producing areas of Nigeria, mixed intercropping with maize and cassava or sorghum is prevalent. Kurt (1984), reported that yam is normally planted after bush clearance early and late yam (D. rotundata and D. alata) are usually planted in the same field, either mixed or sole and interplant with cowpea or low populations of maize, cassava, vegetables and plantain. In terms of crop gender relation, yam is man's crop with the men preparing the land, planting the yam and selling the harvest. Women only help in weeding and interplant their crops at the foot or between the mounds. In the subsequent year, maize and/or rice are planted also intercropped with various minor crops while groundnut and cowpea are the main legumes intercropped with yam. Okigbo and Greenland (1976) reported that over 59% of yams and 75% of maize grown in Nigeria are intercropped. Yam/maize/melon and yam/maize/cassava are the most dominant yam based crop combination in the acid soils of the rain forest zone of Nigeria (Agboola 1979, Ezeilo et. al. 1975).

Yam/maize/cassava intercrop is productive and compatible mainly because maize is a short season crop while cassava and yams are long duration (7-12 months) crops (Ibeawuchi, 2004). The two component crops of yam and cassava provide an example of the presence of competition gap within the period each of the component crops makes maximum demands on the environmental growth resources (soil-moisture, soil nutrients, light etc) and this results in higher total yields than the sole crops (Andrew 1972, Kassam and Stockinger 1973, Okigbo and Greenland 1976, Ikeorgu et al 1989). In most traditional yam based farming systems, yam is usually the first or one of the first crops to be planted after the land is cleared from bush fallow (Onwueme and Sinha 1991, Degras1993). This is because of its high fertility requirements, its relatively long growing season and the high value that farmers attach to the yam crop. In continuous cropping, yam usually occupies a portion in the rotation where it can benefit from high soil fertility, usually after following a legume, except for a nematodes legume crop carrier.

Yam breeding is difficult due to loss of efficient sexual reproduction, a consequence of prolonged vegetative propagation. Another important factor contributing to the paucity in developing new commercial high yielding yam lines may likely be due to limited sustainable attempts at breeding the crop for high yields (Nwachukwu and Igbokwe, 2002). However, some researchers (Nwachukwu and Obi, 1999, 2000, Nwachukwu et al 2002) have shown that yam lines developed by hybridization and mutation induction by gamma ray irradiation of true yam seeds out yielded our local cultivars by more than 50%.

Ikeorgu (2002) while studying the use of maize and Telfairia occidentalis to improve the productivity of irrigated yam grown during the dry season explained that in tropical rain forest of Nigeria growing of yam during the dry season is not yet common. There is hardly any report on dry season yam production apart from the few practiced along Niger River flood plains in Bayelsa and Anambra states. However, maize, Telfairia occidentalis and maize/telfairia occidentalis components depressed yam yield by 34.32%, 11.76% and 16.89%, respectively, indicating that the total calorie productivity and monetary value were highest where the three crops were intercropped. Ibeawuchi et al (2005) examined the effect of Okra and Melon introduction on the productivity of yam mini-sett and they reported that introduction at 10 WAP gave significantly higher tuber yields ($P \geq 0.05$) and low yield of melon seeds than introduction made at 0 and 5 WAP. They observed 44% yam mini-sett tuber depression at 5WAP in Okra /melon plots whereas, combination with melon alone gave a 22% yam mini-sett tuber depression possibly because the melon crop

failed. They also reported that introduction at 0 week depressed yields of yam mini sett tubers by 41,39 and 48% respectively in crop combinations with Okra, Melon and Okra/Melon.

Cassava and Cassava Cropping System

Cassava (Manihot esculenta L. Crantz) is a dicotyledonous plant growing 1-3m high and belonging to the family Euphorbiaceae (Spurge) the Manihot gender. The plant originated in Brazil with Central America as a likely additional center of origin (Onwueme 1978, Howard and Warren 1988, Ustimenko-Bakumovsky 1983, Pierre 1989, Onwueme and Sinha, 1991). Its world production of 136 million tones in 1985 puts cassava in the sixth position after wheat, maize rice, potato and barley. It is widely spread throughout tropical Africa, Asia and South America, being particularly important in Brazil, Thailand, Indonesia, Zaire and Nigeria (FAO Production Year Book 1985). Nigeria is currently the world-leading producer. Cassava is today grown to some extent in practically every country within the tropical belt. The greatest production is found in West Africa and the Congo basin. Nigeria, Zaire, Tanzania, Mozambique and Ghana are the leading countries for cassava production in Africa (Onwueme and Sinha 1991). Cassava a diploid species (2n=36) is one of the principal plants of use to man because of the important role it plays as food (Pierre, 1989). In Nigeria cassava is prepared as cassava fufu and served with vegetable soups. Boiled cassava and cassava chips are eaten with coconut, groundnuts, fish or meat. Salads made with cassava are usually well balanced and nutritious (Onwueme 1978, Pierre 1989). Industrially, cassava chips are used for animal feed. Also, cassava is processed to make syrup and mono-sodium glutamate, which enhances the flavour of other processed foods. It is used in the manufacture of biscuits, ice cream, glue and textile (Ustimenko-Bakumovsky 1983), Mayhew and Penny 1988). Cassava is currently increasing in importance, particularly in drier areas, because it is a hardy drought resistant crop that can give acceptable yields on low fertility soils (Larsen 1984, Thaman and Thomas 1982, Bourke 1982, Richard and Coursey 1981). Cassava is propagated from stem cuttings and requires weeding until canopy is established. The roots mature in 10-14 months, but are not harvested until required (Howard and Warren 1988). The roots deteriorate after 1-3 days exposure to air in the tropics. The plant is unique in that its roots are not organs of dormancy and hence has no natural functions in preservation of the plant through an adverse season (Coursey 1982). The poor storage qualities of cassava present a major problem (Richard and Coursey 1981, Richard 1985). The approach of not harvesting it until required is disadvantageous because large areas of land are occupied in storage of mature cassava (Coursey 1982). The average yield of cassava worldwide is 9.6t/ha which is less than that for sweet potato and yams, but greater than that for taro (FAO Production Yearbook 1985). Pests and diseases of cassava are severe in Africa (Halm et al 1979) but the crop is free of most pests and disease problems in the pacific (Bourke 1982). However, the major confronting problem with cassava is its cyanide content founded in free and bound forms (Howard and Warren 1988) although most of the cyanide can be removed by post harvest treatments and cooking (Conn 1973; Cooke and Coursey 1981). Cultivars of cassava may contain from 1 to 100mg HCN/100g fresh peeled tuber and there are larger amounts present in the peel and the leaves (Howard et al 1988).

According to Kurt (1984) although cassava is most common in the forest region and in the southern Guinea savannah, cassava based cropping systems are mainly found on poor sandy soils of the coastal belt where food crops other than cassava hardly give satisfactory yield except coconut or oil palm. He further explained that cassava is commonly associated with maize and cowpea. With the increasing length of the cultivation period, and decreasing soil fertility, cassava is the predominant staple crop in many regions of the rain forest and southern Guinea Savannah, replacing especially other root and tuber crops like cocoyam and yam, and maize to some extent.

Onwueme (1978) reported that cassava crop is usually relay-intercropped with yam (Dioscorea spp), maize, melon and okra as the last component. Mixture yields of cassava in cassava/maize, or cassava/beans or cassava/groundnuts were reported to be similar to that of sole crop yield (CIAT 1980). Hart (1975) compared cassava/maize/beans mixture with their respective sole crops and reported the highest net economic returns when the three crops were intercropped without fertilizer. The study showed that the variation in crop morphology were such that beans did not allow weed invasion during the first two months, the maize crop also excluded weeds between three and four months after planting during which cassava component developed enough canopy to cover the rows. Adetiloye and Ezuma (1988), while assessing the performance and production of plantain and cassava intercropping systems noted that growth, harvestable yield and productivity of intercrop components were essentially influenced by the population of individual crops more than the population of other components. It is best to introduce maize three weeks after cassava for cassava/maize intercrop while cassava should be introduced 28 days after planting yam/maize and in

each case fertilizer must be applied. Ikeorgu et al (1988) and Jerome et al (1988) reported that in a cassava/maize association, the maize component depressed the fresh storage root yields of cassava by 38%, and that this depression increased with increasing maize population. However, 10,000 plants/ha for cassava and 20,000plant/ha for maize in a cassava/maize intercrop gave the best combination and appeared to be optimum population.

Evaluating the productivity of cassava-yam-maize in the rain forest of Nigeria, Unamma et al (1988) reported that by intercropping, the farmer can obtain the same output as for sole cropping cassava, yam or maize and still have a two year average of 45-67 per cent more land available for other purposes. Similar results were obtained whether the three crops were planted the same day or the cassava component was introduced at 28 or 56 days after planting and maize with or without fertilizer application. The report further observed that on the monetary terms, the mixture in which cassava (Cultivar Abii) was introduced at 56 days after planting maize with fertilizer gave the highest income of N13298.00 per hectare, which was 45 percent greater than the best sole crop income of N6, 818. 00/ha realized from Nwopoko Cultivar that received fertilizer. Based on energy value, the mixture in which cassava was introduced at 56 days after planting yielded 13x104Kcl/ha out-performing the rest of the alternative treatments and produced 38 percent more calories than the best sole crop of cassava as it yielded 8 x 104 Kcal/ha). Ofoh and Lucas (1988) reported that intercropping significantly reduced the yield of cassava and melon but did not affect the yield of maize. However, cassava and maize reduced the yield of melon significantly ($P \leq 0.05$) but cassava had more depressive effect on melon. They reported that there were no significant difference in soil N, P, K Mg Ca, organic carbon and pH resulting from the various cropping systems investigated. They concluded that soil temperatures were significantly low ($P \leq 0.05$) in plots of mixture of legumes and the four crop mixtures. In intercropping of cassava/maize with Mucuna pruriens, Canavalia ensiformis and cowpea, and the corresponding sole cropping of these component, Usman et al. (2002) reported that Mucuna pruriens with application of 100 kg N/ha, 60 kg P_2O_5/ha and 60 kg K_2O/ha, produced significantly higher plant biomass while the lowest was obtained from cowpea with or without application of fertilizers. They reported that cassava/maize/mucuna intercrop produced significantly higher yield of cassava than other treatments and that maize yield was highest when it was preceded by cassava/maize/mucuna with NPK application. The potentials of Mucuna pruriens was observed in Cassava/Maize/mucuna which gave higher yields of cassava roots and high returns at end of the cropping period (Ibeawuchi, 2004)

Maize and Maize-Based Cropping Systems

Maize (Zea mays L.) originated in Mexico in Central America. It is the most important cereal crop in the world after wheat and rice (AID, 1974; Ustimenko-Bakumovsky, 1983; Onwueme and Sinha, 1991; Purseglove, 1972). It is a major item in the diet in many tropical countries whereas in the temperate regions maize is the main grain used for animal feed (Purseglove, 1972). Maize belongs to the family gramineae to which most of the grass species belong. It is an annual monoecious plant. In many African countries, maize is the basic food for subsistence farmers, miners and city dwellers; its importance is as great as that of wheat in the mid East and rice in South East Asia (NRC, 1988).

According to Byerlee and Winkelmann (1981), Africans consume nearly one-fourth of the world's total maize. Onwueme and Sinha (1991) pointed out that the major maize producing countries in tropical Africa are Tanzania, Kenya, Zimbabwe, Zambia, Nigeria, Ethiopia, Malawi, Ghana, Cameroon, Cote d'Ivoire, Mozambique and Zaire. They reported that in 1989 the total maize production was 36.4 million tones. In the tropics, maize is eaten in many different ways. It is prepared and consumed in a multitude of way that can be grouped as follows – ground or pounded and baked or fried, boiled whole, roasted whole, fermented. In Nigeria maize is consumed mainly in two forms (ogi) (pap) and (agidi). Maize is industrially important chiefly for the production of alcohol, oil and starch (Onwueme and Sinha, 1991; Mayhew and Penny, 1988). Ustimenko-Bakumovsky (1983), RRIM (1975), Mayhew and Penny (1988) further stated that in the industry maize is processed for technical oil, ascorbic acid and glutamic acid. They explained that the extracts from maize style (spadix filaments) are used in medicine while the stalks can be used in making papers and cardboards, plastics, menthol and tar.

Due to high variability in climatic conditions, diverse soil types, population density and socio-economic factors, maize cropping systems are very diverse. They include intercropping systems for risk management and efficient use of land and labour resources and sole cropping systems. Sole cropping maize can be produced from high fertilizer inputs to sole cropped maize rotated with legumes or maze produced with integration of maize produced with organic and inorganic inputs (Mafongonya et al., 2003).

They reported that maize intercropping systems are very common in large areas of East and Southern Africa. They reported that maize and beans (Phaseolus vulgaris) are predominant in East Africa while in Southern Africa maize is intercropped with cowpea (Vigna unguiculata), groundnuts and bambara nuts to a less extent. Furthermore, they reported that, the low plant densities of legumes found in most intercrops mean that they can input modest amounts of N and organic matter each year to maintain soil fertility.

According to Okigbo (1977) maize is intercropped with vegetables and other crops in traditional agriculture mainly to satisfy dietary requirements. In Nigeria, maize is often found severally intercropped with assorted crops thereby forming an integral component of various cropping systems. Ikeorgu (1983) reported that maize compatibility in mixtures was attributed to the fact that it is a C4 plant, and giving reasons why C4 plants are successful in most cropping systems. Crookston and Kent (1975) upheld that they have higher temperature requirements for optimum growth and more so, respond to higher light intensities and they remove carbon dioxide from the atmosphere than the C3 plants.

Agboola and Fayemi (1971) reported that in maize/cowpea intercrop, yields of maize were improved with associated cowpea crop, which provided about 25 kg N per hectare to maize crop through nodulation but noted that the intercrop yield of cowpea was lower than the sole crop. Researchers in RRIM (1975) reported that the intercrop yield of maize was within the range of 2.2–4 t/ha in maize-rubber mixture. They also observed that root and stem lodging of intercropped maize was less in maize/melon than in sole maize or even maize/cassava. Also, researchers in IITA (1975) stated that maize grain yield was hardly reduced by intercropping maize with cow pea. However, Wahua et al. (1981) and Eriksen and Whitney (1984) reported that in maize/cowpea mixture, maize is the dominant crop and its shading effects on cowpea have been established to reduce cowpea grain yield

A cropping system or a crop production system consists of the cropping pattern in terms of crop combination, spatial arrangement and sequences of cropping in addition to the resources and input management and technology involved in the production of the desired products (Okigbo, 1978).

For centuries in the tropics, farmers have taken the advantage of the year round favourable temperature and solar radiation when water is available, to produce a number of crops simultaneously on the same piece of land (Ikeorgu, 1983). This system, which involves the practice of growing several crops on the same piece of land, is referred to as multiple cropping (Gomez and Gomez 1986). It is an ancient strategy for crop production among farmers in the tropics. Traditionally, subsistence farmers primarily to increase the diversity of their products and to achieve stability and sustainable agriculture use this method. Since nature consistently integrates her plants and animals into a diverse landscape, a major tenet of sustainable agriculture is to create and maintain diversity (ATTRA 1998). Nature is efficient; there is no waste product since outputs from one organism become inputs for another. The principles by which nature functions explains that the death of one organism becomes food for other organisms and since we are modeling nature, we must understand and utilize these principles to reduce costs and increase profitability, while at the same time sustaining our land resources.

In Nigeria, the traditional farmer finds it more satisfactory to plant a diversity of crops than planting sole. It is cheaper for farmers to grow many of their own requirements than to buy them (Kurt, 1984, Gomez and Gomez 1986). The human environment changed tremendously, when the early human replaced hunting and gathering of food with domestication of crops and animals. By producing a limited selection of crop plants and animals, human kind has greatly reduced the level of biological diversity over much of the earth. Annual crop monocultures represent a classic example and in response to this biological simplification, nature has struggled to restore diversity to these landscapes – that is her tendency (ATTRA, 1998). Our "war" with nature over the tendency towards diversity is what we call "weed control" and "pest management". We could hardly produce any crops if we simply allow our fields to be covered by natural vegetation (weeds) but with the benefit of diversity we can realize some reasonable yields by planting mixture of different crops that help to suppress weeds.

In crop mixtures, cooperation is more apparent than competition and there is far more cooperation in nature than competition. Cooperation is typified by naturally beneficial relationships that occur between species within communities. An example is the relationship that exists among squirrels, fungi and trees in the redesigned forest (Maser, 1990). However, stability tends to increase with increasing diversity because if a crop field is abandoned, it will first be colonized by just a few species of plants, insects, bacteria and fungi. After several years, a complex community made up of many wild species develops and once wild plant and animal community has reached a high level of diversity, it appears to remain stable for many years (ATTRA, 1998). Also, the more complex and diverse communities become, the fewer the fluctuations in numbers of a given species, and the more stable communities tend to be. As the number of

species increases, so does the web of interdependencies, in both lower and higher rainfall years. There are fewer increases in any one species and fewer fluctuations in the community as a whole (Savory, 1998). ATTRA (1998) explained that in pursuing diversity on the farm, we could begin to model them after some natural principles. Some pioneering farmers were able to utilize nature's principle of diversity to their advantage. Some results of their efforts include lower cost of production and higher profits, which is the main target of any farmer.

Most recently, the urban agriculture sprang up and is fast expanding and helping to feed the teeming urban population in Nigeria and West African sub region.. Uncompleted buildings, road sides and fallow lands within the urban areas are seriously being used for intensive farming ranging from rearing of animals to horticultural productions such as ornamentals and vegetable crop production. This system is now feeding a lot of indigenes who at the establishment of the township were displaced and other urban inhabitants who find farming very interesting and economic as an avenue to create wealth and sustain their families.

Intercrop Productivity

One of the most important reasons to grow two or more crops together is the increase in productivity per unit of land. Researchers have designed several methods for assessing intercrop performance as compared to pure stand yield (ATTRA, 1998) but the use of the land equivalent ratio (LER) has become common practice in intercropping studies, because of its relatively simple concept (Kurt, 1984). The land equivalent ratio (LER) may be defined as the relative land area under sole crops that is required to produce the yields achieved by intercropping (Kurt 1984). It is usually stipulated that the "level of management" must be the same for intercropping and sole cropping. In this regard, intercrop and sole crop have to be at their optimum populations as differences in population affects yield responses (Huxley and Maingu 1978).

Kurt, (1984) noted that an important concept inherent in the use of LER is that, whatever be their type or level of yield, different crops are placed on a relative and directly comparable basis. He further explained that based on land areas, LER also reflects relative yields (the numerical yield total is numerical to LER) i.e. the LER can be taken as a measure of relative yield advantage.

The LER is calculated as follows:

$$LER = L_A + L_B + \ldots\ldots + L_N = \frac{Y_A}{S_A} + \frac{Y_B}{S_B} + \ldots\ldots + \frac{Y_N}{S_N} = \sum_{I=1}^{N} \frac{Y_N}{S_N}$$

Where L_A, $L_B \ldots L_N$ is the LER for the individual crops

Y_A, $Y_B \ldots Y_N$ are the individual crop yields in intercropping.

S_A, $S_B \ldots S_N$ is their yields as sole crops

When LER is greater than 1 or more, it signals yield advantage and a ratio of less than 1, is yield disadvantage (ATTRA, 1998, Kurt, 1984).

Soil fertility maintenance: Soil losses and run-off is limited because the practice of intercropping, more especially multi-storey cropping provides a nearly continuous soil cover thus preventing it from the direct impact of the rains (Kurt, 1984; Gomez and Gomez, 1986). They pointed out that intercropping produces a dense and diversified root system and this reduces leaching of nutrients. Okigbo and Lal (1979) reported that relatively simple intercropping system as maize/cassava can increase of CEC (cation exchange capacity), and pH as well as increase Mn content in the soil. According to Ibeawuchi and Ofoh,(2003) in an intercropping of cassava /maize/food legumes the decaying litter form humus and organic acids that form complexes with Iron(Fe) and Aluminum (Al) thus reducing considerably the ability of soil to tie up Phosphorus (P) hence making it available in the soil. Furthermore, the integration of trees into cropping systems in the form of alley cropping is another means of maintaining soil fertility. It reduces soil erosion and leaching with the help of the root systems by "pumping up" nutrients to the surface from layers beyond the root systems of annual crops (NRC, 1984). In a farmer-oriented research, IITA Ibadan Nigeria has for several years developed a method for planting giant leucaena as an intercrop with corn, yam and rice. In the growing season the trees are kept cut and pruned so that they do not shade the nearby crops. The resulting leaves and twigs are used as nitrogen rich mulch while the larger branches serve as poles or firewood. In the dry season, the tree intercrops are allowed to re-grow and draw nutrients from deep soil levels (IITA, 1979).

Soil Nutrient uptake: Several scientists have compared nutrient uptake in crop mixtures and in pure stands and showed that crops extract more nutrients from the soil when grown in mixture than when grown in pure stands Dalal (1974) compared maize and pigeon pea mixture with maize, and observed that the differences in growth duration of the components crops tend to minimize competition. Kassam and Stockinger (1973) shared the same view in reporting that intercropping systems were most rewarding in terms of yield of the component crops when there was a competition gap between the periods the components crops made maximum demand on the micro-environment (soil nutrient, soil moisture, light etc). Agboola and Fayemi (1972) reported the need for intercropping with legume as the tropical legumes were capable of fixing large amounts of nitrogen when grown in mixture with maize. They concluded that the maize component received an equivalent of about 25 kg N/ha from the associated legume.

Water use efficiency: According to (Okigbo, 1978) and Kurt (1984), intercrops have better water use efficiency than sole crops. They explained that this is of special importance for farmers in the semi arid tropics where water is the main limiting factor of production. They reported that one of the reasons for increased water use efficiency of intercrops is the windbreak effect. Okigbo (1978) observed that when low growing crops are interplanted with tall growing ones, this leads to reduced evapotranspiration. Again, there is a low population for the residual moisture at the end of the growing season which is another means of using available soil moisture more efficiently by intercropping (Rao and Willey, 1980; Okigbo, 1978).

Yield Advantage

Intercropping has been reported to have yield advantage over sole cropping. These advantages can occur as a result of complementary use of growth resources such as nutrients, water and light by the component crops (Enyi, 1973). The yield advantage may be in terms of higher yield or higher net income. He further explained that the yield can be quantified in terms of dry matter production, grain or root yields, nutrient uptake, energy or protein production and market value. According to Kurt (1984) and Gomez and Gomez (1986) the yield advantage is measured using land equivalent ratio (LER) or Relative yield totals (RYT). LER is defined as the relative land area under sole crop that is required to produce the yield achieved by intercropping at the same management level. While RYT is the sum of the ratios obtained from the relative yields (intercropping yields divided by respective sole crop yields) of the component crops in a mixture using the above calculations, yield advantage have been reported for cassava/maize and cassava/beans mixtures (CIAT 1979). According to Ikeorgu et al. (1983), cassava/maize intercrop gives higher amount of calories per hectare of land than the pure stands. Also, land equivalent ratio of 1.71 has been reported for cassava/maize intercrop (CATIE, 1977)

Stable yield: Another major advantage of intercropping is yield stability. That means a reliable food production over years that provides a high income for the farmer and enhances diversity of farm products (Rao et al., 1979). Gomez and Gomez (1986) felt that intercropping does not only enhance diversity of farm products but also provides insurance against crop failure. They reported that with diversified crops, intercropping stabilizes yield through the principle of compensation. They explained that when one crop component suffers from pests, diseases, draught etc, the loss of this crop is compensated at least partially by the other component crop(s) since there is now less competition for growth resources, and stated that there would be no compensation if it were to be only a sole crop system.

Pest: Intercropping can play a significant role in integrated pest management. There are many cases where pests and especially weeds are suppressed by certain crop combinations like maize/soybean, maize/black gram, maize/velvet bean (Chaud and Sharma, 1977). They reported that in all the crop combinations there were pest (stem borer) reduction in all intercropping involving maize and another crop when compared to maize grown sole.

According to Moreno (1979) intercropping cassava and maize significantly delayed the onset of the cassava scab (Spaceloma spp) epidemic. Also, when cassava is planted in association with maize and common bean, there is less rust (Uromyces manihotis) on cassava. Arene (1976) and Ene (1977) reported that there was significant reduction of cassava bacteria blight (CBB) (Xanthomonas manihotis) by intercropping with maize, melon or other crops in Nigeria. They concluded that this reduction in CBB could be ascribed to the earlier and better soil cover provided by the intercrops, which at least to a significant level prevented splashing of the bacteria from the soil onto cassava leaves and stems.

Wolfe (2000) reported that farmers in Yemen province of China under the direction of international team of scientists made some simple changes in their rice production methods. They changed from planting their typically pure stand of single rice variety to planting a mixture of two different rice varieties. This technique helped in reducing blast disease and the farmers were able to abandon using chemical fungicides, which they had been using.

Weed and intercropping systems

Onwueme and Sinha (1991) reported that a weed is plant growing where man does not want it to be. They explained that any kind of plant can be a weed as long as it exists in a location or situation where it is considered undesirable. Fadayomi (1979) pointed out that weeds constitute a major limiting factor in food production in Nigeria. After a long research, FDA (1994) reported that one of the major tasks facing Nigerian farmers in their effort to feed the nation is the absence of adequate technologies to control weeds. They stressed that until adequate attention is paid to the weed problems confronting different categories of farmers, real progress cannot be made in agricultural development in Nigeria. The total land area a farmer can cultivate is determined to a great extent by how much labour is available to him for weed control. Also in many cases only a farmer and his wife are available to face this great task because their children of school age are generally away from home.

Akobundu (1987) reported that weeds determine the farm size and limit of crop production potential of peasant farmers and indirectly affect the well being of farm families. According to Lavabre (1991), weeds to some extent affect all crops but how serious this is depends on the species and circumstances. He explained that average crop losses due to weeds are estimated at 25% but may be as high as 50% or over 80% with certain food crops. Also, Reminson (1978), and Nangju (1980) reported on the reductive effect of weed on crop production and indicated that 51% reduction in cowpea yield was due to weed infestation, 65% in cassava, 73% in yam and 80% in maize. Akobundu and Poku (1987) reported that in Nigeria, yield reductions have also been attributed to poor crop establishment and stand reduction in growth, pest and disease infestation.

Kurt (1984) explained that yield losses due to weeds are considerable in the tropics and can exceed 50%. He reported that weed infestation increases with time from clearance onward and after three years the farmers are often forced to abandon a field and clear a new one, because the time needed for weeding is greater than time needed for clearing forest or bush. In Western Nigeria, at least 50% of a farmer's working time is spent on weeding (Moody, 1975), and the situation is similar in other regions.

Most crop combinations suppress weed growth by providing an early ground cover, due to high plant population or fast growing component crop e.g. melon. Even though, the yields of the domesticated crops are often considerably reduced, this is still more than weed would produce in the same place (Evans, 1960). In many intercropping systems, only one weeding is required to produce optimum yields instead of three or more in sole crops. Often times, this weeding is combined planting another intercrop thus further reducing the time required solely for weeding (Kurt, 1984).

Moody (1977) found that in Asia crop associations of maize and groundnut, mung bean or sweet potato were excellent for reducing weed growth, yield losses and weeding time. He explained further that in a maize/sweet potato and maize/groundnut crop combination, weed growth was less than in sole cropped groundnut or sweet potato but higher than in sole maize. Researchers in CIAT (1979) reported that in intercropping systems involving cassava/beans, weed growth was reduced in Central America. They explained that with this result, frequent weeding of pure cassava was no more efficient in weed control than intercropping cassava with beans. Hart (1975) concluded that the success of an intercropping system in suppressing weed growth does of course; depend on soil fertility and climate as well. His result showed that suppression of weed is often higher with low fertility than with high fertility soil and the same was valid for low and high rainfall areas.

Conclusion

Due to the nature of our soils, early weeding should be done to reduce completion for nutrients by the component crops and also weeds in intercropping systems.. Row intercropping is encouraged to give room for mechanization which most medium and large-scale farmers advocate. Timely harvest is also advocated to give room to other component crops with longer harvest period. Intercropping helps the resource poor farmers to produce food in excess of what is required by the family and these surpluses are used to feed the ever-increasing world population. By this, intercropping plays a wonderful role in reducing the hunger gap between the haves and have not. It is therefore by its nature a sustainable way of food production and a strategy for resource poor farmers who produce majority of our foods.

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The Effects of 60Hz Magnetic Fields on Plant Growth

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ABSTRACT: The biological effects of extremely low frequency magnetic fields (ELF MFs) on living organisms have been explored in many studies, but few of them investigate how different waveform MFs act upon their growth. In this study, the biological effects of both a 60Hz sinusoidal MF and a 60Hz pulsed MF on the early growth of plants are presented using mung beans as an example. The sinusoidal MF is produced using a specially-made circuit with a circular iron coil and a triac lamp in series which is driven by 110Vrms 60Hz AC power, and the pulsed MF is produced using the same equipment with the knob of the triac lamp adjusted manually. The results indicate that the 60Hz sinusoidal magnetic field has an enhancing effect on the early growth of mung beans, however some morbid state phenomena were observed on the sprout roots. Also, the effect and morbidity rate increases with MF intensity. In contrast, the 60Hz pulsed magnetic field has a slightly inhibitory effect on mung bean growth. [Nature and Science. 2007;5(1):60-68].

Keywords: biological effect; coil; ELF MF; triac

1 Introduction

As electric appliances such as TV, lights, fans, radiators, etc. are increasingly commonplace nowadays, the effects of the extremely low frequency magnetic field (ELF MF) produced by such equipment on the living organism are much more of concern. Most of the magnetic fields induced are of 60Hz sinusoidal waveform because 60Hz AC power is the most commonly used power rating all over the world. But there are many other occasions where the magnetic fields produced are in the form of a pulsed train. Therefore it is vitally important to find out whether there is any effect on living organisms resulting from exposure to either kind of MF.

Several studies have suggested that ELF MFs may modify plant growth and development (Celestino, 1998; Davies, 1996; Picazo, 1999; Rapley, 1998; Smith, 1993, 1995; Stange, 2002), but exposure to magnetic fields induces quite a variety of biological effects and moreover, knowledge of the effects on living organisms is still not very clear. Nevertheless, the points of view described in (Lednev, 1991; Liboff, 1992) imply that an ELF MF may affect ions in cells, and can be described by the following equation:

$$f = \frac{q}{2\pi m} B_{DC} \qquad (1)$$

where f is the frequency of the ELF MF (Hz), q/m is the charge-to-mass ratio of the ion(C/kg), B_{DC} is the magnetic flux density of the static MF (T) that is superimposed on the ELF MF. From Eq. 1, it can be seen that the motion of ions is severely affected by f and B_{DC}. In other words, different waveform magnetic field sources will result in varied biological effects on living organisms because their frequency spectrums are different.

The present study aims to assess the effects of two different waveforms of magnetic field source (a 60Hz sinusoidal MF and a 60Hz pulsed MF) on plant growth using mung beans as test material, and attempts to relate the effects to the waveform spectrum of the magnetic fields. The sinusoidal MF is induced by a specially-designed electrical circuit which is driven by 110Vrms 60Hz AC power, and the 60Hz pulsed MF is produced from the same circuit, using the knob on the triac (two thyristors in inverse-parallel) for adjustment. Using the exposure system proposed in this paper, the MFs can be produced and mostly concentrated in the coil. Since the MF is concentrated, the affects of the MF on the personnel executing this experiment can be reduced. In the experiment, the exposed mung beans were grown in two places with different magnetic intensity, and additionally the control mung beans were grown in an ambient environment to observe any differences in biological effects on the growth of our test material.

2 Materials and Methods

2.1 Plant material

Mung beans are used as the test material subject in this study, and two tests (one under a 60Hz sinusoidal MF, and the other under a 60Hz pulsed MF) are implemented. In each test, beans of the same weight (0.08g) and similar appearance are selected, and separated into three groups of 10, 30 & 30 beans. Three groups are fertilized with distilled water with initial temperature of 31.5±0.5°C. Two groups of them are grown in a magnetic field (exposed group 1 under higher magnetic intensity and exposed group 2 under lower magnetic field intensity), and the other group is placed in an ambient weak magnetic field (control). The reason only 10 beans are used in exposed group 1 is due to the limited space where they are placed. The environmental conditions such as temperature, humidity and illumination of the three groups of mung beans of two tests are maintained, as shown in Table 1.

TABLE 1 The temperature, humidity and illumination conditions in each group of the two tests

Comment: 調整 illumination data

Item	Sinusoidal MF			Pulsed MF		
	Exposed 1	Exposed 2	Control	Exposed 1	Exposed 2	Control
Position	In the airgap	Adjacent the airgap	Ambient environment	In the airgap	Adjacent the airgap	Ambient environment
Temperature (°C)	28.0±0.2	28.1±0.2	28.2±0.2	25.5±0.5	25.5±0.5	25.5±0.5
Humidity (%)	48.2±0.1	48.2±0.1	48.2±0.1	54.1±0.2	54.1±0.2	54.1±0.2
Illumination (LUX)	608±2	609±2	605±2	598±2	599±2	597±2

[a]The environmental conditions of the two tests are slightly different because they are carried out in the different seasons.

After the three groups of mung beans have been absorbing water for 8 hours, they are taken out, dried and weighed to measure the water absorption rate. The residual water is then poured out and both groups of beans are put back in their original positions to continue growing. After they have been growing for a total of 24 hours, all groups of mung beans are taken out, and each mung bean's sprout diameter and length are measured.

2.2 Exposure system

The purpose of this study is mainly to assess the influence on the early growth of mung beans exposed to a 60Hz sinusoidal MF and a pulsed MF. The equipment needed in this experiment includes a resistor (triac lamp), an inductor (winding coil), oscilloscope, frequency analyzer, etc. The complete set-up is shown in Figure 1.

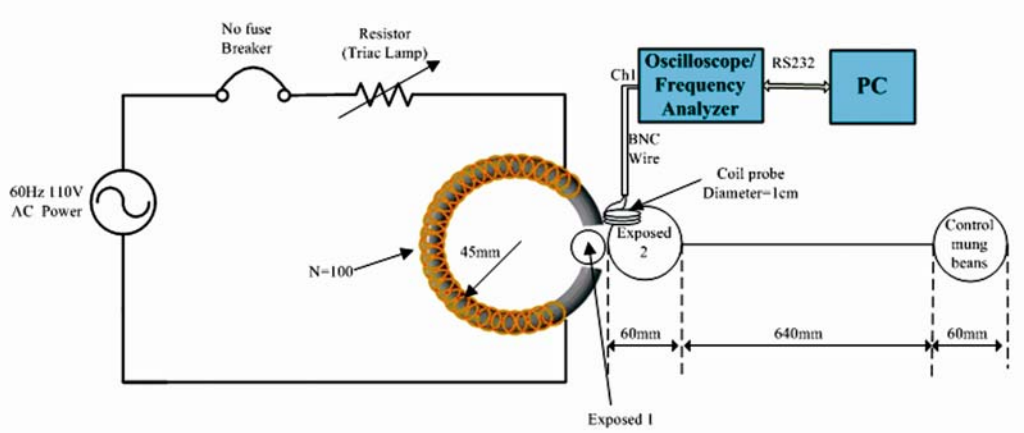


Figure 1. The complete set-up in this experiment

As shown in Figure1, the electrical power used to drive the circuit is 1ψ 110V 60Hz AC power. The circular coil with outer diameter of 130mm and a 24mm air gap (cross section diameter = 28mm) is wound 100 turns with copper wire. The magnetic flux density (B) circulating in the iron core and air gap can be theoretically expressed in the following equations:

$$B = \frac{Ni}{R}$$
$$R = \frac{l_c}{\mu A} + \frac{l_g}{\mu_0 A} \qquad (2)$$

where R is total magnetic reluctance of the core and air gap, μ and μ_0 are the magnetic permeability of the core and air respectively, A is the cross sectional area of the circular core, N is the number of turns of wire, i is the current flowing through the wire and l_c & l_g are the core circumference and air gap distance, respectively.

When the knob of the triac lamp is set to the high level position, the MF flowing in the iron coil is in a 60Hz sinusoidal form. However, when the knob is adjusted to the low level position, the MF produced shows as a pulsed train, as shown in Figure 2 and Figure 3. The locations of the three groups of mung beans are shown in Figure 1. In order to confirm the magnetic flux density (B) in each position of two tests, a magnetic meter is used to measure the B value at each position. These data are listed in Table 2.

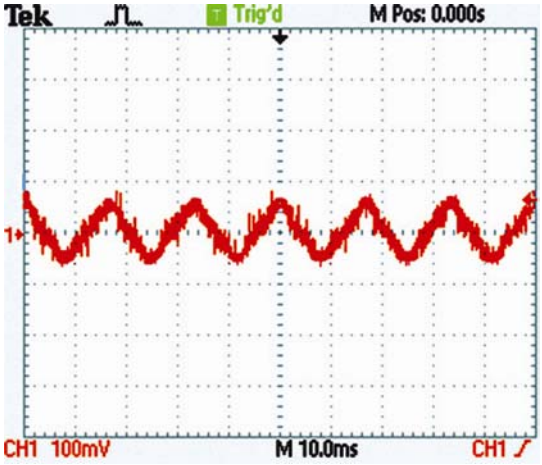


Figure 2 The 60Hz sinusoidal magnetic field produced. Due to the poor quality power used and impedance mismatching between the coil probe and oscilloscope (shown in Figure 1), the waveform of the MF does not look like a perfect sine wave.

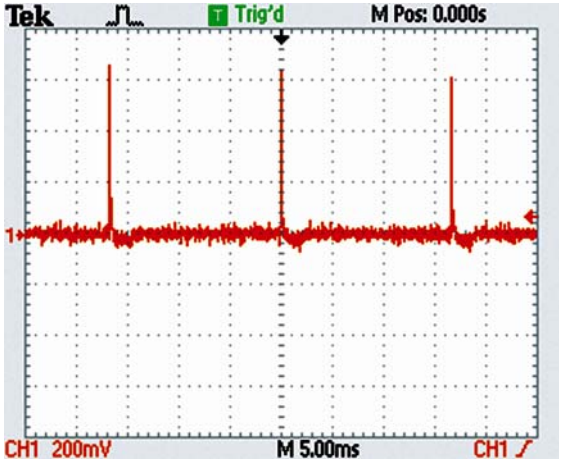


Figure 3. The 60Hz pulsed magnetic field induced

TABLE 2. The magnetic field intensities produced in each group of mung beans of the two tests

Test	Exposed 1	Exposed 2	Control
Sinusoidal MF	235.0 μT	94.1 μT	0.17 μT
Pulsed MF[a]	11.1 μT	6.2 μT	0.20 μT

[a]The pulsed MF intensities are measured using a magnetic meter which has limited bandwidth, so the peak value will be higher.

3 Results

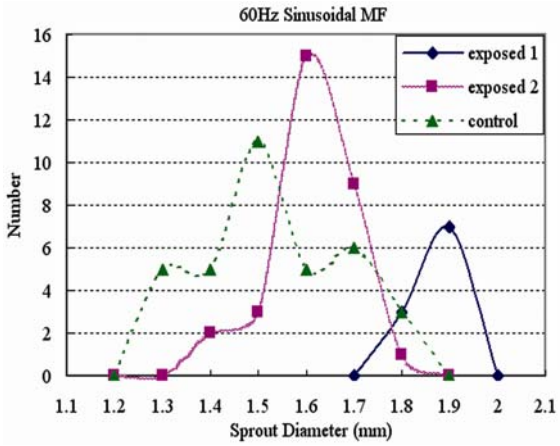
3.1 Water absorption

After 8 hours fertilization with distilled water, the three groups of mung beans were taken out to be observed for germination and weighed. Exposed to the sinusoidal MF, the average weights of the mung beans in both the exposed 1 and exposed 2 groups were 0.180g, and the average weight of the control mung beans was 0.140g. The weight ratio between the exposed and control mung beans was 1.29. This result

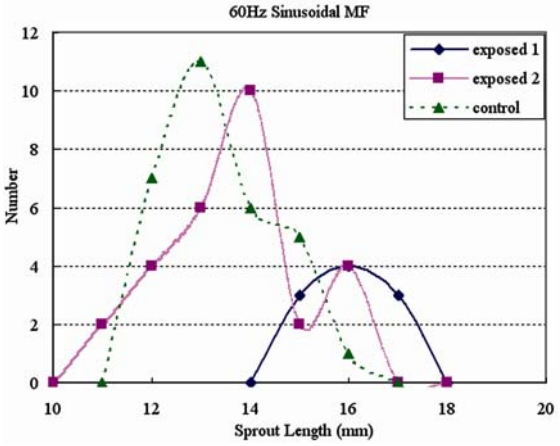
indicates that the mung beans that had been exposed to the sinusoidal MF had absorbed much more water compared with the control mung beans. As for the other test exposed to the pulsed MF, the average weights of the mung beans in both the exposed 1 and exposed 2 groups was 0.136g, and the average weight of the control mung beans was 0.145g each. The weight ratio between the exposed and control mung beans was 0.94. This result shows that the mung beans that had been exposed to the pulsed MF had absorbed less water compared with the control mung beans.

3.2 Early growth

The three groups of mung beans were then put back in their original positions for the remaining 16 hours of the experiment. After they had been growing for 24 hours, the sprout diameter and length of the mung beans in three groups were measured. For the test exposed to the sinusoidal MF, the distribution of the sprout diameters of the three groups with 24 hours growth is sketched in Figure 4(a), and that of their sprout lengths is shown in Figure 4(b). The statistical data are listed in Table 3. As shown, the average sprout diameter and length of the mung beans in exposed group 1 were the greatest of all three groups, and those of the mung beans grown in the ambient environment were the least. Therefore, a sinusoidal MF has an enhancing effect on the early growth of mung beans, and the effect gets stronger as the MF intensity increases.



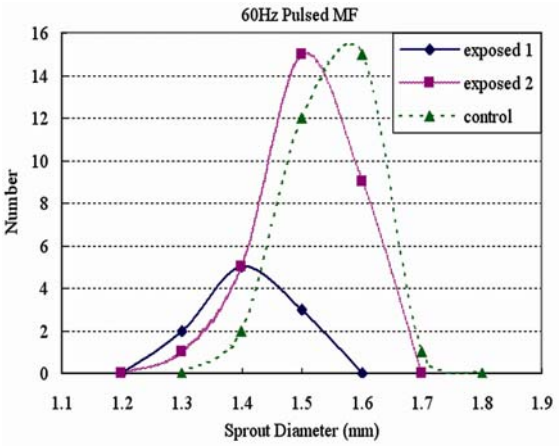
(a)



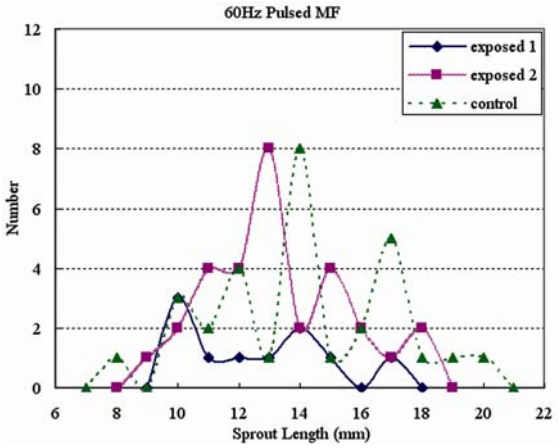
(b)

Figure 4 (a) The sprout diameter distribution of the mung beans exposed to the sinusoidal MF. (b) The sprout length distribution of the mung beans exposed to the sinusoidal MF. Both growth distributions shift higher with increasing MF intensity. (There are 10 sample beans in exposed group 1, which is less than the other groups because of limited space in the iron core airgap)

As for the test exposed to the pulsed MF, the distribution of the sprout diameters of the three groups with 24 hours growth is sketched in Figure 5(a), and that of their sprout lengths is shown in Figure 5(b). The statistical data are also listed in Table 3. As shown, the average sprout diameter and length of the mung beans in exposed group 1 were the least of all three groups, and those of the mung beans grown in the ambient environment were the greatest. The difference is not very significant because the magnetic field intensity used in this test is smaller than that in the sinusoidal test. In any case, a pulsed MF does have an inhibitory effect on the early growth of mung beans.



(a)



(b)

Figure 5 (a) The sprout diameter distribution of the mung beans exposed to the pulsed MF. (b) The sprout length of the mung beans exposed to the pulsed MF. Although the sprout length distribution is quite dispersed, the distribution of both sprout diameter and length shift lower as the MF intensity increases. (There are also 10 samples in exposed group 1)

TABLE 3 The statistical data of the growing mung beans

Item	Sinusoidal MF			Pulsed MF		
	Exposed 1	Exposed 2	Control	Exposed 1	Exposed 2	Control
Sprout diameter (mm)	Avg: 1.87 SD: 0.05	Avg: 1.61 SD: 0.09	Avg: 1.53 SD: 0.15	Avg: 1.41 SD: 0.07	Avg: 1.51 SD: 0.08	Avg: 1.55 SD: 0.07
Sprout length (mm)	Avg: 16.0 SD: 0.77	Avg: 14.0 SD: 1.90	Avg: 13.4 SD: 1.11	Avg: 12.6 SD: 2.29	Avg: 13.3 SD: 2.26	Avg: 14.1 SD: 2.89
Morbid Proportion	4/10	9/30	0/30	1/10	3/30	0/30

[a]Avg refers to average, and SD stands for standard deviation.

3.3 Morbidity

After 24 hours cultivation, the three groups of mung beans were taken out to be observed for germination. Not only are the sprout length and diameter of the mung beans in each group different, but they also differ in external appearance. The morbidity rates of the mung beans in exposed 1, exposed 2 and control groups of the two tests are sketched in Figure 6. It can be clearly seen that, the morbidity rates of mung beans grown exposed to the MF becomes greater as the magnetic intensity increases regardless of magnetic field type.

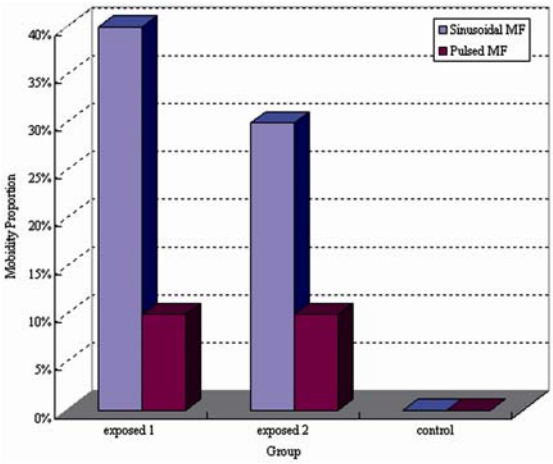


Figure 6 The morbidity rates of the mung beans in exposed 1, exposed 2 and control groups of the two tests

4 Discussion and Conclusion

To investigate how the plant growth was affected by the induced MF, a little probe coil of diameter 1cm (Misakian, 1993) was used to induce an electromagnetic force next to the core where the exposed mung beans were placed. The probe was connected to a frequency analyzer (Tektronix 2012) to obtain the components of the two different kinds of MF in the frequency domain. Figure 7 demonstrates the spectrum of the sinusoidal MF induced next to the iron core. As shown, it can be seen that most of the energy of the MF is concentrated in its fundamental frequency (60Hz), and some distributed among its odd harmonic frequencies. In other words, most energy of the sinusoidal MF is distributed in the lower frequency band. In addition, the spectrum of the pulsed MF produced is shown in Figure 8. Its spectral energy is widely spread in the frequency domain almost reaching 12.5MHz. As mentioned in the introduction, the motion of ions of plants is not only affected by the magnetic intensities but also the frequency. Following this viewpoint, there should exist many natural frequencies of the cells in all kinds of plants, which are dependent on the cell elements, structures, components, alignment, etc. Each cell is capable of "resonating" to an external source of frequencies in the same manner as a guitar string beginning to vibrate when exposed to the

standard pitch of the correct tuning fork (Lakhovsky, 1963; Brown, 1989). Therefore, as the frequency of the applied magnetic field approaches the natural frequency of the plant, some sort of excitation occurs and the original cellular motion is changed. Since there is more than one natural frequency in the plant and there exist different distributions of the natural frequencies with regard to different kinds of plant, the effects of the ELF MF on plant growth is complicated to predict. Nevertheless, this study provides an experimental method to figure out the biological effects.

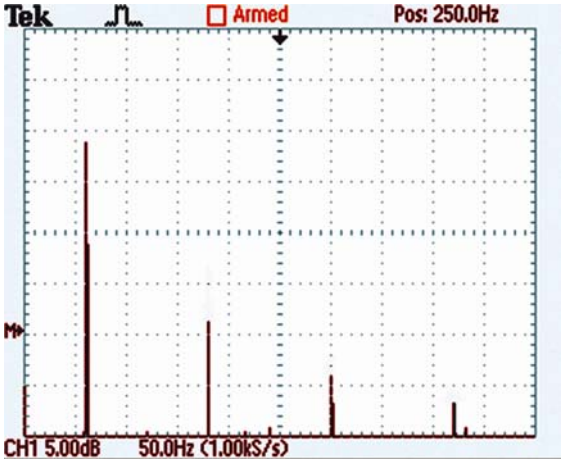


Figure 7 The frequency spectrum of the sinusoidal MF produced. Some harmonic components exist in the spectrum since the MF is not a pure sine.

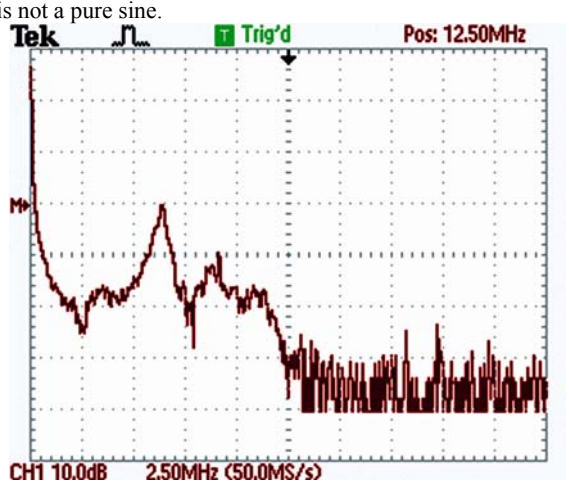


Figure 8 The frequency spectrum of the pulsed MF induced, with bandwidth reaching 12.5MHz.

The enhanced growth of mung beans exposed to the 60Hz sinusoidal MF might result from the fact that 60Hz is close to the natural frequency of mung beans, thus exciting the motion of the Ca^{++} ions within mung beans. It was also presumed that the broad band frequency spectrum of the pulsed MF causes more than one excitation force that turn out to be disturbances. In fact, the kinetic energy of the cellular response to ELF MF exposure can be theoretically calculated, but more work needs to be done to understand the disturbances caused by other ions.

In conclusion we observed that the sinusoidal and pulsed MFs affect the plant growth in different ways (with respect to different kinds of plant). Generally speaking, the 60Hz sinusoidal ELF MF has an apparent enhancing effect on mung bean growth but with the occurrence of some morbid state phenomena. In contrast, the 60Hz pulsed MF inhibits growth. From the results obtained in this experiment, it can be understood that the pulsed MF harms plant growth rather than enhancing growth as the sinusoidal MF does.

This may imply that this kind of MF is detrimental to health.

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Genetic Variability for Biological Nitrogen Fixation Traits in Tropical Soybeans (*Glycine max* (L) Merr)

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Abstract: Twenty five soybean (*Glycine max* (L) Merrill) genotypes were sown at the Teaching and Research Farm of UNAAB in Nigeria during the late planting season in year 2004 to obtain data for grain yield and seven Biological Nitrogen Fixation (BNF) traits. The data were subjected to analysis of variance (ANOVA) and were later used in the computation of heritability estimates for grain weight, days to flower, nodulation rating, days to maturity, number of nodules, nodule weight, primary root length and number of secondary roots. Varieties were observed to be genotypically diverse with respect to the traits evaluated. Large genotypic and phenotypic variations were also observed for the characters. Nodule number and nodule weight that had positive and significant association with nodulation rating, with relatively high broad-sense heritability estimates were adjudged as possible selection criteria for genetic improvement for BNF. Genotype TGx 1921-2F was identified as the only genotype that has the potentials for genetic manipulation of host plant for the improvement of soil nitrogen among the genotypes that were evaluated. [Nature and Science. 2007;5(1):69-74].

Keyword: Native rhizobia, Nodulation, Organic biological nitrogen fixation, tropical soil

Introduction

The initial breeding approach of the International Institute of Tropical Agriculture (IITA) soybean scientists in the improvement of biological nitrogen (N) fixation (BNF) to meet the demand for Nitrogen in soybean in the early 1980s was to breed for promiscuous varieties that would nodulate freely with indigenous soil rhizobia to make inoculation unnecessary (Pulver *et al*., 1982; Kueneman *et al*., 1984). Promiscuous soybean varieties are those that are compatible with and could form effective symbiotic association with a large majority of native rhizobial strains (IITA, 1983) thus, making the use of synthetic fertilizers unnecessary. Compatible lines are lines that have high number of nodules with high biomass production. Shoot dry matter yield (biomass production) in particular has been reported to have strong and positive association with soybean grain yield (Okogun and Sanginga, 2003).

Reports in the last decade (Sanginga *et al*., 1997; Okogun and Sanginga, 2003) have shown that promiscuous soybean varieties derived about 85 kg N from BNF. Positive response of soybean to inoculation (yield increase of 179%) has also been reported to occur in situations where indigenous bradyrhizobial cells were fewer than 10 cells per grain of soil or where the rhizobial populations were not effective (RENEASA, 1996). Sanginga (2003), Okogun and Sanginga (2003) have confirmed that tropical soil rhizobial strains were not effective in fixing enough biological nitrogen to sustain soybean growth and, thus, the need for a starter dose of nitrogen fertilizer or a search for more effective rhizobial strains.

Effectiveness of soil rhizobial strains is influenced by prevailing environmental conditions such as moisture (Nantakorn and Weaver, 1982) and competition for nodule sites (Harold and Fudi, 1992). Excess soil moisture of more than 10% (Osa-Afiana and Alexander, 1979) probably, affects the rhizobial population and, thus, their symbiotic activities (Okogun and Sanginga, 2003).

Efforts have been made to incorporate the promiscuous gene into elite soybean varieties developed between 1991 and the present (FAO, 1999; Ojo, 2002) and selection for promiscuity and nodulation as reflected by the number of nodules and biomass production has been attempted (Sanginga *et al*., 1997; Sanginga *et al*., 2000).

It has been reported earlier that inheritance of promiscuity in tropical soybean was conditioned by a few major genes (IITA, 1980) suggesting that the trait is qualitatively inherited.

Genetic evaluation of BNF traits, particularly, the estimation of the degree of genetic determination of those traits that have significant association with grain yield among selected tropical soybean lines could provide a fast and cost effective means of knowing the traits that can be used as good predictors of yield in this crop. This is because heritability estimates indicate how easy or difficulty it will be to produce a change in a given trait by applying selection (Graham and Welch, 1996) and the amount of grain anticipated from such a selection is best given by heritability (Borojevic, 1990). The exercise could result

in an intelligent choice of the best lines and the right breeding procedure needed for their genetic improvement for effective nitrogen fixation in tropical soils without the use of synthetic fertilizers. Sanginga *et al*. (1997) have observed that the choice of breeding lines could influence the potential contribution of fixed nitrogen to farming system.

This research was intended to (1) examine genetic variability for BNF traits in selected soybean lines (2) examine possible association that exist between grain yield and BNF traits and (3) identify which of the traits that can be used as selection criteria for BNF and thus, grain yield in tropical soybeans.

Materials And Methods

Twenty five tropical soybean genotypes obtained from IITA, Ibadan, Nigeria were sown in the Teaching and Research Farm of the University of Agriculture, Abeokuta, (UNAAB), Nigeria in August 2004.

After land preparation that involved ploughing and harrowing, seeds of each genotype were sown by drilling in four-row plots in randomized complete block design with three replications. Rows were 75 cm apart. On emergence, seedlings were thinned to a plant-to-plant spacing of 5 cm leaving a population of 266, 667 plants per hectare.

Hand weeding was employed as required. Data were collected on number of days to flowering, number of nodules at flowering, weight of fresh nodules, length of primary root, number of secondary roots and nodulating rating. At maturity data were collected for days to maturity and grain yield per plot.

Days to flowering: - this was determined as the period from date of planting to the date when 50% of the plant in the plot were at full bloom stage.

Days to maturity: - was determined as the period between date of planting and the date when plants in the plot had matured physiologically and the pods were brown in colour.

Nodulating rating: - was a rating from 1 to 5, root having very few nodules was rated 1, few nodules rated 2, moderate nodule rated 3, plenty nodules rated 4 while very plenty nodules was rated 5.

Number of nodules: - roots of ten plants were carefully dug up, packed in polythene bags together with the nodules that had become detached during digging and were transported to the laboratory where they were washed and number of nodules were counted. The mean value was then recorded.

Weight of fresh nodules: - after counting the nodules, it was weighted with a sensitive scale.

Grain yield: - grain yield in kg/plot was determined on clean dry grains of plants harvested from two middle rows of each plot.

Length of primary root: - ten plants were randomly picked from boarder rows and tap roots were measured per plant for length of primary root (cm) using a thread and meter rule. The mean length was then determined.

The number of secondary roots was also recorded as the mean number of roots of ten plants selected randomly from the boarder row.

The plot means of each character were subjected to analysis of variance (ANOVA). Simple correlation coefficients were obtained between all possible combinations of traits using Pearson correlation coefficient analysis. Also, estimates of broad-sense heritability for each character were obtained for possible use as selection criteria.

Result And Discussion

Table 1 presents the mean values of the twenty five soybean genotypes that were evaluated for eight BNF traits. Mean grain yield per plot ranged from 160 kg/plot for TGx 1923-4F to 433 kg/plot for TGx 1903-8F. Genotype TGx 1921-2F with highest nodulation rating of 3.4 matured in 87 days just as genotypes TGx 1921-1F, 1921-23F and TGx 1924-1F. Incidentally, genotype TGx 1921-2F recorded the highest number of nodules (21.0), biggest nodule weight (0.3 g/plant), very long primary root (13 cm) as well as highest number of secondary roots (10.0).

Table 1. Mean value of 25 soybean genotypes evaluated for grain weight and biological nitrogen fixation characters

S/N	Variety	GWK g/plot	DF	NR	DM	NN	NW g/plt	RL	SN
1	TG_X 1903-8F	433	40	1.7	83	7.0	0.1	9.8	10.0
2	TG_X 1908-6F	413	42	3.0	83	9.7	0.2	9.6	8.0
3	TG_X 1921-7F	353	41	3.3	81	14.0	0.2	11.0	8.0
4	TG_X 1903-4F	347	41	3.0	84	8.0	0.1	11.0	5.7
5	TG_X 1920-1F	347	42	2.0	84	2.0	0.02	12.0	6.3
6	TG_X 1924-1F	340	47	3.0	87	12.0	0.2	10.0	9.3
7	TG_X 1923-3F	327	41	3.0	84	16.0	0.2	13.0	10.0
8	TG_X 1924-4F	323	46	2.3	91	4.3	0.1	9.5	6.0
9	TG_X 1909-3F	317	42	3.0	81	10.0	0.1	13.0	8.0
10	TG_X 1903-7F	307	41	2.0	81	3.7	0.05	10.0	8.3
11	TG_X 1921-6	307	42	3.3	80	15.0	0.3	12.0	7.7
12	TG_X 1919-1F	293	42	2.3	81	4.7	0.1	11.0	7.3
13	TG_X 1922-1F	260	41	2.3	81	7.7	0.2	11.0	8.3
14	TG_X 1925-1F	259	46	2.0	87	4.7	0.0	10.0	4.7
15	TG_X 1909-2F	253	42	3.3	81	7.0	0.2	13.0	9.0
16	TG_X 1985-1D	240	40	2.3	83	6.0	0.1	11.0	6.0
17	TG_X 1921-13F	240	42	2.3	81	9.7	0.1	11.0	9.0
18	TG_X 1904-2F	220	42	2.0	84	4.0	0.1	14.0	6.0
19	TG_X 1921-1F	220	41	2.7	87	11.0	0.1	11.0	8.7
20	TG_X 1921-2F	220	42	3.4	87	21.0	0.3	13.0	10.0
21	TG_X 1904-4F	220	42	3.0	81	5.0	0.1	12.0	6.7
22	TG_X 1921-23F	200	41	2.7	81	8.0	0.1	11.0	7.0
23	TG_X 1930-20E	173	42	1.3	81	1.3	0.03	12.0	11.0
24	TG_X 1921-20F	173	44	2.0	81	14.0	0.2	13.0	7.0
25	TG_X 1923-4F	160	41	3.3	87	8.0	0.1	13.0	6.3
	Mean	227.3	42.0	2.6	83.6	8.6	0.1	11.5	7.8
	LSD	140.2	1.00	1.44	1.41	7.36	0.11	3.33	3.36

GW = Grain weight, DF = Days to flower, NR = Nodulation rating, DM = Days to maturity, NN = Nodule number, RL = Primary root length, SN = Secondary root number

Mean squares from analysis of variance for BNF traits (Table 2) showed that the twenty five soybean genotypes were highly variable with respect to grain yield, days to flowering, days to maturity, number of nodules, nodule weight and number of secondary roots to confirm the observed variations stated above.

Table 2. Mean squares from analysis of variance of biological nitrogen fixation (BNF) characters in twenty five varieties of soybean

S O V	df	GW	DF	NR	DM	NN	NW	RL	SN
Replication	2	47225.65**	0.37	1.29	0.36	7.72	0.01	7.34	3.21
Variety	24	16305.72**	8.52**	1.04	25.02**	70.61**	0.02**	4.46	8.42*
Error	48	7292.32	0.37	0.77	0.74	20.08	0.01	3.84	4.19

*Significant at 5% probability level, ** Significant at 1% probability level.
SOV= source of variation, df=degree of freedom, GW= Grain weight, DF= Days to flower,
NR= Nodulation rating, DM= Day to maturity, NN= Nodule number, NW= Nodule weight, RL= Root length, SN= Secondary Root Number

Observed correlation coefficients between grain yield and BNF traits as presented in Table 3 revealed that only the primary root length recorded a significant but negative correlation with grain yield. Days to flowering and days to maturity recorded non-significant and negative correlation with grain yield. However, days to flowering and days to maturity were positively and significantly correlated (0.472). Nodulation rating was equally positively and significantly associated with nodule weight (0.674) and number of nodules (0.633). Significant and positive association between two characters suggests that these characters can be improved simultaneously in a selection programme (Hayes *et al*., 1955). This is because a significant positive association shows a mutual relationship as selection for one trait would lead to selection and consequent improvement for the other traits.

Genetic manipulation of selected soybean genotypes for number of nodules, nodule weight and number of secondary roots would be an effective means of rating tropical soybean genotypes for BNF characteristics according to the current study. However, the length of primary root would not have any meaningful contribution to grain yield because both were negatively and significantly correlated.

Table 3. Correlation coefficients between Grain weight and Biological Nitrogen-Fixation characters

	GW	DF	NR	DM	NN	NW	RL	SN
GW	1.000	-0.046	0.077	-0.044	0.054	0.073	-0.561**	0.119
DF		1.000	-0.047	0.472*	0.012	0.038	-0.186	-0.239
NR			1.000	0.093	0.633**	0.674**	0.191	0.017
DM				1.000	0.063	0.040	-0.263	-0.245
NN					1.000	0.856**	0.208	0.392*
NW						1.000	0.212	0.325
RL							1.000	0.002
SN								1.000

*Significant at 5% probability level ** Significant at 1% probability level.
GW= Grain weight, DF= Days to flower, NR= Nodulation rating, DM= Day to maturity
NN= Nodule number, NW= Nodule weight, RL= Root length, SN= Secondary Root Number

Table 4 presents the phenotypic and genotypic variances and estimates of broad-sense heritability (H_B) of eight traits that were evaluated. Days to flowering and days to maturity had the largest estimates of 88 and 92%, respectively and thus were least affected by the effects of the environment. Nodule number and nodule weight recorded average estimates of 46 and 47% respectively, whereas nodulation rating, primary root length and number of secondary roots had H_B estimates that were ridiculously low (5 – 25%)

Table 4. Phenotypic and genotypic variance and heritability of eight traits that were evaluated

S/N	Traits	Phenotypic variance	Genotypic variance	Heritability (H_B)
1	Grain weight	10296.77	3004.47	0.29
2	Days to flowering	3.09	2.72	0.88
3	Nodulation rating	0.86	0.09	0.11
4	Days to maturity	8.83	8.10	0.92
5	Nodule number	36.92	16.84	0.46
6	Nodule weight	0.0088	0.0041	0.47
7	Primary root length	4.05	0.21	0.05
8	Secondary root number	5.60	1.41	0.25

Very low estimates of H_B suggest large effects of the environment and consequently larger phenotypic variances. High soil moisture (Nantakorn and Weaver, 1982), competition among local strains of Rhizobia for nodule sites (Harold and Fudi, 1992) and few number of native Bradyrhizobial cells in a unit grain of soil (Okogun and Sanginga, 2003) have been reported as some of the factors that prevent effective nodulation of soybean lines.

This study shows that number of nodules and weight of nodules were positively associated with nodulation rating. Thus, the two traits could be used as selection criteria for BNF traits among tropical soybean genotypes. As genetic manipulation of host plants offers the greatest potential for the improvement of nitrogen levels in soybean (Sanginga *et al*., 1997) genotype TGx 1921-2F with highest nodulation rating, highest number of nodules, highest nodule weight as well as highest number of secondary roots could be selected for a future genetic improvement of biological nitrogen fixation.

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The Comparison of the Loading Devices on Microstrip Circuit in Patch Antenna

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Abstract: A microstrip band pass filter circuit presented can be used to regulate the characteristic of the antenna. In this article, the circuit effects to the loading devices are investigated to depict the function of the microstrip band pass filter circuit designed for patch antenna. [Nature and Science. 2007;5(1):75-80].

Keywords: VSWR; microstrip circuit; patch antenna

1. Introduction

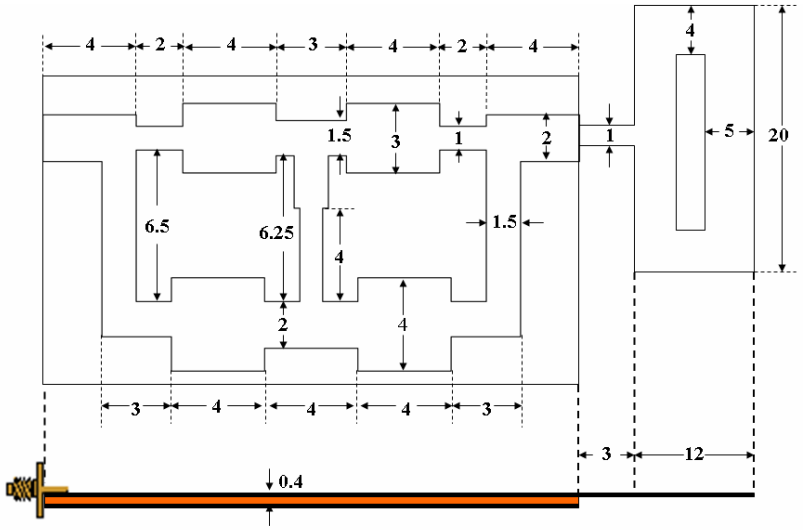
The microstrip circuit can regulate the patch antenna. Design a coupling microstrip circuit connecting the signal source to an effective loading device can control the characteristic of microstrip antenna [1]. Size and shape of the radiator in patch antenna are parameters for design and can be considered as loading impedance regulator for a microstrip circuit in a transmission line model. However, the characteristics of the distributed microstrip elements being coupled to the antenna are the problems to analysis in both Thevenin and Norton equivalents [2]. In this article, we proposed a patch antenna including a microstrip band pass filter circuit to find the circuit behavior on different radiators as the loading devices via a short connection transmission line [4]. Prototype antennas are constructed

2. Configuration

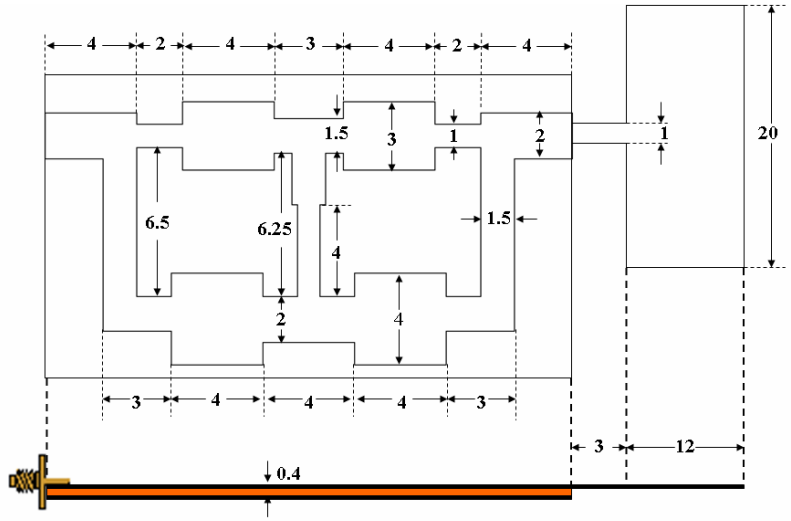
Figure 1 shows the size and shape parameters of the proposed antenna patch antenna with slot and without slot. In Figure 2, the drawing of the return loss depicts microstrip band pass filter causes the similar effect on different resonant loading devices for patch antenna. As shown in the figure, the filter circuit via path connecting to the metal radiator can be analyzed by using transmission line model.

3. Results and Discussion

The proposed antenna is depicted in Figure 3. The thickness and dielectric constant of the FR4 plate are indicated d = 0.4 mm, ε_r = 4.4. Figure 2 depicts the return loss of the measurement of the antenna including microstrip strip circuit coupled to different metal radiators as loading devices. Figure 4 demonstrates the field-patterns of the antenna with different radiators. The presence of the forward and backward scattering current driven by filter circuit delivers the excitation power via path for the modes of resonances of the metal radiator. In Figure 5, the gains of the proposed antenna are depicted. The microstrip circuit can be designed to improve the impedance matching and selecting resonant frequencies for loading devices.

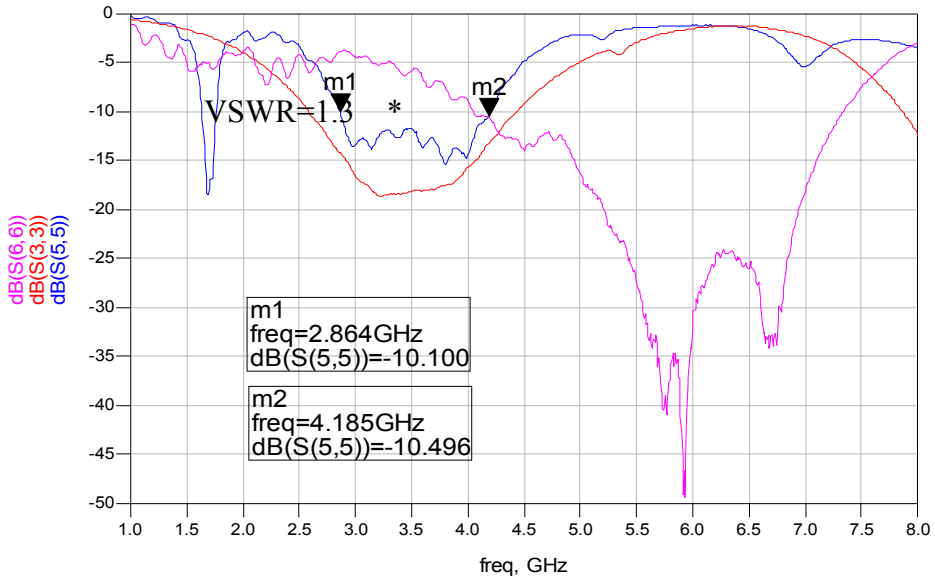


(A)

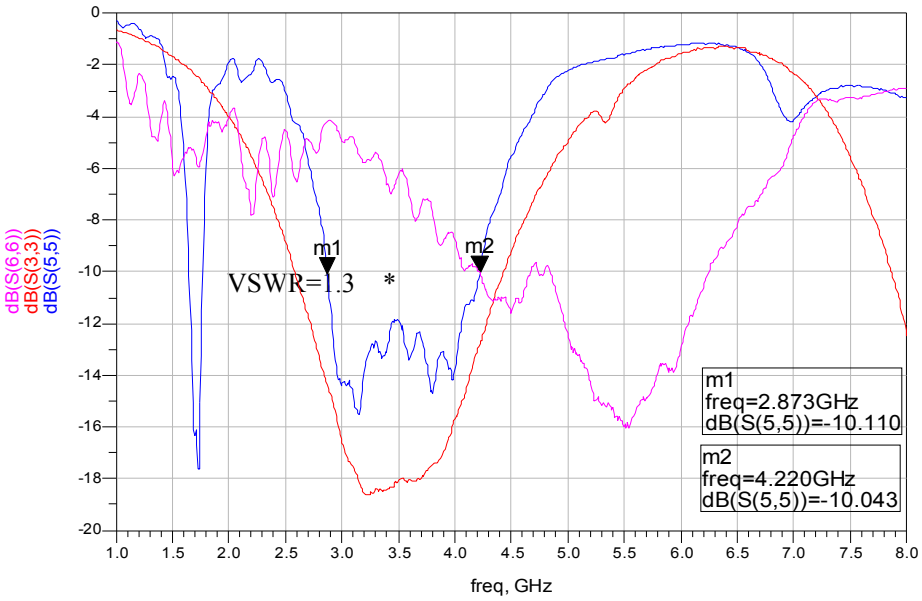


(B)

Figure 1. Size and shape parameters of the proposed antenna patch antenna (A) with slot (B) without slot



(A)

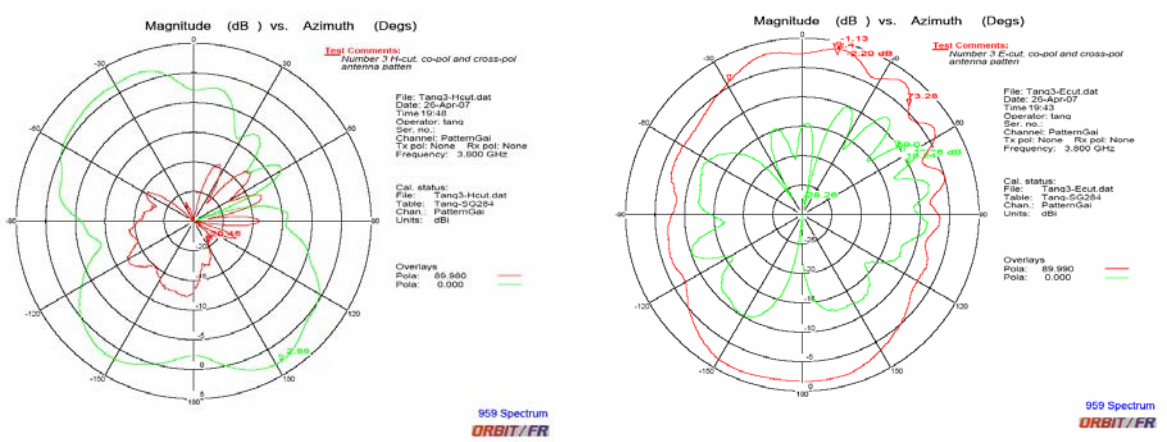


(B)

Figure 2. Measurement of the Return Loss. The impedance matching of the metal antenna at 3.5GHz is shown being improved by coupling to microstrip circuit regulator

(A) **(B)**

Figure 3. Prototype of the proposed antennas in design (A) with slot (B) without slot



(A)

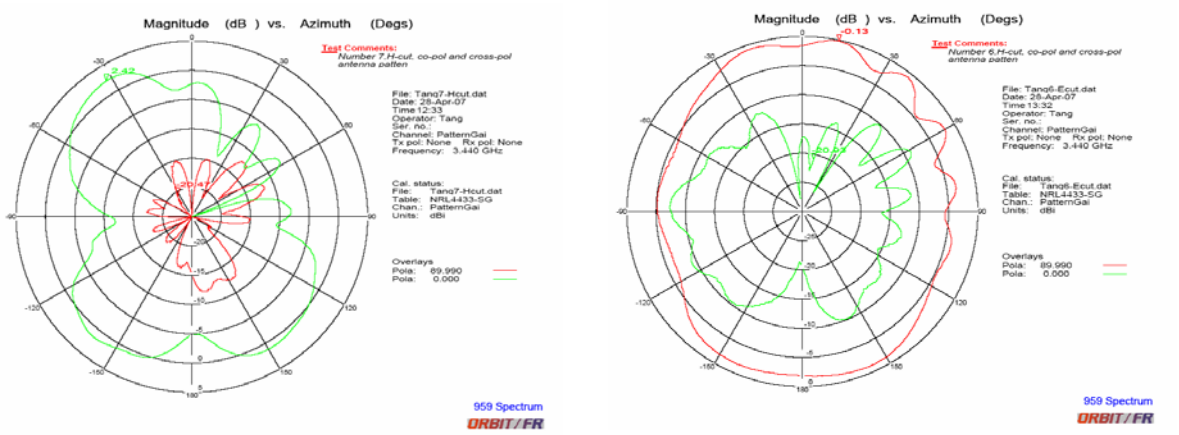
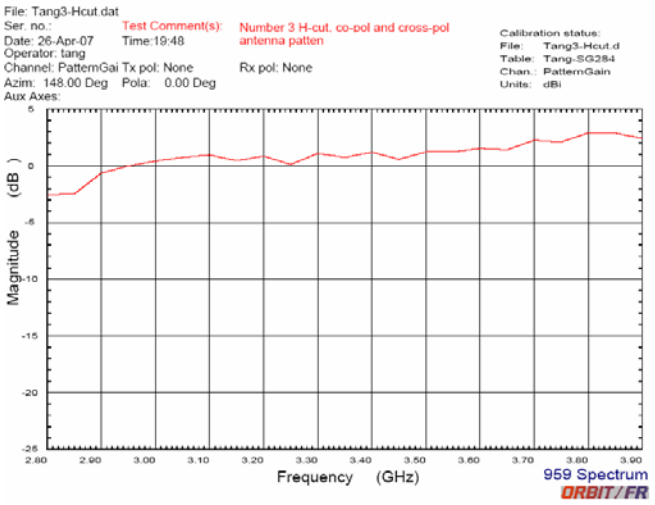
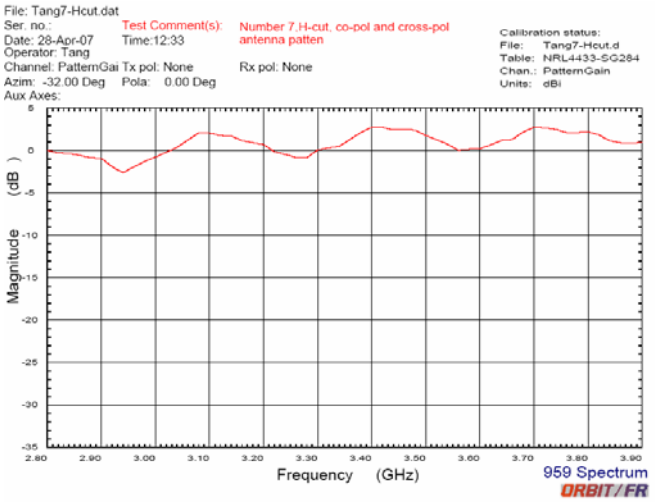


Figure 4. The measured radiation pattern of the proposed antenna at 3.8 GHz (A) with slot and (B) 3.44GHz without slot



(A)



(B)

Figure 5. Antenna Gains for the prototype designs for different resonant radiator connection (A) with slot (B) without slot

4. Conclusion

The proposed antenna with a functional microstrip filter circuit via transmission line coupling to metal radiator can be used to select available frequency-bands for carrying out specific gains for the mobile system. The microstrip circuit functions to regulate the patch antenna with keeping the same characteristic to different loading devices. Under the conditions of different resonant radiators, the optional design of the filter circuit can be conducted to satisfy the resonant specification.

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Eternal Life and Stem Cell

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Abstract: As the nature will, to live eternally is an extracting dream in all the human history. Stem cell is the original of life and all cells come from stem cells. Germline stem cell (GSC) is the cell in the earliest of the cell stage. It is possible to inject the GSC into adult human body to get the eternal life. This article is to try to describe the stem cell and to explore the possibility of the eternal life with the stem cell strategy [Nature and Science. 2007;5(1):81-96].

Key words: DNA; eternal; life; stem cell; universe

1. Introduction

For a person, the most attracting will is to live longer, and the extreme dream is to live eternally. The number two important will for a person is to live happily. Humankind has a history longer than millions of years, and people never stopped the efforts to find a way to live eternally, no matter he/she was a beggar or an emper. There were many ways people considered as the way to keep life longer, even eternal, but people never got the eternal goal.

Stem cell is the origin of an orgnism's life. Stem cells have the potential to develop into all different types of cells in life bodies, tissues and organs. Stem cells can be used in the clinical medicine to treat patients with a variety of diseases (Daar, 2003), and also gives a hope to let us get the eternal life. Serving as a repair system for the living body, the stem cells can divide without limit to replenish other cells as long as the living body is still alive. When a stem cell divides, each new cell has the potential to either remain a stem cell situition or become another type of cell with a more specialized function, such as a muscle cell, a red blood cell, a bone cell, a nerve cell, or a brain cell. Stem cell research is a tipical and important topic of life science.

The definition of stem cell is "an unspecialized cell that gives rise to a specific specialized cell, such as a blood cell" (Stedman's Medical Dictionary, 2002).

Some of the most notable recent findings are as follows: (1) the stemness profile may be determined by approximately 250 genes; (2) organ-specific stem-cell growth and differentiation are stimulated during the reparative phase following transient injury; (3) two bone marrow stem-cell types show a remarkable degree of differentiation potential; (4) some organs contain resident marrow-derived stem cells, and their differentiation potential may only be expressed during repair; (5) the metanephric mesenchyme contains pluripotent and self-renewing stem cells; (6) marrow-derived cells invade the kidney and differentiate into mesangial and tubular epithelial cells, and these processes are increased following renal injury; and (7) epithelial-to-mesenchymal transition generates renal fibroblasts (Oliver, 2004).

Stem cell is totipotent, that means it holds all the genetic information of the living body and it can develop into a mature cell. Stem cell is a single cell that can give rise to progeny that differentiate into any of the specialized cells of embryonic or adult tissue. The ultimate stem cells (fertilized egg) divide

to branches of cells that form various differentiated tissues or organs. During these early decisions, each progeny cell retains totipotency. Through divisions and differentiations the embryonic stem cells lose totipotency and gain differentiated function. During normal tissue renewal in adult organs, tissue stem cells give rise to progeny that differentiate into mature functioning cells of that tissue. Stem cells losing totipotentiality are progenitor cells. Except for germinal cells, which retain totipotency, most stem cells in adult tissues have reduced potential to produce different cells.

Five key stem cells have been isolated from human: (1) Blastocysts; (2) Early embryos; (3) Fetal tissue; (4) Mature tissue; (5) Mature cells that can be grown into stem cells.

2. Germline Stem Cell (GSC)

Testis is the organ for animal to reproduce the generation. As the new generation always has a young feature for the life, no matter how old the parents are, it is possible for the mature life to use the stem cell coming out from the reproduce organ (germline stem cells) to replace the old cells, to keep the mature body always young. The recently developed testis cell transplantation method provides a powerful approach to studying the biology of the male germline stem cell and its microenvironment, the stem cell niche.

In the insect *Drosophila* germline stem cells of the testis, one centrosome remains anchored to the region of the cortex at the interface between germ cells and somatic hub cells, while the other centrosome migrates to the opposite side to establish mitotic spindle orientation. This is important for the germline cells of the testis of *Drosophila.*

Human male germline stem cells, called spermatogonial stem cells (SSCs) in postnatal mammals, are the foundation of spermatogenesis (the process for spermatozoa production) and, together with oocytes from females, are essential for species continuity. SSCs and eggs are the original of the life individual and these kinds of cells are always keep young, no matter how old the parents are. SSCs reside on the basement membrane of the seminiferous tubule in the testis and are almost completely surrounded by somatic Sertoli cells, which form a microenvironment or niche. Within the niche, growth factors and extracellular signals regulate the fate decisions of SSCs either to self-renew or to form daughter cells that will begin the complex differentiation process of spermatogenesis.

The first step in spermatogenesis is the fate decision of an SSC to produce daughter cells committed to differentiation. The availability of a functional transplantation assay and a culture system that allows long-term replication of SSCs made it possible to examine intracellular signals that influence self-renewal and differentiation invitro in a rigorous manner that is not available for most adult stem cells. Stem cell recovery and cryopreservation may be applicable to all mammalian species and could be used to preserve the male germ line of valuable livestock animals, companion animals, and endangered species.

There are three particularly important areas include should be mentioned: (1) Further definition of factors and signals that support self-renewal of SSCs, relative to those that initiate differentiation in order to provide a better understanding of this fate decision; (2) Extension of the serum-free culture system to other species, including domestic animals, endangered species, and humans to confirm that self-renewal signals are conserved among mammals and for relevant applications; (3) Development of methods to allow in vitro differentiation of stem cells to provide mature spermatozoa, which would be enormously valuable in understanding the complex process of spermatogenesis and would have great practical use.

Stem cells are unique cell populations that are able to undergo both self-renewal and differentiation and are found in the embryo, as well as in the adult animal. In the early mammalian embryo, pluripotent embryonic stem cells are derived from the blastocyst stage and have the ability to form any fully differentiated cell of the body. As the embryo develops, stem cells become restricted in their ability to form different lineages (multipotent stem cells). Multipotent stem cells are also found in a wide variety of adult tissues such as bone marrow and brain. However, in the adult animal, the ability of certain stem cells to differentiate can be restricted to only one cell lineage (unipotent stem cells). Examples of mammalian unipotent stem cells include the stem cells residing in the gut epithelium, the skin, and the seminiferous epithelium of the testis.

In the mammalian testis, the germ line stem cells are a small subpopulation of type A spermatogonia that proliferate and ultimately differentiate into sperm under the control of both endocrine and paracrine factors. The ability to isolate, culture, and manipulate the germ line stem cell in vitro would allow us to unravel the molecular mechanisms that drive the first steps of spermatogenesis and to characterize the signaling pathways that induce spermatogonial differentiation versus self-renewal. It is important to know the biochemical and biophysical reasons for the ways how the germline stem cells keep young.

3. Embryonic Stem Cell

Embryonic stem cells hold great promise for treating degenerative diseases, including diabetes, Parkinson's, Alzheimer's, neural degeneration, and cardiomyopathies (Bavister, 2005). Embryonic stem cells are derived from the inner cell mass of blastocyst stage embryos. Embryonic stem cells can replicate indefinitely. This makes it feasible to culture the cells on a large scaled for cell transplantation therapy in clinical application. Embryonic stem cells are pluripotent and have the potential to differentiate into all three germ layers of the mammalian body including the germ cells.

In 2003, scientists in Edinburgh have identified the gene that gives foetal stem cells their ability to multiply without limit and never grow old (Hawkes, 2003). The discovery may make it possible to create foetal stem cells from adult cells, and use them to treat diseases. At present the only way to get such cells is to create embryos. This is controversial, especially in the United States where federal research money cannot be used for embryonic research of this kind. The gene, which the team has named Nanog after the mythical Celtic land where nobody grows old, is a regulator that controls the operation of many other genes. It operates only in embryonic stem cells, which are pluripotent (able to develop into any of the body's specialised cells). Nanog's role, according to papers published in the journal *Cell* by the team from Edinburgh University and Nara Institute of Science and Technology in Japan, is to maintain stem cells and to make them grow. Ian Chambers, of the Institute for Stem Cell Research at Edinburgh, said that nanog was a master gene, which "makes stem cells immortal". Unlike specialised cells, that can only divide a limited number of times before they die, embryonic stem cells can go on dividing for ever. This means that a culture of stem cells can be kept alive for transplantation into patients where they will diversify into necessary cells — brain, muscle, liver or skin, for example. For this to be possible, scientists need to understand how it is that stem cells can either divide without limit, or choose instead to differentiate into specialised cells. Nanog appears to be the key. Nanog does not disappear in adult cells, but it lies dormant. This means that if a way could be found to reactivate it, adult cells could be persuaded to become embryonic cells again.

James Thompson, of the University of Wisconsin, told the *Washington Post*: "As we know`more and more about pluripotency, it will probably be possible to reprogramme cells to make stem cells out

of any cell in the body. This is an important step in that direction." The Edinburgh paper is published alongside a study from Shinya Yamanaka, from the Nara Institute. The two groups realised that they had discovered the same gene last year and have since collaborated in completing the research. The next step is to work out how Nanog is switched on and off. To achieve that it may be necessary to continue working on embryonic stem cells and watching the process as it happens. British scientists have long argued that while work on adult stem cells is important, understanding how they work still requires the use of embryos. Most of the research so far has been conducted in mice, but humans have an almost identical gene. In one experiment the Edinburgh team inserted the human Nanog gene into embryonic mouse cells, and subjected those cells to conditions that would normally make them turn into specialist cells. The human Nanog gene stopped that process. Embryonic stem (ES) cells can be cultured in conditions that either maintain pluripotency or allow differentiation to the three embryonic germ layers. Heparan sulfate (HS).

4. Somatic Stem Cell

Normally to say that somatic stem cells differentiate only into specific tissue cells wherein they reside. However, somatic stem cells can differentiate into cells other than those of their tissue of origin. Adult bone marrow, fat, liver, skin, brain, skeletal muscle, pancreas, lung, heart and peripheral blood possess stem or progenitor cells with the capacity to transdifferentiate. Due to this developmental plasticity, somatic stem cells may have potential in autologous regenerative medicine, circumventing problems like rejection and the ethically challenged use of embryocyte stem cells.

5. Isolation and Characterization of Stem Cells

As the example, the following is describing the isolation and characterization of the putative prostatic stem cell, which was done by Bhatt, Brown, Hart, Gilmore, Ramani, George, and Clarke in 2003. The detail methods have been described by Bhatt, Brown, Hart, Gilmore, Ramani, George, and Clarke in the article "Novel method for the isolation and characterization of the putative prostatic stem cell" in the journal Cytometry A in 2003 (Bhatt, 2003).

5.1 Prostatic tissue collection and culture

When using human tissue, formal consent by the donator must be obtained before tissue collection. Tissue sections are obtained under sterile conditions. Each individual tissue section is bisected with half being sent for histological analysis for diagnostic evaluation and the remainder used for tissue culture. After then, tissue sections are chopped and placed in collagenase type I at 200 U/ml in RPMI 1640 medium with 2% v/v FCS overnight on a shaking platform at 37°C. The digest is then broken down further by shaking in 0.1% trypsin in PBS with 1% BSA and 1 mM ethylenediaminetetraacetic acid (EDTA) for 15-20 min. The cell suspension is then washed three times in PBS with 1% BSA and 1 mM EDTA before resuspending in RPMI 10% v/v FCS. Prostate epithelial cells are separated from fibroblasts by differential centrifugation (360 g, 1 min without braking). This process produced a supernatant enriched for fibroblasts and a pellet enriched for epithelia. The epithelial cell suspension is then spun on a metrizamide gradient (1.079 g/ml), and the cells are isolated from the interface (Bhatt, 2003).

5.2 Ber-EP4/α_2/CD45 labeling of cells

Isolated epithelial cells are labeled at ambient temperature with either anti-human integrin α_2 monoclonal antibody or Ber-EP4 antibody (8 μg/ml in 1% BSA/PBS) for 30 min before the addition of the secondary antibody, RAMBO (2.6 μg/ml in 1% BSA/PBS) for 30 min. After washing with PBS, the cells are incubated for 20 min in the dark with streptavidin PE-Cy7 (20 μg/ml). Samples are then dual labeled with CD45-FITC (1 μg/ml in 1% BSA/PBS) for 30 min (Bhatt, 2003).

5.3 Ber-EP4/α_2 and Hoechst labeling for flow cytometry

Isolated epithelial cells are labeled at ambient temperature with anti-human integrin α_2 monoclonal antibody (8 μg/ml in 1% BSA/PBS) for 30 min before the addition of the secondary antibody, RAMBO (2.6 μg/ml in 1% BSA/PBS) for 30 min. After washing with PBS, the cells are incubated for 20 min in the dark with streptavidin PE-Cy7 (20 μg/ml). Hoechst staining could be performed by using the protocol for HSC as described by Rupesh, et al (Bhatt, 2003). Briefly, epithelial cells are resuspended in Hoechst buffer (Hanks' balanced salts solution, 10% FCS,`1% D-glucose, and 20 mM HEPES) and warmed to 37°C. Hoechst 33342 is then added to give a final concentration of 2 μM and the cells incubated at 37°C for 2 h. Fifteen min before the end of incubation, the cells are labeled with monoclonal anti-human Ber-EP4 directly conjugated to FITC (8 μg/ml). The cells are then washed in ice-cold Hoechst buffer before resuspending in ice-cold Hoechst buffer containing propidium iodide (PI) at 20 ng/ml (Bhatt, 2003).

5.4 Flow cytometry isolation of the SP fraction

Flow cytometry is carried out using a Becton Dickinson FACS Vantage SE flow cytometer. Hoechst 33342 is excited with an argon ion, ultraviolet-enhanced laser at 350 nm, and its fluorescence is measured with a 424/44 BP filter (Hoechst BLUE) and a 675DF20 BP optical filter (Hoechst RED; Omega Optical, Brattleboro VT). A 640 LP dichroic mirror is used to separate the emission wavelengths. PI fluorescence is also measured through the 675DF20 BP (having been excited at 350 nm). A second argon ion laser is used to excite the additional fluorochrome PE-Cy7 at 488 nM. PE-Cy7 is measured using a 787RDF40 (Omega Optical) filter (Bhatt, 2003).

5.5 Cell cycle characterization of SP fraction

Epithelial cells are isolated and all fractions are resuspended in Hoechst buffer and warmed to 37°C. Hoechst 33342 is then added to give a concentration of 2 μM and incubated at 37°C for 45 min. Pyronin Y (250 ng/μl) is added to each tube, and the samples are incubated for 45 min. Monoclonal anti-human Ber-EP4 FITC (8 μg/ml) is added as appropriate 15 min before the end. After this, ice-cold Hoechst buffer is added immediately and the samples are washed then resuspended in ice-cold Hoechst buffer. The samples are analyzed immediately by flow cytometry. Flow cytometry is performed using a modification of the method described above. Cells under study are selected by positive labeling for Ber-EP4 FITC before being analyzed for Hoechst and Pyronin Y staining. These cells are then analyzed by plotting the Hoechst profile on the x-axis and Pyronin Y along the y-axis in a linear scale (Bhatt, 2003).

5.6 Cytokeratin phenotype studies

Samples are processed as above, divided into two fractions, and labeled with either cytokeratin 8 or 14 indirectly conjugated to PE-Cy5. Samples are then dual labeled with Ber-EP4 FITC and integrin

α_2 PE-CY7. Flow cytometry is performed as described and analyzed on forward (FSC) and side (SSC) scatter (Bhatt, 2003).

6. Application of Stem Cells in Clinical Medicine

There are over four thousand registered diseases specifically linked to genetic abnormalities. Although stem cells are unlikely to provide powerful treatment for these diseases, they are unique in their potential application to these diseases.

Indeed, in many research projects, scientists have demonstrated that stem cells can be used to replenish or rejuvenate damaged cells within the immune system of the human body and that damaged stem cells can repair themselves and their neighbors. For example, in what is regarded as the first documented case of successful gene-therapy "surgery", scientists at the Necker Hospital for Sick Children in Paris of French succeeded in treating two infants diagnosed with Severe Combined Immunodeficiency Disease, a life-threatening degenerative disease caused by defects on the male (X) chromosome. With the identification of stem cell plasticity several years ago, multiple reports raised hopes that tissue repair by stem cell transplantation could be within reach in the near future (Kashofer, 2005). In cardiovascular medicine, the possibility to cure heart failure with newly generated cardiomyocytes has created the interest of many researchers (Condorelli, 2005). Gene clone techniques can be widely used in the stem cell researches and applications (Ma, 2004).

7. Renal Stem Cells

Functional recovery in acute renal failure is well known, and the adult kidney is generally recognized to have the capacity to regenerate and repair. The adult stem cells exist in the kidney, including slow-cycling cells, side population cells, CD133+ cells and rKS56 cells. However, in vivo differentiation of bone marrow-derived cells into renal tubular cells may not occur at all, or is at most a minor component of the repair process. Moreover, it is generally accepted that stem cells and multipotent cells contribute to the regenerative process by producing protective and regenerative factors rather than by directly differentiating to replace damaged cells. Therefore, for clinical regenerative medicine in kidney disease, the focus of stem cell biology will shift from multiple differentiation of cells or cell-therapy to multiple functions of the cells, such as the production of bone morphologic protein-7 and other regenerative factors (Hishikawa and Fujita, 2006).

Adult stem cells have been characterized in several tissues as a subpopulation of cells able to maintain generate, and replace terminally differentiated cells in response to physiological cell turnover or tissue injury. Little is known regarding the presence of stem cells in the adult kidney but it is documented that under certain conditions, such as the recovery from acute injury, the kidney can regenerate itself by increasing the proliferation of some resident cells. The origin of these cells is largely undefined; they are often considered to derive from resident renal stem or progenitor cells. Whether these immature cells are a subpopulation preserved from the early stage of nephrogenesis is still a matter of investigation and represents an attractive possibility. Moreover, the contribution of bone marrow-derived stem cells to renal cell turnover and regeneration has been suggested. In mice and humans, there is evidence that extrarenal cells of bone marrow origin take part in tubular epithelium regeneration. Injury to a target organ can be sensed by bone marrow stem cells that migrate to the site of damage, undergo differentiation, and promote structural and functional repair. Hematopoietic stem cells are mobilized following ischemia/reperfusion and engrafted the kidney to differentiate into tubular epithelium in the areas of damage. The evidence that mesenchymal stem cells,

by virtue of their renoprotective property, restore renal tubular structure and also ameliorate renal function during experimental acute renal failure provides opportunities for therapeutic intervention (Morigi, 2006).

Acute renal failure has 50-80% mortality and treatment options for this life-threatening disease are limited. Stem cells offer an exciting potential for kidney regeneration. This review discusses pathogenesis of acute renal failure resulting from ischemia-reperfusion injury and the role of stem cells in reversing or mitigating this disorder. Specifically, the issues of differentiation of kidney cells from embryonic stem cells and bone marrow stem cells, and whether adult kidney stem/progenitor cells exist in the postnatal kidney are discussed. Evidence to support the conclusion that intra-renal cells, including surviving tubular epithelial cells and potential renal stem/progenitor cells, are the main source for renal regeneration is provided. Future research in selecting the type(s) of stem cells and optimizing the dose, frequency and route of administration of the cells will be fundamental in successful cell replacement therapy in acute renal failure. (Lin, 2006).

Repair of inflammatory and/or ischemic renal injury involves endothelial, mesangial and epithelial regeneration. These structures may be rebuilt by resident progenitor cells and bone marrow-derived stem cells. Resident progenitor cells in adult kidney have not yet been conclusively identified. They are likely to be slowly cycling cells located mainly in the outer medulla and renal papilla. In glomerulonephritis with mesangiolysis, mesangial regeneration involves progenitor cells migrating from the juxtaglomerular apparatus and also bone marrow-derived cells. In acute ischemic renal failure, epithelial regeneration of proximal tubules results from the migration, proliferation and differentiation of resident progenitor cells; bone marrow-derived cells may play an accessory role. Molecular mechanisms underlying these repair processes could be targets for new therapeutic approaches (Baud, 2005).

Ischemia causes kidney tubular cell damage and abnormal renal function. The kidney is capable of morphological restoration of tubules and recovery of function. Recently, it has been suggested that cells repopulating the ischemically injured tubule derive from bone marrow stem cells. In GFP chimeras, some interstitial cells but not tubular cells express GFP after ischemic injury. More than 99% of those GFP interstitial cells are leukocytes. In female mice with male bone marrow, occasional tubular cells (0.06%) appeared to be positive for the Y chromosome, but deconvolution microscopy revealed these to be artifactual. In beta-gal chimeras, some tubular cells also appear to express beta-gal as assessed by X-gal staining, but following suppression of endogenous (mammalian) beta-gal, no tubular cells could be found that stain with X-gal after ischemic injury. Whereas there is an absence of bone marrow-derived tubular cells, many tubular cells expressed proliferating cell nuclear antigen, which is reflective of a high proliferative rate of endogenous surviving tubular cells. Upon i.v. injection of bone marrow mesenchymal stromal cells, postischemic functional renal impairment was reduced, but there was no evidence of differentiation of these cells into tubular cells of the kidney. Bone marrow-derived cells do not make a significant contribution to the restoration of epithelial integrity after an ischemic insult. It is likely that intrinsic tubular cell proliferation accounts for functionally significant replenishment of the tubular epithelium after ischemia (Duffield, 2005).

Acute renal failure (ARF) is a common disease with high morbidity and mortality. Recovery from ARF is dependent on the replacement of necrotic tubular cells with functional tubular epithelium. Recent advancement in developmental biology led to the discovery of immature mesenchymal stem cells (MSCs) in bone marrow and several established organs and to the definition of their potential in the recovery from tissue injury (Herrera, 2004).

The kidney has a dramatic capacity to regenerate after injury. Whether stem cells are the source of the epithelial progenitors replacing injured and dying tubular epithelium is an area of intense investigation. Many surviving renal epithelial cells after injury become dedifferentiated and take on mesenchymal characteristics. These cells proliferate to restore the integrity of the denuded basement membrane, and subsequently redifferentiate into a functional epithelium. An alternative possibility is that a minority of surviving intratubular cells possess stem cell properties and selectively proliferate after damage to neighboring cells. Some evidence exists to support this hypothesis but it has not yet been rigorously evaluated. Extratubular cells contribute to repair of damaged epithelium. Bone marrow-derived stem cells have been proposed to contribute to this process but and vast majority of tubular cells derive from an intrarenal source. Interstitial cells may represent another extratubular stem cell niche. It is not clear whether renal stem cells exist in the adult, and if they do where are they located (interstitium, tubule, cortex, medulla) and what markers can be relied upon for the isolation and purification of these putative renal stem cells (Humphreys, 2006).

The kidney has a dramatic capacity to regenerate after injury. Whether stem cells are the source of the epithelial progenitors replacing injured and dying tubular epithelium is currently an area of intense investigation. Studies from our laboratory and others have supported a model whereby many surviving renal epithelial cells after injury become dedifferentiated and take on mesenchymal characteristics. These cells proliferate to restore the integrity of the denuded basement membrane, and subsequently redifferentiate into a functional epithelium. An alternative possibility is that a minority of surviving intratubular cells possess stem cell properties and selectively proliferate after damage to neighboring cells. Some evidence exists to support this hypothesis but it has not yet been rigorously evaluated. A third hypothesis is that extratubular cells contribute to repair of damaged epithelium. Bone marrow-derived stem cells have been proposed to contribute to this process but our work and work of others indicates that the vast majority of tubular cells derive from an intrarenal source. Recent evidence suggests that interstitial cells may represent another extratubular stem cell niche. The fundamental unanswered questions in this field include whether renal stem cells exist in the adult, and if they do where are they located (interstitium, tubule, cortex, medulla) and what markers can be relied upon for the isolation and purification of these putative renal stem cells. In this review we focus on our current understanding of the potential role of renal and extrarenal stem cells in repair of the adult kidney and highlight some of the controversies in this field (Humphreys, 2006).

The capacity of the kidney to regenerate functional tubules following episodes of acute injury is an important determinant of patient morbidity and mortality in the hospital setting. After severe injury or repeated episodes of injury, kidney recovery can be significantly impaired or even fail completely. Although significant advances have been made in the clinical management of such cases, there is no specific therapy that can improve the rate or effectiveness of the repair process. Recent studies have indicated that adult stem cells, either in the kidney itself or derived from the bone marrow, could participate in this repair process and might therefore be utilized clinically to treat acute renal failure. This review will focus on our current understanding of these stem cells, the controversies surrounding their in vivo capacity to repopulate the renal tubule, and further investigations that will be required before stem cell therapy can be considered for use in the clinical setting (Cantley, 2005).

While it remains unknown whether there is a stem cell in the adult kidney, characterization of the cell populations involved in renal repair and misrepair is allowing a new understanding of the mechanisms that are responsible for renal homeostasis (Oliver, 2004).

Ischemia-reperfusion injury (I/R injury) is a common cause of acute renal failure. Recovery from I/R injury requires renal tubular regeneration. Hematopoietic stem cells (HSC) have been shown to be capable of differentiating into hepatocytes, cardiac myocytes, gastrointestinal epithelial cells, and vascular endothelial cells during tissue repair. The current study tested the hypothesis that murine HSC can contribute to the regeneration of renal tubular epithelial cells after I/R injury (Lin, 2003).

The kidney has the ability to restore the structural and functional integrity of the proximal tubule, which undergoes extensive epithelial cell death after prolonged exposure to ischemia. Small numbers of peritubular endothelial cells to be derived from bone marrow cells that may serve in the repair process (Duffield, 2005).

Renal progenitor tubular cells [label-retaining cells (LRC)] are identified in normal kidneys by in vivo bromodeoxyuridine (BrdU) labeling. In normal and contralateral kidneys, LRC are observed scattering among tubular epithelial cells. After unilateral ureteral obstruction (UUO), the number of the LRC significantly increase, and most of them are positive for proliferating cell nuclear antigen (PCNA). In contrast, PCNA+ cells lacking BrdU label are rarely observed. LRC are not only in tubules but also in the interstitium after UUO. Laminin staining showed that a number of the LRC are adjacent to the destroyed tubular basement membrane. Some tubules, including LRC, lose the expression of E-cadherin after UUO. A large number of cell populations expressed vimentin, heat shock protein 47, or alpha-smooth muscle actin in the UUO kidneys, and each population contained LRC. None of the LRC is positive for these fibroblastic markers in contralateral kidneys. When renal tubules from BrdU-treated rats are cultured in the gel, some cells protruded from the periphery of the tubules and migrated into the gel. Most of these cells are BrdU+. Neither the total content of BrdU in the kidneys nor the number of LRC in bone marrow significantly is changed after UUO. LRC is a cell population that proliferates, migrates, and transdifferentiates into fibroblast-like cells during renal fibrosis (Yamashita, 2005).

8. The current scientific, ethical, and policy context of human embryonic stem cell research

New human embryonic stem cell lines are needed if human embryonic stem cells or their products are to be used for transplantation into humans. The twenty or so human embryonic stem cell lines approved for federally funded studies in 2001 by President Bush were derived using nonhuman feeder cells and serum and express the nonhuman antigen Neu5Gc. Thus, they would probably be immunologically rejected by the recipients unless this problem was remedied. Derivation of new human embryonic stem cell lines will be stimulated by the $3 billion in funding for stem cell research authorized by California voters in 2004. This measure will give priority to funding research that cannot be funded by NIH, which is currently the case for derivation of new human embryonic stem cell lines. Other states and private funders have followed suit in providing nonfederal support for human embryonic stem cell research. Outside of the U.S., human embryonic stem cell research is advancing vigorously. In May 2005, researchers from South Korea reported the derivation of 11 human embryonic stem cell lines using somatic cell nuclear transfer, demonstrating that technical obstacles to developing such stem cell lines can be overcome more readily than expected. In turn, such findings will stimulate further research.

Current ethical and policy guidelines for human embryonic stem cell research focus on the derivation of new human embryonic stem cell lines. In May 2005, a National Academy of Sciences (NAS) panel called for voluntary adoption of ethical guidelines in human embryonic stem cell research. Their recommendations included institutional oversight of human embryonic stem cell research

protocols through Embryonic Stem Cell Research Oversight Committees (ESCROs), informed consent from donors of materials for new human embryonic stem cell lines, restrictions on payment to gamete donors, and guidelines for banking stem cells and documentation. The twenty-three NRC recommendations have been endorsed by academic and scientific organizations and adopted as interim regulations for research funded by the state of California. That same month, the FDA issued regulations on screening and testing donors of human cells, tissues, and cellular and tissue-based products (HCT/P). While valuable, these initial efforts do not address crucial ethical issues in clinical trials of human embryonic stem cell transplantation, which have important upstream implications for how human embryonic stem cell lines should be derived, as well as for the conduct of the trials themselves. Our analysis begins with the need both to protect participants in Phase I trials of human embryonic stem cell transplantation and to respect the confidentiality of donors of materials used for derivation of human embryonic stem cell lines. These ethical responsibilities need to be addressed during the initial process of donating materials for new human embryonic stem cell lines. Next we consider challenges confronting informed consent for Phase I trials of human embryonic stem cell transplantation. We present specific recommendations for resolving these ethical issues.

9. Balancing the need to protect participants in Phase I clinical trials against the need to respect donors

The goal of Phase I clinical trials is to assess the safety and feasibility of the investigational intervention and to determine dosages for subsequent clinical trials. Direct therapeutic benefit, although hoped for, is unlikely in early trials, particularly if the first participants receive low doses. The guiding ethical principle of Phase I studies should be “Do no harm.” This ethical responsibility to protect the subjects in Phase I trials has important implications for the derivation of human embryonic stem cell lines. A major safety concern is transmission of infectious agents or serious genetic conditions through transplanted human embryonic stem cell cells or products. The public will expect strong protections against diseases transmitted through human embryonic stem cell transplantation, just as it demands that blood transfusions and solid organ transplants be tested for very rare but serious communicable diseases.

A broader perspective on protecting recipients of transplanted human embryonic stem cell materials is needed because of several clinical features of human embryonic stem cell transplantation. First, there is likely to be a considerable time period between donation of biological materials used to derive human embryonic stem cell lines and clinical trials involving transplantation of human embryonic stem cells or products from them. Polymorphisms and biomarkers associated with risk for specific diseases are being defined at a rapid pace. Second, in human embryonic stem cell transplantation, serious genetic conditions might also be transmitted, some of which may not have been apparent at the time the materials were donated. For instance, after donating, donors may develop cancer or a strong family history of cancer. Third, immunosuppressive drugs, which may be essential after cell transplantation to reduce rejection, will increase the risk of communicable diseases and cancer in recipients. Fourth, if human embryonic stem cell transplantation proves clinically effective, many patients may receive transplantation from a single human embryonic stem cell line over time. Hence many recipients may be at risk for diseases transmitted from donors. In order to safeguard recipients of human embryonic stem cell transplantation, researchers need to recontact persons whose gametes were used to derive the human embryonic stem cell lines at the time of clinical human embryonic stem cell transplantation trials to update information and perhaps do additional testing. Furthermore, if human

embryonic stem cell transplantation becomes a proven clinical treatment, periodic updating of the clinical status of donors would be prudent.

How can screening and testing of donors of materials for human embryonic stem cell lines be updated in an ethically acceptable manner? The responsibility to protect human embryonic stem cell transplant recipients from harm must be balanced against a responsibility to respect donors and protect their confidentiality. To resolve these countervailing mandates, researchers will need to obtain permission to recontact donors if human embryonic stem cell cells or materials derived from their gametes or embryos will be used for transplantation. Researchers need to tell donors about the kinds of information or testing that might be requested later and the reasons the information is needed. Such permission for recontact needs to be obtained when materials are donated for research. Without this permission, it would be a serious invasion of privacy to later recontact the donors. Also, donors who had not agreed to be recontacted might object strongly to a subsequent contact, refuse to provide information about their interim medical history, or undergo additional testing. Previous reports on the consent process for donating gametes and embryos for human embryonic stem cell research have not discussed the issue of recontact in depth. Obtaining permission to recontact will undoubtedly complicate the consent process for donating embryos for human embryonic stem cell research. However, permission for recontact will likely minimize the disqualification of human embryonic stem cell lines late in the development process for use in transplantation studies because of inadequate follow-up with donors. Recontacting donors presents logistical challenges because donors may move and contact may be lost. It would be desirable to ask donors to provide contact information for a relative or friends who will know their new address should they move. Confidentiality must be carefully protected because breaches might subject donors to unwanted publicity or even harassment. Concerns that their identities will not be kept confidential may deter some individuals from agreeing to be recontacted. Because of the intense public interest in and contentiousness over human embryonic stem cell research, it would be prudent for researchers and research institutions to develop stringent mechanisms, extending beyond those employed in routine clinical care, in order to assure donors that their identity and contact information remain protected.

10. Human embryonic stem cell transplantation in Phase I clinical trials

Current procedures for obtaining informed consent are likely to be inadequate to address particular issues faced by recipients of human embryonic stem cell transplantation in Phase I clinical trials. Because the matter is complex and any changes in policy will need careful consideration, discussions of the consent process need to begin now. Problems with informed consent commonly occur in clinical trials. Participants in cancer clinical trials commonly expect that they will benefit personally from the trial, even though the primary purpose of Phase I trials is to test safety rather than efficacy. This tendency to view clinical research as providing a personal benefit has been termed the “therapeutic misconception”. Analyses of consent forms suggest that such misunderstandings in cancer clinical trials do not reflect information in the consent forms. Indeed, cancer patients seeking therapeutic benefit may decide to enroll in a clinical trial before they meet the research staff, before they learn about the risks and benefits of the study or read a consent form.

Several measures may reduce the therapeutic misconception in recipients of human embryonic stem cell transplantation in Phase I clinical trials. First, researchers should frame their discussions with participants in the context of publicity about the potential for human embryonic stem cell to treat serious diseases. Second, investigators in human embryonic stem cell clinical trials must discuss a

broader range of information with potential participants than in other clinical trials. Informed consent requires researchers to discuss with potential participants information that is pertinent to their decision to volunteer for the clinical trial. Third, and most importantly, researchers should verify that participants have a realistic understanding of the study. The crucial ethical issue about informed consent is not what researchers disclose in consent forms or discussions, but rather what the participants in clinical trials understand. Lack of attention to the special ethical concerns raised by clinical trials of human embryonic stem cell transplantation and their implications for the derivation of new human embryonic stem cell lines may undermine or delay progress towards stem cell therapies (Bernard, 2005).

11 Why Are Embryonic Stem Cells So Valuable?

While grown in a dish, human embryonic stem cells can maintain their *"stem-cellness"* and provide an unlimited supply of more stem cells, as well as specialized cells that can be used for experiments and for the development of therapies. Apart from their potential to treat or cure diseases, human embryonic stem cells also provide a model to study very early human development and some of the disorders that lead to birth defects and childhood cancers. Many of these disorders develop in early pregnancy and are impossible to study in humans. Also, human embryonic stem cells also can be used to examine the genes that are turned "on" or "off" as stem cells generate more specialized cell types, permitting a unique understanding of the genetics of human development. The specialized cells derived from human embryonic stem cells also can be used to study the effectiveness of potential new drugs to treat diseases. This provides a human cellular model and can reduce animal experimentation and drug development costs. Additionally, embryonic stem cells can be derived from human blastocysts with specific genetic abnormalities. These types of blastocysts are identified through genetic diagnosis during IVF treatment, to screen out genetically abnormal blastocysts, and are usually discarded. The stem cells from them can provide a unique resource to understand genetic diseases and to develop cures. Human embryonic stem cells also could be used to understand the origin or causes of various diseases such as Alzheimer's disease or Parkinson's disease, which are currently unknown. Stem cells derived through *nuclear transfer* (more info below) from patients with such afflictions would provide special tools to study these diseases and possibly develop drugs for treatments.

12. Embryonic Stem Cells in the Clinic

Embryonic stem cells have not yet been used in treating humans. But numerous animal studies have shown that many of the specialized cells derived from them can indeed integrate into damaged tissues and function properly. Thus, diseases such as myocardial infarction, severe immune deficiency, diabetes, Parkinson's disease, spinal cord injury, and demyelination have been successfully treated in animal models. But the pathway from animal models to the clinic is still complex and burdened with obstacles to be overcome. First, not all specialized cells derived from human embryonic stem cells have been shown to integrate into animal tissue and function properly. This can be due to the poor quality of the specialized cells derived in culture, or to a lack of adequate communication between the human cells and the animal environment in which they are placed. Then there is the problem of scaling up to yield enough of the specialized cells to treat a human, since this requires many more cells than to treat a tiny mouse. Such cells will have to be produced under specific conditions to ensure safety for use in patients. Most human embryonic stem cells are still grown on a layer of mouse feeder cells, a potential source of contamination. Last, there's the problem of immune rejection by the patient. While the drugs

used in the organ transplantation field to suppress immune rejection have been improved over the years, rejection is still a major problem.

13. Debates on Stem Cell Research

There are a lot of debates on the stem cell research. Stem cell research is a high-tech question and the people involved in this rebates should have certain scientific knowledge on the stem cell. It is OK for the politicians or religionists to show their opinions on any topic they are interested in, but not suitable for them to make decisions (or make laws) that will significantly influence the scientific research as this field the politicians or religionists are not specialized. Such as, it is not suitable for the American President George W. Bush to show the power in the stem cell research. It is scientists' job. When politics and science collide, science should do scientific way, rather political way. Major ethical and scientific debates surround the potential of stem cells to radically alter therapies in health care (Williams, 2005).

14. Eternal Life

The production of functional male gametes is dependent on the continuous activity of germline stem cells. The availability of a transplantation assay system to unequivocally identify male germline stem cells has allowed their in vitro culture, cryopreservation, and genetic modification. Moreover, the system has enabled the identification of conditions and factors involved in stem cell self-renewal, the foundation of spermatogenesis, and the production of spermatozoa. The increased knowledge about these cells is also of great potential practical value, for example, for the possible cryopreservation of stem cells from boys undergoing treatment for cancer to safeguard their germ line

According to Greek mythology, the hapless mortal Tithonus mistakenly asked the goddess Eos to confer eternal life rather than eternal youth, and he thus found himself condemned to immortal decrepitude. A new report suggests that if Tithonus had cut a side deal with Dionysus, the god of wine, he might have fared much better.

The study knits together threads of recent molecular research on aging, the venerable antiaging strategy of calorie restriction, and, surprisingly, the health benefits of moderate tippling. David Sinclair of Harvard Medical School in Boston and colleagues identify several naturally occurring small molecules that extend the life of yeast cells by approximately 70% and offer some protection to cultured human cells exposed to radiation. The molecules activate genes known to extend life span in laboratory animals. They belong to a family of chemicals known as polyphenols, some of which are prominent components of grapes, red wine, olive oil, and other foods.

The work by Sinclair and collaborators at the biotech firm BIOMOL Research Laboratories in Plymouth Meeting, Pennsylvania, including Konrad Howitz, is the latest in an increasingly hot field exploring the molecular biology of calorie restriction, a phenomenon first demonstrated in the 1930s. Laboratory rats fed a limited diet live about 40% longer than normal and are resistant to many chronic illnesses typical of aging. The observations have been replicated in yeast, fruit flies, nematodes, fish, spiders, and mice, with hints from ongoing experiments that they hold true for primates. These findings have fueled interest in understanding how calorie restriction works--and an increasingly spirited search for molecules that might mimic the process without requiring a draconian diet.

Research in the Massachusetts Institute of Technology laboratory of Leonard Guarente, for example, has shown that increasing the activity of a single gene, called *SIR2*, can extend the life span

of yeast. And without the gene, calorie restriction doesn't prolong life. The new research shows that certain molecules activate *SIR2* in yeast, as well as an analogous gene, *SIRT1*, in human cells. Sinclair says that preliminary data from experiments in nematodes and fruit flies are "encouraging," in terms of whether similar activation of *SIR*-like genes, known collectively as sirtuins, can occur in those organisms, too. The study "establishes that you can get activation of *SIR2*," says Guarente, who has co-founded a company called Elixir Pharmaceuticals, which is searching for drugs that target the Sir pathway.

Working with colleagues at Harvard, BIOMOL researchers began screening a library of compounds about 2 years ago for molecules that trigger *SIRT1* activity. The initial screen yielded two polyphenols, quercetin (found in apples and tea) and piceatannol. The team then searched for other molecules with similar structures. That canvass yielded another 15 compounds, the most potent of which turned out to be resveratrol, found in grapes and red wine. It increased *SIRT1* activity 13-fold, the team reports online 24 August in *Nature*.

Resveratrol's *SIRT1*-activating power adds another dimension to the work, because it suggests a link to the so-called French paradox, the observation that despite a high-fat diet, people in France suffer about 40% less cardiovascular disease than expected; epidemiologists have linked this effect to the moderate consumption of red wine. Sinclair and colleagues speculate that these benefits may derive from activation of *SIR*-like genes. Increased *SIRT1* activity in human cells seems to blunt the activity of the tumor-suppressor gene *p53*, blocking programmed cell death. Sinclair suggests that the *SIR*-activating compounds buy time for cells to heal themselves rather than commit suicide.

In addition to its immediate implications for aging and life extension, the new work bolsters the notion that there is an evolutionarily conserved mechanism to stall the aging process during times of stress, such as when food is scarce. It also raises the possibility that the sirtuin-activating compounds reflect an interaction between plant and animal species. According to this hypothesis, which Sinclair calls "xenohormesis," plants increase their own production of polyphenols in response to environmental stresses such as drought, and that message of impending crisis may be passed on to animals that eat the plants. "Other unrelated, nonplant species can get chemical clues from the plant world," Sinclair says, "which causes them to mount their own defense response." Alternatively, he adds, the plant compounds may simply be similar to analogous, unidentified molecules in human biology.

Richard Weindruch of the University of Wisconsin, Madison, who is conducting calorie-restriction experiments in monkeys and other animals, applauds the new report but adds, "I think one needs to be very cautious about making dramatic leaps from the yeast model into mammals." He notes that it was unclear, for example, whether resveratrol affected the aging process in the kind of cells in the heart and brain that are particularly susceptible to degeneration with age.

"It's kind of romantic that red wine contains something that could extend your longevity, don't you think?" says Cynthia Kenyon, who researches aging at the University of California, San Francisco, after seeing the data presented at a meeting in Switzerland last week. But the results have not caused Sinclair to renegotiate his relationship with Dionysus. "I'd already increased my red wine consumption prior to this discovery," he confesses with a laugh (Hall, 2003).

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