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CONTENTS

IDENTIFYING POPULATIONS OF PAPUA NEW GUINEA'S INDIGENOUS CHICKENS FOR PRIORITY CONSERVATION

Gariba Danbaro [1], S Zhao [2,3,4], J Han [3,4]

ABSTRACT

The possible decline in genetic diversity of Papua New Guinea's (PNG) indigenous chickens and its consequences for food production and other concerns has necessitated a discussion of measures to conserve this genetic resource. As an initial step to investigate the genetic diversity and provide a theoretical basis for a conservation program of PNG's indigenous chickens, calculation and analyses of genetic diversities were carried out in this study using mitochondrial DNA (mtDNA) D-loop sequence variations in three populations of indigenous chickens. The results indicate that all of the Alotau, Madang and Port Moresby chicken populations in PNG have unique haplotypes and high genetic diversity. Priority for conservation of these populations has been suggested on the basis of their contributions to genetic diversity.

Key words: *Papua New Guinea, indigenous chicken, DNA, genetic, conservation*

INTRODUCTION

Genetic diversity in indigenous chickens will be required in the future to meet production needs in various environments, to allow sustained genetic improvement and to facilitate rapid adaptation to changing breeding objectives. However the need for conserving animal genetic resources (AnGR) is generally not well appreciated. According to the FAO (2007a) even though 321 world-wide chicken breeds have been found to be not at risk, 467 breeds are either extinct, critical, critical-maintained, endangered or endangered-maintained. FAO (2005, 2007a, 2007b) has therefore included indigenous chicken breeds in its AnGR conservation programs. However, the production potential of indigenous breeds in some developing countries is often inadequately documented and utilized (Philipsson and Okeyo 2006). The indigenous chickens of PNG have never been considered in AnGR conservation programs. Indigenous chickens were probably introduced into PNG between 2000 to 3000 years ago (Bellwood, 1978). The breed is currently found distributed throughout all parts of PNG and is the most important poultry species in the rural areas for food security and other socio-economic purposes. Currently these populations of indigenous chickens are threatened by many factors including genetic erosion by crossbreeding with imported exotic breeds, intensification of production systems and loss of habitat due to increasing human population and activity (Turner, 1972; Bilong 1990; Moat and Bilong 1999). Genetic characterization of these indigenous breeds for conservation and rational use is therefore necessary and urgent. In this study therefore, the genetic diversity of some populations of indigenous chickens of PNG were analysed and assessed for the first time using mitochondrial DNA (mtDNA) D-loop sequences and priorities for conservation of these indigenous chicken populations are discussed.

MATERIALS AND METHODS

Sampling

Blood samples were collected on FTA cards (Whatman, Inc.) from a total of 92 indigenous chickens in three different geographical regions of Papua New Guinea: Alotau (35 birds), Madang (35 birds) and Port Moresby (22 birds). Within each region, samples were collected from several birds from multiple households in different villages. To minimize the chances that the birds used from each village were closely related, a single bird was sampled from each household. The households within each village from which birds were used were approximately 0.7–1.5km apart.

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PCR amplification, purification and DNA sequencing

This work was done at the CAAS-ILRI Joint Laboratory on Livestock and Forage Genetic Resources, Beijing and the PNG University of Technology, Biotechnology Centre.

The D-loop region was amplified directly from the genomic DNA by polymerase chain reaction (PCR). The primer pair, L16750 (5'-AGGACTACGGCTTGAAAAGC–3') and H547 (5'- ATGTGCCTGACCGAGGAACCAG-3'), described by Niu et al. (2002), was used to amplify the first 510bp segment of the D-loop hypervariable region. In the primer names, L and H refer to the light and heavy chains, respectively, and the number designates the position of the 3'-end of the primer on the complete chicken mtDNA sequence (Desjardins and Morais, 1990). PCR reactions were carried out in 50µl volumes using 1× buffer, 1.5 mM MgCl2, 2.5 mM dNTP, 10 pM of each primer and 1 unit Huitian Taq polymerase. The PCR cycle included the initial denaturation at 94°C for 10 min followed by 30 cycles of denaturation at 94° C for 30 sec, annealing at 61°C for 30 sec and extension at 72°C for 30 sec with a final extension at 72°C for 10 min using GenAmp 9700 (Applied Biosystems, CA, USA). The PCR products were purified with Tiangen® PCR purification kit according to the manufacturer's instructions. Sequencing of the DNA was performed by using Big Dye Terminator Cycle Sequencing Ready Reaction Kit (v3.1, Applied Biosystems, CA, USA) and electrophoresis was done by a ABI3130XL DNA Genetic Analyzer (Applied Biosystems, CA, USA).

Data analysis

The mtDNA nucleotide sequences obtained in this study were aligned by using the ClustalX program (http:// www.igbmc.ustrasbg.fr/pub/ClustalX; Jeanmougin *et al.*, 1998) and identical sequences were considered as the same haplotypes. Calculation of haplotype frequency and Genetic diversity analyses were performed using Dnasp software version 4.10.3 (http:// www.ub.es/dnasp).

RESULTS AND DISCUSSIONS

Variant sites analysis

Analysis of the mtDNA D-loop sequences from the 92 samples showed a total of 28 nucleotide changes which could be grouped into 22 haplotypes (Table 1). The two largest haplotype groups consisted of 34 and 13 individuals while the remaining 20 haplotypes contained less than 10 individuals each. The nucleotide changes were characterized by transitions at 26 sites and transversions at 2 sites and no deletions or insertions.

Twenty eight polymorphic sites were found in the 397bp sequenced giving an average of 7.05% polymorphic sites in the 92 samples. This value is higher than those reported by other authors. *Liu et al.* (2004), Niu *et al.* (2001) and Fu *et al.* (2001) who reported the average percentage of polymorphism in D-loop region to be 6.4 and 4.45 (33 and 21 polymorphic sites in the same fragment of 397bp), respectively, for a Chinese native chicken breed. *Lee et al.* (2007) reported that the average percentage of polymorphic sites was 3.53 for 510bp (17 polymorphic sites in the same fragment of 397bp) for Korean Ogol chickens and attributed this lower level of polymorphism to sampling from one location where selection pressure continued for a long time. Moreover, low percentage of polymorphic sites in mtDNA D-loop sequences may be due to an evolutionary bottleneck during the course of domestication (Moritz, 1994). Therefore the higher level of polymorphism found in this study suggests that the samples have wide representation among the indigenous chickens of Papua New Guinea, and that these indigenous chickens could have experienced a milder bottleneck compared with those of other localities.

Distribution of haplotypes

The frequency of 22 haplotypes (H1-H22, Table 1) found in this study ranged from 1.09% to 36.96%, and the diversity of haplotypes was 23.91%. Only one haplotype, H4, was found in all the three regions of PNG while four haplotypes were observed in two of three regions and 17 haplotypes were detected in only one region. The wide distribution of haplotype H4 in all three regions suggests that this haplotype had higher fitness during the long history of domestication and so it might be an ancestral haplotype among PNG's indigenous chickens. Haplotypes 4, 12 and 1 were unique to Alotau, Madang and Port Moresby regions respectively, giving a total of 17 unique haplotypes. This distribution pattern of unique haplotypes indicates the genetic distinctiveness of the three indigenous chicken

Table 1: Polymorphic sites of mtDNA D-loop of indigenous chicken in Papua New Guinea

Haplotypes	Variable Sites*	Frequency			
	11112222222222223333333333 67791122445668990011234566 9 97179275836615136690524243 71 GTTATGTCCCCCTCGTCCCTCTG	Alotau	Madang	Port Moresby	Total
H1	GATCTC		1	5	6
H2	.....T.....		1		1
H3	.....T.....		9		9
H4	.....T.T.....	20	7	7	34
H5	.....CT.T.....		2		2
H6	A.....T.T.....	1**			1
H7	.....T.TC...G....	9		4	13
H8	.....T.TC..AG....	1			1
H9	A.....T.TC...G....	1			1
H10	.....T.TC.....		2		2
H11	.....T.T....G....	1	1		2
H12	.....T....G....		1		1
H13	...T.....T....T....G.T..		1		1
H14	.....TC.....T.....		1		1
H15	...C.C.....A.TT.....		4		4
H16	....C.....A.TT.....C.		2		2
H17	....C.....A.TT.....	1		5	6
H18	....AC.....A.TT.....		1		1
H19	..C.A.....A.TTT..A.CT..		1		1
H20	..C.A.....A.TTT..A.CT.T		1		1
H21	.C...TT.TC.A.TTC.....	1			1
H22	....A...TTTC.A.TTCT.....			1	1
	Total	35	35	22	92

*Numbers indicate nucleotide base position in mitochondrial D-loop region and hyphen represents the identical nucleotide with the H1 sequence. ** The italic number indicate the unique haplotypes.

populations. Thus the Madang chicken population with 12 unique haplotypes is the most distinctive followed by the Alotau and Port Moresby chicken populations.

Genetic distinctiveness of populations is an important criterion used when populations are selected for conservation. The highest priority for conservation is often given to population with highest genetic distinctiveness Moritz (1994). Parker *et al.* (1999, 2001) selected distinct highest priority for conservation followed by the Alotau and Port Moresby chicken populations.

Genetic diversity

Genetic diversity indices calculated for the three chicken populations are shown in Table 2. Nucleotide diversity indices ranged from 0.00446 to 0.00862 while haplotype diversity indices were between 0.620 and 0.889. Values of both indices were lower than those calculated

by Silva et al (2008) for indigenous chicken populations of Sri Lanka The difference in diversity indices between PNG and Sri Lankan indigenous chickens could be explained by the fact that Sri Lanka, located in Southeast of the Indian subcontinent, is an important centre of origin of indigenous chicken (Fumihito *et al.* 1996; West and Zhou 2002; Liu *et al.* 2006) and forms a confluence or exchange centre of other centres from where indigenous chickens were distributed to other parts of Southeast Asia (including PNG) and Africa (Muchadeyi *et al.* 2008). Therefore Sri Lankan indigenous chickens can be expected to have generated and accumulated higher levels of genetic diversity after several centuries of domestication. However, nucleotide diversity indices observed in this study were higher than those estimated by Liu *et al.* (2006) for certain clades of chickens from Europe, Middle East, Southeast and East Asia, and by Oka *et al.* (2007) for Japanese native chickens. Higher genetic diversity in chickens is indicative of center of species origin and confluence (Chen *et al.* 2002) where genetic variation has been generated and accumulated over long periods of time. Japanese native chickens are believed to have been established from native chicken populations of other East and Southeast Asian countries and this account for the lower genetic diversity of the foundation populations of Japanese native chicken compared to original populations of other Asian countries. Furthermore, most Japanese native chickens are ornamental breeds that have low productivity. Therefore the number of individuals in some of these Japanese breeds could be decreasing, and this could be one of the causes of reduction in genetic diversity (Oka *et al.* 2007). Several authors have suggested that Southeast Asia is another centre of origin of indigenous chicken (Fumihito *et al.* 1996; West and Zhou 2002; Liu *et al.* 2006) and that one of three major maternal lineages of the modern Chilean chicken breeds is from the South Chinese/ Indonesian/Japanese area (Gongora *et al.* 2008). Because PNG has a common border with Indonesia to the west it might be part of the Southeast Asian centre of origin of indigenous chickens. This could explain the higher genetic diversity of the indigenous chickens of PNG observed in this study. The genetic diversity generated because of originality of centre is the most important for conservation (Chen *et al.* 2002).

Another approach used by some authors is to give higher priority for conservation to populations that show higher genetic diversity (Chen *et al.* 2002). In this study, the diversity indices Pi, k and Hd for the Madang chicken population were 0.00862, 3.422 and 0.889 respectively (Table 2) and these were the highest values among the three populations. The Port Moresby chicken population had the next highest indices while the Alotau population had the lowest. These results also indicate the order of genetic richness in the three indigenous chicken populations of PNG and consequently the order to be followed in prioritizing these populations for con-

Table 2 Diversity parameters of Indigenous chicken in Papua New Guinea

Population	S	Hn	Hu	Pi	k	Hd+SD
Alotau	35	8	4	0.00446	1.771	0.620±0.074
Madang	35	15	12	0.00862	3.422	0.889±0.034
Port Moresby	22	5	1	0.00835	3.316	0.797±0.039

S; The size of populations' Hn: The number of haplotypes; Hu: The number of unique haplotypes, Pi; Nucleotide diversity, k; Average number of nucleotide differences; Hd: Haplotypes (gene) diversity, SD; Standard deviation

Genetic contribution analysis

Due to differences in the size of populations, scarcity of funds for species conservation and conflict between conservation and economic development, deciding what and where to conserve is an essential step in managing important species. Generally, the main aim is usually to protect the genetic resources as much as possible, on the basis of both genetic distinctiveness and diversity. However, the genetic distinctiveness-based approach chooses populations with more genetic uniqueness for priority conservation. It does not consider genetic variation within populations (Chen *et al.* 2002; Pennock and Dimmick 2002), while the genetic diversity-based approach chooses populations with high genetic variation for priority conservation without considering genetic distinctiveness. Thus some haplotypes unique to some populations with low genetic variation may not receive sufficient attention for conservation in some cases. Therefore, Petit *et al.* (1998) put forward the approach of genetic contribution, a synthesis that considers genetic diversity. This approach appears to be the most appropriate for selecting populations for conservation (Chen *et al.* 2002; Ping *et al.* 2004). The contributions of genetic diversity ($R_{S\,(k)}$) and genetic distinctiveness ($R_{D\,(k)}$) are combined to get the total genetic contribution ($R_{T\,(k)}$) of the k^{th} population

$$R_{S(k)}=\frac{R_k}{n};\ R_{D(k)}=\sum_i^{R_k}\frac{n-n_i}{nn_i}\ \text{and}\ R_{T(k)}=R_{S(k)}+R_{D(k)}=\sum_i^{R_k}\frac{1}{n_i}$$

Where n represents the total of populations studied and n_i represents the number of populations with the i^{th} haplotype. Similarly the rates of contribution attributed to genetic variation ($C_{S(k)}$) and genetic distinctiveness ($C_{D(k)}$) to the total genetic contribution rate ($C_{T(k)}$) of the k^{th} population with R_k haplotypes are obtained by using the formulae:

$$C_{RS(k)}=\frac{R_{S(k)}-\overline{R}_S}{R_T};\ C_{RD(k)}=\frac{R_{D(k)}-\overline{R}_D}{R_T}\ \text{and}\ C_{RT(k)}=\frac{R_{T(k)}-\overline{R}_T}{R_T}$$

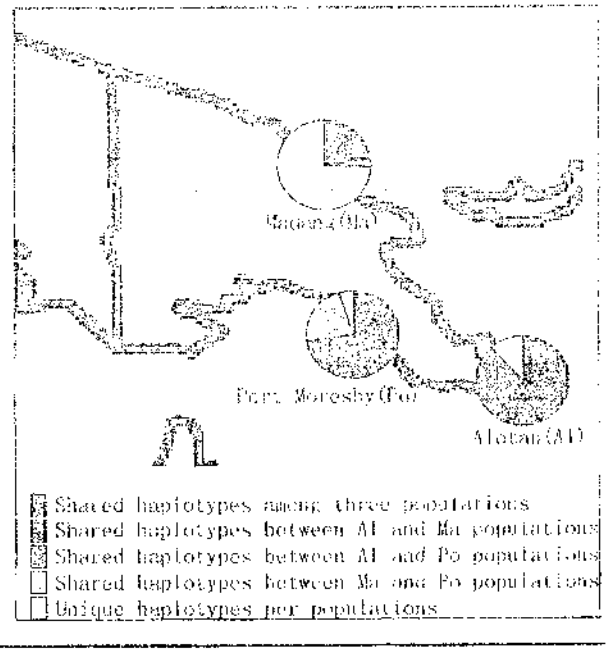
Where R_T represents the total of haplotypes, $\overline{R}_S=\sum_k R_{S(k)}/n$, $\overline{R}_D=\sum_k R_{D(k)}/n$, $\overline{R}_T=\sum_k R_{T(k)}/n$. The total contribution rate $C_{RT(k)}$ can be partitioned into two components, $C_{RS(k)}$, which is the rate of contribution of the k^{th} population due to its own diversity and $C_{RD(k)}$, the contribution due to its divergence, i.e. $C_{RT(k)}=C_{RS(k)}+C_{RD(k)}$, $\sum_k C_{RS(k)}=0$, $\sum_k C_{RD(k)}=0$, $\sum_k C_{RT(k)}=0$ (Table 3).

Table 3 Genetic contribution of three indigenous chicken populations in Papua New Guinea

Populations	$R_{S(k)}$	$R_{D(k)}$	$R_{T(k)}$	$C_{RS(k)}$ (%)	$C_{RD(k)}$ (%)	$C_{RT(k)}$ (%)
Alotau	2.667	3.167	5.834	-0.020	-0.048	-0.068
Madang	5.000	8.334	13.334	0.086	0.187	0.273
Port Moresby	1.667	1.167	2.834	-0.066	-0.139	-0.205

The values obtained for R_S and R_D (and therefore R_T) were highest for the Madang chicken population and lowest for the Port Moresby chicken population. The value of $C_{S(k)}$, $C_{D(k)}$ and $C_{T(k)}$ provide relative overall criteria for setting priorities for conservation of PNG's indigenous chicken populations. The positive values of $C_{S(k)}$, $C_{D(k)}$ and $C_{T(k)}$ in Madang chicken population show that the genetic variation contribution rate, genetic distinctiveness contribution rate and the total of genetic contribution rate were higher than the average of the three populations, thereby indicating that the Madang chicken population could contribute most to improving the genetic variation and haplotypes richness of PNG's indigenous chicken followed by the Alotau and Port Moresby indigenous chicken populations respectively.

Figure 1: Geographical distribution of three chicken populations in Papua New Guinea



This order could therefore be followed in the conservation of PNG's indigenous chicken population given that human, material and financial resources are limited. This order is the same as the order based on genetic distinctiveness and implies that the priorities for conservation of PNG's chicken genetic resources depends to a great extent on the genetic distinctiveness of the chicken populations.

CONCLUSION

Papua New Guinea's indigenous chicken populations have unique haplotypes and high genetic diversity and probably belong to the Southeast Asia centre of domestication of indigenous chickens and are therefore an important genetic resource which needs to be considered for conservation. This need is all the more urgent in the light of global climate change and its consequences for food production especially in developing countries, genetic erosion and intensification of production systems in the country. This study has concentrated on three indigenous chicken populations and suggests that the highest priority for conservation should be given to the Madang chicken population followed the populations from Alotau and Port Moresby in that order.

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PANICLE CHARACTERISTICS IN HIGH-YIELDING *japonica* RICE LINES CARRYING *Ur1* (UNDULATED RACHIS-1) GENE

ABSTRACT

Ur1 (Undulate Rachis -1), an incompletely dominant gene on chromosome 6, being characterized by undulation of primary and secondary branches at the lowest part of a panicle, has the principal effect of increasing number of secondary branches per primary branch, and additional effects of increasing both spikelet number per single secondary branch and number of primary branches per panicle. This genic effect can increase grain yield by enlarging sink size. We examined the panicle traits of two high-yielding Ur1-carrying lines (MR79 and MR53) and two commercial varieties ('Hinohikari' and 'Nishihikari'). They were grown at high, middle and low fertilizer levels in a paddy field. In addition to the traits mentioned above, the numbers of differentiated (developed + degenerated) primary and secondary branches were measured. MR53 was outstandingly higher than the two commercial varieties in spikelet number per panicle, number of secondary branches per primary branch and number of differentiated secondary branches per primary branch. In each of these traits, MR79 was higher than the two commercial varieties, but lower than MR53 more or less. By number of differentiated secondary branches per primary branch, the spikelet twinning and the panicle opening in total, plants with the Ur1/Ur1 genotype could be selected in a segregating population like F_2 in a process of breeding by the use of Ur1, even if undulation is little or absent like in MR79.

Keywords : *rice, Ur1 gene, panicle, spikelet number, number of differentiated secondary branches.*

INTRODUCTION

Ur1 (Undulate Rachis -1), an incompletely dominant gene on chromosome 6 (Nagao *et al.* 1958 and 1963; Sato and Shinjyo 1991), being characterized by undulation of primary and secondary branches at the lowest part of a panicle (Fig.1), has the principal effect of increasing number of secondary branches per primary branch, viz acceleration of secondary branching from primary branches, and additional effects of increasing both spikelet number per single secondary branch and number of primary branches per panicle (Murai and Iizawa 1994; Murai *et al.* 2012).

In addition to the panicle traits mentioned above, number of differentiated (developed + degenerated) secondary branches, pedicel length and other traits were investigated for an isogenic line of Taichung 65 carrying both *Ur1* and *sd1-d* (dee-geo-woo-gen dwarf), the *Ur1* isogenic line of 'Nishihikari', together with the *sd1-d* isogenic line of Taichung 65 and 'Nishihikari' (their respective genetic backgrounds) (Murai *et al.* 2012). Effect of *Ur1* on number of differentiated secondary branches per primary branch was higher than number of (developed) secondary branches per primary branch. They found twined spikelets at the tip of the uppermost primary branch of a panicle in the *Ur1/Ur1* genotype (Fig. 2), because *Ur1* shortened the pedicel length of the top spikelet but it elongated that of the second spikelet. This visual characteristic was recommended as one of indicators for the *Ur1/Ur1* genotype.

Murai *et al.* (2005) preliminarily reported that a *japonica Ur1*-carrying line Murai 79 (hereafter "MR79") had a higher yielding-ability exceeding the levels of ordinary *japonica* varieties in southern Japan, due to its higher sink size caused by more spikelets per panicle, which was derived from the cross of a Japanese commercial variety and an isogenic line of Taichung 65 carrying both *Ur1* and *sd1-d* (dee-geo-woo-gen dwarf). Another *Ur1*-carrying line "MR53" was developed from the same cross by Murai (Malangen *et al.* 2013). MR79 and MR53 possess extremely late and rather early heading times, respectively (Table 1). These two lines

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Fig. 1. Undulation of primary and secondary branches at the lowest part of a panicle in MR53 (*Ur1/Ur1*).

Fig. 2. Twinning of the top spikelet and next one in the uppermost primary branch of a panicle in an isogenic line of Taichung 65 carrying both *Ur1* and *sd1 -d* (*Ur1/Ur1*).

Table 1. Fertilizer application levels and heading dates of the two *Ur1*-carrying lines and two commercial varieties.

Fertilizer level	Basal dressing ($N^{2)}g/m^2$)	Top dressing [1] ($N^{2)}$ g/m^2)	80%-heading date in 2003			
			MR 53	MR79	Ni	Hi
High	8	8 [3]	July 29	Aug. 22	Aug. 13	Aug. 9
Middle	4	4 [3]	July 28	Aug. 22	Aug.13	Aug.9
Low	2	2 [3]	July 29	Aug. 22	Aug.13	Aug.9

[1] A slow-release coated fertilizer (LONG ®, 100-day type presented from Chisso Asahi Fertilizer Co., Ltd.), about 7% of each element of which was readily available, was used.

[2] P_2O_5 and K_2O elements were supplied at the same level as N element, an ordinary chemical fertilizer was used.

[3] Applied on June 30 for MR53, July 27 for MR79, July 9 for Hi, July 14 for Ni in 2003.

and two commercial varieties were grown under various fertilizer levels in a paddy field of Kochi University in southern Japan in 2003 and 2005 in the previous study (Malangen *et al.* 2013). High yields of 728 and 723 g/m^2, respectively, were obtained by MR79 and MR53 through a heavy fertilizer application. Furthermore, MR79 was the highest-yielding in every combination of fertilizer levels and years, suggesting that MR79 involves a high yielding-ability stable to variation of fertilizer level.

In the present study, panicles of MR79, MR53 and the two commercial varieties grown at three fertilizer levels in 2003 (Malangen *et al* 2013) were used for measurements of above-mentioned panicle traits inclusive of number of differentiated secondary branches. On the basis of the data, we examine:

1) Which traits can classify the two *Ur1*- carrying lines from the two commercial varieties?

2) Whether the classification was influenced by fertilizer level or not?

3) Which differences were detected between MR79 and MR53? and

4) Combination of measurable trait(s) and visual characteristics enable gentypic determination at the *Ur1* locus in a segregating population like F_2?

MATERIALS AND METHODS

Ur1-carrying lines

The highest-yielding F_1 in the yield tests for various F_1 hybrids with the *Ur1/* + genotype (Murai *et al.* 1997 and 2003) was used for developing recombinant inbred lines with and without *Ur1* (Murai *et al.* 2005; Malangen *et al.* 2013). Its maternal and paternal parents were 'Nishihikari' and an isogenic line of Taichung 65 carrying both *Ur1* and *sd1-d* (dee-geo-woo-gen dwarf), respectively. The F_2 population was grown in 1992, and the generation was progressed to F_8 without selection in glasshouse condition. In 1999, the 108 F_9 lines originating from the respective 108 F_2 plants were grown in a paddy field; the two well-ripened lines carrying *Ur1*, viz. MR53 and MR79 were selected from *Ur1*-carrying lines by field observation. The uniformity (non-segregation) in MR53 and MR79 was confirmed from 2000 to 2003 (F_{10} to F_{14} generations).

Fig. 3. Panicles of MR53, MR79 and Hi ('Hinohikari'), from left to right. MR 53 and MR 79 carry *Ur1* gene (*Ur1/Ur1*).

Appearances in panicles of two *Ur1*-carrying lines

Fig. 3 exhibits matured panicles of MR53, MR79 and 'Hinohikari'. The undulation of primary and secondary rachis branches at the lowest part of panicles was noticed in MR53 (Fig. 1), whereas little or no undulation was observed in MR79. The panicles of the two *Ur1*-carriers, particularly MR53, were more open at maturity than the ordinary, being in agreement with the observation by Murai *et al*, (2002). Moreover, twined or semi-twined spikelets at the tip of the uppermost primary branch, which is caused by *Ur1* (Murai *et al.* 2012), were observed in at least one panicle within every hill of both MR 53 and MR 79 (Fig. 3). Such spikelets were more frequently observed in MR53 than in MR79. Accordingly, MR53 had typical panicle appearance of the *Ur1/Ur1* genotype. On the other hand, MR79 had many panicles which were hardly decided as *Ur1*–carrying from their appearances.

Commercial varieties for comparison to *Ur1*-carriers

'Nishihikari' and 'Hinohikari', (abbreviated as "Ni" and "Hi" respectively) were used for the field experiments. Ni is a short-culm and panicle-number type variety possessing the highest lodging tolerance in southern Japan (Nishiyama 1982). Hi is a leading variety in southern Japan, which possesses rather late heading, rather long culm and rather many panicles.

Cultivation and sampling

The two *Ur1*-carrying lines and the two commercial varieties were seeded on April 16 and transplanted on May 9 in 2003 to a paddy field of the faculty of Agriculture, Kochi University, Japan (Malangen *et al*, 2013). Two seedlings per hill were transplanted at a spacing of 30.0 × 15.0cm. They were grown under high, middle and low fertilizer levels (Table 1). The randomized block design with three replications was adopted for all combinations of the lines-varieties and fertilizer levels. Each plot comprised 108 hills (4 rows × 27 hills). The largest panicle in each of ten hills from each plot (a total of 30 panicles per line/variety at each fertilizer level from three replications) was used for the measurements of traits described below.

Panicle traits measured

Table 2 shows 12 panicle traits adopted in the present study. There is the following relationship between spikelet number per panicle and the four components (Murai and Kinoshita 1983; Murai and Iizawa 1994). NS = SB1×NB1 + SB2×NB2×NB1 (NB2×NB1 = number of secondary branches per panicle). Thus, spikelet number per panicle can be equated by NB1 (the number of primary branches), NB2 (the number of secondary branches per primary branch), SB1 (the number of spikelets per primary branch) and SB2 (the number of spikelets per secondary branch). Beside the numbers of developed primary and secondary branches, the numbers of degenerated primary and secondary branches were measured, being denoted by DNB1 and DB2, respectively. The number of differentiated primary branches (TNB1) is calculated by NB1 + DNB1. Similarly, TB2 is calculated by B2 + DB2. TNB2 equals TB2/NB1.

Table 2: List of panicle traits assessed in this study

Trait (abbreviation)	Explanation
1. No. of spikelets per panicle (**NS**)	Measured by the largest panicle in each hill.
2. No. of primary branches (**NBl**)	Ditto
3. No. of secondary branches (**B2**)	Ditto
4. No. of secondary branches per primary branch (**NB2**)	No. of secondary branches / No. of primary branches per panicle.
5. No. of spikelets per primary branch (**SB1**)	No. o f spikelets setting on primary branches / No. of primary branches.
6. No. of spikelets per secondary branch (**SB2**)	No. of spikelets setting on secondary branches / No. of secondary branches.
7. **SB2 %**	Percentage of spikelet setting on secondary branches to No. of spikelets per panicle.
8. **DNB1**	No. of degenerated primary branches per panicle.
9. **DB2**	No. of degenerated secondary branches per panicle.
10. **TNB1**	Total number of differentiated primary branches = **NB1 + DNB1**
11. **TB2**	Total number of differentiated secondary branches = **B2 + DB2**
12. **TNB2**	Total number of differentiated secondary branches per primary branch = **TB2 /NB1**

Statistical analysis

Analysis of variance was conducted using EXCEL Toukei Version 5.0 (ESUMI Co., Ltd., Tokyo, Japan), and Least Significant Difference (LSD) was calculated from error variance.

RESULTS

Table 3 shows results of analysis of variance for the panicle traits of the two *Ur1*- carrying lines and two commercial varieties at the three fertilizer levels. For NS, the effects of the lines and the fertilizer levels were statistically significant whereas the interaction was not significant. This trait was in the order MR53 > MR79 > Hi ≧ (or >) Ni at each of the fertilizer levels (Table 4). At every fertilizer level, MR53 and MR79 were significantly higher than Hi and Ni. MR53 and MR79 were 34 to 43% and 23 to 37%, respectively, higher in NS than Hi in all fertilizer levels. There was non-significant or small difference between Ni and Hi. Ns of each line/variety increased with the enhancement of fertilizer level.

For NB1, the effects of the lines and the fertilizer levels were statistically significant but the interactive effect was not significant (Table 3). At every fertilizer level, MR79 was the highest among all lines-varieties in this trait, while Ni was the lowest. Differences between MR53 and Hi at the middle and low fertilizer levels were not statistically significant. Hence, the two *Ur1*-carriers were not distinctly classified from the two non-carriers by this trait. Every line/variety showed positive fertilizer response.

The effect of lines alone among the three factors was significant in B2. This trait was in the order MR53 > MR79 > Ni ≧ (or ≦) Hi at each of the fertilizer levels. The B2s of MR53 and MR79 were about twofold and about one and half of that of Hi, respectively, at every fertilizer level. Ni was not significantly different from Hi at every fertilizer level.

Regarding NB2, the effect of lines was significant, while those of fertilizer levels and the interaction were not significant. It was in the order

Table 3. Analysis of variance for all panicle traits of the two *Url*-carrying lines and two commercial varieties at the three fertilizer levels

	Source of Variation [1]			
Trait (Abbreviation)	**Lines (A)**	**Fertilizer levels (B)**	**Interaction (A X B)**	**All combinations of lines and fertilizer levels**
NS	186.69 ** [2]	10.95 ** [2]	1.02 [2]	53.46** [2]
NB1	52.31 **	6.58**	2.03	16.57**
B2	306.92 **	2.61	<1	84.48**
NB2	381.50 **	<1	<1	104.35**
SB1	278.10**	<1	<1	76.25**
SB2	58.54**	<1	<1	16.62**
SB2 %	97.59**	<1	2.24	27.96**
DNB1	4.41*	<1	<1	1.38
DB2	210.27**	6.93**	3.70*	60.62**
TNB1	50.01**	8.76**	2.54	16.62**
TB2	383.49**	7.06**	1.14	106.49**
TNB2	371.97**	<1	<1	102.01**

[1] Degrees of freedom for fertilizer levels, lines, interaction, all combinations of lines and fertilizer levels and error are 3,2,6,11 and 22, respectively.

[2] Numbers in the table indicate F-values.

*,** Significant at the 5% and 1% levels, respectively.

MR53 > MR79 > Ni ≧ (or >) Hi at every fertilizer level. MR53 and MR79 were 107 to 117% and 36 to 43%, respectively, higher in this trait than Hi in all fertilizer levels. Ni was not significantly different from Hi at middle and low fertilizer levels.

As for SB1, the effect of lines alone was significant. At every fertilizer level, it was in the order MR53 < MR79 < (or ≦) Ni ≦ Hi. According to Murai *et al* (1994 and 2012), *Url* accelerates secondary branching toward the tip of each primary branch, resulting in decrease of spikelets directly set on a primary branch. This may cause the lower SB1s in the two *Url*-carriers.

As for SB2, the effect of lines alone was significant. It was in the order MR53 > MR79 > (or ≧) Ni ≧ (or >) Hi at each of the fertilizer levels. Thus, the two *Url*-carriers were higher in SB2 than the two non-carriers.

Regarding SB2%, the effect of lines alone was significant. At every fertilizer level, it was in the order MR53 > MR79 > Ni ≧ (or >) Hi. Thus, the two *Url*-carriers were distinctly higher than the two non-carriers; particularly, MR53 was the highest.

Table 6 shows the panicle traits concerning degeneration and differentiation of branches (Table 2) in the two *Url*-carrying lines and two commercial varieties at the three fertilizer levels. For DNB1, the effect of lines alone was significant at the 5% level (Table 3). This trait ranged from 0.03 to 0.30 overall combinations of lines-varieties and fertilizer levels; no or at most one degenerated primary branch per panicle was observed in all measured panicles of all lines-varieties. MR79 had little more degenerated primary branches than the other three lines-varieties at each fertilizer level.

Regarding DB2, the effects of lines, fertilizer levels and the interaction were all significant. It was in the order MR79 > MR53 > Hi > Ni at every fertilizer level. MR53 and MR79 were 35 to 74% and 105 to 140%, respectively, higher in this trait than Hi in all fertilizer levels. This trait was higher at the high fertilizer level than at the low fertilizer level in both MR53 and MR79, but

Ni and Hi showed no significant fertilizer responses.

The result in TNB1 was similar to that in NB1 (Table 4), since every measured panicle had no or at most one degenerated primary branch.

Regarding TB2, the effects of the lines and the fertilizer levels were significant but the interactive effect was not significant. It was in the order MR53 > (or ≧) MR79 > Hi ≧ (or >) Ni at every fertilizer level. MR53 and MR79 were 91 to 109% and 75 to 81%, respectively, higher in this trait than Hi in all fertilizer levels. Significant and non-significant positive fertilizer responses were detected in the two *Ur-1* carriers and the two non-carriers, respectively.

The effect of lines alone was significant in TNB2. This trait was in the order MR53 > MR79 > Hi ≧ (or >) Ni at every fertilizer level. MR53 and MR79 were about twofold and about one and half of Hi, respectively, in this trait at every fertilizer level. There was non-significant or small difference between Ni and Hi at each of the fertilizer levels.

DISCUSSION

Regarding NB1, MR53 was similar to or lower than Hi; on the other hand, MR79 was higher than the two non-carriers (Table 4). According to Murai *et al.* (2012), *Ur1* increased NS by 38 and 35%, NB1 by 13 and 5%, NB2 by 45 and 58% and SB2 by 10 and 20%, but it decreased SB1 by 5 and 18%, respectively, on the genetic background of Ni and that of Taichung 65 with *sd1-d*. Thus, *Ur1* increased NB1 more on the former genetic background than on the latter genetic background. It is assumed that gene(s) accelerating primary branching in the cooperation with *Ur1* inherited from Ni to MR79 in the process of development.

MR53 was outstandingly higher than the two non-carriers in NS, NB2, SB2 and B2%, as well as TB2 and TNB2. In each of these traits, MR79 was higher than the two non-carriers, but

Table 4: No. of spikelets per panicle, its components and other traits of the two *Url*-carrying lines and two commercial varieties at the three fertilizer levels.

Trait	Fertilizer level	MR53	MR79	Ni	Hi	$LSD_{(5\%)}$
NS	High	134.7 ^{a} (134)[1)]	123.9 ^{bc} (123)[1)]	92.0 ^{e} (91)[1)]	100.6 ^{d}	7.5
	Middle	129.9 ^{ab} (143)	122.1 ^{c} (134)	88.7 ^{e} (97)	91.0 ^{c}	
	Low	122.9 ^{bc} (141)	120.1 ^{c} (137)	87.0 ^{e} (99)	87.4 ^{b}	
NB1	High	10.37 ^{c} (93)	11.90 ^{a} (107)	9.53 ^{d} (86)	11.10 ^{b}	0.65
	Middle	10.27 ^{c} (103)	11.50 ^{ab} (115)	9.53 ^{d} (95)	10.00 ^{cc}	
	Low	9.77 ^{cd} (102)	11.53 ^{ab} (121)	9.47 ^{c} (99)	9.53 ^{d}	
B2	High	29.86 ^{a} (205)	21.57 ^{b} (148)	14.33 ^{c} (98)	14.60 ^{c}	2.15
	Middle	29.50 ^{a} (225)	21.87 ^{b} (166)	13.70 ^{c} (104)	13.13 ^{c}	
	Low	27.90 ^{a} (216)	21.63 ^{b} (167)	13.17 ^{c} (102)	12.93 ^{c}	
NB2	High	2.87 ^{a} (217)	1.81 ^{b} (137)	1.51 ^{c} (114)	1.32 ^{d}	0.18
	Middle	2.87 ^{a} (217)	1.90 ^{b} (143)	1.45 ^{cd} (110)	1.32 ^{d}	
	Low	2.86 ^{a} (207)	1.88 ^{b} (136)	1.40 ^{cd} (101)	1.38 ^{d}	
SB1	High	3.84 ^{d} (67)	5.40 ^{bc} (94)	5.61 ^{ab} (98)	5.74 ^{a}	0.27
	Middle	3.76 ^{d} (66)	5.25 ^{c} (91)	5.58 ^{ab} (97)	5.74 ^{a}	
	Low	3.87 ^{c} (67)	5.18 ^{c} (90)	5.53 ^{ab} (96)	5.78 ^{a}	
SB2	High	3.18 ^{a} (126)	2.76 ^{bc} (110)	2.69 ^{bcd} (107)	2.51 ^{c}	0.16
	Middle	3.08 ^{a} (119)	2.82 ^{b} (109)	2.59 ^{dc} (100)	2.59 ^{dc}	
	Low	3.04 ^{a} (122)	2.81 ^{b} (112)	2.63 ^{cde} (105)	2.49 ^{c}	
SB2%	High	70.0 ^{a} (193)	47.8 ^{b} (132)	41.8 ^{c} (115)	36.4 ^{d}	4.0
	Middle	69.9 ^{a} (188)	50.7 ^{b} (137)	39.9 ^{cd} (108)	37.0 ^{d}	
	Low	68.8 ^{a} (188)	50.7 ^{b} (139)	39.7 ^{cd} (108)	36.6 ^{d}	

[1)] Percentage to Hi in parentheses

Values followed by the same letter within each trait are not significantly different at the 5% LSD level.

Table 5: Traits concerning degeneration and differentiation of primary and secondary branches of the two *Url*-carrying lines and two commercial varieties at the three fertilizer levels.

Trait (abbreviation)	Fertilizer Levels	MR53	MR79	Ni	Hi	LSD (5%)
DNB1	High	0.03 ^{b} (50)[1)]	0.30 ^{a} (450)[1)]	0.03 ^{b} (50)[1)]	0.07 ^{ab}	0.24
	Middle	0.10 ^{ab} (100)	0.23 ^{ab} (233)	0.03 ^{b} (33)	0.10 ^{ab}	
	Low	0.17 ^{ab} (125)	0.27 ^{ab} (200)	0.03 ^{b} (25)	0.13 ^{ab}	
DB2	High	9.83 ^{c} (159)	14.90 ^{a} (240)	2.70 ^{f} (44)	6.20 ^{e}	1.49
	Middle	9.83 ^{c} (174)	12.13 ^{b} (214)	3.13 ^{f} (55)	5.67 ^{e}	
	Low	7.70 ^{d} (135)	11.70 ^{b} (205)	3.17 ^{f} (56)	5.70 ^{e}	
TNB1	High	10.40 ^{c} (93)	12.12 ^{a} (109)	9.53 ^{e} (85)	11.17 ^{b}	0.71
	Middle	10.37 ^{cd} (103)	11.73 ^{ab} (116)	9.53 ^{e} (94)	10.10 ^{cde}	
	Low	9.93 ^{cde} (103)	11.77 ^{ab} (122)	9.50 ^{e} (98)	9.67 ^{de}	
TB2	High	39.70 ^{a} (191)	36.47 ^{b} (175)	17.03 ^{e} (82)	20.80 ^{d}	2.78
	Middle	39.33 ^{a} (209)	34.00 ^{bc} (181)	16.83 ^{e} (89)	18.80 ^{de}	
	Low	35.60 ^{bc} (191)	33.33 ^{c} (179)	16.33 ^{e} (88)	18.63 ^{de}	
TNB2	High	3.83 ^{a} (203)	3.06 ^{b} (162)	1.80 ^{cd} (95)	1.88 ^{cd}	0.25
	Middle	3.83 ^{a} (203)	2.96 ^{b} (156)	1.78 ^{cd} (94)	1.89 ^{cd}	
	Low	3.65 ^{a} (183)	2.90 ^{b} (145)	1.73 ^{d} (87)	1.99 ^{c}	

[1)] Percentage to Hi in parentheses

Values followed by the same letter within each trait are not significantly different at the 5% level, determined by LSDs in the table.

lower than MR53 more or less. Yamagishi *et al.* (2002) found a QTL controlling number of secondary branches per panicle in a genetic difference between two ordinary Japanese varieties; even though its effect seems to be smaller than that of the major gene *Ur1*. It is inferred that allele(s) with minus-direction effect(s) at such QTL(s) diminished these traits in MR79, resulting in its less typical panicles compared with those of MR53.

MR53 had its typical panicle appearance caused by *Ur1*, while MR79 had panicles which cannot be distinguished distinctly from those of non-carriers (see Materials and Methods, Fig. 2). TB2, including degenerated secondary branches, can divide MR79 from the two non-carriers more distantly than B2 (Table 4 and Table 5). Similarly, TNB2 can divide the former from the latter more distantly than NB2. Besides, any lines-varieties did not show significant fertilizer response in this trait, suggesting that TNB2 can be an indicator for the *Ur1/Ur1* genotype stable to variation of fertilizer level. In a field observation, easier measurement than TNB2 would be needed; more conveniently, number of differentiated secondary branches per primary branch could be measured by the longest two or three primary branches in the largest panicle of a plant. Accordingly, number of differentiated secondary branches per primary branch could be a prospective tool to select high-yielding segregants of the *Ur1/Ur1* genotype like MR79 in a process of breeding by the use of *Ur1*. By this trait, and the spikelet twinning and the panicle opening in total, genotype of a plant or a line could be estimated to select those with the *Ur1/Ur1* genotype (Murai *et al.*, 2012), even if undulation is little or absent like MR79.

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PERFORMANCE OF PAPUA NEW GUINEA NATIVE PIGS GIVEN IMPROVED MANAGEMENT CONDITIONS. (*ORIGIONALE PEPA*)

James BcGatch Duks

ABSTRACT

Pig is an important traditional livestock species in PNG agriculture. Pigs are reared for various reasons such as prestige, bride prices, feasts, initiation ceremonies, for wedding events, for compensation payments, funeral activities, for food as protein source and quiet recently for some cash income. Despite the importance of pigs in this country, there is minimum report on the native pig. The Labu Research Centre bought some native pigs from the villages within the Huon Gulf, Nawae and Mumeng Districts of Morobe Province in 1992 for a study on the performance of native pigs given improved management conditions. The pigs were bred and raised in confinement sufficient for a five sow and one boar piggery under a modern cement galvanised iron roof building with nipple drinkers. *The pigs were fed various feed resources depending on availability and funding situations. This paper reports in two parts; the reproductive (part 1) and productive (part 2) performances of the native pig given improved management for a 3 year period data.*

Key: *Native pigs, Reproductive performance, Productive performance, oestrus cycle, gestation period, litter size*

INTRODUCTION

Pig is an important traditional livestock species in PNG agriculture. A large proportion of the rural population own pigs. ANZDEC, (1994) report estimated village pig population in PNG to be between 1.6 – 1.8 million at an estimated value of K68,400,000.00 and this amounts to 97% of total pig production. These pigs are reared for various reasons such as prestige, bride prices, feasts, initiation ceremonies, for wedding events, for compensation payments, funeral activities, for food as protein source and quiet recently for some cash income.

The Papua New Guinea (PNG) native pig is called Sus scrofa papuansis. This pig has a long snout, erect ears and strong hind legs for leaping. Most native pigs are brown, black or have stripes of brown-black colouring similar to the Philippines native pig (Penalba, etal: 1990). The indiscriminate breeding programme in the 1940's and 1970's has resulted in various coat colour combinations. Pure native pigs may be found in the fringes of the country in isolated regions such as the Star Mountains and the border areas along the West Irian Province of Indonesia. These pigs thrive under free-ranging management systems if reared, while their counterparts in the wild feed by rooting, grazing and/or searching for wild nuts and fruits in abandoned old gardens close to human gardening activities.

Despite the importance of pigs in this country, there is minimum report on the native pig. Labu Research Centre had bought some native pigs from the villages within the Huon Gulf, Nawae and Mumeng Districts of Morobe Province in 1992. These pigs were bred and raised in confinement sufficient for a five sow and one boar piggery under a modern cement – galvanised iron roof building with nipple drinkers.

The piggery has two boar pens, five dry sow and five farrowing pens, three weaner and three fattener pens. A tank and pump were installed to facilitate drinking and flushing floors. The pigs were fed various feed resources depending on availability and funding situations. The feed resources included commercial pig breeder and grower pellets, mill run-copra meal -village pig concentrate, staples (sweet potato/ cassava) – village pig concentrate, wastes from Unitech mass, broken biscuits and fresh coconuts.

The sows were mated and their reproductive and productive data were collected and recorded over a three year time period (1992-1995).

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This paper reports in two parts, the reproductive (part 1) and productive (part 2) performance of the native pig given improved management for a 3 year period data.

Part 1: The reproductive performance of native pigs given improved management conditions.

Methodology

Five sows and one boar were introduced to Labu from Huon, Nawae and Mumeng districts of Morobe Province. The pigs were mated when heat was detected from swollen-reddish vulva and from riding test when the sows stood to pressure on the hump. Sows were brought to boar pens (4m square) for mating. During mating, inexperienced boars were assisted by inserting the penis into the vagina. The native boar had high libido, good and strong hind legs and was a docile animal. It was noted that sows were receptive for about forty eight hours.

After three weeks, sows were confirmed impregnated if they did not return to heat. The sows were feed and maintained in dry sow pens ($1m^2$) until one or two weeks from farrowing when they were moved to the farrowing pens of 0.5m by 2m ($1m^2$) that also had creep pens (0.5m square) on both sides. These pens were thoroughly washed and disinfected before sows were moved.

Farrowing times were detected from records, prolapse of stomach, general swelling and enlargement of vagina as well as squeezing of sows nipples to detect milk. At farrowing, sows were assisted in parturition by attendant and mesenteric coatings were removed from piglets. The piglets that died at birth and placenta were buried after parturition that usually took two hours.

After farrowing, wood chips were spread over the creep pens to provide brooder for warmth of piglets against chilling.

After three days from farrowing the piglets were given intra-muscular iron injection in the hip area with dexavin, while canine teeth were clipped with tooth clippers to prevent teat damage to sows during suckling. Piglet ears were also notched using a ear notcher indicating sow and piglet numbers for identification purposes.

Sows were replaced depending on reproductive ability while still maintaining the five sow status. Piglets were weaned after 21 to 28 days from birth and sows were returned to dry sow pens for reconditioning and subsequent mating.

Data recorded include oestrus cycle, time on heat, gestation period, littersize at birth and weaning, piglet weight at 3 days from farrowing, mortalities, functional teats of weaners and weaning to service interval in sows.

Part 2: The productive performance of native pigs given improved management conditions

Methodology

The pigs were weaned by removing the piglets from the sows into weaner pens (2m square) after 3 to 4 weeks from farrowing. The weaner pigs were moved to larger grower/finisher pens (4m square) after one to two months from weaning.

Weaner pigs were dewormed with Nilverm Pig wormer at weaning and repeated after 3 to 4 months. Pigs were identified form ear-notches. Tactic Ec was used to spray for Sacoptic mange infections while sulfamez was administered orally for *E.coli* infection that causes piglet diarrhoea.

Pigs were weighed weekly and their records were kept. When pigs reached about 35 to 40kg in 8 to 9 months they were sold to customers.

RESULTS

Part 1: Reproductive Performance.

Table 1: Reproductive Performance of Native Pigs*.

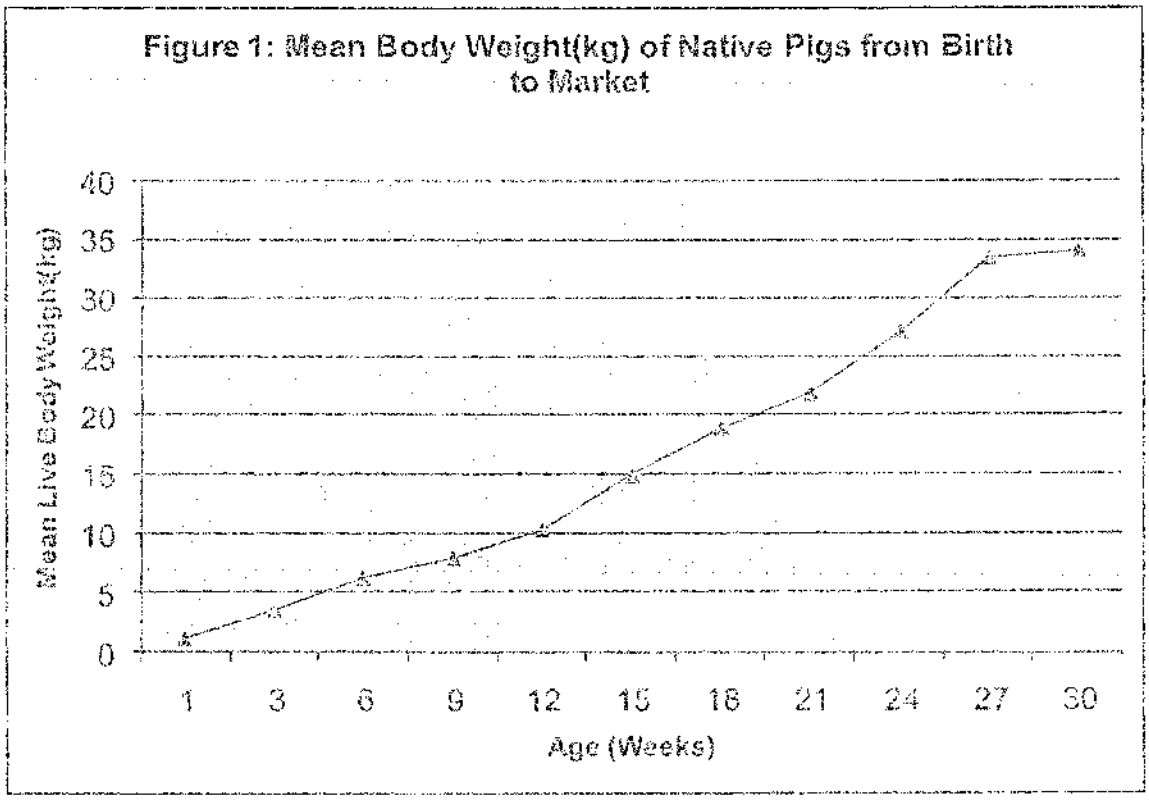
Performance	Sample size #	Mean
First sign of oestrus in gilts (months)	20	8
Oestrus cycle in gilts, sows (days)	23	21
Time on heat (hrs)	20	48
Gestation period in sows (days)	20	114
Litter size at birth in sows (3 days)	20	8
Piglet weight at birth in kg (3 days)	122	1.24
Litter size at weaning in sows (1 month)	20	6
Functional teats of weaner	144	11
Mortality in piglets/weaner (%)	20	21
Weight at weaning in kg (30 days)	132	4.6
Weaning to service interval in sows (days)	7	8

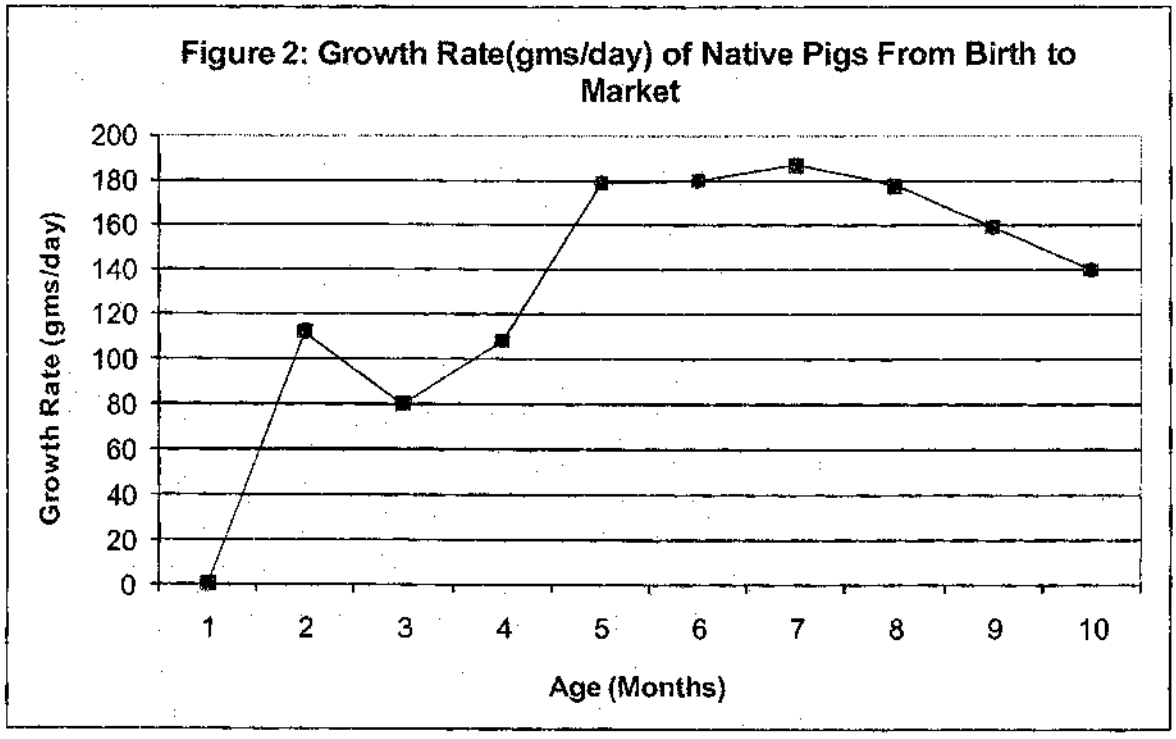
* About 75% Native and 25% Exotic. # number of observations.

Part 2: Productive Performance

Table 2: Mean Body Weight (kg) of Litters of Native Pigs from Birth to Market

Age (weeks) Birth	Sample Size	Mean Body Weight (kg)
3	16	1.2
6	16	3.6
9	17	6.3
12	15	8.00
15	17	10.40
18	17	15.00
21	15	19.00
24	17	22.00
27	14	33.60
30	8	34.50





DISCUSSION

The results presented here are weekly data over a 3 year time period. Table 1, gives the female reproductive performance while the productive performances are shown in tables 2.

The reproductive performance of the native pigs with various sample sizes or number of observations and their arthematic means are given in table 1. The mean oestrus cycle, time on heat, gestation period and weaning to service intervals are similar to improved breeds. Low litter size is common in native pigs as reported elsewhere (Cheng, *etal*: 1990 & Penalba, *etal*: 1990) and subsequent weaning coupled with low birth and weaning weights including high mortalities may to some extent give the impression that native pigs are inferior to the improve pig breeds. However, large numbers of functional teats, may mean there is scope to improve litter size with good breeding while mortalities may be decreased given sound management.

The weight gaining performances of the litter is given in table 2 and also presented in figure 1 as a sigmoid growth curve. The graph shows that weight gains increase gradually from birth to 27 weeks of age at about 30kg and plateaus thereafter when most pigs were sold due to good taste and customers demand. The steep growth region is between week 12 to 27. It should be remembered that native pigs can be improved by good breed and / or nutritional inputs. Dr. A. Quarterman remarked that Native pigs can make 70% improvement given good husbandry and nutrition (pers. com.).

The productive performances at post-weaning are low (table 2). The growth potential is relatively low only reaching 34kg in 9 months after birth (table 2). This is similar to the Taiwanese native pig that reaches 40-70kg in 1 year (Lee, etal; 1990). A decline in growth rate at 60 days is expected due to weaning time in 1 month (figure 2) and subsequent weaning stress. A quadratic response on growth is shown in figure 2 and this indicates that native pigs reach a maximum growth rate of 187 gms per day in the 6 month and thereafter the growth rate declines.

The native pig with such low growth potential could be breed related and Malynicz (1973) postulated this to be a fitness character. Work on native pigs in the Philippine (Penalba, etal: 1990) and Taiwan Native pig, Taoyuan, (Cheng, etal: 1990) also reported low growth potential.

CONCLUSION

Native pigs on the whole are reproductively similar to those of the exotic pig and if litter size is to be increased some crossbreeding pro-

grammes be initiated. Alternatively identify from the local pool of native pigs in country with large litter size and do crossbreeding to increase little size.

The productive performance may be increased with good nutrition and sound management if low growth potential is fitness character that is breed related. However, native pig can be sold after 6 to 7 months of age at an average live weight of 30 kg as smaller carcases. Furthermore, native pigs reach maximum growth rate of 187gms per day in 5 to 6 months and then drops.

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UNDERSTANDING BIOCHEMISTRY OF COCOA FERMENTATION TO ARREST BEAN ACIDITY IN PAPUA NEW GUINEA COCOA

Noel Y.Kuman

ABSTRACT

The dominant microbial species observed in a commercial fermentation of Papua New Guinea cocoa were Yeast, Acetobacter, Lactobacillus and Bacillus species. Maximum growth of different species of microbial was observed starting from day 2 of the fermentation. The microbial activities and their rate of metabolism during the fermentation correlated to total acid production, rate of pulp sugar utilization, oxygen concentration and temperature regime. Better understanding of cocoa biochemistry would enable devising of better interventions to arrest acid production during the fermentation to reduce residual bean acidity to improve the overall cocoa flavour.

Key words: *Cocoa microbiology, fermentation and biochemistry*

INTRODUCTION

Cocoa beans are essential raw material in chocolate production. They are produced as seeds in pods of the plant *Theobroma cacao*. The first stage in processing of the bean is fermentation. The beans are removed from pods and placed in mass in heaps or boxes where fermentation developed naturally. Fermentation is regarded as being essential for initiating the reaction that leads to the formation of substance that confer characteristic of chocolate flavour (Lehrian and Patterson, 1983).

Fermentation involves two distinct processes. First, microorganism grow in the pulp surrounding the bean, leading to a physical solubilisation of the pulp and the generation of metabolic end products and enzymes that have the potential of diffusing into the beans and affecting its chemical composition. Secondly, the environment created by microbial growth causes death of the beans and initiates an internal autolytic process that is conducted by endogenous enzymes. Numerous studies in different countries have demonstrated the contribution of yeast, lactic acid, acetic acid bacteria and other bacteria to the process (Ostovar and Keeney, 1973). Different studies conducted intended to determine the essential of microbial species to the development of good chocolate quality and to control fermentation conditions such that the growth of desirable species may be encouraged or alternately, the growth of undesirable species may be prevented (Hansen, 1975 a, b; Sanchez *et al.*, 1985). Another goal has been to link the presence of particular microorganism with key chemical and biochemical changes with the pulp and bean, such as changes in turning of the beans being related to the sensory properties of chocolate (Lopez and Quesnel 1973b; Hansel 1975 a, b; Passos *et al.*, 1984b). Despite numerous studies that have been conducted, a clear link between the microbial ecology of fermentation, biochemical changes within the beans and bean quality have not been established, though the important of microbial activities is precursor to flavour development.

Generally, beans produced from South East Asia countries including PNG are deemed to be inferior in quality than those produce from Africa. The decrease in appeal in use is the result of development of an unacceptable acidic character, which in some way, is connected to the microbial ecology to fermentation (Chong *et al.*, 1978; Karen *et al.*, 1983). Genetic materials have shown to have very limited influence on bean acidity problem. Bean acidity is measured in pH and Titratable Acids (TA). Detailed investigation confirmed a very strong correlation ($r=0.98^{**}$) between pH and total TA (Chong *et al.*, 1978). A study of the citric acid cycle and carboxylic acid carried out to determine the cause of the low pH of cocoa, indicated that carboxylic acid (acetic and lactic) appears to be the group of acids that would contribute most to low pH (Rohan and Steward, 1965).Traditional box fermentation produced beans that have excessive acidity (pH 4.4-4.7). Chocolate derived from these beans were not entirely acceptable, hav-

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ing bitter taste and lacking chocolate strength (Shepherd and Yap, 1984).

To understand the relationship between the microbial ecology and biochemistry of fermentation, it is important to have quantitative information about the growth of individual species of microorganism throughout fermentation, as well as some knowledge about their biochemical proprieties. The concentration of metabolites and potential chocolate flavour precursors produced by individual microbial species during the fermentation will depend upon the quantitative extent of their growth (Ardhana, 1990).

Many variables are considered to affect the content of cocoa bean fermentation. These include the scale and physical conditions of fermentation, sanitation of fermentation containers and surrounding environment, degree of bean mixing during fermentation, maturity of beans at harvest and cultivar of the beans (Rohan 1963; Minifie 1980). The literature suggested the participation of a range of microbial species in the fermentation including yeast, fungi and bacteria, which is influence by different factors including environment, location and country and fermentation practices.

Cocoa is an important export commodity of Papua New Guinea, however some quality attributes of the bean are considered inferior to those produced in some other countries and there is an urgent need to address these problems. One such initiative involved obtaining more knowledge about the fermentation process and practices and understanding the involvement and influence of microbial activities and its effect on cocoa flavour. This would lead to development of improved processing techniques to improve the quality of PNG cocoa. This paper intends to identify the general ecology of microbial species present in fermentation of PNG cocoa and its influence on general cocoa biochemistry, in particular, bean acidity and to make recommendations on how the residual acidity can be reduced to improve overall cocoa flavour.

OBJECTIVES

Identify general profile of microbial species in commercial fermentation of Papua New Guinea cocoa, and its influence on fermentation pattern and cocoa biochemistry; with an attempt to arrest acid production to reduce residual acidity of the bean to improve overall flavor attributes of cocoa.

MATERIAL & METHODS

Experimental Design

Fermentation process

Cocoa pods of mixed cultivar were harvested in the field and their pods were removed and bagged before they were transported to the fermentry. The wet beans were fermented inside a standard commercial fermentation box (120 x 90 x 90) cm and dried on a conventional commercial dryer. The dried beans from each of the 25 fermentation replicates conducted were collected, bagged for quality assessment.

Sample collection and preparation

The bean acidity, sugar concentration of the pulp, and sample for flavour assessment were prepared following the standard cocoa quality laboratory procedure (PNGCCRI, 1995). The oxygen concentration was measured using an oxygen logger. The laboratory mercury thermometer was used to measure temperatures of the fermenting mass inside the fermenting box.

Flavour assessment

Organoleptic evaluation was conducted by the trained taste panel following the procedure described by BCCCA (1996); Sukha (2001).

Microbial analyses

Microbial isolation and enumeration was determined following procedures described by Carr and Passmore, (1979)

RESULTS

Figure 1, shows that all microbial activities reached their maximum level during day 2 of fermentation, before decreasing afterwards. The Yeast population increased between days 2-3 before decreasing sharply until it disappeared at day 4 of fermentation. The Acetobacter followed a similar pattern as Yeast, but continuo to maintain its population at a low level towards the end of the fermentation. As for Lactobacillus species, its population decreased after day 2 of fermentation, but maintained a relatively high

Figure 1: *Profile of average microbial population of commercial cocoa fermentation*

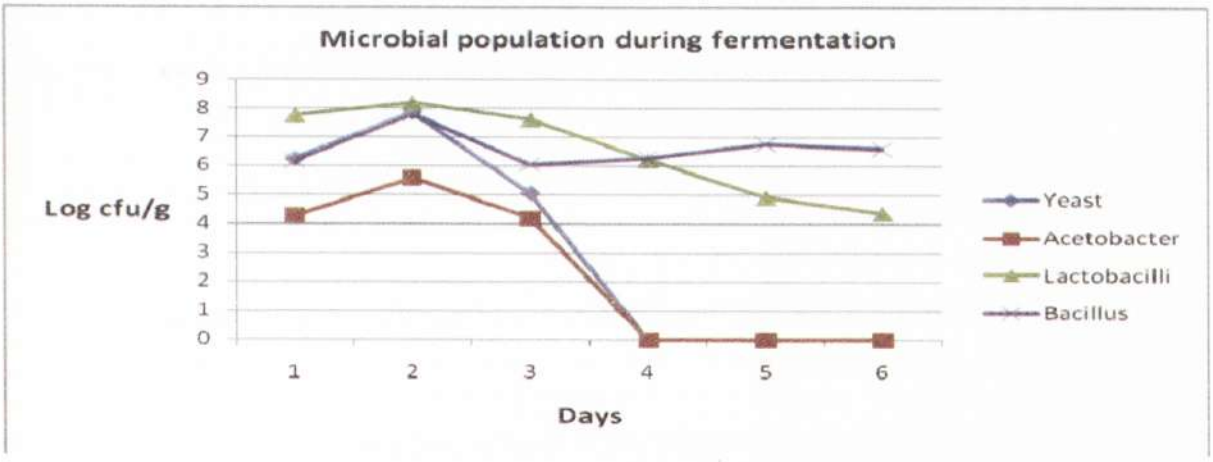
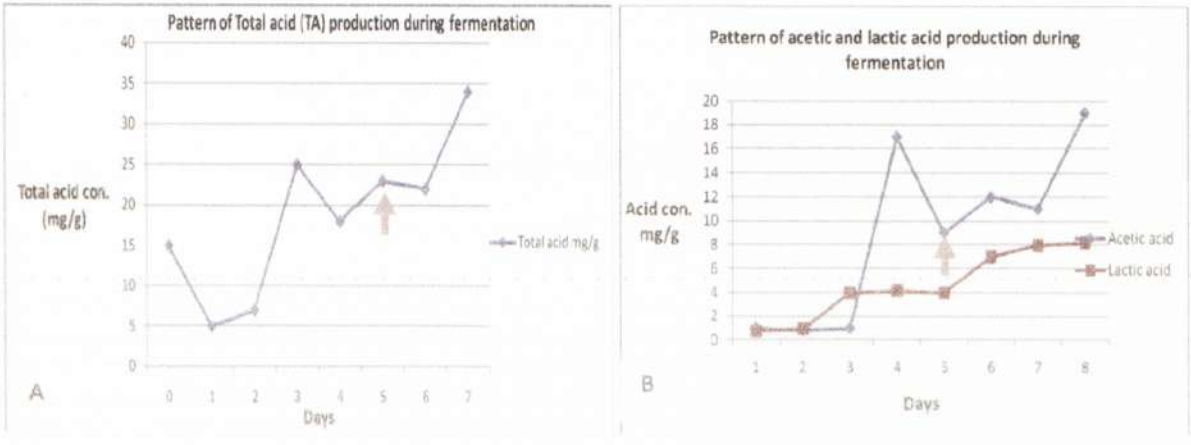


Figure 2: *Pattern of average total acid production (a) and major acids: Acetic and Lactic acid (b) in a commercial fermentation*



population toward the end of fermentation unlike Yeast and Acetobacter. The Bacillus population decreased after day 2 of fermentation, before increasing from day 3 onwards and maintained a large population throughout the rest of fermentation.

Figure 2 a, shows the average total acid (Oxalic, Citric, Ethanol, Succinic, Lactic and Acetic) concentrations produced during the fermentation. The individual concentration of minor organic acids is shown in Figure 3. The total acid concentration began to increase from days 1 – 6, with a sharp increase between days 2 - 3 coincided to the increase microbial population and activities (Figure 1). Among the acids produced, acetic (range 0.6 - 18.5 mg/g wet beans) and lactic acid (range 0.5 - 8 mg/g wet beans) were dominant throughout the fermentation, thus become the potential candidates responsible for high level of residual acids found in the beans. Acetic acid was produced in large quantity than lactic acid, showing a sharp increase from day 3 - 4 (range 0.7 - 16.5 mg/g wet beans) before decreasing (8.5 g /mg wet beans) in day 5, and increased afterwards to reach its maximum level (18.5mg /g wet beans) at the end of the fermentation. As for Lactic acid, it shows a gradual increase in concentration, but remains at a lower level than acetic acid throughout the fermentation.

For average minor organic acid concentration ***(Fig. 2);*** Succinic and Oxalic acids were present in small quantities with an average concentration ranging from 0.1 - 2mg/g wet beans. Ethanol was present in large quantity with an average concentration ranging from 0.1 - 17.5 mg/g wet beans during the fermentation. However, its concentration fluctuated at each day of the fermentation and this can be related to the acid being used mainly for microbial activities. The citric acid concentration decreased starting from days 1 – 3 (from 10 to 1 mg/g) before increasing towards the end of the fermentation with acid concentration of 3 mg/g wet beans.

Note: Higher the Titratable acidity (TA) value, the higher the bean acidity.

The average acid profile of the fermenting beans ranged from 0.05 - 0.26 mg/g beans. From the start, this was gradual, followed by a sharp decrease in pulp TA after day 3 of fermentation; corresponding to a sharp build up of acid concentration in the bean kernel; an indication of acid migration from the pulp to the kernel.

production as the result of microbial activities (Fig.5)

The oxygen concentration during the fermentation (Fig. 6) ranged from 18 - 75 % saturation. At day 1, the oxygen concentration was high (70 % saturation) for fresh beans placed inside the fermentation box, but decreased as the fermentation condition becomes anaerobic at day 2-4 of fermentation. However, the oxygen con-

Figure 3: Average minor organic acid concentration in a commercial fermentation

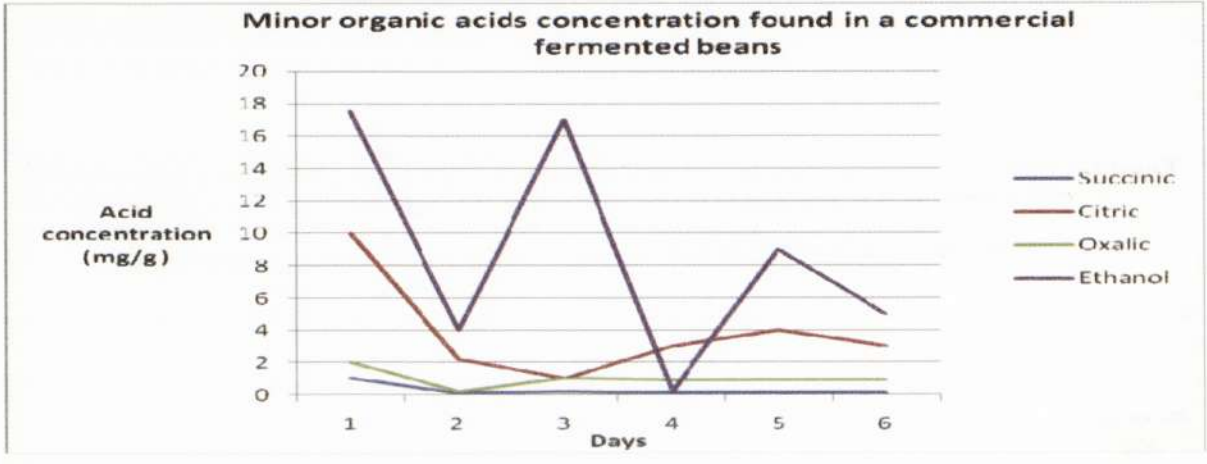
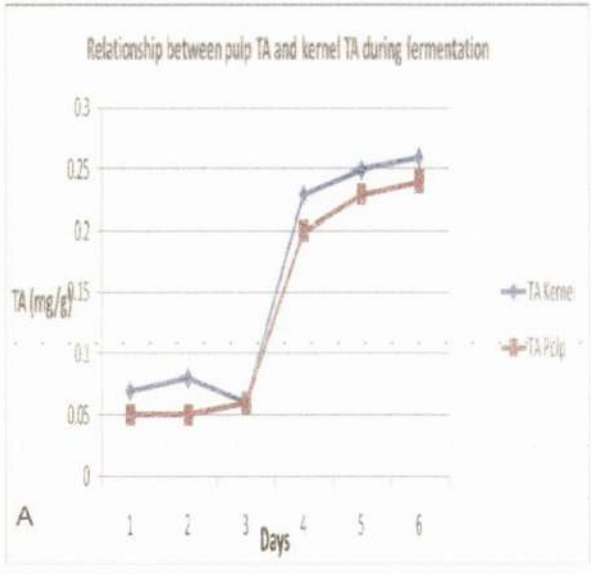
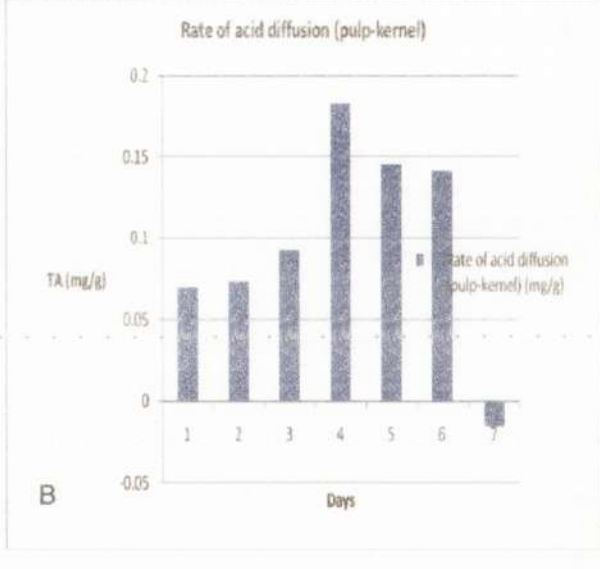


Figure 4: Relationship between average pulp and kernel TA (a) and rate of acid diffusion between bean pulp and kernel (b).





The pulp sugar production decreased during fermentation with the highest concentration (82mg/g wet beans) at the beginning of the fermentation and reached the lowest concentration (7mg/g wet beans) on day 3, before showing a slight increase towards the end of the fermentation; indicating a possible sugar

centration increased after day 4, reaching the maximum concentration (75 %) at the end of the fermentation as the result of maceration (physical solubilisation of pulp) and daily turnings of beans, introducing aeration into the fermentation box. Aeration is required for oxidation reactions to take place to produces acids that is required to cause the death of the bean.

***Figure 5*: Profile of Pulp sugar production during the fermentation**

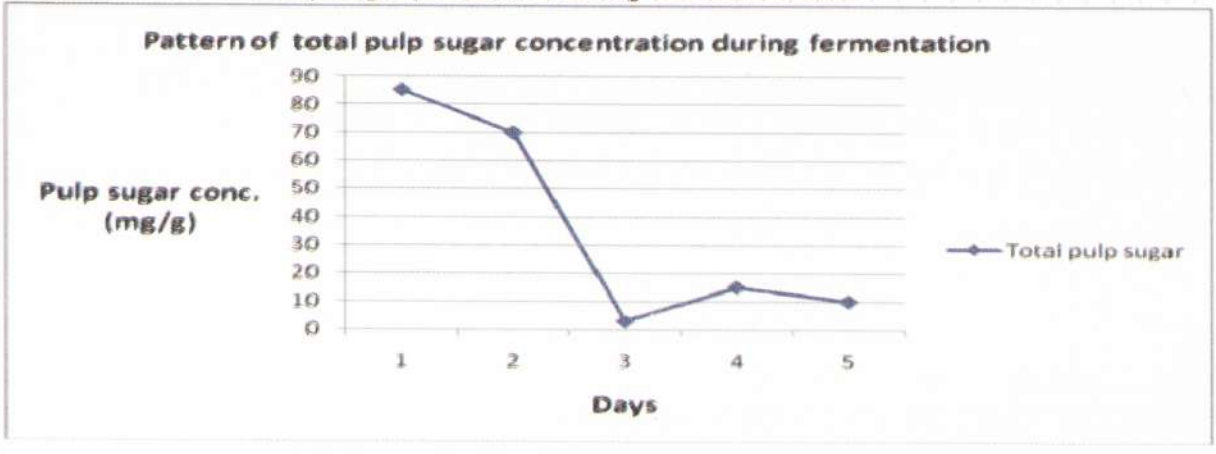
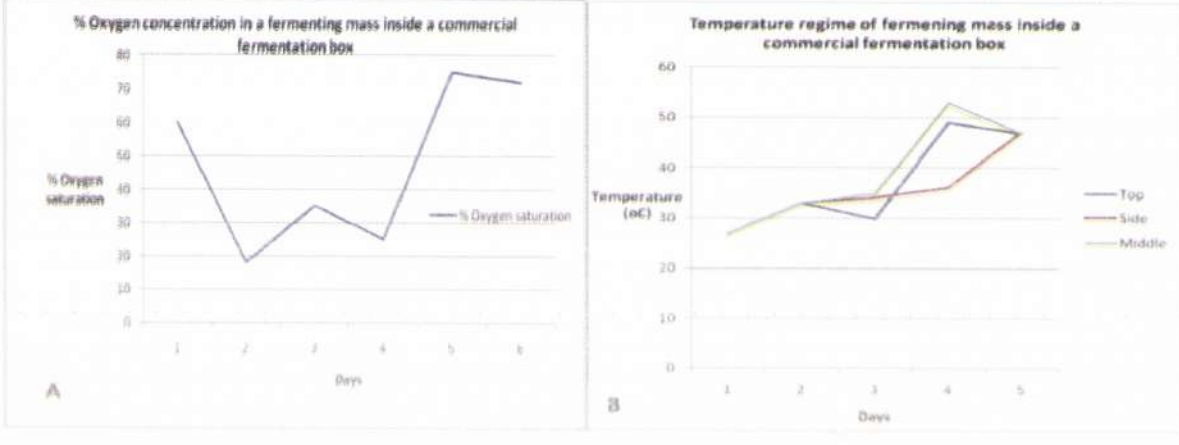


Figure 6: Oxygen saturation (%) (a), and temperature regime (b), during the fermentation



The temperature range of fermentation mass at the top, sides and middle of the fermenting mass was between 27 - 49, 27 - 47 and 27 - 53 ^{0}C respectively (Fig. 6b). The temperature of the fermenting mass at all parts of the box increased during the fermentation, reaching their maximum temperatures at day 4, before decreasing towards the end of the fermentation. A slow increase in temperature was observed for fermenting mass at the sides of the box during the initial phase of fermentation before the temperature increased rapidly from day 4 - 5. The temperature profile of the fermentation indicated that the maximum temperature of 53 ^{0}C was reached in the middle of the fermentation mass with lower temperatures recorded at the top and sides of the fermentation box.

The average flavor attributes of commercial fermented beans generated was compared against Ghana cocoa, which is considered as universal standard. PNG cocoa produced low level of chocolate flavour intensity with pronounced acidity, bitterness and astringency (Table 1).

DISCUSSION

Acid production during the fermentation period coincided with microbial activities. The microbial activities and their rate of metabolism during the fermentation correlated to total acid production, rate of pulp sugar utilization, oxygen concentration and temperature regime as shown by the result of this study. By understanding the role and presence of each of the microbial activities during the course of the fermentation, acid production can be arrested to reduce the level of residual acid in the beans to improve the overall flavour cocoa.

Yeast

Yeast population was predominant from day 0 - 2 of fermentation with a population range of 10^{7}-10^{8} cells/g, before decreasing to a population of 10^{4} cells/g at the end of the fermentation. Similar results were obtained by Ostava and Keeney (1973). The proliferation of yeast population (Figure 1) corresponded to decrease in pulp

Table 1: **Average sensory assessment result of commercial fermented beans**

Sample	Chocolate	Acidity	Bitterness	Astringency
Commercial fermented Beans	6.0	3	1.0	2.3
Ghana (Control)	8.4	7	1.6	1.0

The flavour intensity was estimated using 0-10 scale, with 0 being weak and 10 being strong. The lower the acidity value, the higher the acidic taste in flavor scoring or vice versa.

sugar concentration (Figure 3a). High population of yeast was observed during the period when the pulp sugar concentration was high, but decreased as the pulp sugar being utilized. The dominant of yeast population in the early stage of the fermentation could be responsible for converting pulp sugar primarily into ethanol that appears to be used in the later part of the fermentation to produce carboxylic acids as well as enzymes to aid pulp maceration. The initial low temperature, high pH and aerobic condition favour yeast proliferation. The presences of high yeast population in the beginning of the fermentation could be the result of pulp being cross contaminated with remnant of yeast present inside the fermentation box from previous fermentations. Yeast is also responsible for loosening physical structure of the pulp and to some extend metabolize citric acid to give a slight increase in pH. In figure 3, it shows concentration of citric acid decreasing, coincided to the same period when the yeast population was highest. Sequence of different yeast species identified to be present at different stages of fermentation (Carr and Passmore, 1979). The pulp sugar or its metabolites are used by Acetobactor and Lactobacillus species to produce acetic and lactic acid respectively.

Lactobacillus

Lactobacillus population remains high throughout the duration of the fermentation. The maximum population was reached in day 2 of fermentation with a population range of 10^4-10^8 cells/g. They are responsible for high acetic acid production, which eventually migrate into bean, hence increasing the acid concentration of the kernel (Figure 2 & 3). Similar results were obtained by Carr and Davies, (1980) for Malaysian cocoa. Qualitative and quantitative analyses of volatile and non-volatile low molecular acids have indicated that acetic and lactic acid are primarily responsible for the excessive acidity of beans. Individual species of Lactobacillus varies through out the course of fermentation of which some play predominant role than others. Both acids are products of fermentation of pulp sugar.

Acetobacter

The Acetobacter growth started in day 1 of the fermentation, with a population range of 10^4 - 10^6 cells/g. The maximum population was reached in day 2, before decreasing and represents less than 1 % of the total microflora population during rest of the fermentation (Figure 1). Acetic acid was prominent during fermentation of cocoa beans at estates in Ghana and Malaysia by days 3-5 and present at a population of 10^6-10^8 cells/g. They were present at the commencement of the fermentation being more prevalent on the beans in Ghana than those in Malaysia (Carr and Davies, 1980). Their growth coincide with conversion of ethanol into acetic acid, aeration of the bean mass by turning and collapse of the physical structure of the pulp, which also promoted aerobic condition. The oxygen concentration in the fermenting mass fluctuated due to daily turning of the beans as well as being utilized for oxidative reaction. The oxygen concentration started to increase in day 2, reaching a maximum concentration of 75 % saturation at day 5 and remain at relatively high concentration throughout the rest of the fermentation (Figure 5 a). The diminished of Acetobacter population towards the end of the fermentation could be attributed to their inability to tolerate the increasing temperature, which increased to 47-53 ^{0}C by days 4-5 (Figure 5b). Similar result was reported by Forsyth and Rombouts (1951). Acetic acid bacteria appears to make significant contribution to cocoa bean fermentation involving different species accounting for high concentration of acid, though lower than lactic acid (Figure 2b).

Bacillus

The Bacillus species appears on day 2 of fermentation with an initial population of 10^6 cells/ g, and continuo to increase and reached a

maximum population of 10^8 cells/g in day 2, before decreasing, and maintained reasonably high population of 10^6-10^7 cells/g towards the end of fermentation (Figure 1). Similar profile of Bacillus population was observed by others studies during the fermentation (Ostavar, K., 1971; Ostavar and Keeney, 1973). The contribution of Bacillus to quality of bean is not fully understood, but their contribution may not be significant. However, with an increase of Bacillus population towards the end of the fermentation that coincided to high acid production (Figure 2), their role needs to be further investigated.

Pattern of acid production

The initial decrease in pulp acidity between day 0-1 (Figure 3a) could be result of pulp sweating; acid in the pulp are lost through sweating. Bean acidity started to increase from day 1 of fermentation and this is when acid migration from pulp to kernel started (Figure 3 a & b). Large amount of total acids and acetic and lactic acids (9-10 mg/g) were produced towards the end of the fermentation (Figure 2 a & b shown by arrows), which may be an ideal period to arrest the acid production to reduce residual acid in the bean.

Acid migration

Total acid concentrations build up from day 1 and continue to increase during the fermentation with a sharp increase between days 2 and 3. The concentration of minor acids (Figure 3) may not contribute significantly to total acidity as they were presented in low concentration throughout the fermentation except for Ethanol. The acid migration from the pulp to the kernel started at day 1 of the fermentation, showing a sharp increase after day 2 and reached the maximum rate at day 4, before decreasing slightly and remains at a reasonably higher rate towards the end of the fermentation. The importance of acid production is, it contributes towards causing death of the beans.

Pulp sugar concentration

The pulp sugar started to decrease from day 1 of fermentation concided to yeast activity. In the initial stage of the fermentation (day 0-1), the yeast was responsible for metabolising the pulp sugar to ethanol, which is then utilised by the Lactobacillus and Acetobacter species to produce mainly large quantity of lactic and acidic acid respectively. Besides yeast, the other microbial activities started from day 1 of the fermentation and this is when the acid production commenced.

Oxygen and temperature regime

The initial aerobic condition of the fermenting mass could be the result of freshly harvested beans been saturated with oxygen. The aerobic condition changes to anaerobic from days 2-4 of fermentation, as the result of rigid compact structure of the fermenting mass formed inside the confinement of the fermentation box after the collapse of pulp, which limit oxygen flow into the fermenting mass. The fermentation condition changes to aerobic after day 4 of the fermentation as the result of physical solubilisation of pulp. The slight increase in oxygen concentration between days 3-4, could be the result of daily turning of the beans. Under aerobic condition, it favours proliferation of Acetobacter resulting in high acetic acid production, while Lactobacillus population is not being affected since it favours aerobic condition.

Temperature regime

The temperature of the fermenting mass can be related to the microbial activity, as fermentation is an exothermic reaction. Higher temperature was measured for all sides (top and sides) of the fermentation mass between days 3-5 coincided with proliferation of Acetobacter population. The temperature at the centre of the fermenting mass increased steadily throughout the fermentation. Lower temperatures were measured at the sides and top of the fermenting mass due to beans exposed to the external environment. Slower temperature increase was also observed at these sides of the fermenting mass compared to centre of the fermenting mass, which is less affected by the external environment. The fluctuating in temperature shown throughout the fermentation could be the result of daily bean turning

CONCLUSION

The results of this study indicated that maximum microbial population were observed from days 1 - 3, which coincided with sharp increase in acid production and migration of acid produce from the pulp into the kernel. During the same period, there was a sharp decrease in total pulp sugar indicating the conversion of pulp sugar by yeast to ethanol or other metabolites which was used by other microbial population to produce acids as the main byproducts. Besides, yeast also

produced enzyme to aid pulp maceration. The study highlighted several important aspects of fermentation process that can be manipulated to reduce residual bean acidity, while retaining the overall flavor attributes of cocoa. The acid production can be arrested by reducing the fermentation days from 7 to 5 days. On average, large quantity of total acids (84 %) were produced from day 3-7 of which (45 %) was produced between day 5-7 (Figure 2 a & b). From the total acid produced, large amount (66 %) migrated from the pulp into the kernel (Figure 4 a & b) as the result of high level of microbial activities. The high temperature and oxygen concentration in the fermenting mass (Figure 6 a & b) correlated to increase microbial activities; converting ethanol and other metabolites (by products of pulp sugar) into organic acids. Heat and acid produced during the fermentation combined to kill the bean. The bean death causes loss of cell membrane and integrity of intercellular compartments resulting intra and intercellular mixing of water-soluble compartment. This reaction is a prerequisite to development of flavor precursors, which develop into the chocolate flavor plus other ancillary flavours. Therefore, the fermentation process can only be arrested towards the end of the fermentation and the most ideal time would be at day 5. This is when fermentation can be stopped to reduce residual bean acidity by preventing further migration of acid into the bean kernel. At that point, fermentation process is complete and the large amount of volatile acids produce is expected to remain in the pulp that can easily be removed by drying as compared to acids locked inside the kernel. In the later case, it will be difficult to remove most of the acids especially the none-volatile acid. The 5 days fermentation has shown to proceduce similar quality attributes as 7 days fermentation (Hollywood, 1994). The fermenting mass also needs to be turned and mixed properly daily during the fermentation to maintain homogeneity. This will allow all beans to be properly fermented because of temperature variation observed at different sides of the fermentation mass. Similarly, adequate mixing and turning would be required to ensure adequate aeration inside the fermenting mass especially during the anaerobic phase (before day 4) to facilitate oxidative reactions to generate sufficient heat and acid to kill all the beans to reach full fermentation status. Maximum temperature of 48-50 ^{0}C is required to cause the death of the beans. The temperature range between 47 - 53 ^{0}C was reached for all sides of the commercial fermentation, which is sufficient to cause the death of the bean.

Furthermore, residual acidity can be further reduced by applying slow drying initially to give sufficient time for acids within the kernel to make its way out to the bean pulp to be removed before the shell hardens. Commercial dryer temperature can easily be adjusted to meet this requirement, while initial slow drying can be applied for klin dryers used by majority of smallholder farmers. This requirement suits the solar dryers. This process adjustments would improve chocolate flavour and reduce bean acidity to reach quality attributes near to Ghana cocoa (Table 1) since high level of bean acidity usually tainted chocolate flavour.

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INFLUENCE OF POST HARVEST PROCESS, GENOTYPE AND ENVIRONMENTAL FACTORS ON SELECTED COCOA QUALITY ATTRIBUTES

Noel Y. Kuman[1]

ABSTRACT

The cocoa bean quality attributes are influenced by genotype, environmental factors, and plant size. The fat content of the bean is influenced by pod ripeness and affected by pod storage period. The clonal material produce desirable physical quality parameters; however there is negative correlation

Key words: *Post harvest process, quality parameters, genotype and environmental factors*

INTRODUCTION

Fat is an important quality attributes that decides in most case, the end price of cocoa. The standard fat content most manufacturers prefer is greater than 56 %; expressed in dry weight basis. Fat content of the beans increases during pod development. Fully ripe pods usually have maximum fat level as compared to unripe and partially ripe pods. Fat is lost if pods are not harvest on time. Overripe pods can lose fat through germination. Fat loss during fermentation is not significant as shown by work carried out by Roelofsen (1959); Forsyth and Quesnel (1963). Also, chemicals studies of germination showed that decrease in fat, starch and protein occur in the cotyledon over period of several days. When germination occurs, it reduces the value of cocoa by reducing the fat content and opens the testa.

That fat content varies between different genotypes, and is influenced by environmental factors. The fat content of the cocoa beans is determined by the genetic composition of the plant .The influence of genotype was demonstrated by Forastero genotypes, with a fat content between 56-58 %, in contract to some Amazonian crosses from Ghana and Ivory Coast with a fat content range between 58-61 % . Criollo beans have a low fat content around 53 % (Wood, 1975). It appears that there is no genetic correlation between cocoa butter content and yield or bean weight (Lockwood and Pang, 1995), but there is a correlation between bean weight and cocoa butter within the clones (Beek *et al*., 1977). The genetic effect on cocoa butter hardness is small compared to the environment factors (Lockwood and Eskes, 1995). Low fat content was associated with beans developing during the dry season. However, results of some studies proposed that, in addition to rainfall, temperature could influence the fat content. Some of the variation in cocoa butter content may also be due to xenia effects. Ehrencron and Heemskerk (1976) found that change of pollen parent could alter cocoa butter content by up to 4.8 %; a similar, but smaller effect was reported by (Beek *et al*., 1977).

Bean weight is the average weight of one bean and bean count is the total weight of 100 beans. The standard bean weight preferred by most manufacturers is between 0.8-1.0 g/bean. Bean weight is negatively correlated with shell content. The average bean weight is determined by environment, but the there is no evidence of interaction between season and genotype (Lockwood and Edward, 1990). Tan, (1990) have shown high heritability of bean weight, an additive component of genotype variance and absences of material effects.

The shell content of bean varies depending on genotypes, and negatively correlated with bean weight (Vello *et al*., 1972). The shell content of the beans was significantly influenced by month and genotype. The shell content is also significantly influenced by plant size (Kuman *et al*., 2005a). The shell content of the bean could be genetically controlled.

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Recovery rate is the ration of dry fermented beans to wet unfermented beans expressed as a percentage. Recovery rate can be influenced by season, genotype or method of processing. Pod storage prior to fermentation increased the recovery rate (Kuman, 1998). Howat *et al.*, (1957) reported the influence of cocoa genotype on recovery rate (i.e. Amelonado 44 % and Amazon 38 %). The recovery rate varies considerably from 30 % to well over 40 % (Kuman, 1998). Rohan (1963) published data on recovery rates varying from 31.5 % in Ecuador to 46 % from Zaire. Season is another major factor affecting the recovery rate. Recovery rate rises during the dry season, and fall in the wet season (Wood, 1975). Are and Atanda (1972) reported a 46.3 % recovery rate in the dry season and 38 % in the wet season. Pod ripeness also influences recovery rate; recovery rate increases as the pods ripens (MacLean and Wickens, 1951).

OBJECTIVE

The objective of this study is to assess the influence of post harvest process, pod ripeness and environmental factors on bean quality attributes.

MATERIAL AND METHODS

Location

The quality data of genotype (Kuman, 2005) were collected from a breeding trial conducted at the Papua New Guinea Cocoa and Coconut Research Institute, Tavilo Rabal; East New Britain. The environmental condition of the trial site was reported in cocoa breeding report in the Papua New Guinea Cocoa and Coconut Research Institute (1998) Annual Report. The average rainfall of 134.9 mm with a maximum of 343.9 mm and minimum (12.5 mm) was recorded during the 24 months when this trial was conducted. The clonal material assessed were the progenies of original Trinitario and Amazonian parents (Kuman, 2005).

Experimental Design

The breeding trial was divided into three sub trials based on the three size: small trees (1-1.5 m), intermediate (1.5-3 m) and large (>5 m). The trees of each class were of the same age uniform in each replicate and were planted in split-plot design. Four replicates were used with 12 trees in a (3 m x 3 m) plot.

Effect of harvest and pod storage

The effective harvest and pod storage experiement was conducted to assess the effect of harvest interval pod storage on fat content. A factorial experimental design was set up with harvest intervals of 1, 2 and 3 weeks combined with pod storage time of 0, 5 and 10 days as shown in Table 1. The experiment was conducted for a period of 6 months.

Table 1: Effect of harvest intervals and pod storage on fat content

Treatment	PS0	PS5	PS10
HI Week	Ti	T2	T3
HI Week	T4	T5	T6
HI Week	T7	T8	T9

Key:
HI=Harvest Interval
PS=Pod storage
T= Treatment

Data collection

a) Quality analysis

Ripe pods of approximately 1 kg (equivalent to 6 pods) were harvested twice every month from each of the genotype. The pods were transported to the laboratory and their husks were removed. The wet beans of approximately 1 kg were collected from each genotype, labeled, wrapped inside the shade cloth bags and fermented using a procedure described by Clapperton *et al.*, (1994).The beans were dried at the end of 6 days fermentation and their quality attributes (shell content, fat content, bean size including recovery rate) were determined over a period of 12 months.

b) Effect of harvest and pod storage

Wet beans collected from each treatment (Table 1) were micro-fermented (Kuman, *et al.*, 2010), dried and their fat content was analyzed. The beans processed using fermentation methods described by Clapperton *et al.*, (1994) and Kuman *et al.*, (2010) produce same quality attributes. The two fermentation methods were used alternative to generate samples for quality assessment.

Sample collection and Preparation

Approximately 600 g of dried beans from each genotype was dried inside the oven (Contherm, New Zealand). At 115 ^{0}C for 15 min to standardize moisture content to less than 7 % before being emptied into a mixing container, and thoroughly mixed. Any foreign materials and debris were removed before passing the samples into a funnel of a quartering, which randomly divided the sample into four quarters. Approximately, 150 g of beans received from each quartering devise was used to analyze the bean size, fat content and shell content.

Sample Analysis

Fat content of the beans was extracted using the standard fat extraction method (AOAC, 1970). The other quality attributes (bean size, shell content, recovery rate) were determined using the standard cocoa quality analysis procedure described by Kuman *et al.*, (2001).

Data Analysis

Quality data were subjected to analysis of variance to determine the influence on bean quality attributes and reported (Kuman *et al.*, 2005a).

RESULTS

There was a significant amount of fat loss recorded during pod storage and harvest intervals. There was no economical benefit in prolonged pod storage beyond two days, as the pods were shown to be heavily infected by fungus resulting in heavy loss of pods (75-80 % loss to fungus infection).

Pods harvested at week 2, has the maximum average fat content of 58.3 %, an increase of 1 % fat. This is when fully matured pods were harvested and fermented. Large amount of fat (1 % on average) was lost between week 2 and 3, which coincided to an increase in bean germination from week 2 to week 5 (Figure 1) as the result of harvesting overripe pods.

Table 2: Effect of harvest interval and pod storage on fat content

Treatment	PS0	PS5	PS10	Mean
HI Week 1	57.69 (± 0.2)	56.95 (± 0.3)*	56.36 (± 0.2)**	57.0
HI Week 2	58.54 (± 0.2)	58.46 (± 0.4)*	58.03 (± 0.3)**	58.3
HI Week 3	57.51 (± 0.2)	56.78 (± 0.2)*	56.18 (± 0.1)**	56.8
Mean	58.2	57.4	56.9	

Key: HI=Harvest PS=Pod storage T= Treatment
*P≤ 0.05 **P≤ 0.01 *** P≤ 0.001 (NS) Not significant

Figure 1: Effect of harvest interval on fat content

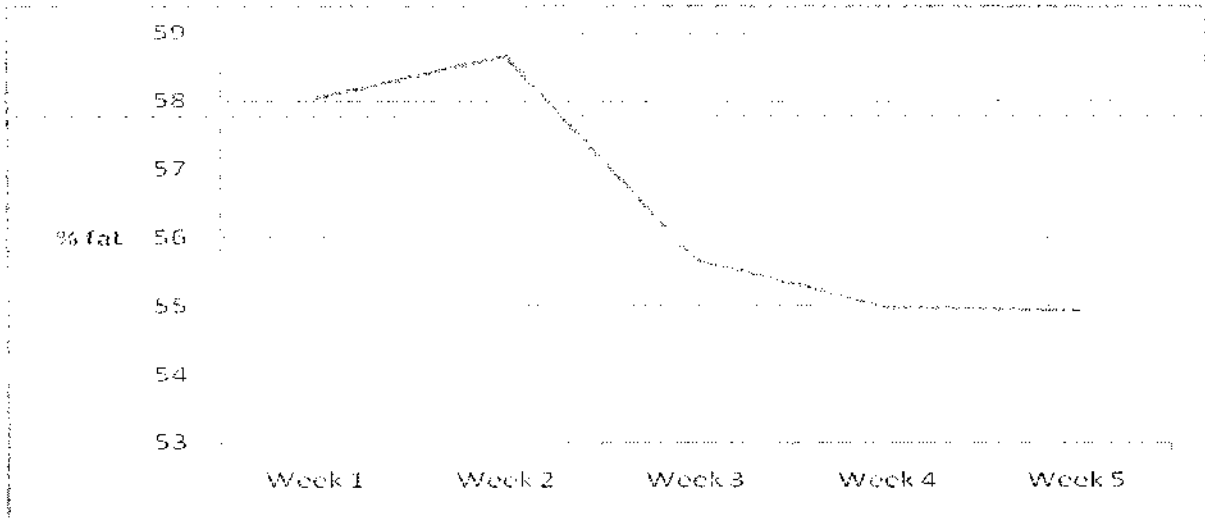
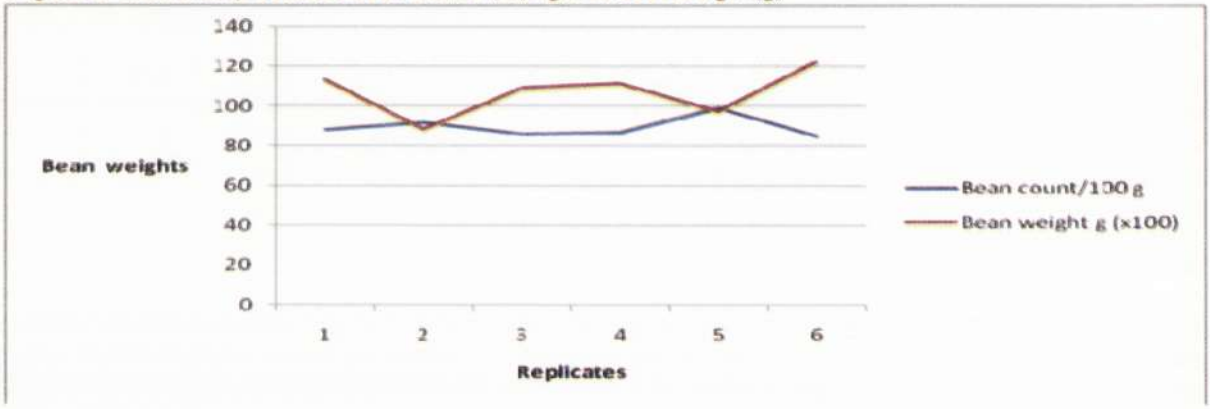


Figure 2: Relationship between bean counts (%) against bean weight (g)



The bean count is expressed in percentage (weight of 100 beans) and average bean weight (average weight of single bean) expressed in percentage as shown in the figure 2 to illustrate the relationship between the two attributes. There is a negative correlation between bean count and bean weight. The lower the bean count, the bigger the bean or vice versa.

Table 3: Effect of pod storage on recovery rate

Clone	Recovery rate	
	Beans fermented straight after harvest	Bean fermented after two days of pods storage
38-3/4	39.5	46.3
38-8/2	32.7	42.5
33-15/1	29.2	42.5
34-13/2	31.3	46.5
23-3/1	32.5	40.5
KA2-101	30.0	43.4
16-2/3	38.7	45.0
67-9/3	31.3	43.6
23-6/1	32.5	44.4
67-9/3	35.0	40.3
23-6/1	32.5	40.5
Mean	33.2	43.2
Range	29-39.5	40-46.5
Std	3.3	2.2

**Minibox fermentation method described by Kuman et al., (2009)*

The average recovery rate was 33.2 %, and range from 29-39.5 % for pods not stored. Pods stored two days before fermentation yield higher recovery rate (average increase of 10 %) than beans fermented straight after harvest. Similar results were obtained for commercially fermented beans.

Table 4: Assessment of individual quality attributes of the hybrid clones-Recovery rate

a) Plant density: Big plants

Clone	16-3/2	16-2/3	73-2/2	38-8/2	K82	38-3/4	34-14/4	36-3/1	37-13/3	36-8/3	KA2-101	37-13/1
Range	27-32.1	28-33.6	28.5-32	28.5-31.7	31-39.3	32-36.8	29-34.2	26-31.7	26-34.1	30-32.7	21-32.9	26.2-31.3
Mean	30	30	30	30	35	34	33	29	30	32	28	29
Std	2	2	2	2	2	2	2	2	3	1	3	2

b) Plant density: Intermediate plants

Clone	67-9/3	25-6/3	73-6/1	73-3/3	17-14/4	33-8/3	23-3/1	37-6/4	33-15/1	38-10/3
Range	26-30.4	31-36.8	29-36.4	31-37.3	29-33	29-32.6	28-32.8	30-34.8	25-29.1	26-32.2
Mean	29	33	34	33	32	32	32	32	31	30
Std	1	2	2	2	1	1	1	2	2	2

c) **Plant density:** Small plants

Clone	24-4/1	17-3/1	24-9/1	23-6/1	17-7/4	34-13/2	73-14/1	24-7/1	63-7/3
Range	28-32.6	27-32.6	26.6-30.3	23-28.9	30-34.1	21-27.3	30-31.9	30-32.6	25-29.6
Mean	30	29	29	26	32	25	31	32	27
Std	1	2	2	2	1	2	1	1	2

The quality attributes of individual clones are presented in Table 4, 5, 6 & 7 to assess the relationship between each bean quality attributes, its influence by the environmental factors and plant size (density).

The recovery rate of the clones varies with an average of 30.6 %, range from 21-39.4 %. The recovery rate was shown to be influenced by genotype and month, but not tree size (Kuman, 2005a).

Table 5: Quality attributes of the hybrid clones-Shell content

A. Plant density: Big

Clone	16-3/2	16-2/3	73-2/2	38-8/2	K82	38-3/4	34-14/4	36-3/1	36-8/3	KA2-101	37-13/3	37-13/1	34-14/4
Range	14-17.4	16-18.2	15-18.5	13-15.7	15-18.8	14-17.7	13-16.2	15-18.8	14-14.8	14-17.4	15-18.6	14-16.4	13-16.2
Mean	16.1	17.4	17.0	14.6	17.3	16.4	14.9	17.1	14.0	15.8	15.3	15.5	14.8
Std	1	1	1	1	1	1	1	1	1	1	1	1	1

B. Plant density: Intermediate

Clone	67-9/3	25-6/3	73-6/1	73-3/3	17-14/4	33-8/3	23-3/1	37-6/4	33-15/1	38-10/3
Range	16-18.9	12-16.2	15-17.5	15-18.8	14-16.5	14-17.5	15-17.6	15-18.1	15-16.5	14-16.6
Mean	17.9	14.7	16.2	17.5	15.4	16.1	16.6	17.1	15.5	16.0
Std	1	1	1	1	1	1	1	1	1	1

Plant density: Small

Clone	24-4/1	17-3/1	24-9/1	23-6/1	17-7/4	34-13/2	73-14/1	24-7/1	63-7/3
Range	14-16.6	15-17.7	17-18.0	5-17.2	6-18.0	5-17.8	16-18.7	17-15.6	15-18.8
Mean	15.8	16.4	17.8	16.4	16.9	16.6	17.4	14.9	17.7
Std	1	1	1	1	1	1	1	1	1

The shell content of the clones varies with an average of 15.5 %; range from 14-17.9 % .The shell content of the beans size was shown to be influenced by month, genotype and tree size.

Table 6: Quality attributes of the hybrid clones-Bean size

Plant density: Big

Clone	16-3/2	16-2/3	73-2/2	38-8/2	K82	38-3/4	34-14/4	36-3/1	36-8/3	KA2-101	37-13/3	37-13/1	34-14/4
Range	1.0-2.1	1.2-1.5	1.0-1.59	1.0-1.8	0.92-1.2	1.3-1.7	1.4-1.8	1.2-1.7	1.3-1.6	1-1.5	1.0-2.0	1-1.2	1.2-1.7
Mean	1.7	1.4	1.5	1.6	1.1	1.5	1.5	1.6	1.5	1.4	1.6	1.1	1.5
Std	0.3	0.2	0.1	0.2	0.3	0.1	0.1	0.4	0.1	0.1	0.1	0.1	0.2

Plant density: Intermediate

Clone	67-9/3	25-6/3	73-6/1	73-3/3	17-14/4	33-8/3	23-3/1	37-6/4	33-15/1	38-10/3
Range	1.2-1.5	1.3-1.6	1-.3-1.7	1.6-2.0	0.8-1.3	1.1-1.4	1-1.3	1.05-1.5	1.1-1.3	1.0-1.8
Mean	1.3	1.3	1.3	1.4	1.2	1.2	1.2	1.3	1.2	1.6
Std	0.5	0.4	0.2	0.5	0.1	0.2	0.1	0.2	0.1	0.2

Plant density: Small

Clone	24-4/1	17-3/1	24-9/1	23-6/1	17-7/4	34-13/2	73-14/1	24-7/1	63-7/3
Range	1.3-1.5	0.8-1.1	1.1-1.2	0.8-1.0	1.0-1.2	0.9-1.4	0.8-1.2	1.1-1.2	0.9-1.8
Mean	1.2	1	1.1	0.9	1.1	1.2	1.1	1.1	1.3
Std	0.2	0.1	0.1	0.1	0.1	0.2	0.1	0.1	0.3

The bean weight of the clones varies with an average of 1.3 g, range from 0.9-1.7 g. The bean weight was shown to be influenced by genotype and plant size, with no effect of monthly environmental factors.

Table 7: Quality attributes of the hybrid clones-Fat content

Plant density: Big

Clone	16-3/2	16-2/3	73-2/2	38-8/2	K82	38-3/4	34-14/4	36-3/1	36-8/3	KA2-101	37-13/3	37-13/1	34-14/4	Across Clone Mean
Range	55-59.3	56.8-59.7	56-59.7	48.8-52.2	56.5-57	52.3-53.4	54.1-56.1	54.9-55.4	57.2-59	52.4-54.8	54.5-56.6	56.4-57.7	52.5-56.1	
Mean	57	58	56	50	57	53	54	55	58	54	56	57	54	55.6
Std	2	1	1	2	1	1	1	1	1	1	1	1	1	

Plant density: Intermediate

Clone	67-9/3	25-6/3	73-6/1	73-3/3	17-14/4	33-8/3	23-3/1	37-6/4	33-15/1	38-10/3	Across Clone Mean
Range	55.1-58.9	56.2-57.1	59.3-60.6	55.5-58.7	58.2-58.6	53.1-53.9	54-55.8	56.8-58.9	57.3-59.2	56.5-59	
Mean	57	57	60	57	59	53	54	58	58	57	57
Std	2	1	1	1	1	1	1	1	1	1	

Plant density: Small

Clone	24-4/1	17-3/1	24-9/1	23-6/1	17-7/4	34-13/2	73-14/1	24-7/1	63-7/3	Across Clone Mean
Range	55.2-55.5	60.1-60.6	51.6-55.7	55.4-57	57-58.6	54.1-53.9	54.4-55.7	56.5-58.5	58.2-59.1	
Mean	55	60	54	56	58	54	55	58	58	56.4
Std	1	1	1	1	1	1	1	1	1	

The fat content of the clones varies with an average of 56.3 %, and range from 53-60 % on dry weight basis. The fat content was shown to be influenced by genotype and environment variations but not tree size (Kuman, 2005*a)*

DISCUSSION AND CONCLUSION

Pod harvest & storage

Pod harvest can be delayed up to a maximum period of two weeks. Pods can be left on the tree, until they are fully ripped before harvesting, and this is when the maximum fat content of the beans is reached (Figure 1). When pod harvest is delay beyond two weeks, the beans started to loss significant amount of fat as the result of seed germination; seeds used food reserve (fat) to germinate.

There is no economical benefit to harvest, and store pods beyond 2 days as they were shown to be infested with fungus, resulting high pod loss. Pods though, can be harvested and stored up to a maximum of 2 days in order to increase the recovery rate. Higher average recovery rate of 40 % was achieved for beans from storage pods compared to 30 % for beans fermented straight after harvest; an increase of 10 % recovery rate.

Fat content

Fat content of the beans is influenced by genotype and genotype x environment (G x E) interaction. Fat content contributed 28 % to the total sum of squares (SS). The plant size does not affect the fat content (Kuman *et al.*, 2005a). The results in (Table 7) indicated that fat content is not influenced by bean weight and shell content. For example, clone 16-2/3 has an average bean weight of 1.4 g and shell content of 17.4 %, but has a high fat content of 58.0 %. Contrary, clone 34-14/4 has the average bean weight of 1.5 g and shell content of 14.8 %, but has a low fat content of 54.0 %. This means, clones with large bean size and low shell content does not necessary produce high fat content. Pearson correlation coefficient indicated that there is no association between any of the quality parameters, except between recovery rate and the fat content (Kuman, 2009).

Shell content

The shell content of the bean was significantly influenced by monthly environment variation and genotype. Genotype contributed (25 %) to the total SS followed by month (3 %) (Kuman *et al.*, 2005a). The average shell content of the clones was 15.5 %, and range from 14-17.9 %. The shell content of the beans does not influence the fat content or the bean weight. Beans with high shell content still have large bean weight containing high fat content. The shell content of the bean could be genetically controlled. Moretzsohn *et al.*, (1999) showed that shell thickness of the seed was likely to be controlled by a single locus with two alleles (sh^+ and sh^-) showing co-dominant expression. There is no economical value to identify clones with low shell content as low as 13-14 %, which has been the focused of research in the past. Similarly, there is no need to make further effort or adopt post harvest methods to reduce shell content as there are no economical benefits; this can only incur additional overhead cost to the farmers.

Bean weight

The bean weight of the clones varies with an average of 1.3 g and range from 0.9-1.7 g. All the genotypes produce large bean weight greater than the normal required bean weight of 0.9-1 g. The weight is influenced by genotype and less affected by monthly environmental variation and tree size. Genotype contributed largest percentage of sum of square (35 %) to the total SS (Kuman *et al.*, 2005a). There is no positive correlation between bean weight, shell and fat content. A large bean

does not necessarily have high fat content, and low shell content. For instant, clone 36-3/1 (Table 5,6 & 7) has an average bean weight of 1.6 g, but has high shell content of 17.1 % and low fat content of 55 %, while clone 38-10/3 has smaller bean weight of 1.3 g, with reasonably high shell content of 16 .0 %, but still produce high fat content of 57.0 %.

Recovery rate

Recovery rate is significantly influenced by monthly environment variation and genotype. Genotype contributed (22 %) to the total SS followed by month (3 %). The recovery was not influenced by the tree size (Kuman *et al.*, 2005a). The mean recovery rate was 30.6 % with a range from 21-39 %. Some clones that shown to have high recovery rates above 30 % (Table 3 & 4). The recovery rate is also influenced by post harvest process; beans from stored pods yield high average recovery rate of 43.4 % compared to 33.2 % for bean fermented straight after harvest (Table 3 & 4). The possible reason for difference observed in the recovery rate could be related to the bean composition. Beans that developed during wet season have large pulp volume to bean ratio, and would normally have a lower recovery rate than beans produced in the dry season (Wood and Lass, 1985). The increase in the recovery rate of beans as the result of pod storage could be the results of increase in fat due to pod ripeness as demonstrated in figure 1.This also confirms the strong correlation exist between recovery rate and fat content.

CONCLUSION

The selection of the clones for breeding and distribution to the farmers obviously has to be based on what the manufactures prefers. The manufacturers prefer bean quality attributes based on their specific quality requirements and operational specification. The quality requirements may vary between manufacturers, but fall with the following acceptable standards. The manufacturers prefer bean fat content above 56 %, shell content between 11-12 % (beans offering a large edible proportion and less waste). The shell content of the beans have to be as low as possible, but of adequate thickness to protect the beans from mould and insect invasion. However, thick shell content is also valuable as it protects the beans from cracking during storage and handling. The bean weight prefers by most manufacturers is between 0.8-1.0 g/bean. The selection of the ideal clones has to be based on the combination of the quality attributes preferred by the manufacturers.

Pod harvest can be delayed for two weeks to allow fat content in the beans to reach its maximum level before harvest. This practice is economical viable and convenient to smallholder farmers, who need time to collect sufficient pods for fermentation. There is a risk of significant pod loss, if pods are harvested and stored beyond two days; prolonged storage pods was shown to be heavily infected by fungus.

The recovery rate is another value adding attribute that needs to be included in selection of the clones, as it is an in direct measure of yield. An increase in recovery rate is an economical gain. The high recovery rate is also an indication of beans containing fat content as there is a strong correlation between recovery rate and fat content.

In this study, it shows that nearly all the clones have large average beans weigh around 1 g / bean. Most of the clones produce high fat content above 56 %, while few containing higher fat content from 59-60 %. The shell content of the beans is high, well above the standard (11-12 %) preferred by the manufacturers. However, this should not be a concern because manufacturers prefers large edible portion of the beans, which the clones can produce in the form of high fat content and large bean weight. The results from this study indicated that there is no correlation between each of these qualities attributes, therefore beans with high shell content can still produce high fat content and large bean weight to meet the requirements of the manufacturers.

FUTURE RESEARCH

The future quality research needs to be focused on breeding and distribution of clones with high fat content and large bean size. The shell content becomes secondary importance. The important quality parameters (fat and bean size), which dictate the final price of cocoa can be produced by the clones. This study shows that clonal materials produce beans with high shell content still produce large beans weight, and high fat content. Likewise, smaller beans with high shell content can still produce high fat content. The recovery rate is another attribute that can add value to other physical quality parameters, which is important in terms of economical

gain; therefore this parameter needs to be included in selection of the clones.

Clones with high fat content large than 56 % and large bean size (around 1 g) can be selected for breeding and distribute to the farmers. The shell content of the beans becomes secondary importance as clones with high shell content produce large bean weight, and contain high fat content.

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EFFECT OF HOT WATER PRE-TREATMENT, LIGHT AND STORAGE ON GERMINATION OF *SCHLEINITZIA NOVOGUINENSIS* VERD SEEDS

Haron Jeremiah[1], Ruth Turia[2]

ABSTRACT

S. novoguinensis is a multi – purpose legume tree that is indigenous to the Melanesian group of islands in the pacific region. Though commonly used locally in these islands, there has been very little study regarding its use, management and seed germination. This study assesses the germination rate of two seed types (stored and fresh) treated with hot water under different immersion times and germinated under three shade levels. Stored seeds immersed between 30 to 40 seconds and germinated in 100% sunlight achieved the highest germination as compared to other treatments. Most viable seeds germinated between 3 – 9 days and gradually declined after 18 days.

Keywords: *Schleinitzia novoguinensis, Papua New Guinea, germination, shade level, boiling water immersion.*

INTRODUCTION

Multi purpose tree legumes (MPTL) have been widely used to improve farming systems as land becomes scarce in many parts of Papua New Guinea (ACIAR 2004). MPTL increase productivity when integrated into cropping systems, and are very useful in managing composition of fallow vegetations (Bourke 1997). Species such as *Casurina oligodon* and *Leucoena* have been widely used to sustain and provide soil nutrient as well as cover crops and forage for livestock.

Schleinitzia novoguinensis is commonly used in the coastal communities of New Guinea (Hoft 1992). Local subsistence farmers grow the species in newly cleared gardens by transplanting wild saplings and rarely from seeds due to the hard seed coat dormancy. Verdcourt (1979) describes the botanical attributes of the species, however there is little information published regarding its indigeneous use, and seed germination potential (Bourke 1997). Like other legumes, Schleinitzia *novoguinensis* has a hard seed coat that causes dormancy in normal environmental conditions (Krugman *et al.* 1974).

Many studies have used mechanical scarification, acid treatment and hot water at controlled lower temperatures to break dormancy in legume seeds (Bonner et al. 1974, Emery 1987). This study however opted to use boiling water at reduced immersion times as the findings will be greatly adopted in rural areas where highly automatic temperature control devices are rarely available. The main objective of the study is to assess the potential to improve seed germination by this simple seed pretreatment technology; which will in turn increase its ecological and social benefit to the subsistent farming environment and communities in PNG coastal areas.

MATERIALS AND METHODS

Seeds of *S. novoguinensis* were collected from Milne Bay Province of Papua New Guinea. First collection was done in November 2006, while second collection was in July 2007. Seed trees were located on fallow secondary forests along the coasts of the mainland and atoll Islands of the Province. The earlier collected seeds were stored at room temperature for the period of nine (9) months. Germination assessment was undertaken at the PNG University of Technology's Forestry Nursery in

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Morobe Province. The two seed groups were treated through immersion in 100 ^{0}C (boiling water) for 0, 10, 20, 30, 40 and 60 seconds then sowed under 0, 50 and 80 percent (%) shade levels.

A factorial arrangement of treatment was applied consisting of 2 seed groups, 6 immersion times, 3 shade levels and 3 replications consequently 108 experimental units in a randomized complete block design. Each experimental unit (seed tray) contains 100 seeds sowed on treated soil with an average of 65% seed viability for the stored seeds and 60% for the fresh seeds. Germination counts were undertaken in 3 days interval for a period of 30 days. Non immersed seeds sown in 100% sunlight (0% shade level) were used as controls. Average number of germination of the 3 replications were taken for the 10 counts and entered into SPSS for mean differences and ANOVA analysis at 95% CI.

RESULTS AND DISCUSSIONS

Seed germination performance between seed groups and shade levels

During the germination period, the following results were obtained. Stored seeds' germination differ significantly compared to fresh seeds, indicated by mean differences and ANOVA (Table 1 and Figure 1).

Assessment of Germination by immersion time and germination period

General trend of germination (Figure 2), indicates that seeds immersed at shorter times germinate better in lower shade levels. Seeds immersed for longer times on the other hand performed better in higher shade levels. There is high significance of variance ($P > 0.05$) in germination between shade levels and between the 6 immersion times. Least Significant Difference (LSD) analysis showed that germination performance of seeds in 0% shade level were significantly higher to those in 50% and 80% shade levels. Similarly, LSD analysis for immersion times indicates that immersed seeds perform significantly better than the untreated seeds (control).

Treated seeds immersed between 10 - 40 seconds germinated with less significant difference at lower shade levels however as shade level increases, 30 – 40 seconds immersed seed displayed a significant germination performance from the other treatments. An average combine regression analysis is made to identify the best germination performance of the immersion times (Figure 3). The analysis confirms that on average, immersion times between 10 – 40 seconds achieved better germination than the other lower and upper immersion times. The R^2 values for linear (0.073) and quadratic (0.216) are very low and can not be used to make precise germination prediction using the immersion time however, it shows a general trend of germination with increasing immersion times.

Table 1: ANOVA between Seed type and Shade levels

	Sum of Square	df	Mean square	F	Sig.
Between Seed Groups	1198.84	1	1198.84	43.44	.000
Between Shade Levels	518.75	2	259.38	8.77	.000

Table 1 indicates that there is a significant difference in germination performance of the two seed groups and shade levels. Stored seeds performed better than fresh seeds at significance level of 0.00 (F value of 43.44). There is also significant germination performance between the 3 shade levels. Seeds germinated under 0% shade level (exposed to 100% sunlight) performed better followed by 50% and 80% shade levels (Figure 1), where averages of 14%, 10% and 6% of the total stored seeds sown germinated under 0%, 50% and 80% shade levels respectively. Fresh seeds germination performance indicates a significant difference between 0% shade level and an insignificance difference between 50% and 80% shade levels with germination percentages of about 7% and 8 % respectively.

Most seeds germinated between 3 -10 days then progressively subsided after 10 days (Figure 4). There was no germination in all the samples after 20 days. The R^2 values ($R^2 < 0.5$) show that germination projection on daily basis can not be made using the regressions (linear and quadratic) in Figure 4. This may be factored by the obvious germination differences between the treatments and their replications, though general trends are indicated.

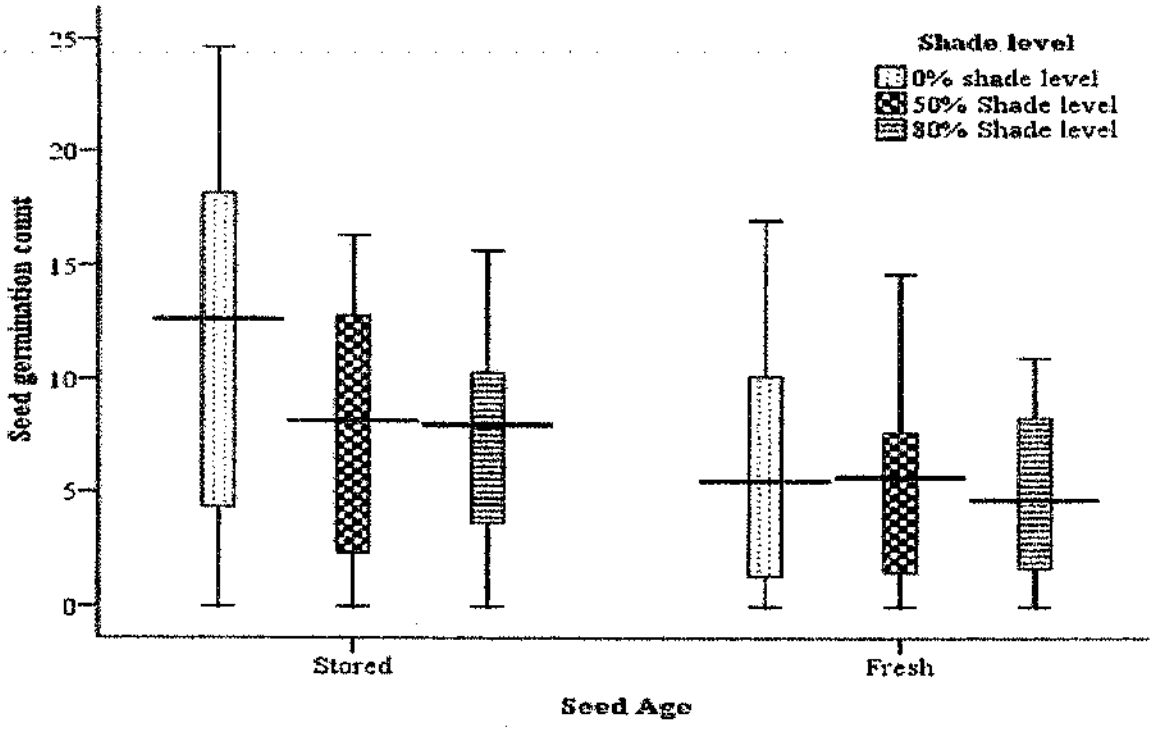

Figure 1: Germination performance of stored and fresh seed groups under different shade levels

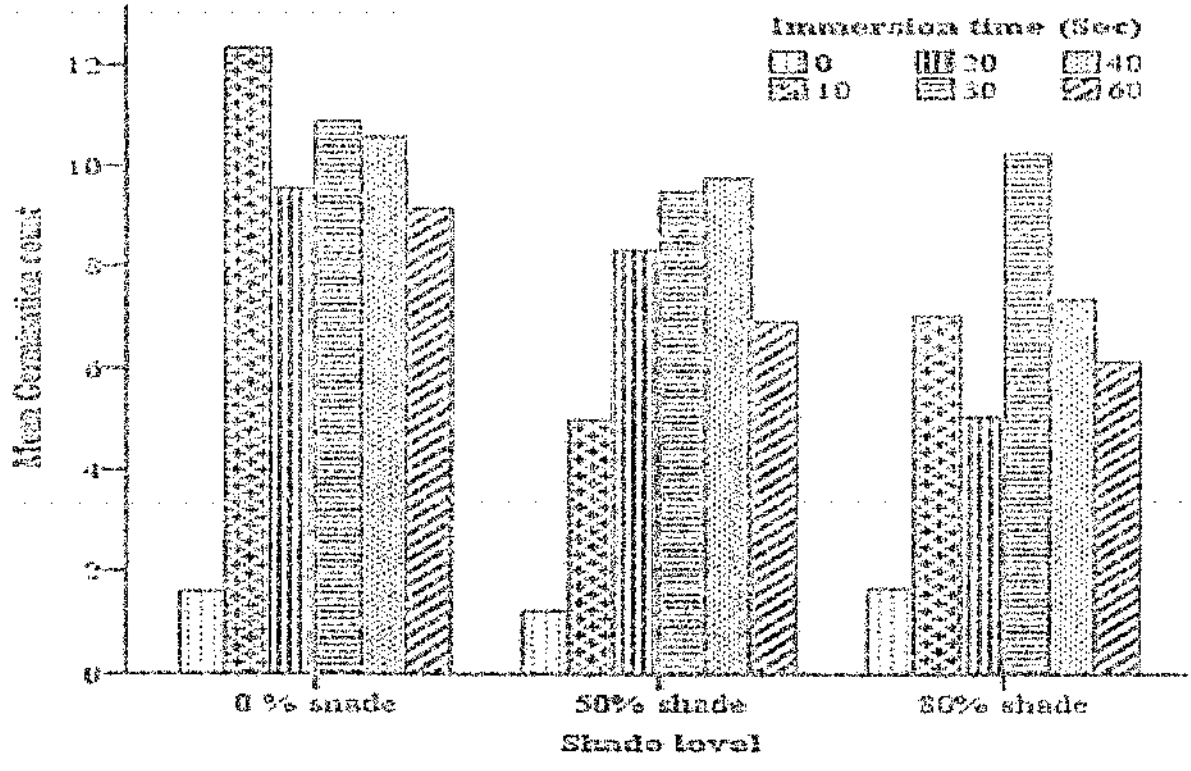

Figure 2: Germination performance of the immersed seeds under different shade levels

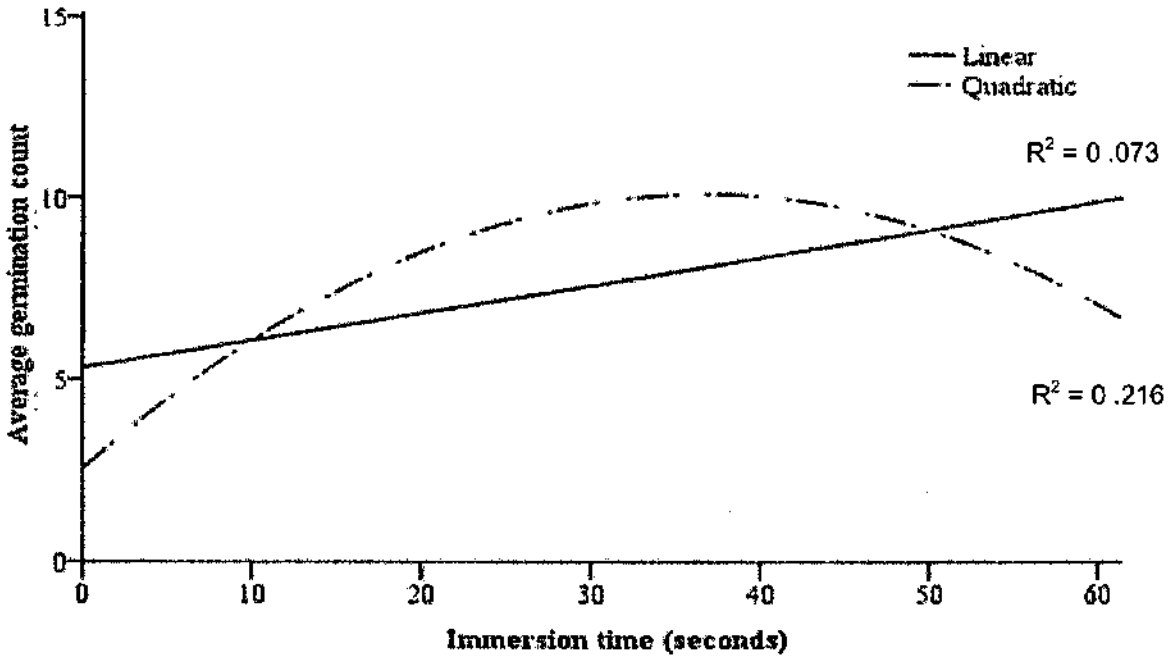

Figure 3: Germination performance of seeds under different immersion times

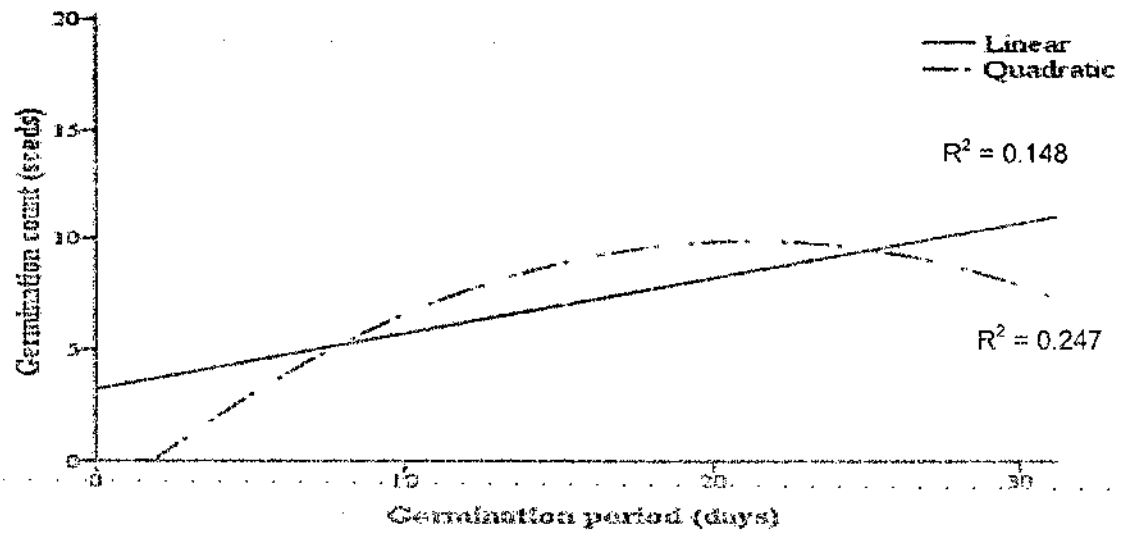

Figure 4: Overall average germination rate over the 30 days

CONCLUSIONS AND FURTHER RESEARCH

Stored seeds germinate better than freshly collected seeds. Shading of seeds from sunlight during germination does not improve germination performance. In addition, germination improved significantly in treated seeds as compared to non treated seeds (0 seconds). General average trend indicated the best germination at 20 – 40 seconds immersion times for the three shade levels however, there was no significant variance to 10 and 60 immersion times. These results also reconfirmed Oomen and Koppe (1969) findings that most tropical and sub tropical hard coated seeds germinate well between temperature ranges of 10^0C – 35^0C. Basra (1995) approves this theory by the development of automatic cabinet incubator for seed germination with standard temperature range of 10^0C – 35^0C. Most germination occurred within the first two weeks and no seeds germinated after the third week.

General germination trends indicated in this study can be utilized by the practicing farmers who mostly grow the tree by transplanting naturally germinated seedlings. Seeds picked from matured trees can be stored for months until the next cropping season. This seed can then be immersed in boiling water for half a minute and germinated on a prepared open seed bed for transplanting in due time.

This is a preliminary study into the seed germination of the species using boiling water. Results from this study indicated a very low ratio of actual seeds germinated to the quantity of viable seeds. Thus, there is a need for more research into other germination inducement techniques to improve germination quantities and rates. Lower shade levels should be trialed to increase sunlight and reduce shifting erosion in seed trays from direct raindrops as experienced in this study.

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t - tonne
l - litre
ml - millilitre
ha - hectare
mm - millimetre
cm - centimeter
M - metre
a.s.l. - above sea level
yr - year
wk - week
h - hour
min - minute
s - second
k - kina
n.a. - not applicable or not available
n.r. - not recorded
var - variance
s.d. - standard deviation
s.e.m.- standard error of difference
d.f. - degrees of freedom

Levels of significance

n.s. - not significant
* - 0.01 $p < 0.05$
** - 0.001 $p < 0.01$
*** - $p < 0.001$

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