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Comparative Nutritional Analysis of *Tylosema esculentum* (Marama Bean) Germplasm Collection in Namibia

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Abstract: Malnutrition is a medical condition caused by an unbalanced diet, typically characterised by stunting and wasting in children. Malnutrition causes approximately a third of all deaths in children between 0-59 months mostly in developing countries. In Namibia, 24% of children under the age of 5 years are stunted while 6.2% are wasted. *Tylosema esculentum*, commonly known as marama bean is an underutilised legume of high nutritious value. Indigenous to Namibia, marama bean seeds have comparably high protein and lipid content. Marama bean is an appealing crop to Namibia in particular due to its low cultivation demands as it grows in sandy soils with minimal water requirements and no need for fertilisers. Ten accessions of marama bean seeds were analysed for their nutritional composition. The results indicate that ash content was found ranging between 2.13% and 3.46%. Minerals analysed were calcium, iron, magnesium, phosphorus and zinc. Their range of concentrations were 750.1-2306.2 $mgkg^{-1}$, 53.9-322.4 $mgkg^{-1}$, 1764.1-7415.0 $mgkg^{-1}$, 4300.8-5267.9 $mgkg^{-1}$ and 32.2-48.8 $mgkg^{-1}$ respectively with no significant difference in concentration among the ten accessions. Correlation analysis of the minerals within the accessions showed that the correlations between zinc-magnesium and zinc-phosphorus concentrations were significantly different as compared to the rest of the pairs for all accessions. When analysed, the maximum and minimum amounts of crude fat and carbohydrates were 29.9%-44.1% and 19.4%-39.0% respectively which were found to not have a significant difference. However, the protein analysis determined that there was a significant difference with PMBC2 (mean content 34.6%) being the most significant accession. Therefore, PMBC2 was found to be the most suitable accession for crop development and domestication. This study's main contribution with respect to the domestication of marama bean was the identification of the most superior accession based on nutritional composition.

Keywords: Malnutrition, Marama Bean, Nutritional Composition, Biofortifier, Crop Domestication

1. Introduction

1.1. Malnutrition

Malnutrition is a medical condition that arises from an unbalanced diet characterised by two extremes of nutrition-dependent health complications being undernutrition and over nutrition. Conditions most commonly associated with malnutrition are stunting (low height for age), wasting (low weight for height), underweight (low weight for age), and morbidity (excess weight) [1]. In cases where it goes untreated, conditions such as marasmus and kwashiorkor may arise resulting in a condition known as severe acute malnutrition [2]. It is estimated that as a result of it, one-third of all child and infant deaths in developing countries are due to malnutrition with African and Asian countries having the highest cases [3].

The causes of child malnutrition vary but are largely influenced by social, economic and cultural shortcomings. The most significant and constant cause is education level of the child's caregivers, observing that less educated mothers would most likely have malnourished children. Poor maternal health also contributes negatively to child health together with a low income in a family [3, 4]. However, the main signs and symptoms of malnutrition may also be linked to food insecurity and the absence of varieties of food choices [5].

1.2. Effects of Malnutrition

Prolonged malnutrition leads to long term mental and physiological ailments. Some of the common effects include protein-energy malnutrition (PEM), typically characterised by kwashiorkor or marasmus, and micronutrient deficiencies including deficiencies in iron, iodine, zinc and vitamin A [6]. Iron deficiency (ID) in particular is associated with poor neurodevelopment, retarded growth and impaired immune response increasing susceptibility to infections [7]. The effects of malnutrition alter the natural gut microbiota causing the diminution of *Bifidobacterium longum* in the gut among others, indicating severe acute malnutrition. If prolonged, death may occur [8, 9]. Secondary effects of malnutrition include developmental or intellectual delays and susceptibility to infections due to immune dysfunction which may contribute to lower chances of survival and increased risks of morbidity [10, 11]. Furthermore, malnutrition puts a physiological strain on children with cardiovascular and metabolic complications arising at later stages [12].

1.3. Solutions to Malnutrition

Clinical treatment options for malnutrition include treating symptoms which may require hospital admission (especially in cases of severe acute malnutrition), so that provision of nutrition rich food and counselling of caregivers is availed [2]. The first treatment choice is usually a nutrition-based intervention were the children are given nutrition-rich foods in order to restore balance. However, feeding programs may be unsuccessful in treating malnutrition in communities due to cultural factors among others [13]. Research, therefore, has gone into developing other ways to curb malnutrition since 24% of children in Namibia are stunted while 6.2% are exhibit signs of wasting. The studies include incorporating plant proteins into cereals and initiatives of providing seed material to poor communities and interactive workshops with farmers to encourage the farming of sustainable crops [14].

This research will be based on *Tylosema esculentum*, (Burchell) Schreiber, (marama bean) leguminous plant indigenous to Botswana, Namibia and South Africa with the ability to survive and thrive in arid conditions. It is known to grow in poor sandy soils with evident heat tolerance [15]. Marama bean is an underutilised food crop despite its nutritional and economic advantages. Marama bean contains an estimated 29 – 39% protein [16], significantly higher than soya bean and chickpea (which are estimated to contain approximately between 34.3 - 36.3% and 23% respectively) [17, 18]. Therefore, this research will reference indigenous knowledge systems of the San and Otjiherero people of Namibia who have been known to use *T. esculentum*, known to them as ozombanui.

2. Materials and Methods

Marama bean (*Tylosema esculentum*) seeds from ten accessions were collected for nutritional analysis from the Otjozondjupa Region, Namibia. The ten accessions were chosen based early flowering, high number of seeds per pod and high number of seeds per plant. For each sample, the seeds were dehulled and ground to a flour to allow for ease of analysis. The macronutrients quantified were proteins and fats and carbohydrates, while micronutrients analysed were minerals (calcium, iron, magnesium, phosphorus and zinc using spectrophotometry and spectrometry).

A modified method for sample preparation was used [19]. Air dried marama been seeds were de-hulled using a hammer. They were stored in Ziploc bags at -20°C to reduce the possibility of spoilage, moisture absorbance and to prevent oxidation. The samples were ground into a flour using a laboratory mill.

2.1. Ash Content

The determination of ash content in marama bean was done using marama bean cotyledons which were weighed at approximately 3.0g each and placed in porcelain crucibles and incinerated at 500°C for 24hrs and then 650°C for 4hrs until there was no change in weight indicating complete removal of all organic material [20]. The crucibles and sample were weighed before and after ashing. Samples were treated in triplicates and determination of percentage ash was done following the equation below:

$$\% \, Ash \, (dry) = \frac{M_{c+a} - M_c}{M_{c+s} - M_c} \times 100 \tag{1}$$

Were:
M_{c+a}= Mass of crucible + ash residue
M_{c+s}= Mass of crucible + sample
M_c= Mass of empty crucible

2.2. Crude Protein Analysis

Crude protein determination was done using the LECO TruSpec® Micro N-Nitrogen/Protein Analyzer (CHN628) following the Duma combustion method [21]. Approximately 140.0 mg of each sample was weighed into tin foil cups. Results were determined as percentage crude protein.

2.3. Mineral Analysis

Approximately 300mg of sample was digested for calcium, iron, magnesium and zinc analysis using the PerkinElmer Titan MPS™ Microwave system using a mixture of HCl and

HNO_3 (3:1). The mineral analysis of the samples was carried out using a PerkinElmer® Optima™ 8000 Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) instrument.

The analysis of phosphorus was done via a colorimetric technique using Hach® Lange DR6000 Benchtop Spectrophotometer following a method described by Agri Laboratory Association of Southern Africa (6.2.5) [22].

2.4. Crude Fat Analysis

Crude fat was determined after the method developed by Agri Laboratory Association of Southern Africa (6.2.5) [22].

2.5. Carbohydrate Determination

The total carbohydrate content was determined following the method described by Holse, Husted and Hansen [23].

$$Carbohydrate\ content = Ash + Crude\ Protein + Crude\ Fat \quad (2)$$

2.6. Data Analysis

The data collected were analysed using SPSS (Version 22). Parametric tests were done using one-way analysis of variance (ANOVA) while Kruskal Wallis was employed as the non-parametric test of choice. Probability was accepted at $p<0.05$.

3. Results

3.1. Ash Content

The percentage of ash content was found to be between 2.13% and 3.46%. Test for normality was done by means of the Shapiro-Wilk test. The data were not normally distributed ($p=0.002$). The subsequent Kruskal Wallis test revealed no significant differences of ash percentages among the marama bean samples ($\chi^2= 9.267$; $p=0.413>0.05$).

3.2. Crude Protein Content

The data were subjected to the Shapiro-Wilk test for normality, which showed the data to be normally distributed ($p=0.631>0.05$). The maximum and minimum protein content observed from all accessions were 34.8% and 30.1% respectively. To determine significance, one-way ANOVA was performed. The results showed that a statistically significant difference among samples with respect to protein content ($p<0.001$). A Tukey post hoc test revealed that the highest mean difference was observed between PMBC2 and PMBC8 ($p< 0.001$). Therefore, accession PMBC2 had the highest content of crude protein.

3.3. Mineral Concentration

The minerals concentrations (in $mgkg^{-1}$) are shown in Table 2. The difference between the highest and lowest mean concentrations (being from PMBC7 and PMBC9 samples) was 47.3%, while the mean concentrations of phosphorus had a 19.6% difference with PMBC2 having the maximum mean concentration and PMBC10 having the lowest. However, magnesium had the largest percent difference of means of 79.9% (PMBC3 and PMBC5).

Data obtained for calcium, magnesium, phosphorus and zinc concentrations were not normality distributed (Shapiro Wilk, $p<0.05$). Therefore, non-parametric treatments were applied to all data sets. The Kruskal Wallis Test was performed in order to determine significance. The p values for calcium, magnesium, phosphorus and zinc were 0.538, 0.621, 0.111 and 0.961 respectively (therefore, $p>0.05$) in all cases. As a result of the Kruskal Wallis H values of calcium, magnesium, phosphorus and zinc being noted as high ($p>0.05$), it was revealed that there was no significant difference among the concentrations of the individual elements among the 10 accessions.

Iron concentrations values were first subjected to the Shapiro-Wilk test for normality, which was found to be normally distributed ($p = 0.598>0.05$) and subsequently subjected to one-way ANOVA. The test revealed that there was no significant difference among the samples for the concentration of iron ($p=0.099>0.05$). Marama bean from the accession labelled PMBC4 had the highest mean concentration of iron at 322.4 $mgkg^{-1}$ while the lowest recorded concentration was 53.9 $mgkg^{-1}$ from PMBC7.

3.4. Crude Fat Content

Data obtained from fat analysis was presented as a percentage of samples' weight. The mean crude fat content for all samples was 39.3%, while the lowest fat content was 29.9% and the highest being 44.1% (Table 1). The Shapiro-Wilk test for normality showed that the data were not normally distributed and therefore the non-parametric test, Kruskal Wallis had to be applied to the data. The Kruskal Wallis H test result was observed to be $\chi^2 = 22.934$, $p = 0.006$ ($p<0.05$), $df = 9$. This indicates that the data collected on the amount of crude fat in marama bean samples were significantly different.

3.5. Carbohydrate Content

Carbohydrate content was determined by using the difference from the total proximate content. Carbohydrate content determined ranged from 19.4% to 39.0% as shown in Table 1. The Shapiro Wilk test for normality showed the data to be not normally distributed ($p<0.001$). Therefore, a non-parametric test (Kruskal Wallis) was applied to the data. The Kruskal Wallis H test results in indicated that there was a significant difference ($\chi^2 = 20.215$, $df = 9$, $p = 0.017$) among the samples of marama bean accessions. The mean carbohydrate content for all marama bean accession was 25.1%.

Table 1. *Concentrations of nutrients analysed.*

Sample	Ash (%)	Calcium (mg/kg)	Magnesium (mg/kg)	Phosphorus (mg/kg)	Iron (mg/kg)	Zinc (mg/kg)	Carbohydrate (%)	Crude Fat (%)	Protein (%)
PMBC1	2.87	1503.866	3405.528	4580.480	90.306	46.152	24.57	39.86	32.70
PMBC1	3.01	1298.535	2883.221	4583.920	62.083	37.509	25.14	39.35	32.50
PMBC1	2.83	1070.856	2410.523		37.999	30.854	24.78	39.59	32.80
PMBC1		1819.630	2643.020			35.190			
PMBC1		1807.900	2689.980			34.880			
PMBC2	2.28	2200.968	4860.110	5238.400	150.980	67.054	25.80	37.42	34.50
PMBC2	2.65	814.501	2528.307	5267.930	118.640	23.797	25.21	37.54	34.60
PMBC2	3.18	1316.585	3247.004		75.334	38.912	24.22	37.80	34.80
PMBC2		1360.990	2856.820			32.820			
PMBC2		1355.220	2893.000			31.080			
PMBC3	2.77	2306.222	7415.043	4655.960	251.250	78.246	25.57	39.46	32.20
PMBC3	3.12	1039.001	2588.071	4739.110	67.452	36.647	25.98	39.00	31.90
PMBC3	2.89	1000.568	2428.520		56.161	31.518	26.07	38.94	32.10
PMBC3		1758.260	2523.390			35.130			
PMBC3		1784.370	2656.410			34.980			
PMBC4	2.83	1263.368	2787.311	4613.930	247.493	45.120	24.76	39.91	32.50
PMBC4	3.18	1545.620	3415.185	4656.850	618.174	57.990	23.62	40.40	32.80
PMBC4	2.95	1076.069	2409.407		101.500	30.124	24.14	40.01	32.90
PMBC4		1809.320	2589.350			35.600			
PMBC4		1861.580	2591.560			35.090			
PMBC5	2.99	1187.839	2459.578	4608.060	181.171	41.997	24.61	38.20	34.20
PMBC5	2.93	1037.333	2220.133	4591.990	56.307	27.141	24.59	38.28	34.20
PMBC5	3.11	1025.628	2231.216		34.716	27.438	24.96	38.43	33.50
PMBC5		1703.700	2701.520			35.190			
PMBC5		1701.010	2808.890			35.690			
PMBC6	2.92	1094.300	2979.119	4375.900	57.000	34.688	24.70	40.78	31.60
PMBC6	2.83	1335.948	3505.163	4427.830	66.126	41.009	36.04	29.93	31.20
PMBC6	2.89	1013.237	2807.363		57.240	33.170	25.77	40.34	31.00
PMBC6		1185.120	2766.900			27.940			
PMBC6		1172.280	2853.080			28.250			
PMBC7	3.03	1188.135	2720.970	4566.430	42.752	31.674	19.41	44.06	33.50
PMBC7	2.94	1288.481	3012.969	4574.050	53.621	35.746	23.48	40.28	33.30
PMBC7	2.84	1387.159	3051.758		65.421	37.095	23.82	40.44	32.90
PMBC7		1827.490	2756.490			32.890			
PMBC7		1820.040	2798.760			33.930			
PMBC8	3.12	1061.378	2985.532	4300.810	112.983	34.422	24.89	41.89	30.10
PMBC8	3.10	1020.318	2833.174	4360.810	65.717	33.949	24.14	41.46	31.30
PMBC8	3.46	967.142	2787.033		66.274	33.988	23.66	41.78	31.10
PMBC8		1252.160	2715.170			28.610			
PMBC8		1271.030	2754.520			30.990			
PMBC9	2.91	1106.277	2391.143	4497.600	48.976	28.406	25.89	38.20	33.00
PMBC9	2.13	1369.251	2964.160	4373.140	74.356	39.212	27.38	37.59	32.90
PMBC9	3.08	2015.383	4611.358		102.791	57.399	25.20	38.92	32.80
PMBC9		1732.080	2737.960			31.280			
PMBC9		1700.840	2697.130			30.170			
PMBC10	2.95	1272.426	2865.124	4371.000	98.515	38.374	24.60	39.55	32.90
PMBC10	2.94	750.1082	1764.121	4412.600	46.200	20.313	25.19	39.57	32.30
PMBC10	3.28	1385.828	3118.590		123.825	42.515	23.81	39.81	33.10
PMBC10		1693.750	2789.220			29.750			
PMBC10		1716.650	2767.410			30.630			

4. Discussion

Analysis of the different important nutrients of marama bean populations has been carried out on bean populations from 3 countries namely Botswana, Namibia and South Africa with the sole purpose of identifying populations with highest concentrations of various nutrients. The ash content of a crop is used to provide a percentage content by mass of minerals in samples of interest [24]. It reflects the total amount of minerals in a sample, however, it does not show the concentrations and is not selective of the minerals that are left, therefore, even toxic heavy metals (if present) are included in the ash mass [25].

4.1. Ash Content

The results for ash content were between 2.7% and 3.2%, values consistent with previous studies on marama bean samples from Botswana, Namibia and South Africa where ash contents of 2.5% - 3.7% have been reported [23]. The ash content of treated marama bean flour indicates similar values, although an increase in ash content was noted in partially or fully defatted marama bean flour. These treated marama bean flour samples had ash values between 2.7% and 2.9% for full fat marama bean flour to 4.2% and 4.7% for partially defatted flours with the higher values in each case being for unheated samples [19].

4.2. Crude Protein Content

The protein content of marama bean determined was found to be between 30.1% - 34.8%. These values correspond with a previous study that found the crude protein content to be between 29% - 38% [27]. An analysis of 3 marama bean samples harvested between 2001 and 2004 found the crude protein content ranging between 34.0% and 36.9% with an average value of 35.2%. The study found that the climate during the time the samples were collected had a lesser impact on nutritional composition compared to the seasonal influence on plant growth [26]. The protein content parallels closely to that of soya bean which has a protein content of approximately 37.7% with a range between 36.9% - 40.1% [28]. Comparison of marama bean with soya bean shows that they fall within range of each other with marama bean falling short of soya bean by 2%. Due to their high protein contents, both marama bean and soya bean are suitable candidates for nutrient supplements and food alternatives [27]. When compared with other legumes, it is observed that marama bean is superior to other commonly consumed legumes. Cowpea (*Vigna unguiculata* L. Walp) has a crude protein content range between 23.2 - 28.1% while kidney beans or common beans (*Phaseolus vulgaris*) have an average crude protein content of 20.1% (±0.52) [18, 29]. Therefore, the protein content of marama bean accessions within this study compares favourably with other legumes with a crude protein content range of 30.1 - 34.8%. Statistical analysis revealed that PMBC2 had the highest crude protein content of the marama bean accessions analysed.

4.3. Mineral Content

Marama bean, as an underutilised legume with great potential, is highly comparable to other legumes such as soybean and chickpea while peanuts are a common entrant as a nutritional source. Soybean is nutrient rich with significant values of the major minerals. The content of zinc and iron (essential trace elements) from one study on samples from Benin City, Nigeria were approximately 27.0 $mgkg^{-1}$ and 164.0 $mgkg^{-1}$ respectively. Calcium, magnesium and phosphorus were found to be 3003.6 $mgkg^{-1}$, 2582.4 $mgkg^{-1}$ and 6952.0 $mgkg^{-1}$ respectively [28]. Cowpeas have mineral values much less than that of soybean [29]. However, their mineral content is worth mentioning as cowpeas also provide significant amounts of nutrients and minerals. The content of calcium in cowpeas compared to that of soybean is significantly low with ranges between 0.1-0.2 $mgkg^{-1}$. The concentrations of magnesium and phosphorus in cowpeas are higher with ranges between 1856-2274.0 $mgkg^{-1}$ and 4625.0-5924.0 $mgkg^{-1}$ respectively. Iron and zinc content ranged from 60.6-106 $mgkg^{-1}$ and 32.6-51.1 $mgkg^{-1}$ [29]. It can be seen that in both legumes, soybean and cowpea, the most abundant macro element is phosphorus with a sharp contrast being observed in the amount of calcium which may be attributed to the type of crop and soil conditions [30].

The mineral content of marama bean is highly comparable to that of soybean. Calcium, in particular, was observed to range between 750.1-2306.2 $mgkg^{-1}$. Though lower compared to the mean calcium content of soybean it was considerably higher than that observed in cowpea varieties. Unlike in soybean and cowpea tests, the highest concentration of any macro element observed in marama bean was magnesium were the minimum concentration observed was 1764.1 $mgkg^{-1}$ and the maximum was 7415 $mgkg^{-1}$. Phosphorus concentration ranged from 4300.8-5267.9 $mgkg^{-1}$, the lowest values observed among the three legumes mentioned in this section, the highest being observed in soybean. Compared to soybean and cowpea, mean iron concentrations in marama bean were lower to that of soybean. It should be noted that there was a 1666% difference between the lowest and highest concentrations of iron in marama bean alluding to the difference observable between accessions of a single plant species. The mean zinc concentrations were also higher in marama bean compared to both soybean and cowpea. Museler and Schonfeldt [26] when analysing marama bean seeds from Namibia and Botswana found that the mean concentrations for zinc and calcium were 62 $mgkg^{-1}$ and 2410.0 $mgkg^{-1}$ respectively, values close to double of mean concentrations determined in this study. However, the concentrations of iron, phoshorus and magnesium (39.5 $mgkg^{-1}$, 4540.0 $mgkg^{-1}$ and 2745.0 $mgkg^{-1}$ respectively) in the same study were lower than those determined in this study with the greatest difference being observed in the concentrations of iron.

The values of mineral content in marama bean indicate a strong similarity compared to data collected on varieties from Botswana (BO0603), Namibia (NA0701) and South Africa (SA0703) shown in Table 2 [23]. Both iron and zinc concentrations from PMBC samples (Namibia germplasm collection) were the highest in comparison to the other samples analysed from Botswana, Namibia and South Africa in a past study, an indication of the superiority of marama bean accessions in that respect [23]. The table also includes the values observed for accession PMBC2 which contained the highest protein content.

Table 2. *Mineral content ranges (mgkg^{-1}) of marama bean varieties and marama bean accessions.*

Mineral	NA0701*	BO0603*	SA0703*	PMBC	PMBC2**
P	4050–4576	3307–3383	5488–5594	4301-5268	5238-5268
Mg	3580–3593	2330–2647	3712–3783	1764-7415	2528-4860
Ca	937–1462	2038–2176	1313–1361	750-2306	814-2201
Zn	31–39	33–33	38–39	20-78	24-67
Fe	12–14	13–14	35–40	35-618	75-151

*Ranges of mineral content of marama bean samples analysed by Holse, Husted and Hansen [23].
**Ranges of mineral content of accession PMBC2.

4.4. Crude Fat Content

Comparing marama bean with other similar legumes, soybeans are the most nutritionally competitive legumes however, fat content of marama bean is typically twice that of soybeans. Soybeans are known to contain between 17.0% - 20.0% fats while marama beans previously analysed were found to contain between 32.0% - 42.0% fats [23], values that correspond with the crude fat content determined within this study (29.9% - 44.1%). However, edible species seeds of *Rynchosia* have a far less crude fat content ranging between 3.3-4.4% compared to marama bean accessions [31]. Compared to a previous analysis of marama bean seeds from Namibia and Botswana, (39.9% and 40.2%, respectively) [26], marama bean seeds analysed in this study had similar content values of 39.3%. The difference in crude fat content may allude to the possible use of these legumes' plant oils in maintaining optimal cardiovascular health. Plant-based oils which are known to be low in saturated fats, thereby reducing and reversing the effects of coronary diseases, a trait most crucial to the health of all individuals [32].

4.5. Carbohydrate Content

The carbohydrate content determined was presented as an estimate from the proximate content of marama bean accessions. Moisture content was not considered in marama bean accession samples used in this research as the samples were dried before analysis, therefore, there were minuscule amounts of moisture. Carbohydrate content determined ranged from 19.4% to 36%, with the highest calculated percentage being taken as an anomaly as it deviated from the previously determined maximum amount of carbohydrates in marama beans of 24%. Prior studies have estimated the carbohydrate content of marama bean samples from Botswana, Namibia and South Africa to be between 19 – 24% [23]. This estimation found dietary fibre to be the most abundant within the carbohydrates. Another study found the mean carbohydrate content to be approximately 14.07 g100g^{-1} [26]. A calculated estimation much lower compared to the values obtained in this study.

5. Conclusions and Recommendations

It was found that phosphorus was the most abundant mineral while zinc had the lowest total concentration across all accessions. Analysis of the minerals (calcium, iron, magnesium, phosphorus and zinc), carbohydrates and crude fats did not find a significant difference among the accessions. Protein content, on the other hand, was observed to be (statistically) significantly different among the 10 accession samples. The greatest difference was observed between PMBC2 and PMBC8 with the former having a higher concentration of proteins. Therefore, this suggests that with respect to protein content accession PMBC2 is most suitable for crop development. It is recommended to investigate the nutritional composition of marama bean accessions in South Africa and Botswana where marama bean also grows indigenously. It is also suggested that a full vitamin analysis be done on the accessions done with special concentration on vitamin A and E. Trials on composite flours with marama bean added as a biofortifier are also recommended to be initiated with PMBC2.

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