SEQUENTIALLY PARTITIONED POPULATION ATTRIBUTABLE FRACTION AS AN ESTIMATE OF IMPACT OF TICK PRESENCE ON INFECTION

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ABSTRACT

The impact of *Rhipicephalus appendiculatus* tick presence (exposure) on *Theileria parva* infection seroprevalence (outcome) on a farm was assessed in a group of cattle using population attributable fractions (PAF). The analysis was based on a representative sample of 80 traditional smallholder mixed farms from Mbeere District, Kenya. The PAFs were estimated using sequentially partitioned PAF approach that estimated a PAF associated with the exposure after adjusting for any effect that the confounder (agro-ecological zone [AEZ]) may have had by influencing the prevalence of the exposure. The resultant PAF was compared with Bruzzi approach PAF that estimated the proportion of *T. parva* infection cases directly attributable to the exposure after controlling for confounding by AEZ. The estimated PAF on the Bruzzi approach was 26.4% [95% CI: 9.6%, 43.2%]) whereas the partitioned PAF was 15.5% [95% CI: 1.5%, 29.6%]) implying that about 11% of the estimated impacts was driven by AEZ effects. Both approaches were consistent in estimating a relatively low impact of farm vector tick presence with a relatively high level of uncertainty. Overall, the results suggested that under endemic instability in Mbeere District, (1) presence of *R. appendiculatus* was not a good indicator of *T. parva* infection occurrence on a farm, and (2) ecological variation could play a role in determining infection impacts. This study provides a preliminary basis for evaluating the potential value of estimating PAFs for variables amenable to control in tick-borne diseases epidemiological studies.

INTRODUCTION

Population attributable fraction (PAF) is an epidemiological measure of the proportion of cases in a population that are due to a risk factor (Rothman and Greenland, 1998). Population attributable fraction represents combined information on both the exposure probability and the strength of association between the exposure and outcome of interest. Thus, PAF is applied in impact measurements at a population level and has epidemiological interpretation as a measure of preventable disease (Dohoo *et al.*, 2003). The PAF estimation in veterinary epidemiology has previously been applied in studies involving calf health problems (Sanderson and Dargatz, 2000), animal feet injuries (Gillman *et al.,* 2009) and broiler chicken flock health problems (Bouwknegt *et al*., 2004) among others. Previous studies on tick-borne diseases (TBDs) have not attempted to estimate PAF from the identifiable risk factors.

Since 1953 (Levin, 1953), researchers have progressively developed PAF estimation techniques based on different assumptions and strategies. The main strategies include estimating a PAF for one risk factor while simultaneously and statistically controlling for other variables to account for confounding and interaction (Bruzzi *et al.*, 1985), use of stratification approaches to control for confounding, and considering the sequence in which risk factors may influence each other to produce an outcome (Mason and Tu, 2008).

In population studies, a variable may explain a relation or a causal link between other variables. This study utilizes this concept in the epidemiology of East coast fever (ECF), TBD of cattle caused by a parasitic protozoan, *T. parva,* and transmitted by the three-host tick, *R. appendiculatus* (Norval *et al.,* 1992). Agro-ecological zone (AEZ) is an important environmental variable associated with the epidemiology of ECF (Norval *et al*., 1992). Epidemiologically, simultaneously adjusting for the mutual association between AEZ effects and the vector tick presence when examining the effect of these variables upon *T. parva* seroprevalence is different from a model in which AEZ effects influence tick presence which then impacts on *T. parva* seroprevalence. Ignoring such sequential ordering of risk factors assumes that they are independent which is unrealistic.

The primary objective of this study was to assess the impact of *R. appendiculatus* tick presence (exposure) on *T. parva* infection seroprevalence (outcome) using a sequential PAF partitioned approach (Mason and Tu, 2008) in the cattle population of Mbeere District,

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Kenya. The sequentially partitioned approach estimated a PAF associated with the exposure after adjusting for any effect that the AEZ may have had by influencing the prevalence of the exposure. The sequentially partitioned PAF was compared with the Bruzzi approach (Bruzzi *et al*. 1985) that estimated the proportion of *T. parva* infection cases directly attributable to the exposure after simultaneously adjusting for the mutual association between AEZ effects and the vector tick presence. The purpose of the study was to apply the PAFs as a practical tool in applied epidemiology to contribute to animal health policy in terms of disease control and in identifying new research initiatives.

MATERIALS AND METHODS

Data

Data used in this study comprised of population-level cross-sectional data collected from cattle in Mbeere District in Kenya (Gachohi *et al.,* 2010). Briefly, cattle were selected by multistage random sampling from 80 farms in 8 sub-locations within the four administrative divisions in Mbeere District. *Theileria parvasero* prevalence and its 95% confidence limits were19% [14%, 25%] indicating endemic instability. The variable 'division', representing agro-ecological effects, was analysed and suspected to be a confounder. The sampled farms fell in two AEZs in the district: the Lower midlands 4 (LM4 zone) and Lowlands 5 (L5 zone) having different ecological characteristics (Jaetzold and Schmidt, 1983). Based on ecological requirements of *R. appendiculatus*, anecdotal hypothesis is that LM4 zone is more suitable for the tick relative to L5 zone (Norval *et al*., 1992).

To remove the effect of correlated observations at the farm-level (Gachohi *et al*., 2010), the data was analyzed at the farm level. A farm was considered infested if at least one animal on a farm was found to carry *R. appendiculatus* during the farm visit. For the outcome, a farm with at least one *T. parva* seropositive animal was considered seropositive.

Data analysis

Frequencies of farm-level variables and AEZ variable were first generated. Univariate (P≤0.1) and multivariate (P≤0.05) logistic regressions were implemented on the farm *T. parva* infection serostatus against farm-level and AEZ variables. These analyses were implemented in STATA 10. At the multivariable analysis, only presence of the vector tick on a farm variable was significant (P=0.01). Nevertheless, the current study accounted for confounding by AEZ from the a priori subject matter knowledge (Hernán *et al*., 2002).

PAFs estimation

Estimation of PAF necessitates computation of two components: (a) measure of association of the factor in the population (relative risk (RR)) and, (b) the probability of exposure (Morgenstern, 2008; formula 7, page 58).

The sample of 80 farms was placed in j mutually exclusive strata formed by cross-classifying the confounder (AEZ) and the significant risk factor levels (Table I). Table I illustrates the notations used in the analysis. This step facilitated the computation of prevalence of exposure among seropositives (Table I). The referent categories were 'farm vector tick absent' and 'L5 zone' (jth stratum = 0) (Table I) which were hypothesized to be the low risk categories.

Sequentially partitioned PAF approach

In using the sequentially partitioned PAF approach (Mason and Tu, 2008), AEZ was hypothesized to influence the 'prevalence' of the presence of vector tick on the farm. In the original data, more farms in LM4 zone were infested with *R. appendiculatus* relative to L5 zone. Sequentially, infested farms were more likely to be *T. parva* seropositive. Consequently, more *T. parva* seropositive farms were likely to be found in the LM4 zone.

The first step aimed to remove the relational effect of LM4 zone upon tick presence or absence by adjusting the prevalence of the latter variable. To achieve this, the increased risk of a farm being infested with *R. appendiculatus* given that the farm was found in the high risk LM4 zone was computed as an *RR* (and denoted $RR_{B|A}$). This was done using a 2×2 frequency table by

TABLE I - STRATUM-SPECIFIC NOTATIONS UPON CROSS-CLASSIFICATION OF THE CONFOUNDER AND RISK VARIABLE LEVELS

	Confounder	Risk variable		
jth stratum	AEZ	Vector tick status	n_i	*T. parva* seropositives
1	LM4	Present	n_1	x_1
2	LM4	Absent	n_2	x_2
3	L5	Present	n_3	x_3
0	L5	Absent	n_4	x_4
Total			n	x

Prevalence of exposure among seropositives =(x1+x3)/x.

treating tick presence or absence as the 'outcome'.

To remove any effect that the LM4 zone may have had in terms of increasing the prevalence of tick presence, an adjusted frequency table was created by adjusting frequencies among farms in LM4 zone (n_1 and n_2 in Table I) as follows:

$$n`_1 = n_1 * RR_{B|A}^{-1}$$

where$n`_1$ was the expected number of farms with both risk factors after the adjustment (stratum j=1 in Table II).

In this approach, the stratification approach in Table I was used and initially computed an unadjusted RR in stratum j as $(x_j * n_4)/(n_j * x_4)$. Bruzzi adjusted PAF for vector tick presence was computed as follows: (Bruzzi *et al*., 1985, equation 8)

$$Bruzzi\ PAF = 1-(\Sigma_j\ (xj/RR_j°))/x$$

Where x_j represented the number of positive cases in each stratum, RRj° represented the relative risk for stratum j compared with relative risk for stratum that would be observed if the risk variable of interest (exposure) were eliminated (i.e., were at baseline levels) while the confounder variable retain the actual levels and x represented the total number of seropositive farms.

TABLE II - NOTATIONS FOR EXPECTED NUMBER OF FARMS IN STRATA WITH HIGH RISK AEZ ON ADJUSTING FOR THE RELATIONSHIP BETWEEN THE CONFOUNDER AND THE EXPOSURE

jth stratum	AEZ	Vector tick	*T. parvaseropositives*	*T. parvaseronegatives*	Total
1	LM4	Present	n^{∞}	$n`_1 - n^{\infty}$	$n`_1$
2	LM4	Absent	$n^{ø}$	$n`_2 - n^{ø}$	$n`_2$
3	L5	Present	x_3	$n_3 - x_3$	n_3
0	L5	Absent	x_0	$n_0 - x_0$	n_0
Total			$x`$	$[n - x`]$	n

The total number of farms in LM4 zone was maintained in the adjusted table (Table II) as follows:

$$n`_2 = n_1 + n_2 - n`_1$$

where$n`_2$ was the number of farms in stratum j=2 in the adjusted data (Table II) and n_1 and n_2 were the number of farms in strata j=1 and j=2 respectively in Table I. The adjusted table (Table II) reflected the predicted frequencies given no association between AEZ and *R. appendiculatus* presence or absence.

The adjusted PAF for 'vector tick present' (given no association between AEZ and *R. appendiculatus* presence or absence) was calculated as follows (Mason and Tu, 2008):

$$PAF`_B = \{[x` - p`_{Case|notB} * n]/x\}.$$

Where x` was the total number of cases in the adjusted table (Table II), $p`_{Case|NotB}$ was the probability of the outcome among farms in the adjusted table in Table II in which the tick was absent $(\{n^{ø}+x_0\}/\{n`_2+n_0\})$, n was the total number of farms in the sample and x was the total number of *T. parva* seropositive farms in the original data.

Bruzzi's approach

Bruzzi *et. al*. (1985) proposed a strategy for estimating an adjusted PAF using logistic regression in case-control studies but can also be applied in cross-sectional studies.

Variance estimation for PAF point estimates

Variance for PAFs was calculated using ajack-knife re-sampling method called 'delete-a-group' (Wagner *et al*., 2001) under the multistage random sampling.

RESULTS

Farm-level strata-specific *T. parva* seroprevalence

The overall farm-level seroprevalence was 45% with strata comprising of the exposure (tick present) having the largest seroprevalence (Table III).

Logistic regression analysis

Univariate analysis returned five significant variables (P≤0.1) from the eleven variables screened. During multivariable analysis, only presence of the vector tick on a farm variable was significant (P<0.0021). The AEZ was not associated with exposure (P<0.27), an indication of information loss after analysing the data at the farm-level relative to findings at the animal-level in the original study.

PAFs

Predicting vector tick present based upon the level of high risk AEZ yielded an RRB|A of 1.9 [95% CI: 0.7, 3.1] indicating that contrary to the original hypothesis, farms in LM4 zone were not significantly more likely to have *R. appendiculatus* relative to farms in the L5 zone. The adjusted PAF for vector tick present after removing any relationship between the exposure and the

TABLE III - DISTRIBUTION OF THE RISK VARIABLES AND T. PARVA SEROPREVALENCE FOR EACH RISK VARIABLE STRATA

	Confounder	Risk factor			
jth stratum	AEZ	Vector tick status	n_j	*T. parvaseropositives*	*T. parvaseroprevalence*
				n	*(%)*
1	LM4	Present	17	15	88.2
2	LM4	Absent	31	11	35.5
3	L5	Present	8	3	37.5
4	L5	Absent	24	7	29.2
Total			80	36	45.0

confounder was calculated to be 15.5% [95% CI: 1.5%, 29.6%]. On the other hand, the Bruzzi adjusted PAF of farm vector tick present adjusted for AEZ was 26.4% [95% CI: 9.6%, 43.2%].

DISCUSSION

Conceptually, adjusting for the mutual association between tick presence and ecological variables simultaneously when evaluating the effect of these variables upon *T. parva* seroprevalence, as in Bruzzi approach, is different from a model in which favourable ecological effects leads to heightened risk for tick presence which in turn leads to elevated risk for *T. parva* seroprevalence. The assumption of sequential ordering of effects is more realistic as an earlier risk factor can impact subsequent risk factors by increasing their prevalence (Mason and Tu, 2008). In the original study, LM4 zone was hypothesized to be a higher risk zone relative to L5 zone, probably due to the ecological differences between the two zones (Jaetzold and Schimdt, 1983). In this context, the objective was to derive an adjusted PAF for farm *R. appendiculatus* presence that was unrelated to AEZ effects.This strategy can provide important information on contribution of each of the variable types (independent and intervening) and, therefore assist in designing more sustainable risk-based disease interventions.

Both PAF approaches estimated a relatively low impact of farm *R. appendiculatus* presenceon *T. parva* seroprevalence under endemic instability despite the fact that the tick is the sole vector for the infection. Under endemic instability, these findings suggested that presence of *R. appendiculatus* is a poor indicator of *T. parva* infection on a farm.

These results, however, need to be interpreted with caution as the estimated PAFs are from a cross-sectional study in which there was no follow-up and, thus, the estimates represented the proportion of prevalent and/or previous *T. parva* infection cases as well as single time tick presence/absence information. Further, these findings need to be interpreted in the context of additional limitations of PAF estimation. The PAF values are based on the assumption that the exposure has a causal relationship on the outcome of interest (Benichou, 2005). Only infected *R. appendiculatus* can transmit infection and, therefore, presence of the vector has no causal connection with *T. parva* infection. Considering that a susceptible cattle population is likely to build up under endemic instability, presence of the tick on the farm is likely to trigger off tick control. This is what motivated this study- to quantify the impact of finding the vector on a farm on exposing the animal to *T. parva* infection.

The main ECF control methods combine treatment of clinical cases, tick control using acaricides, and immunization (Norval *et al*., 1992). In a population where a large proportion of cattle are susceptible such as that in Mbeere District (~81%) (Gachohi *et al*., 2010), and with the finding that vector tick presence is not a reliable indicator of occurrence of infection, tick control and early treatment of clinical cases would be the most appropriate control strategies. Disease control under such conditions requires additional innovative approaches such as (a) farmer awareness of the biology, ecology and identification of the tick species on their farms, and (b) ECF clinical picture in cattle in case of successful parasite transmission. This would optimize both the use of acaricides by applying the most appropriate frequency and early management of infection.

CONCLUSION AND RECOMMENDATIONS

This study successfully and quantitatively estimated the impact of farm vector presence that was both related and unrelated to the AEZ, justifying the confounding effects of AEZ. The relatively low impact of the exposure suggested that presence of the *R. appendiculatus* on a farm is not a good indicator of *T. parva* infection occurrence under endemic instability. Although causal references to the exposure and outcome could not be made in this study, presence of *R. appendiculatustick* is a priori known risk factor for the outcome and is modifiable (through tick control) leading to a greater utility of the estimated PAFs. Limitations included lack of information about tick infection prevalence and seasonality. These preliminary findings have

implications on disease control in ECF endemic unstable areas. However, more studies are needed to confirm the potential value of estimating PAFs in tick-borne diseases epidemiological studies in contributing to disease control policies.

ACKNOWLEDGEMENTS

The authors are indebted to Craig Mason of the College of Education and Human Development, University of Maine, USA for his constructive comments. The Director KARI for support and facilitation in data collection and analysis.

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WILD EDIBLE PLANTS POTENTIAL TO ENHANCE AGROBIODIVERSITY AND RESILIENCE IN A CHANGING ECOSYSTEM

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ABSTRACT

Wild edible plants are accessible, available and cheaper to diversify household diet in drylands. However, the diversity and resource base are not properly documented to inform policy makers for proper utilization in food security. Therefore, a study was conducted in the semiarid part of East Shewa in Fantalle (transhumance pastoralists) and Boosat (settled farmers) Districts of Ethiopia to determine the diversity, abundance and densities of wild edible plants. Species data was collected by field inspection and participatory observations with recording of the dominant vegetation (shrubs and trees) and wild edible plants (WEPs) along six transects. Ninety plant species were identified as a major component of vegetation of the study area in which 40 wild edible plants are distributed. The wild edible plants were identified by interview, focus group discussions and through key informants' field walks. Shannon-Weiner diversity indice measured for the species diversity and evenness of species distribution of six study sites showed no difference between the study sites ($P>0.05$). Whithaker beta (βw) diversity calculated for turn-over of species composition was not different ($P>0.05$) between the study sites. Jaccard's coefficient similarity between transhumance and settled farmers districts was 66.67%. The results indicated that the districts are potentially similar in terms of plant biodiversity. However, mean values of abundance and densities vary ($P>0.05$) across land uses. The transhumance land use has better abundances and densities for some species of wild edible plants. *Lantana camara, Ziziphus spina-christi* and *Acacia senegal* ranked 1st to 3rd, respectively in abundance and densities. Being in the arid region within a fragile ecosystem, the study area; east Shewa, is not free from climate variability and change consequences. Hence, the resilience of human livelihoods could be enhanced by integrating conservation and sustainable use of wild edible plants in dryland agro-biodiversity.

Keywords: Changing ecosystem, resilience, diversity indices, Jaccard's, agro-biodiversity.

INTRODUCTION

Useful plants are distributed in various habitats; world-wide, continentally and nationally and in specific localities. Knowledge of distribution of these plants is essential for conservation and management. Wild edible plants (WEPs) which are essential for rural people's diet are distributed in forests, woodlands, grasslands and wetlands. Description of habitat distribution of WEPs is one of the research gaps in Ethiopia (Teketay and Eshete, 2004).

In Ethiopia a number of published reports that provide information on indigenous edible plants, including fruit trees and shrubs have accumulated over the years. However, the documents mainly focused on botanical descriptions (Teketay and Eshete, 2004). The information was scanty on different uses, processing of the edible parts for food and their habitat distribution (Asfaw and Nigatu, 1995; Asfaw, 2009). Attention on research and development on various aspects of the plants, e.g. biology, ecology, silviculture, conservation both *in situ* and *ex situ* management, nutritional value, propagation techniques, domestication, processing, seed storage behaviour and marketing (local, national and international) of edible parts, value addition on edible parts and other aspects is inadequate (Teketay and Eshete, 2004; Wube, 2009). Research supported development activities are important, for sustainable utilization of wild edible fruit tree species. Therefore, the aim of this present study was to identify the potential resource base, and local habitats distribution of the WEPs.

The knowledge of WEPs habitats is helpful for their conservation and sustainable utilization. This must be followed by identification of potential and preferred WEPs in terms of their food and multiple uses. It is also useful for domestication and mass cultivation efforts. Specific objectives were to (1) identify habitats where WEPs were distributed in the study area and describe the suitability of the habitats for their growth and (2)

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measure the abundance, density and diversity of in the same in the habitats. Therefore, this study has focused on identification of the habitats, abundance, densities and suitability of the habitat for the WEPs.

MATERIALS AND METHODS

Study area

The study was conducted in Fantalle and Boosat Districts, in East Shewa Zone of Oromia National Regional State, Ethiopia. East Shewa is located in the northern part of the Great East African Rift Valley. It has an area of about 14,050 km^2. The population of East Shewa is about 1,800,000, the urban population being 28 % with average density of 128.11 persons per km ^{2}.

Sampling procedures in selecting sampling sites

The study sites in Boosat and Fantalle were selected based on topographic diversity, vegetation and lifestyle of people living in the zone (transhumance and settled farmers) to capture diverse landscapes and people's Indigenous Knowledge. Lowland areas and highland areas, watershed alignments were also carefully considered.

Characterization of habitats of wild edible plants

Data on characterization of vegetation was collected through field inspection, and participatory observations of the study area. The dominant vegetation of the study area was recorded and collected along six transects of 5 km length with 400 m^2 plot size laid 200m apart following the procedures of Mueller-Dombois and Ellenberg (1974) and Cook and Stubbendieck (1986). Habitats of WEPs were characterized by emic (using information drawn from the way people perceive vegetation and classify it) and etic (records taken from the field data) approaches following Martin (1995) and Cotton (1996).

Sampling strategy

The study area was sampled using a randomized systematic method. Sample sites were located after reconnaissance survey of Boosat and Fantalle districts. Sampling was made on a floristically homogeneous surface area with a plot size of 400 m^2 as used by Eshaghi *et al.* (2009). In each plot, a full floristic list of each woody vascular plant (trees and shrubs) were recorded using a standard scale (Mueller-Dombois and Ellenberg, 1974; Cook and Stubbendieck, 1986)

Measuring abundance, densities and diversity

Frequency and density of WEPs were measured following procedures of Mueller-Dombois and Ellenberg (1974); Cook and Stubbendieck (1986) in the quadrats sampled. Specifically, Density = # of individuals / area sampled (number of individuals/sum of all plot areas) and, relative density = species density / total density for all species ×100. Frequency = # of quadrants in which species occur (Number of plots which have at least 1 individual of a species) / total # of quadrats sampled and relative Frequency = species frequency / total of frequency values for all species × 100. Species diversity is a measure of the number (richness) and abundance (in the sense of number of individuals/species) of species in a community.

Alpha diversity

Alpha diversity (α-diversity) is the biodiversity within a particular area, community or ecosystem, and is usually expressed as the species richness of the area. This can be measured by counting the number of taxa (distinct groups of organisms) within the ecosystem (e.g. families, genera, and species). However, such estimates of species richness are strongly influenced by sample size, so a number of statistical techniques can be used to correct for sample size to get comparable values.

Alpha (α) and Beta (β) Biodiversity- Biodiversity was described by two parameters:

(i) point or α diversity represented by the number of species in a specified area and
(ii) β diversity represented by the turnover of species across space.

Data analysis

Qualtitative data on observations, key informants discussions were narrated carefully. Frequency, density, abundance were stored in Excel spread sheet and analysed through SPSS Software Version 16.

Measuring plant diversity

Species richness index was estimated as the number of species inventoried in the plot. To quantify the diversity of the plant species, the Shannon Weiner diversity index (*H'*) and Simpson's index were used as a measures of species abundance and richness. The latter index which takes both species abundance and species richness into account is sensitive to changes in the importance of the rarest classes (Heuserr, 1998). Weiner index (used as Shannon diversity index in this research) is the most commonly used index (Kent and Coker, 1992). For any sample it is calculated as:

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

Where, is equals the number of species and pi is the relative cover of ith species (Whittaker, 1972; Pielou, 1975).

The variables, Shannon diversity index (*H*) is another index that is commonly used to characterize species

diversity in a community. Shannon's index accounts for both abundance and evenness of the species present. The proportion of species *i* relative to the total number of species (*pi*) was calculated, and then multiplied by the natural logarithm of this proportion ($\ln p_i$). The resulting product was summed across species, and multiplied by -1 to get Shannon Diversity Index which has minimum value of 0 and maximum of 5.

$$H = -\sum_{i=1}^{s} p_i \ln p_i$$

Shannon's equitability (*EH*) was calculated by dividing *H* by H_{max} (where $H_{max} = \ln S$). Equitability assumes a value between 0 and 1 with 1 being complete evenness. , $E_H = H/H_{max} = H/\ln S$ where, S= total number of species in the community (richness), p_i = proportion of *S* made up of the *i*th species and E_H= equitability evenness(Beals *et al*., 2000).

Relative species abundance is a component of biodiversity and refers to how common or rare a species is relative to other species in a defined location or community (Hubbell, 2001; Baker, 2002; Wikipedia, 2010). Relative species abundances tend to conform to specific patterns that are among the best-known and most-studied patterns in macroecology (Fidelibus and Mac Aller, 2010).

Beta (β) diversity is a measure used to characterize the patterns of species diversity across heterogeneous regions (Perlman and Adelson, 1997). There are different beta (β) diversity measures which: (1) estimate richness and evenness differences over a range of habitats or sites, (2) indicate diversity changes along a gradient, or (3) compare the species composition of different communities (Wilson and Shimida, 1984; Magurran, 1988). Although there are different ways to calculate beta diversity, all methods determine species turnover (replace one another) between different sites or along environmental gradients (Perlman and Adelson, 1997). In the present study, beta diversity was determined using the Whittaker (1972) and Wilson and Shmida (1984) methods.

The Whittaker (1972) method is: βw = (Sc/S)-1, where βw is Whittaker beta diversity, Sc is the number of species in the composite sample (number of species in the whole data set), and S is the average species richness in the sample units. If βw is 0 then all sample units have the same species. βw <1 is rather low and βw >5 can be considered high (McCune and Grace, 2002). The maximum value of βw is obtained when no species are shared among sample units.

Comparison of plant diversity

A Tukey test was conducted to test for differences in the species richness, diversity and evenness indices among the two districts using SPSS version 16.0. This was also used by Eshaghi *et al.* (2009) to assess diversity of vegetation of North Tehran.

RESULTS

Diversity of WEPs identified from the vegetation of the study area

A total of 40 WEP species that belonged to 23 families and 27 genera were found in the local vegetation. The WEPs constituted 44.4% of the major tree and shrub woody plants of the area (Table I). Wild edible plants identified in the study area out of transects are not included in the table.

Species diversity, richness and evenness for each study site

An inventory of dominant vegetation component revealed that the area has 90 tree and shrub species identified in the study plots [(20×20) ×11 ×6] = 26400 m^2. Diversity indices were computed for six study sites (Table I). The results showed that the vegetation of semiarid East Shewa has HE (EH) ranging from 0.91-0.93 (Table II). It indicates good evenness and abundance. This was also compared with Simpson Diversity Index (D) and Simpson Evenness (ES) (Table II).

Comparison of mean values of diversity indices

Tukey's test for the mean values of diversities revealed that, there is no divergence (P>0.05) in six of the indices across the two districts (Table III) in diversity of plant species. Whithaker beta (βw) diversity also indicated that there is low turnover of species composition (Table III).

Comparative analysis of causes of natural resources degradation in the study area

Among the causes identified, communities ranked human population pressure as first (5) accompanied by agricultural expansion (4 and 5). Based on a mean value, adverse climate change, land use change, overexploitation of resources, poverty/hunger all ranked third (4). Tribal conflict and restriction of mobility are severe problems at transhumance land use system with average rank values of four while settled farmers identified land use system with ranked value of two. The mean values indicated that expansion of agriculture is more intense while tribal conflict is less intense in settled farmers' areas. High livestock population was a relatively low factor while tribal conflicts were severe factors for resources degradation in transhumance land use system (Table IV). T –test values indicated lack of statistical differences between the two land use systems. One of the reasons may be that as neighbours they exchange knowledge and experience of resource use

TABLE I - NUMBER OF FAMILIES, GENERA AND SPECIES OF WEPS FOUND IN THE VEGETATION

No	Family	Total genera	% of total	Total species	% of total
1	Amaranthaceae	1	3.70	1	2.5
2	Anacardiaceae	1	3.70	1	2.5
3	Apocynaceae	1	3.70	1	2.5
4	Balanitaceae	1	3.70	1	2.5
5	Boraginaceae	1	3.70	3	7.5
6	Burseraceae	2	7.41	2	5
7	Cactaceae	1	3.70	1	2.5
8	Capparidaceae	1	3.70	1	2.50
9	Combretaceae	1	3.70	1	2.5
10	Ebenaceae	1	3.70	1	2.5
11	Fabaceae	2	7.41	5	12.5
12	Lamiaceae	2	7.41	2	5
13	Loganiaceae	1	3.70	1	2.5
14	Moraceae	1	3.70	3	7.5
15	Oleaceae	1	3.70	1	2.5
16	Polygonaceae	1	3.70	1	2.5
17	Rhamnaceae	3	11.11	3	7.5
18	Salvadoraceae	1	3.70	2	5
19	Sterculiaceae	1	3.70	2	5
20	Tiliaceae	1	3.70	5	12.5
21	Ulmaceae	1	3.70	1	2.5
22	Verbenaceae	1	3.70	1	2.5
23	Capparidaceae	1	3.70	1	2.5
Total	23	27	100	40	100

TABLE II - DIVERSITY INDICES, SPECIES RICHNESS, AND EVENNESS OF EACH STUDY SITE

Study sites	Species richness	Shannon Diversity index(H')	H'max	Evenness(HE)	Simpson Diversity (D-1)	Index Evenness
DT	70	3.90	4.25	0.92	0.97	0.40
TB	76	3.95	4.33	0.91	0.98	0.44
XA	71	3.89	4.26	0.91	0.97	0.40
GA	76	3.90	4.33	0.90	0.97	0.39
QO	72	3.94	4.28	0.92	0.97	0.40
DH	77	4.34	4.34	0.93	0.98	0.48

DT= Digalu Tyo, TB=Trii Bireti, XA=Xadacha.GA=Galcha, QO= Qobo, DH= Dheebiti

The study sites lie within the altitude range of 948-1468 m.

and management that somehow counters the causes and threats, rather both communities share a similar agroclimatic region.

Statistically, there was no difference (P>0.05) in the causes of natural resources degradation between Fantalle and Boosat districts.

Comparison of abundances and densities of commonly used WEPs relative to major vegetation components

Analysis of average relative abundance and densities of three study sites for each district indicated variations for all WEPs between districts (Table V). *Acacia senegal* was the same in density but higher in abundance in Boosat district. *Acacia tortilis* and *Berchemia discolo*r

TABLE III - MEAN VALUES OF DIVERSITY INDICES OF THE STUDY DISTRICTS

Diversity Indices	Districts	No of sites	Mean	Std Error	Minimum	Maximum	P
Species richness	Boosat	3	72.33	1.86	70.00	76.00	
	Fantalle	3	75.00	1.53	72.00	77.00	
	Total	6	73.67	1.23	70.00	77.00	0.329
Shannon Diversity Index	Boosat	3	3.91	0.02	3.89	3.95	
	Fantalle	3	4.06	0.14	3.90	4.34	
	Total	6	3.99	0.07	3.89	4.34	0.357
Shannon Max	Boosat	3	4.28	0.03	4.25	4.33	
	Fantalle	3	4.32	0.02	4.28	4.34	
	Total	6	4.30	0.02	4.25	4.34	0.327
Shannon evenness	Boosat	3	0.91	0.00	0.91	0.92	
	Boosat	3	0.92	0.01	0.90	0.93	
	Total	6	0.92	0.00	0.90	0.93	0.775
	Fantalle	3	0.97	0.00	0.97	0.98	
	Total	6	0.97	0.00	0.97	0.98	0.855
βw ††	Boosat	3	-0.20	0.02	-0.22	-0.16	
	Fantalle	3	-0.17	0.02	-0.20	-0.14	
	Total	6	-0.18	0.01	-0.22	-0.14	0.306

††= No divergence in diversity indices between study districts (P>0.05),

TABLE IV - PAIRED SAMPLE T-TEST OF THE POOLED MEAN VALUES OF DIRECT MATRIX RANKING OF CAUSES OF NATURAL RESOURCES DEGRADATION IN STUDY AREA

Causes	Boosat	Fantalle mean	Combined	Std. Deviation	S E M	t	P
Adverse climate change	4	4	4.00	0.000	0.000	5.000	0.126
Expansion of agriculture	5	4	4.50	0.707	0.500	3.000	0.205
High livestock population	2	5	3.50	2.121	1.500	2.000	0.295
Human population pressure	5	5	5.00	0.000	0.000	7.000	0.090
Lack of alternative livelihoods	3	5	4.00	1.414	1.000	5.000	0.126
Land use change	4	4	4.00	0.000	0.000	5.000	0.126
Over exploitation of resources	4	4	4.00	0.000	0.000	5.000	0.126
Poverty/hunger	4	4	4.00	0.000	0.000	5.000	0.126
Restriction of mobility	2	4	3.00	1.414	1.000	3.000	0.205
Tribal conflicts	2	4	3.00	1.414	1.000	3.000	0.205

SEM= Standard error of the mean.

were higher in both abundance and density in Fantalle. *Balanites aegyptiaca* was highest in Fantalle in terms of abundance and density. This proves the respect that transhumance give to these plants which serve as a site for women to celebrate spiritual ceremonies. Similarly, *Lantana camara, Grewia flavescens* and *Grewia tenax* were fairly abundant in both districts. *Lantana camara, Rumex nervosus* and *Opuntia ficus-indica* were available in higher abundance in Boosat than in Fantalle district IV. Density was not calculated for WEPs identified in transects.

The densities of most of the WEPs were higher in transhumance than settled farmers land uses system especially for *B. aegyptiaca* and *Z. spina-christi* (Table V). High values were obtained for Galcha, Qobo and Dheebiti kebeles of Fantalle district.

DISCUSSION

Status of habitats of WEPs

The habitats of these valuable WEPs are increasingly threatened by continued destruction of indigenous vegetation. The fact that most of the WEPs have multiple uses, have posed a big threat to their existence as long as destruction of their habitats and overharvesting continues. Over harvesting and destruction of habitats resulted in the rarity of a majority of the WEPs. Information

TABLE V - ABUNDANCE OF WEPS IN THE STUDY AREA RELATIVE TO MAJOR VEGETATION COMPONENTS

NO	Spp	Boosat AV Frqu	Boosat Av % Rel Frqu	Boosat Av Dens	Boosat Av %Rel Dens	Fantalle Av Frequ	Fantalle Av %Rel Frqu	Fantalle Av Dens	Fantalle AV % Rel Dens
1	*Acacia senegal*	7.33	66.67	18.9	2.64	5	45.45	18.94	2.60
2	*Acacia seyal*	2.33	21.21	6.06	0.85	1.33	12.12	3.03	0.40
3	*Acacia tortilis*	4.00	36.36	12.1	1.69	4.67	42.42	17.42	2.31
4	*Balanites aegyptiaca* .	2.67	24.24	6.82	0.96	3.67	33.33	17.42	2.27
5	*Berchemia discolor*	3.33	30.30	9.09	1.27	2.00	18.18	6.82	0.93
6	*Carissa spinarum*	2.33	21.21	7.58	1.06	3.33	30.30	9.09	1.17
7	*Celitis toka*	0.00	0.00	0.00	0.00	1	9.09	3.03	0.42
8	*Combretum molle*	1.67	15.15	3.79	0.53	1.00	9.09	3.03	0.40
9	*Commiphora africana*	4.33	39.39	12.12	1.69	2.67	24.24	8.33	1.11
10	*Commiphora schimperi*	0.00	0.00	0.00	0.00	1.67	15.15	4.55	0.62
11	*Cordia africana* ††	0.33	3.03	0.76	0.11	0.00	0.00	0.00	0.00
12	*Cordia monoica* .	0.00	0.00	0.00	0.00	2.00	18.18	4.55	0.59
13	*Dobera glabra*	0.00	0.00	0.00	0.00	1.67	15.15	3.79	0.51
14	*Euclea racemosa*	1.67	15.15	5.30	0.74	1	9.09	5.30	0.73
15	*Ficus vasta*	0.00	0.00	0.00	0.00	0.67	6.06	1.52	0.21
16	*Ficus thonningii*	0.00	0.00	0.00	0.00	0.67	6.06	1.52	0.19
17	*Grewia bicolor*	0.67	6.06	1.52	0.21	1.00	9.09	3.03	0.38
18	*Grewia flavescens*	4.67	42.42	13.64	1.91	5.33	48.48	15.15	2.01
19	*Grewia tenax*	1.67	15.15	7.58	1.06	3.67	33.33	18.18	2.43
20	*Grewia villosa*	5.00	45.45	19.70	2.74	4.67	42.42	15.91	2.13
21	*Greiwia schweinfurthii*	1.33	12.12	3.79	0.52	3.33	30.30	9.09	1.23
22	*Flueggea virosa*	2.00	18.18	6.06	0.84	3.00	27.27	8.33	1.11
23	*Lantana camara*	8.67	78.79	36.36	5.07	7.33	66.67	27.27	3.79
24	*Meriandra bengalensis*	0.33	3.03	0.76	0.11	0	0.00	0.00	0.00
25	*Myrica salicifolia*	1.33	12.12	3.03	0.42	1	9.09	2.27	0.33
26	*Opuntia ficus-indica*	8.00	72.73	28.79	4.01	5.33	48.48	14.39	1.89
27	*Premna resinosa*	1.00	9.09	2.27	0.32	0.33	3.03	0.76	0.11
28	*Rhus natalensis*	2.67	24.24	6.06	0.86	1.33	12.12	3.79	0.50
29	*Rumex nervosus*	4.67	42.42	14.39	2.01	2.00	18.18	5.30	0.74
30	*Salvador persica*	0.00	0.00	0.00	0.00	1.67	15.15	5.30	0.70
31	*Tamarindus indica*	0.00	0.00	0.00	0.00	1.33	12.12	3.79	0.50
32	*Ximenia americana*	0.33	3.03	0.76	0.11	1.33	12.12	3.79	0.52
33	*Ziziphus spina-christi*	6.00	54.55	28.03	3.91	3.67	33.33	10.61	1.47
34	*Cleome gynandra* ††								

††= WEPs identified out of transects in the study districts with the help of key informants

informants and field observation along landscapes revealed that, WEPs such as *X. americana, B. discolor, D. glabra* and, *C. spinarium* were rarely encountered in the area. The future fate of wild edible tree species like *Z.spina-christi, B.aegyptiaca, A. tortilis, X. americana, B. discolor* might be restricted to near settlements, in or on the borders of farms, in relic forests, rocky hillsides and in spiritually protected areas where the households are conscious/ aware about their uses. Asfaw and Nigatu (1995) reported elsewhere in Ethiopia that, some WEPs were used as live fence, left in crop field and open spaces for multiple uses. The present study indicated that, in the settled farmers' area of Boosat, *B. aegyptiaca* was less abundant and dense than the Fantalle transhumance while *Z. spina-christi* was very dense near and in settled farmers land use systems. The reason for this is the natural forest is more degraded in settled farmers' area due to charcoal and firewood production, expansion of agriculture and settlements. The key informants and interview information revealed that WEPs were declining in all areas with greater decline in the settled farming land use systems.

Availability of WEPs

The availability of WEPs in these habitats is also influenced by seasonal variation. Some of the annual herbs such as *Amaranthus* were only available during the main rainy season and their spatial distribution was restricted to a few places near the shades of trees making their collection and use difficult. According to key informants' group discussion, 25 to 40 years ago there were many WEPs for food, medicine and other uses. Collecting WEPs near by was very easy in those early years. An earlier study reported a decreasing trend of diversity as the population increases (Marsden and Pilgrim, 2003). In recent years, because of the deforestation to expand agricultural land, commercial fire wood collection and charcoal production, encroachment by invasive- alien species, cutting the trees for construction, overgrazing/ browsing and other development activities, some WEPs are not easily available and accessible. For instance the availability of *X. americana, T. indica, D. glabra* were restricted to some secluded areas. Restricted resources which can not be accessed for people's livelihoods are available for enhancing the assets of people (Daregne, 1988). Hence, dryland cropping must move in tandem with proper utilization of the diversity of WEPs.

Lack of knowledge by the local people about the multiple uses and management of WEPs has contributed less to degradation of local vegetation and WEPs. Among the examples raised by key informants were that policies in force were inconsiderate to communities in the valid management of WEPs. Hence, WEPs were available in the natural vegetation than under human managed habitats except a few WEPs such as *Z. spina-christi, B. aegyptiaca* and *G. flavescens* which are found in human managed habitats (farm borders, live fences, traditional agroforestry and home gardens). According to transhumance WEPs like *B. discolor, D. glabra, C. spinarium, T. indica, X. americana* were not found within the reach of most people. They were found at distances of 2 to 3 hours from home. WEPs such as *X. americana, D. glabra, T. indica* were rare in terms of availability, accessibility and abundance. The abundance of these plants was less compared to other vegetation components like *Acacia spp, Commiphora africana, Grewia spp.* which were abundant. For other WEPs there is relatively, little problem of availability and accessibility.

Local consumption of WEPs as food was not regarded as a threat to the survival of WEP species unless when demand becomes higher than sustainable harvest in the future. Key informants from the transhumants system pointed at many reasons that make overgrazing the most important threat. They described the shrinking of pastureland by expansion of agriculture, shortage of forage due to drought and increasing livestock population. Overgrazing/browsing has especially affected WEPs that have forage value. For instance, *Z. spina-christi, G. flavecens, B. aegyptiaca, G. tenax, X. americana, G. villosa, B. discolor* and *T. indica* are among the forage species. Mechanized agricultural expansion for the sugar plantation and small scale household farming in the area increased and cleared the natural vegetation on the plains and across the Awash River bank. More than 80% of the deforestation or forest degradation is attributable to agricultural expansion (FAO, 1999). These factors, combined with the natural vulnerability of the area by climate variability and change, may lead to further reduction of the WEPs available.

The differences were ascribed to: 1) intensive agriculture in the settled farmers' area which has eliminated large natural vegetation areas and the population pressure was more intensive than on transhumant land. Transhumances are more knowledgeable and practice environmental friendly living given their indigenous customary rules and regulations of natural resources conservation. Commercial charcoal production was not a common practice of the transhumance; it was a new activity and an alternative livelihood. However, it has been practiced for decades in settled farmers'areas. This was because of the transhumant nature of Fantalle livestock producers that give chance for vegetation to rest and regenerate and relatively continue reproducing and evolving in nature while providing them with livelihood. This happens when they move their livestock to other places for certain periods of the year.

All these indicated the management practices that need

integration in settled farmers land use system at least in the form of rotational grazing and dryland agroforestry and enclosure to conserve vegetation. This is an old sustainable use practice which also sustained the use and management of nutritious wild edible plants in the area.

CONCLUSIONS

The socioeconomic and environmental problems could be partly reduced by integrating rehabilitation, conservation and enhancing climate adaptation strategies with plant biodiversity in general and WEPs in particular. Maintaining the diversity of the entire vegetation will allow the survival of WEPs in general. Domestication is urgent for rare plants before they become locally endangered and extinct. Implementing *in situ* and *ex situ* conservation measures such as enhanced dryland agroforestry, extensive enhancement of traditional closure (KALO), live fencing, community forestry and environmental education are recommended.

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ON FARM PERFORMANCE OF SELECTED ORANGE- FLESHED SWEET POTATO VARIETIES IN HOMA BAY COUNTY OF KENYA

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ABSTRACT

Sweetpotato [*Ipomoea batatas* (L.) Lam], is a major root crop for high dietary energy. Orange fleshed sweet potato varieties are higher in provitamin A and can contribute significantly to vitamin A nutrition. In Kenya the crop is grown by small-scale farmers who encounter several constraints namely low yields, susceptibility to viral diseases and marketing. The objective was to introduce, evaluate and select improved varieties in major production zones for increased production. Five selected orange fleshed sweet potato varieties; Kakamega 004 (Spk004), Odinga, 292-H-12, Zapallo and Nyawo were compared with two local checks for yields, Nyathi Odiewo and Kuny kibuonjo, one for each site, respectively. The experiment was conducted for two seasons, long and short rainy seasons of 2005 at two sites of Kabondo and Ndhiwa in a randomized complete block design with four replications. The six genotypes were evaluated at each site for fresh root yield, above ground biomass, dry matter content, virus disease score and tuber weevil damage. Statistically, significant differences were observed between the sites and among the varieties. All the varieties had dry matter above 30% except Zapallo which recorded 21.6 %. The improved varieties were all tolerant ($P<0.05$) to viruses as compared to Kuny kibuonjo one of the controls. Results also indicated that 292-H-12, Nyawo, Odinga, Spk 004 gave better yield than the controls on fresh root yields. These varieties are recommended for scaling up for adoption by farmers.

Key words: orange fleshed sweet potato, varieties, performance, Kenya.

INTRODUCTION

Sweet potato (*Ipomoea batatas*) is a food security and drought tolerant crop of high nutritive and energy value and wide adaptability. Sweet potato generates large amounts of food per unit area per unit time (Wolfe, 1992). It tolerates occasional dry spells and yields even on less fertile soils in contrast to other crops such as maize (Ewell, 1990). Compared to other crops, sweet potato requires few inputs and relatively less labour (Norman *et al*., 1984). Once the plants cover the ground, the crop tends to smother further weed growth. In Kenya, over 75% of production is concentrated in western, central and coastal areas of the country. Out of this, over 80% is grown in western Kenya (Woolfe, 1992; Andima *et al.*, 2003; Grũneberg *et al.,* 2004; Jaetzold *et al*., 2006).

In Rachuonyo South and Ndhiwa counties of Kenya, most of the farmers produce white fleshed varieties that are low in provitamin A (Low, 1995; Omosa, 1997). The orange fleshed varieties contain large quantities of provitamin A (β-carotene) and their consumption is considered important for improved vitamin A intake (Low, 1995). The edible roots are high in dietary energy and contain minerals such as calcium, phosphorous and iron (Woolfe, 1992). It also has ascorbic acid (vitamin C), thiamin (B1) riboflavin (B2), niacin (B6) folic acid and vitamin E. The leaves are rich in iron, protein and vitamins A, B2 and C (Amenyenu *et al*., 1998). Nutritionally it constitutes 60 – 70 % water, 15 – 25 % starch, 1 – 2 % protein and 1 – 2 % sugar fat 0.3%, carbohydrates 26%, fibre 1.0 calcium 0.25, iron 1.0 Provitamin A 0-4000, Thiamine 0.1, Riboflabin 0.04 Nicitinemide 0.7 (Onwueme and Shinha, 1991; Woolfe, 1992; Hagenimana *et al.*, 2001; Degres, 2003). In Kenya, increased sweet potato production has been realized through introduction and selection of breeding lines and land races that have been virus cleaned and their traits improved through selection (Gichuki *et al*., 2003). The national farmer average root yield is 5.6 tonnes per hectare while the potential can be up to 50 t/ha (Kihurani, 2004). The aim of the study was to introduce and evaluate introduced orange fleshed sweet potato varieties in the two zones for improved root yields, dry matter content and reaction to viruses and weevils.

MATERIALS AND METHODS

Site descripion

The experiment was conducted in two locations (Table I): Kabondo in Rachuonyo South county situated in Agro Ecological Zones (AEZ) of Upper Midland 2 (UM2) at an altitude of 1450 metres above sea level (masl), to the South 00 26 026^{O} and to the East 34 57 282^{O}.

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The soil type is Chromoluvic phaezems. The site enjoys a mean annual temperature of 20.1^{O}C, with a bimodal rainfall pattern.The mean annual rainfall is 1509 millimetres (mm) (FAO/ UNESCO 1990; Jaetzold *et al*., 2006).

Ndhiwa site is in AEZ of Lower midland 2 (LM2) at an altitude of 1337 masl, to the South 00 43 918^{O} and to the East 34 22 854^{O} (FAO/ UNESCO 1990; Jaetzold *et al.,* 2006) with soil type of Vetro-phaezems.The site has a bimodal rainfall with an annual mean of 1372 mm and a mean temperature of 21.1^{O}C (Andima *et al.,* 2003; Jaetzold *et al*., 2006). The trials were planted in May 2005 during the long rainy (LR) season and also in October, 2005 during the short rainy (SR) season. Ndhiwa and Kabondo sites received a total of 896.5 and 634.1 mm of rainfall, respectively during the long rains. While in the short rains Ndhiwa received 782.0 mm and Kabondo received 316.5 mm for the period the crops were in the field respectively.

Experiment design

The experiments were laid in a randomised complete block design (RCBD) replicated four times at the two sites with each plot measuring 3.3 × 4 m, that made four ridges spaced at 1.0 m, an inter-row spacing of 0.3 m. The gross plot had four rows each having twelve sweet potato vines planted giving a total of 48 plants per plot. The net plot harvested for analysis comprised of the two middle rows with twenty plants leaving one plant at the start and one at the end of every row as guard row plants thus resulting in an area of (2 ×2.27 m (4.54 m^2) harvested at 150 days from planting.

The treatments comprised of five orange fleshed sweet potato varieties namely Kakamega 004 (Spk004), 292 - H-12, Zapallo, Nyawo, Odinga and one local check in each site namely Nyathi Odiewo for Kabondo and Kuny kibuonjo for Ndhiwa with a total of six treatments per site.

Data collected during the experimentation included: number of plants at the start of the experiment as stand count to give the optimum number recommended for optimum production per unit area and uniform stand, at harvesting, above ground biomass yields, roots yields and dry matter percent. Weevil damage on roots and vine crown were scored on a five point scale (5 = over 75 % damage, 4 = 50 – 75 % damage, 3 = 26 – 50 % damage, 2 = less than 25% damage and 1 = no damage). The virus infection was scored on a five point scale (5 = severe damage and stunted growth in most plants, 4 = severe damage on a few plants, 3 = mild symptoms detected on several plants, 2 = mild symptoms detected on few plants and 1 = no virus symptoms). The data were taken on ten randomly chosen plants per net plot (CIP, 1999). To determine the above ground biomass, foliage was weighed in kg at harvesting per net plot. The total number of roots per plant were counted. The fresh weights were taken at harvest 150 days from the date of planting at the maturity of the crop. The weights were recorded in kg per plot. During harvesting a sample of roots weighing 200 g per plot (variety) were picked at random, cut into small cubes of 1 cm^3, oven dried at 80^{O}C for 24 hours and dry matter content determined. This was expressed as percentage of root dry weight to fresh weight as dry matter percent according to the International Potato Centre (CIP, 1999).

Data analysis

Data were analysed by a general linear model (GLM) using the Statistical Analysis for Scientists (SAS) statistical package for the analysis of variance (ANOVA). Mean separation using least significant difference (LSD) at 5% probability was applied to determine whether there were significant differences among the varieties (Gomez and Gomez, 1984).

TABLE I - ORANGE FLESHED SWEETPOTATO VARIETIES EVALUATED

Variety	Site*	Origin	Flesh Colour	Skin Colour	Remarks
292-H-12	1, 2	(CIP) (Peru)	Orange	Red	Breeding line
Kuny kibuonjo (LC)	2	Ndhiwa (Kenya)	White	Red	Local variety
Nyathi Odiewo (LC)	1	Kabondo (Kenya)	Orange	Red	Local variety
Odinga	1, 2	Siaya (Kenya)	Orange	Red	Improved
Spk004	1, 2	Kakamega (Kenya)	Orange	Orange	Improved
Zapallo	1, 2	(CIP) (Peru)	Orange	Red	Breeding line
Nyawo	1, 2	Kabondo (Kenya)	Orange	Red	Improved

*Sites (1 = Kabondo, 2 = Ndhiwa), LC = Local check variety

Source: (Hagenimana *et al.*, 2001; Ndolo *et al.*, 2001; and; Grũneberg *et al*., 2004

RESULTS

Stand count at harvesting

There were differences ($P<0.05$) in stand count at harvest among the varieties and between the sites. The crop stand count for all the varieties ranged from 55 - 95 % (Table II). This might have been attributed to the adequate rainfall received when the crops were in the

field. Dhiwa had higher stand count in long rains than Kabondo with the variety Spk004 leading while Nyawo had the lowest stand. During short rains the variety Zapallo and 292-H-12 had the highest stand count while the variety Zapallo had the lowest stand count.

Fresh root yields

There were differences (P<0.05) in fresh root yield among the varieties and between sites. The highest fresh root yields was realised for the local check variety, Kuny Kibuoni, at Ndhiwa LR season of 25.3 t /ha whilst the lowest mean yield of 5.5 t /ha was for variety Zapallo at Ndhiwa during the SR season (Table III).

Above ground biomass yields

There were differences (P<0.05) on above ground biomass yields among the varieties and between the sites. In the LR season at Ndhiwa, the variety Kuny kibuonjo had highest above the ground biomass accumulation yields of 19.2 t /ha whilst variety, Zapallo had the least biomass with 4.9 t /ha during SR season at Ndhiwa (Table IV). The long rains had higher biomass than short rains

Root dry matter contents

There were differences (P<0.05) on root dry matter content among the varieties and between the sites. The variety Odinga had the highest dry matter content of 37.3% at Kabondo site SR season as compared to the variety Zapallo with the lowest of 16.5% at Ndhiwa site LR season (Table V).

Virus and weevils evaluations for orange fleshed sweet potato varieties

Virus infectiion was observed to be higher especially on controls as these checks were taken from farmers field without selection. There were differences (P<0.05) on weevil damage on roots among the sweet potato varieties and between the sites. At harvesting the SR season, the local check variety Nyathi odiewo had the highest weevil (*Cylas* sp) damage with a mean score of 5.0 whilst variety 292-H-12, had the lowest damage with

TABLE II- ORANGE FLESHED SWEET POTATO VARIETIES PERCENT STAND COUNT PER PLOT AT HARVESTING

Season 2005	LR % stand count		SR % stand count	
Site / Variety	Kabondo	Ndhiwa	Kabondo	Ndhiwa
292-H-12	62abcd	72bcd	72ab	78a
Kuny kibuonjo (Local Check)	-	77abcd	-	75a
Nyathi Odiewo (Local Check)	68abc	-	60b	-
Nyawo	46d	72bcd	71ab	73ab
Odinga	47d	67cde	70ab	62ab
Spk004	58bcd	81abcd	73ab	61ab
Zapallo	68abc	52e	78a	56b
Mean	58	70	70	68
CV%	18	21	17	14
LSD (5%)	19	18	15	17

Means followed by the same letter in the same column are not different as separated by LSD at P≤0.05

TABLE III- ORANGE FLESHED SWEET POTATO VARIETIES FRESH ROOT YIELDS T /HA

Season 2005	LR		SR	
Site / Variety	Kabondo	Ndhiwa	Kabondo	Ndhiwa
292-H-12	21.4 a	16.7 c	8.5 c	6.8 cd
Kuny kibuonjo (Local Check)	-	25.3 ab	-	13.2 ab
Nyathi Odiewo (Local Check)	18.7 ab	-	12.1 abc	-
Nyawo	13.4 b	24.2 abc	14.2 abc	14.2 abc
Odinga	17.6 ab	20.3 abc	9.6 bc	10.2 bc
Spk004	15.6 ab	20.6 abc	13.9 abc	8.4 cd
Zapallo	16.2 ab	6.6 d	17.8 a	5.5 d
Mean	17	19	13	10
CV%	24	27	14	29
LSD (5%)	8	8	6	4

Means followed by the same letter(s) in the same column are not different as separated by LSD P≤0.05

TABLE IV- ORANGE FLESHED SWEET POTATO VARIETIES ABOVE GROUND BIOMASS YIELDS /HA

Season 2005	Long rain		Short Rain	
Site / Variety	Kabondo	Ndhiwa	Kabondo	Ndhiwa
292-H-12	14.8abcde	18.1ab	5.5c	6.6a
Kuny kibuonjo (Local Check)	-	19.2ab	-	8.6a
Nyathi Odiewo (Local Check)	11.0def	-	6.6bc	-
Nyawo	8.8f	14.3bc	6.6bc	6.8a
Odinga	12.1cdef	15.4bc	8.8abc	7.1a
Spk004	12.1cdef	16.5bc	9.3abc	8.2a
Zapallo	9.8ef	5.2d	7.9bc	4.9a
Mean	11	15	7.4	7.0
CV%	29	22	18	19
LSD (5%)	6	5	5	NS

Means followed by the same letter(s) in the same column are not different as separated by LSD at (P<0.05)

TABLE V- ORANGE FLESHED SWEET POTATO VARIETIES PERCENT DRY MATTER CONTENT FOR THE YEAR

Season 2005	LR%dry matter		SR%dry matter	
Site / Variety	Kabondo	Ndhiwa	Kabondo	Ndhiwa
292-H-12	32.7ab	31.6ab	34.8abc	33.3abc
Kuny kibuonjo (Local Check)	-	32.3ab	-	33.5bc
Nyathi Odiewo (Local Check)	29.3abc	-	34.8abc	-
Nyawo	26.7cd	31.3a	32.8bc	34.2ab
Odinga	31.5abc	28.7b	37.3 a	36.0 a
Spk004	30.3abc	29.7ab	33.3bc	31.3bcd
Zapallo	22.6d	16.5c	27.2 d	23.0 e
Mean	28	28	33.3	32
CV%	11	14	9	5
LSD (5%)	5	6	5	2.4

Means followed by the same letter(s) in the same column are not different as separated by LSD at P<0.05

TABLE VI - VIRUS SCORE ON VINES AND WEEVILS SCORE ON ROOTS FOR ORANGE FLESHED SWEET POTATO VARIETIES

Season 2005	Long Rains				Short Rains			
	Virus score		Weevils score		Virus score		Weevils score	
Site / Variety	Kabondo	Ndhiwa	Kabondo	Ndhiwa	Kabondo	Ndhiwa	Kabondo	Ndhiwa
292-H-122.2	2.0 b	1.0b	1.0	1.2	1.0d	4.2ab	3.2ab	
Kuny kibuonjo (LC)	-	4.0a	-	1.5	-	3.7a	-	3.5ab
Nyathi Odiewo (LC)	1.7	-	1.5ab	-	1.0	-	5.0a	-
Nyawo	2.0	2.0 b	2.0ab	2.0	1.0	2.0bc	2.0bc	4.7a
Odinga	1.5	2.0 b	1.5ab	1.7	1.2	1.7cd	2.0bc	4.0ab
Spk004	2.0	2.0 b	1.5ab	1.5	1.0	1.2cd	3.0abc	3.7ab
Zapallo	1.7	2.0 b	1.5ab	2.0	1.0	1.0d	2.2bc	4.0ab
Mean	1.8	2.3	1.5	1.6	1.0	1.7	3.0	3.8
CV %	24	24	16	28	15	15	16	23
LSD (5%)	NS	1.2	1.6	NS	0.6	0.9	2.3	2.6

Means followed by the same letter(s) in the same column are not different as separated by LSD at P<0.05, LC = Local check variety

a mean score of 1.0 (Table VI).

DISCUSSION

There were differences ($P<0.05$) in above biomass yield among the varieties and between the sites. Each site had varieties with above ground biomass yields that were preferably advantageous for livestock farmers. However, the local varieties Nyathi Odiewo and Kuny kibuonjo were as good as the introduced orange fleshed varieties. The differences among the varieties might have been due to genetic makeup and environmental factors (Onyango, 1993; Gichuki, *et al*., 2003). The LR season had higher biomass yields than SR season most likely due to the higher amount of rainfall received in the LR season. Higher above ground biomass yields in Ndhiwa site may also be due to higher rainfall received than in Kabondo site.

The selected orange sweet potato varieties had higher fresh root yields than the local checks. This means that they have the potential of improving production and nutritional status of the farmers. In Phillipinnes, new sweet potato varieties doubled the annual sweet potato fresh root yields. In Kenya earlier studies reported newly introduced sweet potato out yielded the local check varieties (Ndolo *et al*., 2001; Mcharo *et al*., 2001) Farmers may realize better returns from the newly improved orange fleshed varieties since some varieties had fresh root yields above 20 t/ha compared to the Kenya national farmer root biomass yield average of 5.6 t/ha (Abalo *et al*., 2001; Kihurani, 2004).

The orange sweet potato varieties evaluated had higher dry matter content during SR season than in LR season. As moisture level increased there were significant decreases in dry matter content of sweet potato varieties (Kosambo *et al*., 1998). Similarly, water stress can increase dry matter content (Onyango, 1993)

Variety Zapallo was introduced because of its high β-carotene which is a precursor of vitamin A and therefore has a high nutrition value. The variety may be good for Kabondo site as relates to yields of fresh roots yield, however, it gave very low fresh root yields in Ndhiwa site (Ekanayake *at al*., 1988; Gester, 1993; Hagenimana *et al.*, 1999). There were significant differences on above ground biomass produced among the orange fleshed sweet potato varieties. Two varieties Odinga, Zapallo had lower biomass yields. The ability of a variety to intercept and transform solar energy controls the biomass yields and varies with the plant variety (Onyango, 1993). The significant differences in fresh root yields accumulated amongst the varieties of sweet potato may be attributed to varietal differences in their ability to utilise environmental resources (Constantin *et al*., 1977; Onyango, 1993). Several factors influence this ability including differences in leaf angle, leaf size, branch number, leaf number, length of vines and genetic constitution between varieties. There is variation in various plants species in their ability to extract nitrogen from soil and that genetic differences exist in the use of this nitrogen as various plant species also intercept light differently (Constantin *et al.*, 1977). The differences in inherent biomass yield ability could arise from genetic constitution (Onyango, 1993). This may account for the variation observed among the orange fleshed sweet potato varieties.

Two orange fleshed sweet potato varieties, 292-H-12, Odinga were higher in their dry matter content this has attributes to the mealyness of the variety a factor preferred by consumers (Low, 1995; Omosa, 1997). The orange fleshed variety Zapallo had the lowest dry matter content. The orange fleshed sweet potato varieties evaluated had higher dry matter content during SR season than in LR season, this concurs with other researchers elsewhere. Kosambo *et al.* (1998) reported that with increased moisture level there were significant decreases in dry matter content of sweet potato varieties. Similarly water stress can increase dry matter content (Onyango, 1993).

The orange fleshed sweet potato varieties were tolerant to viruses than the local checks. The variety Zapallo was the most tolerant among them. This was probably due to selection for tolerance as has been confirmed earlier (Ndolo *et al*., 2001). Results indicate that a local check variety Kuny Kibuonjo was susceptible to viruses. This was probably due to virus build up within the site since the planting materials were taken from farmers fields. Sweet potato farmers usually do not rogue or select planting material which has virus infection due to lack of awareness for virus. Sweet potato being a vegetatively propagated crop, has no certified seed producer thus making the farmers to rely on their previous own crop as seed vine for planting material or from their neighbours (Kwach, 2008). The recycling of the previous planting materials within a locality contributes to sweet potato virus innoculum build up.

There was higher weevil damage in drier, SR season for both sites than in wetter LR season. The amount of rainfall received which is evenly distributed during crop growth creates an environment unfavourable for weevil damage to the roots. Sweet potato planted on ridges, creates an environment less favourable for weevils damage. In deep soils, roots can be covered completely using soils to create unfavourable conditions for weevils attack The varieties which tend to root deeply may escape the weevils damaged (Ndolo *et al*., 2001; Ssebuliba *et al.,* 2001). Compact soils with enough moisture will favour less soil cracks thus creating unfavourable condition for

weevil entry into the soil to reach sweet potato roots. The less damaged varieties might have been due to their genetic make up and rooting system as those with deeper storage roots in the soil leading to reduced chances of weevil entry. (Ssebuliba *et al*., 2001).

CONCLUSION

The improved varieties were better than the local check varieties in terms of above ground biomass yields, fresh root yields and tolerance to weevil damage and viruses. These varieties have high potential of increasing farmers' production per hectare and can meet nutritional needs of consumers, especially children. Variety Zapallo had low above ground biomass yields but is of higher nutrition value being orange fleshed and high in provitamin A (β-carotene). Farmers may realise better returns in terms of yields and cash from these varieties since varieties 292-H-12, Nyawo, Odinga and Spk 004 out yielded local check varieties above the Kenyan national farmer average root yield of 5.6 tons per hectare. Adoption of these varieties might improve sweet potato productivity in Kenya.

ACKNOWLEDGEMENTS

The authors wish to sincerely thank Director, Kenya Agricultural Research Institute for the financial support. The Centre Director KARI-Kisii for the enabling environment and the Kabondo and Ndhiwa farmers that we worked with.

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RESPONSE OF MAIZE TOP CROSS HYBRIDS TO LOW PHOSPHORUS ACID SOILS IN WESTERN KENYA

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ABSTRACT

Phosphorus (P) deficiency is considered one of the major factors limiting plant growth in many natural ecosystems. The objective of this study was to evaluate the effect of low P on newly developed maize genotypes across two sites. Fourteen test hybrids and two commercial checks were evaluated in acid soils at Bumala and Sega in western Kenya. The experiment was set up in a randomized complete block design with three replicates. Planting was done in two row plots of 10 plants spaced 0.3 m apart with an inter-row spacing of 0.75 m. Phosphorus was applied inform of Triple Super Phosphate (TSP) at the rate of 36 kg P/ha and a check with 0 kgP/ha for each genotype. Analysis of variance revealed an interaction ($P<0.05$) between variety, treatments and site for yield, plant height and days to 50% tasseling and a non-significant interaction between site ×variety and treatment × variety. The mean grain yield increased by 75.9 and 60 % when P was applied at Sega and Bumala respectively while the mean ear height increased by 10.2 and 17.9 % with P application at Sega and Bumala respectively. The mean plant height increased by 5.7 and 12.3 % when P was applied at Sega and Bumala respectively. The mean number of days to 50 % pollen reduced by 4.1 and 4.3 % with P application at Sega and Bumala respectively. The results indicate that there is potential to improve maize yields in acid soils by developing varieties which can extract soil available P and also utilize the P applied to the soil more efficiently.

INTRODUCTION

Acid soils in the tropics are often characterized by high concentration of aluminium (Al), low total and available P contents and high P retention capacity. Phosphorus deficiency in plants suffering from Al- toxicity is due to a combination of reduced root elongation and interruption of cell metabolism, as well as immobilization of P by Al on or within the root surface. Surface applied P has been found to improve root penetration into acidic sub-soils (De Miranda and Rowell, 1987). Phosphorus is therefore of central importance to agricultural productivity and sustainability in both developing and high-income economies (Lynch, 1998; Obura et al., 2001). The importance of low P availability in the plant biology is reflected in a variety of adaptations to low P manifested by nearly all higher plants. These include mycorrhizal symbioses, morphological features such as root hairs, induction of phosphates, RNAses and other P-scavenging enzymes and P-mobilizing organic acid root exudates (Kochian, 1995). Up to 75% of applied fertilizer may revert to poorly soluble mineral phosphates; consequently, P fertilizers are applied in great excess (Goldstein, 1992; Raposeiras et al., 2006).

The concentration of available phosphorus in the soil solution of P deficient soils is extremely low because most soil-P is bound in forms of low solubility (Sanchez and Uehera, 1980). Due to this low mobility, P uptake is generally considered to be proportional to the surface area of the plant organs involved in P uptake (Sattelmacher et al., 1994). Price increases of phosphatic fertilizer are considered to be imminent due to the rapid depletion of high grade, low cost, non-renewable phosphate rock reserves throughout the world (Cathcart 1980; Steingrobe et al., 2006). It has been estimated that at least 5.8 billion hectares of land accounting for about 45% of the total cultivated land in the world suffer from the low P problem (Lu, 1987). Plants absorb P from the soil as inorganic orthophosphate (Pi) ion. In most soils, the concentration of available Pi is in soil solution (2µM) is several orders of magnitude lower than in plant tissues (5-20µM) (Raghothama, 1999). To develop P-use efficient maize cultivars that are characterized by an improved ability to access soil bound P or by a more efficient internal use of P in biomas production may, therefore, be a cost-effective solution to this problem. Low phosphorus availability may also restrict nitrogen cycling by limiting symbiotic nitrogen fixation which is a phosphorus intensive process (Norman et al., 1984).

Phosphorus deficiency is considered one of the major factors limiting plant growth in many natural ecosystems (Liu et al., 2004). Phosphorus deficiency is perhaps the most important factor that limits maize yields on many soils (Chaubey et al., 1994). In addition to low soil P content in absolute terms, P deficiency can arise if P is strongly adsorbed by soil colloids (Sanchez and Salinas, 1981; Dobermann *et al*., 1998).

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These authors estimated that more than 90% of the added P fertilizer may rapidly be transformed into P forms that are not easily available to plants. The plant's ability to absorb P from the soil and its ability to utilize absorbed P may both influence P-deficiency tolerance of the crop or plant. The development of maize cultivars capable of using a higher proportion of the fixed P already present in the soil may be an attractive and cost-effective alternative, with positive effects on the long term sustainability of agricultural systems. Deficiencies can be alleviated by fertilizer application, but farmers are constantly facing financial difficulties with increasing fertilizer costs, especially in developing countries. The problem is aggravated by the high P fixing capacity of many soils found in maize growing regions. On these soils only 10 to 20% of the P supplied in fertilizers is available to plants (Wada *et al.*, 1990), the rest is bound in the soil mainly to Al and Fe sesquioxides. Phosphorus deficiency tolerance has either been measured directly as dry weight or grain yield produced on low P soils (Fageria, 2006). The critical limit for P deficiency is about 5 mg P/kg of soil (Chaubey *et al*., 1994). Although P-deficient tropical soils could have a total P concentration of 700 mg /kg of soil, available P may only constitute 2 mg/kg of such soils (Yoshida *et al*., 1976).

Available data indicate that maize growing areas in Kenya require application of P for good yields and this requirement is even higher in regions with acid soils (Okalebo, 1987; Okalebo *et al.*, 1993). It has been observed that P-deficiency is one of the major yield limiting factors in all the maize growing areas of Kenya (Kisinyo *et al*., 2009). The specific objective of this study was to evaluate the effect of phosphorus on newly developed maize genotypes under low P soil conditions in Kenya.

MATERIALS AND METHODS

Genetic material

Maize germplasm used in this study were developed in 2011 at KARI-Kitale in collaboration with Rongo University College (Table I). Fourteen newly developed top cross hybrids with gray leaf spot resistance and two commercial checks (WH505 and WH502) were tested at two sites (Sega and Bumala). Sega is located at 0°15'N and 34°20'E. It is at an elevation of 1140-1400 m above the sea level and has bimodal rainfall pattern with minimum average annual rainfall of 800-1200 mm. The mean temperature is 15-17°C while the maximum temperature is 27-30°C. The soils are orthic Acrisols characterized by low pH (4.5) and aluminium saturation of 43.1% with P levels of 2.2 mg/kg (Kisinyo 2011). Bumala is located at 00°19'N and 34°12'E. It has an elevation of 1135-1500 m above the sea level and has bimodal rainfall pattern with average annual rainfall of 900-2000 mm. Mean annual temperature range is 20.5-22.7°C. The soils are orthic Ferralsols characterized by low pH (4.6) and aluminium saturation of 26.5 % with P levels of 2.74 mg/kg.

The experiment was set up in a Randomized Complete Block Design (RCBD) with three replicates. Each variety received 2 levels of P fertilizer (36kgP/ha and 0kgP/ha). Each genotype was represented in 2 row plots of 10 plants spaced 0.3m apart. The inter-row spacing was 0.75m, thus giving a plant population of 44,444 plants per hectare. Triple Super Phosphate (TSP 0-46-0) was applied at the rate of 36 kg P per hectare. Top dressing was done 6 weeks from planting using calcium ammonium nitrate (CAN 26-0-0) at the rate of 80 kg N per hectare. The crop was protected from stalk borer (*Busseola fusca*) damage using Permethrin (5% dust) applied into the whorl of each plant after thinning. Data was taken on grain yield, ear height, plant height and days to 50% pollen shed. Data analysis was done using GenStat version14.1 and Mean separation done using Turkey's Range Test.

RESULTS

Differences $P<0.001$ were observed between the varieties and P levels for yield, plant height, ear height and days to 50% pollen shed (Table II). The two sites were siginificantly different for all the parameters measured. However there was no ($P>0.05$) interaction between site and P level, site and variety and site by variety by P levels (Table II).

When P was added at Sega the yield increase ranged from 22.2% in WH502 to 206% in Mul 817 × Mul 991 × R12C9 (Table III). The plant height increased by 1.1% in Mul 1007 × S558-2—2-3-7 × R12C10 to 21.4% in Mul 817 × Mul 991 ×R12C7 while the ear height

TABLE I- LIST OF THE TOP CROSS HYBRIDS USED IN THE STUDY

No	Top cross hybrid	No	Top cross hybrid
1	MUL 211×S558-27-2-1×R12C9	8	MUL1007×S558-2-2-3-7×R11C10
2	MUL125×MUL863×S558-2-2-3-7	9	MUL852×G×R12C12
3	MUL 211×MUL822×R12C12	10	MUL863×MUL996×R11C10
4	MUL211×MUL822×R12C11	11	MUL211×S558-27-2-1×R12C7
5	MUL211×MUL216×R12C7	12	MUL211×S558-27-2-1×R12C12
6	MUL817×MUL991×R12C9	13	MUL817×MUL991×R12C7
7	MUL817×MUL991×R12C9	14	MUL125×MUL863×R11C10

increased by 3.4% in Mul 125 × Mul 863 × R11C10 to 28.8% in Mul 817 × Mul 991 × R12C7. The number of days to 50% pollen shed was reduced by 10% in Mul 817 ×Mul 991 × R12C7 when P was applied while there was no reduction in Mul 211 × S558-27-2-1 × R12C7 and in Mul 852 × G × R12C12. Yield increase due to P application at Bumala ranged from 10% in Mul 125× Mul 863×R11C10 to 79.5% in Mul 211×S558-27-2-1×R12C9 (Table IV). The plant height increased by 0.4% in WH502 to 17.4% in Mul 211× Mul 216×R12C7 while the ear height increased by 3.4% in WH 502 to 29.7% in Mul 211 × Mul 216 × R12C7. The number of days to 50% pollen shed was reduced by 1.4% in Mul 211×S558-27-2-1 ×R12C12 to 10.8% in Mul 863 × Mul 204 × R12C7.

Vasconcelos and Raghothama, 2006). Wissuwa and Ae (2001) working with rice, observed that P- deficiency delayed heading by 2 weeks. Application of P resulted in significant increase in grain yields and it is evident that using P alone in these low P acidic soils is currently not viable unless farmers in these regions access improved P-efficient maize varieties. Phosphorus application increases grain yield due to increase in dry matter accumulation (Evans and Kamprath, 1970) and Pearce and Sumner, (1997). The significant increase in yields when P was applied was also due to the complexing of part of Al+ with P, from the fertilizer leading to the formation of $Al(OH)_2H_2P0_4$, which precipitates, reducing the Al+3 available in the soil solution. The same was observed by Geopfert and Freire, (1972) and Ernani *et.*

TABLE II - MEANS SQUARES FOR GRAIN YIELD AND YIELD COMPONENTS OF MAIZE TOP CROSS HYBRIDS TESTED FOR P-EFFICIENCY ACROSS 2 ENVIRONMENTS.

Source of variation	d.f	Yield (t/ha)	Plant height (cm)	Ear height(cm)	Days to 50% tasseling	Days to 50% silking
Site	1	0.78	2913.38***	4700.52***	871.25***	1944.38***
Rep	2	9.67	163.5	133.38	84.98	143.53
Treatment	1	200.92***	5094.38***	2552.08**	287.63***	27.75
Site*Treatment	1	1.92	1944.38	507.00	0.13	24.79
Variety	15	10.81**	6307.87***	3444.25***	193.72	212.37***
Site*Variety	15	2.15	838.81	249.12s	19.63	61.55**
Site ×Treatment × Variety	1	1.96	19304.40	250.86	17.63	10.25
Error	126	2.41	662.63	267.72	25.93	24.7
CV		39	12.094	17.86	7.32	6.97
Grand mean		3.95	212.80	91.61	69.53	7
R2		0.61	0.66	0.67	0.60	
Root MSE		1.55	25.75	16.36	5.09	

NB * , **and *** indicates significance at $P \leq 0.05$, $P \leq 0.01$ and $P \leq 0.001$ levels, respectively

DISCUSSION

The observed difference ($P<0.05$) between the varieties tested is useful for maize improvement in acid soils. Such variations among maize genotypes have been reported by other authors (Parentoni *et al*., 2010) and have been associated with genetic variation among various maize hybrids. Significant difference between the treatments was expected and is an indication that P application increased maize performance under acid soil conditions. The two sites also differed siginificantly and this can be attributed to their differential P sorption and available P levels. Non-significant interaction between site × variety, treatment × variety indicated that selection for P-efficient topcross varieties could be effective at the two sites and under both P levels. Application of phosphorus reduced the number of days to anthesis. This observation supports the idea that phosphorus deficiency results in a delay in flower development (Marschner, 1995; *al*.(2000). Generally, P is known to improve plant root development, plant vigour and form and hence increased yields. There were significant increases in plant and ear height with P application at both sites. This shows that there was variation for ear height under P and no P conditions. Greater ear heights and fewer days to silk and to reach anthesis under P application compared to low- P acid-soil environments has been reported by Granados *et. al*. (1993), Duque Vargas *et. al.* (1994) and Pandey *et. al*.(1994).

CONCLUSIONS

There is genetic variability for phosphorus use efficiency among the tested genotypes and there is a potential to develop varieties which can extract soil available P and

TABLE III -PEFORMANCE OF TOP CROSS MAIZE VARIETIES UNDER ACID SOIL AT SEGA

	Grain yield (t/ha)		Plant height (cm)		Ear height (cm)		Days to 50% Tasselling		% yield Reduction	
Top Crosses	P	Cntrl	P	Cntrl	P	Cntrl	P	Cntrl		
MUL 211×S558-27-2-1×R12C9	9.7a	5.3ab	228.3a	212.3a-d	104.7ab	89.0b-e	74a-d-	75a-c	45.88	
MUL125×MUL863×S558-2-2-3-7	6.6a	3.5b	182.0c-e	166.3e	75.7c-g	65.7fg	64d	65cd	46.68	
H505	6.2ab	3.5b	185.3c-e	174.0e	70.7e-g	68.0fg	66cd	69a-d	44.53	
MUL 211×MUL822×R12C12	4.3ab	3.3b	214.0a-c	210.7a-d	95.7a-c	92.3b-e	74a-d-	76a-c	22.01	
MUL211×MUL822×R12C11	5.1ab	3.1b	209.0a-d	202.3b-e	100.0ab	93.0a-d	70a-d	75a-c	39.45	
MUL211×MUL216×R12C7	4.2ab	3.0b	219.3a-c	216.0a-c	100.0ab	93.3a-d	75a-c	79a	28.27	
MUL817×MUL991×R12C9	9.2a	3.0b	210.7a-d	208.3a-d	95.0a-d	91.0b-e	67b-d	68b-d	67.39	
MUL863×MUL204×R12C7	3.7ab	2.9b	214.3a-d	202.0b-e	98.3ab	86.3b-f	72a-d	75a-c	22.19	
H502	3.3b	2.7b	178.0de	168.7e	59.7g	56.7g	67b-d	69a-d	19.34	
MUL1007×S558-2-2-3-7×R11C10	5.5ab	2.6b	191.3b-e	189.3b-e	86.7b-f	80.0b-f	66cd	69a-d	53.21	
MUL852×G×R12C12	4.5ab	2.5b	246.3a	232.0a	119.3a	109.0a	78ab	78ab	43.30	
MUL863×MUL996×R11C10	4.2ab	2.4b	189.0b-e	183.3c-e	77.0c-g	74.3c-g	67b-d	71a-d	42.38	
MUL211×S558-27-2-1×R12C7	4.2ab	2.4b	229.0a	208.7a-d	108.7ab	91.7b-e	80a	80a	44.08	
MUL211×S558-27-2-1×R12C12	4.0ab	2.3b	209.3a-d	199.0b-e	102.3ab	91.0b-e	73a-d	78ab	42.78	
MUL817×MUL991×R12C7	3.4b	2.1b	207.3a-d	170.7e	86.3b-f	67.0fg	63d	70a-d	37.09	
MUL125×MUL863×R11C10	4.3ab	1.9b	183.7c-e	175.0e	73.7d-g	71.3d-g	67b-d	73a-d	55.58	
Treatment mean	5.1	2.9	206.1	194.9	90.9	82.5	70	73		
Grand mean	4.02	4.02	200.50	200.50	86.70	86.70	72	72		
CV	45.20	45.20	45.20	45.20	12.20	12.20	7	7		
SE	0.74	0.74	6.59	6.59	4.31	4.31	2	2		
SED	1.05	1.05	9.31	9.31	6.10	6.10	3	3		

TABLE IV - PEFORMANCE OF TOP CROSS MAIZE VARIETIES UNDER ACID SOIL AT BUMALA

	Grain yield (t/ha)		Plant height (cm)		Ear height (cm)		Days to 50% Tasselling		% yield Reduction	
Top Crosses	P	Cntrl	P	Cntrl	P	Cntrl	P	Cntrl		
MUL 211×S558-27-2-1×R12C9	7.0a	3.9ab	249.0a-c	237.3a-d	109.0a-c	101.3a-c	67a-c	71a-c	43.31	
MUL125×MUL863×S558-2-2-3-7	4.6ab	2.8b	201.0b-e	158.7ab	69.7c-e	54.7e	62c	65bc	38.61	
H505	5.0ab	2.7b	214.3a-e	174.7de	81.7b-e	64.0de	64c	66a-c	46.71	
MUL 211×MUL822×R12C12	5.2a	2.8b	239.7a-d	212.3a-e	107.7a-c	88.3a-e	72ab	77a	46.51	
MUL211×MUL822×R12C11	5.4a	4.0ab	253.0ab	251.3a-c	117.0ab	109.3a-c	67a-c	65bc	26.43	
MUL211×MUL216×R12C7	5.0ab	3.1ab	290.0a	247.0a-c	132.7a	102.3a-c	67a-c	69a-c	37.02	
MUL817×MUL991×R12C9	6.1a	3.9ab	246.3a-c	213.0a-e	115.0ab	85.3b-e	63c	64c	35.53	
MUL863×MUL204×R12C7	4.7ab	2.6b	277.3a	248.7a-c	133.0a	113.7ab	66a-c	74a	44.61	
H502	3.5ab	2.6b	167.0e	166.3e	61.0e	59.0e	63c	65a-c	24.50	
MUL1007×S558-2-2-3-7×R11C10	5.6a	3.2ab	241.3a-c	199.3b-e	106.0a-c	79.3b-e	64c	68a-c	42.53	
MUL852×G×R12C12	4.9ab	2.2b	274.0a	234.7a-d	132.3a	116.3ab	67a-c	72ab	55.06	
MUL863×MUL996×R11C10	4.3ab	2.8b	229.3a-d	203.0b-e	85.7b-e	82.0b-e	65bc	67a-c	35.60	
MUL211×S558-27-2-1×R12C7	3.4ab	1.8b	250.3a-c	216.3a-e	115.3ab	94.0a-e	68a-c	80a	46.51	
MUL211×S558-27-2-1×R12C12	4.4ab	2.1b	247.0a-c	213.0a-e	124.3a	96.3a-d	70a-c	71a-c	51.70	
MUL817×MUL991×R12C7	3.7ab	3.0ab	209.0b-e	202.0b-e	88.0a-e	81.3b-e	63c	65bc	18.63	
MUL125×MUL863×R11C10	4.4ab	4.0ab	222.0a-e	215.7a-e	93.3a-e	91.0a-e	63c	66a-c	9.61	
Treatment mean	4.8	3.0	238.2	212.1	104.5	88.6	66	69		
Grand mean	3.9	3.9	225.1	225.1	96.6	96.6	67	67		
CV	30.8	30.8	12.5	12.5	18.9	18.9	7	7		
SE	0.5	0.5	11.5	11.5	7.4	7.4	2	2		
SED	0.7	0.7	16.3	16.3	10.5	10.5	3	3		

also utilize the P applied to the soil more efficiently. Selection for tolerance to low P in the topcross hybrids can be done under low and high P conditions.

RECOMMENDATIONS

There is need for further studies on inheritance for P-uptake or internal P-use in segregating populations and also upscale and test the maize varieties in soils with less heterogeneity.

ACKNOWLEDGEMENT

GCP, AGRA and USAID SO7 for providing research funds and KARI Kitale and Rongo University College for providing logistical support.

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CASSAVA STARCH AS AN ALTERNATIVE LOW COST GELLING AGENT FOR SLOW GROWTH *IN VITRO* CONSERVATION OF POTATO

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ABSTRACT

Lack of cost-effective protocols has hampered wide-scale application of slow growth *in vitro* conservation of potato. Therefore, the potential of cassava starch as an alternative low cost gelling agent for potato *in vitro* conservation media at normal propagation temperature was investigated using a two factor experiment in a randomized complete block design for a period of 18 months. Three gelling agents: i) cassava starch (8%) + agar (0.25%), ii) cassava starch (8%) and iii) agar (0.8%) were evaluated using three varieties (Arka, Dutch Robijn and Tigoni). Plantlet survival and condition of plantlets after 18 months of conservation was similar when cassava (8%) + agar (0.25%) and agar (0.8%) were used as gelling agents for all the three varieties and were higher than survival and condition of plantlets grown on media gelled with cassava starch (8%) alone. Plantlets grown on media gelled with cassava starch (8%) + agar (0.25%) and agar (0.8%) alone, respectively, had higher numbers of usable single node cuttings per culture than plantlets grown on cassava starch (8%) regardless of the variety. Gelling costs were reduced by 16.6 and 24.4 % when cassava (8%) + agar (0.25%) and cassava (8%) alone were used, respectively, as the gelling agents compared to agar (8%) alone. However, media gelled with cassava starch (8%) alone had poor clarity and gel strength indicating its unsuitability for conservation. All plantlets that survived the 18-month conservation period had 100% viability irrespective of the type of conservation media used. Therefore, cassava starch (8%) + agar (0.25%) may be used as a cheaper alternative for agar in potato conservation media.

Key words: Cassava starch, gelling agent, *in vitro* conservation,

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INTRODUCTION

Potato (*Solanum tuberosum L.*) cultivars are generally maintained through vegetative propagation mainly to maintain their genetic integrity because the crop is highly heterozygous and segregates on sexual reproduction. Maintenance of potato germplasm through field genebanks is time-consuming, requires large amounts of space and is labour- intensive. Field genebanks also expose the plants to risks of losses due to diseases, pests, abiotic stresses and natural calamities (Withers *et al*., 1990). Hence, many potato genebanks conserve potato as *in vitro* propagated plantlets under disease-free conditions (Golmirzaie *et al*., 1999; Gopal *et al*., 2002). When grown under optimum propagation conditions (e.g. Murashige and Skoog (MS) medium with 30 g/lsucrose, 16 hr photoperiod, 22-25^{O}C), the plantlets require sub-culturing every 4-8 weeks. In order to reduce the frequency of sub-culturing, the growth of the plantlets is restricted by using growth retardants or osmotic stress in combination with reduced energy source, low temperatures, low light intensity and varied photoperiod (Ishige, 1995; Siddiqui *et al.*, 1996; Lopez-Delgado *et al*., 1998; Gopal and Chauhan, 2010). However, conservation based on a decrease in temperature is a costly option in the tropical and developing countries (Gopal *et al.*, 2002) and other additional stresses may cause undesirable effects on plants under *in vitro* conservation (Lizarraga *et al.,* 1989; Lopez-Delgado *et al*., 1998). Use of low temperatures (6-8^{O}C) and 16 hr photoperiod (15-30 µmol m-2 /s light intensity from cool white fluorescent lamps) is almost universal in potato genebanks for conservation. However, maintenance of plantlets at 6-8^{O}C can be energy demanding and costly due to the high electricity costs especially in developing countries like Kenya. Gopal *et al.* (2002) demonstrated that *in vitro* plantlets can be effectively conserved at 24±1^{O}C using MS medium supplemented with 20 g/l sucrose and 40 g/l sorbitol.

Recurring costs of micropropagation and *in vitro* conservation include costs of culture media ingredients such as gelling agents, carbon sources, inorganic and organic supplements, and growth regulators. Agar is usually used as the gelling agent and sucrose as the source of carbon, and together they constitute the most significant cost of the culture media. In a conventional propagation media, agar can represent up to 70% of

the total cost of media followed by minerals, water, sucrose and other minor media components (Prakash *et al.*, 2004). Cassava starch has shown potential for use in *in vitro* propagation media. Cassava starch powder at 10% (w/v), has been reported to give satisfactory setting typical of solid nutrient medium in pour plates and microbial growth (Dabai and Muhammad, 2005). Kuria *et al*. (2008) reported that 8 % cassava starch mixed with 0.25 % agar provided the same firmness as 0.8% agar and maintained gel integrity throughout the culturing period of 84 days during micropropagation of potato. There is, however, little information on the use of cassava starch in slow growth *in vitro* conservation media for potato. Therefore, the objective of this study was to evaluate the potential of cassava starch as an alternative low cost gelling agent and low cost carbon source, respectively, in *in vitro* conservation media for potato.

MATERIALS AND METHODS

Study site and plant materials

The study was carried at the Plant Tissue Culture Laboratory of the University of Nairobi, Faculty of Agriculture between January 2009 and September 2010. Three potato varieties differing in maturity periods (Arka-early maturing, Dutch Robijn-medium maturity and Tigoni-medium late maturity) were used in the study. The *in vitro* plantlets had previously been initiated through meristem and shoot tip culture from plants that had been certified as disease free. The plantlets were then routinely cultured every 3-4 weeks on normal propagation media (Espinoza *et al.*, 1992) in test-tubes (25 ×150 mm) using nodal cuttings in order to attain sufficient quantities for the experiments. Growth conditions during the *in vitro* plantlets multiplication phase were: 16-h photoperiod; 3,000 lux light intensity and 24±1^{O}C.

Treatments and experimental design

Treatments consisted of media with three gelling agents [(cassava starch (8%) + agar (0.25%), cassava starch (8%) and agar (0.8%)] and three cultivars (Arka, Tigoni and Dutch Robijn). All the conservation media had sucrose (3%) as the carbon source, and mannitol (4%) as the osmoticum. The treatments were laid out in a randomized complete block design with a factorial arrangement and replicated four times. Ten culture bottles were set up per experimental unit.

Preparation of conservation media

The conservation media consisted of MS (Murashige and Skoog, 1962) supplemented with 30 g/l sucrose, hormones, vitamins and 40 g/lmannitol with various gelling agents described above. The pH for all the media was adjusted to pH 5.8 with either 0.1N HCl or 0.1N NaOH before autoclaving. The media containing agar were then boiled until the agar had dissolved. However, in the case of media containing cassava starch, the cassava starch was incorporated as a thick slurry into the preheated media. About 10 ml aliquots were then dispensed into universal media bottles measuring 2.8 × 8.5 cm (and a capacity of 30 ml). The media were then autoclaved at 15 pounds per square inch (Psi) for 15 minutes and used for the experiments after cooling.

Inoculation, incubation and culture conservation

Inoculation was carried out in a sterile laminar flow hood. Nodal cuttings each measuring 2 cm were dissected from four-week old *in vitro* plantlets. Single nodal cuttings were then inoculated into a universal bottle containing the sterile experimental media. Surface sterilization was achieved with 70% ethanol. Growth conditions during the conservation period plantlets were: 24-h photoperiod; 1,000 lux light intensity provided by cool white fluorescent lamps and 24±1^{O}C.

Data collection

Plant height

Plant height (cm) was measured beginning at 1 month up to 6 months after the start of conservation. After 6 months of storage some of the treatments had overgrown the entire length of the universal bottle and it was not to accurately determine the plantlet height.

Number of shoots/culture and number of roots/culture

Number of shoots/culture and number of roots/culture were determined by counting after 18 months of conservation.

Condition of plantlets

The condition of plantlets was evaluated after 18 months of storage using a visual rating on a scale of 0-3 where, 0=dead, 1= very poor, 2= average and 3= good.

Survival and viability of plantlets

The number of surviving plantlets per treatment was recorded on a monthly basis and survival calculated as a percentage of the initial plantlets cultured. After 18 months of conservation, the cultures that had withstood slow growth were transferred to the propagation medium for assessment of their viability after storage. Eight shoot tip explants from each conservation treatment were sub-cultured. The viability was assessed as the ability to regenerate a minimum of one shoot from a culture.

Gel strength, media clarity and quantity of media remaining after conservation

The gel strength of various media was visually rated as good or poor where good was the ability of the media not to shake upon movement and poor was the inability of the media to shake upon movement. Media clarity was visually rated as good or poor, where good

was clear media that permitted easy observation of the roots and poor was opaque media that did not permit easy observations of the roots. The quantity of media remaining after storage for 18 months was observed on surviving cultures and was visually rated as exhausted, more than half of initial amount remaining and less than half of the initial amount remaining.

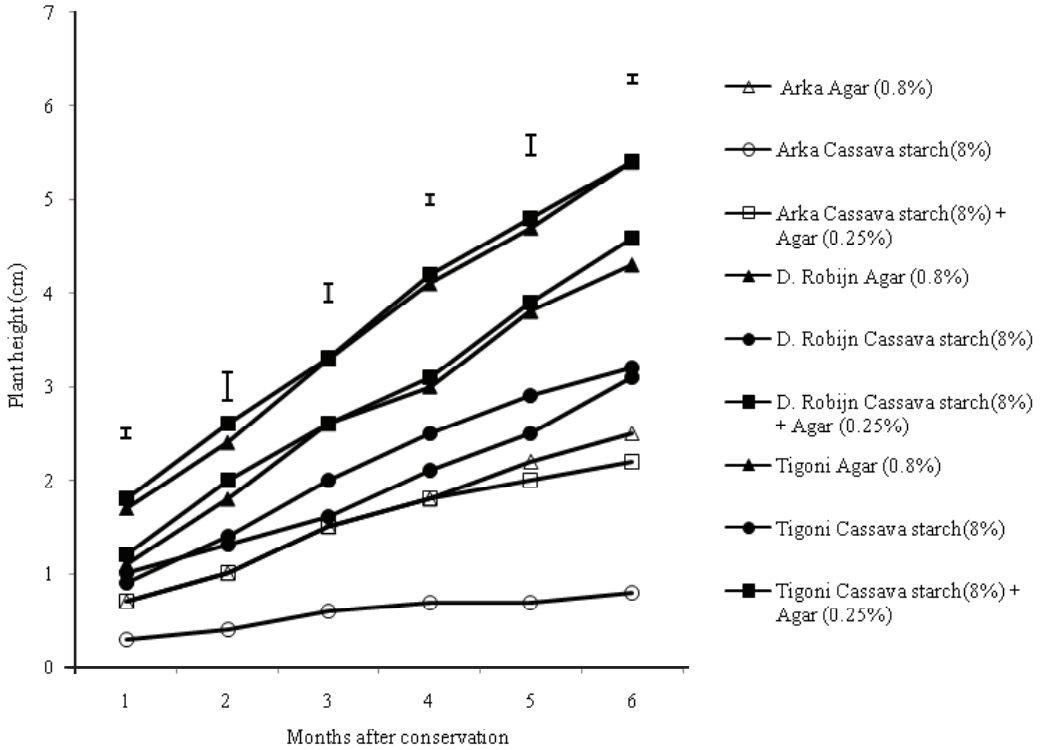
Data analysis and comparison of costs of media

Data were subjected to analysis of variance using Genstat 11.1 software (2008). Differences among the treatment means for all data were compared using Fisher's protected least significant differences (LSD) test at P<0.05 level (Steel *et al.*, 1997). Costs of gelling agents (agar and cassava) were calculated on the basis of prevailing costs in Kenya Shillings (KES) and the actual amount used to prepare 1 L of media. Because equal amounts of other chemicals were used in each treatment, the costs of these chemicals were considered constant for all media tested and were not included in the cost comparison.

RESULTS

The effect of conservation media and variety on plant height is shown in Figure 1. The effects of cultivar, media type and their interaction were significant (P<0.05) for plant heights measured at between 1 and 6 months of storage. The differences in plant height tended to be more pronounced as the conservation period increased. Beyond six months of storage, cultivars Tigoni and Dutch Robijn grown on media gelled with agar (8%) alone and cassava starch (8%) + agar (0.25%) had grown the entire length of the universal bottle and it was not possible to accurately determine the plant height. Cultivar Arka had the shortest plants during most of the six months regardless of the media on which it was conserved. After six months of storage, plant height varied from 0.8 to 5.4 cm. The shortest plants were those of cultivar Arka grown on media gelled with cassava starch (8%) (0.8 cm) alone followed by Arka grown on media gelled with cassava starch (8%) + agar (0.25%) (2.2 cm). The tallest plants were those of variety Tigoni grown on media gelled with agar (0.8%) alone and cassava starch (8%) + agar (0.25%). After six months of conservation, plants grown on media gelled with cassava starch (8%) + agar (0.25%) and agar (0.8%) alone were taller than those grown on media gelled with cassava starch (8%) alone regardless of the variety.

The effect of cultivar on the number of shoots/culture was significant (P<0.05) but that of media type and the interaction between cultivar and media type was not. Cultivar Arka had significantly the lowest number of shoots/culture while D. Robijn had the highest.



[1]Error bars refer to least significant difference (P<0.05) values

Figure. 1:Effect of media containing various gelling agents on plant height of three potato cultivars

TABLE I - EFFECT OF VARIOUS CONSERVATION MEDIA (AFTER 18 MONTHS OF STORAGE) ON NUMBER OF SHOOTS AND ROOTS PER CULTURE OF THREE POTATO CULTIVARS

Cultivar	Number of shoots/culture			
	Agar (0.8%)	Cassava starch (8%)	Cassava starch (8 %) + agar (0.25%)	Mean of cultivars
Arka	2.9	3.0	2.9	2.9
Dutch Robijn	9.2	8.3	8.4	8.6
Tigoni	5.6	5.2	5.2	5.3
Mean of gelling agent	5.9	5.5	5.5	5.3
$LSD_{cultivar}$ (P=0.05)				0.5
LSD_{media} (P=0.05)				0.5
$LSD_{cultivar\ x\ media}$ (P=0.05)				0.9
Cultivar	Number of roots/culture			
	Agar (0.8%)	Cassava s starch (8%)	Cassava starch (8 %) + agar (0.25%)	Mean of cultivars
Arka	3.3	1.7	3.1	2.7
Dutch Robijn	7.6	5.2	7.4	6.7
Tigoni	5.7	4.3	5.7	5.2
Means of gelling agent	5.5	3.7	5.4	4.9
$LSD^{1}_{cultivar}$ (P=0.05)				0.2
LSD_{media} (P=0.05)				0.2
$LSD_{cultivar\ x\ media}$ (P=0.05)				0.4

[1]Fisher's protected least significant difference

The number of shoots per culture after 18 months of storage ranged from 2.9 to 9.2 (Table I).

The effects of cultivar, media type and their interaction were significant for number of roots per culture. Cultivar Arka had significant the lowest number of roots/culture while Dutch Robijn had the highest. There was no difference between agar (0.8%) and cassava starch (0.8%) + agar (0.25%) in the number of roots per culture in all the varieties. Cassava starch (0.8%) alone had the lowest number of roots/culture in all the varieties. The number of roots per culture after 18 months of storage ranged from 1.7 to 7.6 (Table I).

All the three media evaluated supported growth of almost 100% shoot-forming cultures up to 6 months after inoculation. With the increase in age of the cultures beyond 6 months, survival rates began to decline depending on the cultivar and type of conservation media. The effects of cultivar and media type on % survival were significant at 9, 12, 15 and 18 months after storage but their interaction had no effect on this parameter ($P<0.05$). At 9 months of storage, cultivar Tigoni had the lowest % survival which ranged between 87.5 and 97.5% (Table II). The highest survival in 12- month old cultures was observed on storage media gelled with agar (0.8%) and cassava starch (8%) + agar (0.25%) (Table II). In general, % survival at 15 months of storage was lowest for cultivar Tigoni for all the media evaluated. At 18 months of storage, none of the cultures had 100% survival.

Cultivar, media type and their interaction had significant effects on condition of plantlets at 18 months of storage ($P<0.05$). Cultivar Tigoni had the poorest condition of plantlets for all media evaluated (Table III). The condition of the plantlets was similar for cultivars Arka and Dutch Robijn on media gelled with agar (0.8%) and cassava starch (8%) + agar (0.25%). However, plantlets of cultivar Arka were in a better condition than those of cultivar Dutch Robijn on media gelled with cassava starch (8%) alone. The effects of cultivar and media type on the number of usable single node cuttings /culture after 18 months of storage were significant ($P<0.05$) but the interaction between cultivar and media type had no effect on this parameter (Table IV).

Cultivars Dutch Robijn and Arka gave the highest and lowest number of usable single node cuttings/culture, respectively (Table IV). The number of usable single node cuttings/culture ranged from 3.7 to 13.4. All plantlets that survived the 18-month conservation period had 100% viability irrespective of the type of conservation media that was used. Agar (0.8%) media had a higher number of usable single node cuttings/culture than cassava starch (8%). However, the two media were not different from cassava starch (8%) + agar (0.25%) in

TABLE II - EFFECT OF VARIOUS CONSERVATION MEDIA (GELLING AGENTS) ON PERCENT PLANTLET SURVIVAL OF THREE POTATO CULTIVARS

Cultivar/gelling agent	Plantlet survival (%)											
	12 months after storage				15 months after storage				18 months after storage			
	Agar (0.8%)	Cassava starch (8%)	Cassava starch (8%) + Agar (0.25%)	Mean	Agar (0.8%)	Cassava starch (8%)	Cassava starch (8%) + Agar (0.25%)	Mean	Agar (0.8%)	Cassava starch (8%)	Cassava starch (8%) + Agar (0.25%)	Mean
Arka	100	83.8	98.8	94.2	97.5	80.0	96.3	91.3	92.5	76.3	92.5	87.1
Dutch Robijn	100	77.5	100	92.5	98.8	76.3	96.3	90.4	91.3	70.0	92.5	84.6
Tigoni	81.3	73.8	87.5	80.8	71.3	61.3	75.0	69.2	65.0	47.5	62.5	58.3
Mean	93.8	78.3	95.4	89.2	89.2	72.5	89.2	83.6	82.9	64.6	82.5	76.7
LSD[1]cultivar (P=0.05)				4.2					4.1			2.8
LSDgelling agent (P=0.05)				4.2					4.0			2.8
LSDcultivar x gelling agent (P=0.05)				NS					NS			NS

[1]Fisher's protected least significant difference; NS – Not significant

usable single node cuttings/culture.

The characteristics of the various conservation media and the quantity of media that remained in the culture bottles after 18 months of storage is presented in Table V. Like media gelled with agar (8%), media gelled with cassava starch (8%) + agar (0.25%) had good clarity, remained stable and did not show any softening throughout the conservation period. However, media gelled with cassava starch (8%) had poor clarity with poor gel strength. The amount of media in all the treatments had not been

TABLE III - CONDITION OF PLANTLETS AFTER CONSERVATION PERIOD (18 MONTHS) OF THREE POTATO CULTIVARS

Cultivar	Agar (0.8%)	Cassava starch (8%)	Cassava starch (8 %) + agar (0.25%)	Mean
Arka	2.4	2.1	2.5	2.3
Dutch Robijn	2.4	1.8	2.5	2.2
Tigoni	1.5	1.4	1.6	1.5
Mean of gelling agents	2.1	1.8	2.2	2.0
LSD[1]cultivar (P=0.05)				0.09
LSDmedia (P=0.05)				0.09
LSDcultivar x media (P=0.05)				0.15

[1]Fisher's protected least significant difference

TABLE IV - EFFECT OF VARIOUS CONSERVATION MEDIA (AFTER 18 MONTHS OF STORAGE) ON NUMBER OF USABLE SINGLE NODE CUTTINGS PER CULTURE OF THREE POTATO CULTIVARS

Cultivar	Agar (0.8%)	Cassava starch (8%)	Cassava starch (8 %) + agar (0.25%)	Mean
Arka	4.8	3.7	4.7	4.4
Dutch Robijn	13.7	11.0	13.4	12.7
Tigoni	10.9	8.6	8.8	9.4
Means of gelling agent	9.8	7.8	9.0	8.8
LSD1cultivar (P=0.05)				1.4
LSDmedia (P=0.05)				1.4
LSDcultivar x media (P=0.05)				NS

[1]Fisher's protected least significant difference; NS-Not significant

TABLE V -THE GELLING CLARITY AND QUANTITY OF MEDIUM AFTER 18 MONTHS OF STORAGE

Media	Gel strength	Clarity	Quantity of media remaining after 18 months of conservation
Agar (0.8%)	Good	Good	More than half remaining
Cassava starch (8%)	Poor	Poor	More than half remaining
Cassava starch (8 %) + agar (0.25%)	Good	Good	More than half remaining

TABLE VI - COMPARATIVE COSTS OF CULTURE MEDIUM FOR IN VITRO CONSERVATION WITH VARIOUS GELLING AGENTS

Gelling agent	Cost/kg	Concentration/l(% w/v)	Cost/L (Kshs)	Decrease (%) in comparison to agar (0.8%) based media
Agar (0.8%)	15,000	0.8	492	-
Cassava starch (8%)	400	8	372	24.4
Cassava starch (8 %) + agar (0.25%)		8.0a,0.25b	412	16.6

a refers to concentration of cassava starch (%w/v) in media containing both cassava and starch; b refers to concentration of agar (%w/v) in media containing both cassava and starch

Kshs 73=I USD as at December 2008

exhausted by the time the experiment was concluded at 18 months after conservation.

Cost of media

Media gelled with either cassava starch (8%) or cassava starch (8%) + agar (0.25%) were cheaper than agar (0.8%) gelled media (Table VI). A 24.4% cost reduction in gelling costs was achieved using cassava starch (8%) alone instead of agar (0.8%) alone while a 16.6% reduction was achieved when agar (0.8%) was replaced with cassava starch (8%) + agar (0.25%).

DISCUSSION

Results from the present study indicate that media gelled with cassava starch (8%) + agar (0.25%) had good clarity, remained stable and did not show any softening throughout the conservation period which indicated that the media did not metabolize during the conservation period. In contrast, media gelled with cassava (8%) had poor clarity with poor gel strength as indicated by its low viscosity upon shaking of the culture bottles. Clarity of plant tissue culture media is necessary for prompt detection of microbial contamination. Thus, the cassava (8%) gelled media may not be ideal for conservation of potato cultures despite giving acceptable survival of plantlets and good plant condition. Satisfactory results were obtained when cassava 8% was mixed with agar (0.25%). After 18 months without sub culturing, survival rates varied between 62.5 and 92.5% with good plant condition for varieties Arka and Dutch Robijn.

Cassava starch mixed with agar is likely to be a more cost effective alternative to agar for *in vitro* conservation potato because cassava starch is cheaper than agar and a lot of cassava is grown in Kenya. Agar is widely used as a gelling agent owing to its stability, high clarity, resistance to metabolism (not digested by plant enzymes and remains stable at all feasible incubation temperatures), limited diffusion of media components (does not react with media components) and water (McLachlan, 1985; Henderson and Kinnersly, 1988) and

gel strength (Deberg, 1983).

The presence of cultivar x media interaction suggested that cultivars may respond differently to different media. This observation is in agreement with Wilkins *et al*. (1988) who reported that slow growth storage methods are extremely variable and genotype dependant. Screening of a large number of cultivars may, therefore, be necessary before development of cultivar specific protocols to improve the conservation and subsequent regeneration of conserved material. Gopal *et al.* (1998) made similar observations regarding the need for cultivar specific protocols for conservation. However, this may be difficult and costly when a large number of genotypes need to be conserved. Therefore, media that may not be optimum but give good enough survival rates and plant condition for a broad range of genotypes may be more practical than developing specific media for a large number of genotypes.

The amount of media in all the treatments of both experiments had not been exhausted at 18 months after storage. It would be interesting to see how long cultures would stay before the media are exhausted or until the cultures die. In general, none of the treatments had poor root growth in both experiments. This is probably attributable to the non-metabolizable sugar alcohols such as mannitol, which was an ingredient in all the media, which reduce the water availability to growing cultures by imposing water-deficit stress (Thompsom *et al*., 1986). This stress is perhaps responsible for stimulating the plantlets to develop more roots which enhance water uptake.

This study showed that plantlets had normal phenotypes with thick stems and broad leaves and it was easy to sub-culture them with 100% regeneration. Plantlets conserved at low temperatures generally look abnormal with stunted growth, thin stems and leaves that are reduced in size or absent. Lizarraga *et al.* (1989) and Lopez-Delgado *et al*. (1998) reported that such plantlets tend to have a high incidence of abnormalities such as chlorosis, vitrification and flaccidity, particularly when low temperature is combined with media with osmotic stress or growth retardants. Recovery of normal plants from plants with such morphological abnormalities is difficult during sub-culture (regeneration) and the genetic stability of plants raised in this way has also been questioned (Dodds *et al*., 1991; Harding, 1999). Therefore, slow growth at normal propagation temperature is a good alternative for *in vitro* conservation of potato. Reduction of light intensity from the 3000 lux used in normal propagation (Espinoza *et al*., 1992) to 1000 lux used in this study, can also contribute to cost reduction of conservation.

Results showed that by substituting agar with cassava starch (8%) + agar (0.25%), the cost of the gelling agent could be decreased by 16.6%. This media supported the survival of between 62.5 and 92.5% of the cultures with good plantlet condition and is therefore recommended for *in vitro* potato conservation. It would be interesting to find out if this medium would support conservation with the same level of plantlet survival and condition if growth hormones such as gibberellic acid are excluded from the medium.

CONCLUSION

This study demonstrated that potato plantlets could be stored for over 18 months in conservation medium gelled with cassava starch (8 %) + agar (0.25 %). The use of cassava starch can easily save 16.6 to 24.4% of the gelling agent costs of conservation. The similar performance of cassava starch (8 %) + agar (0.25 %) with that of agar for medium solidification without any adverse effects on plantlet survival and condition strongly indicates that this treatment is suitable for potato conservation.

ACKNOWLEDGEMENT

The authors are grateful to the Kenya Agricultural Productivity Project (KAPP) for funding this study.

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BAR CODING SOYBEAN *BRADYRHIZOBIUM* STRAINS INDIGENOUS TO KENYAN SOILS.

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ABSTRACT

While soyabean is an exotic crop introduced in Kenya early in the last century, promiscuous (TGx) varieties which nodulate with indigenous rhizobia have only been recently introduced. Since the majority of farmers in Kenya cannot afford or access inorganic fertilizer, the identification of effective indigenous *Bradyrhizobium* strains which nodulate promiscuous soyabean could be useful in the development of inoculant strains. The objectives of this study were to assess the genetic diversity and phylogeny of indigenous *Bradyrhizobium* strains nodulating introduced promiscuous soyabean varieties grown in contrasting sites in the Kenya and to determine genetic relatedness of the indigenous rhizobia with type and other strains in the GenBank.

Genetic diversity was assayed using the Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) of the 16S-23S rDNA intergenic spacer region and 16S rRNA gene sequencing. The differences in fingerprints were used to group strains into intergenic spacer (IGS) groups. Strains nodulating varieties in lowland sites were grouped into 8 while those in highland sites were grouped into 18 IGS groups respectively. Predominant groups were A, B and D and I, III and II in the lowland and highland sites respectively. The IGS groups were specific to sites but not varieties. Phylogenetic analysis of the 16S rDNA gene sequences showed that all indigenous strains belonged to the genus *Bradyrhizobium*. Sequencing of 16S rDNA gene also showed that 37.5% of the strains nodulating soyabean in all sites were related to *Bradyrhizobium elkanii*, 30% to *Bradyrhizobium japonicum*, 25% to *Bradyrhizobium* spp and 7.5% to *Bradyrhizobium yuanmingense*. The polymorphism in *Bradyrhizobium* populations from these sites represents a valuable genetic resource that has potential variability for the selection of more effective and competitive strains for use as inoculants to facilitate soyabean production at low cost.

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INTRODUCTION

Indigenous rhizobia associated with leguminous crops are diverse. They exhibit this diversity in their genetic constitution as well as competitiveness and effectiveness with and between hosts (Padzemik *et al*., 1977; Pueppke and Broughton, 1999). A variety of methods exist for the assessment of genetic diversity in closely or distantly related bacterial species. Traditionally, variation has been determined using characteristics such as growth rate, colony morphology (size, shape, color, texture and general appearance) and antibiotic resistance methods (Graham *et al.*, 1991; Somasegaran and Hoben, 1994). However, these methods are not sufficiently discriminative to account for all the variation exhibited in the target species. They cannot delineate sources of observed phenotypic variation into its components that may be due to environmental factors or underlying genetic factors. In recent years, DNA techniques have been used to detect sequence polymorphisms within and between strains of bacteria (Stern *et al*., 1984; Nakamura *et al.*, 1987; Williams *et al*., 1990; Hulton *et al*., 1991; Laguerre *et al*., 1994; Vos *et al*., 1995; Lanham and Brennan, 1999; Virdi and Sachdeva, 2005).

The application of PCR-RFLP analysis of the 16S-23 rDNA intergenic region and sequence analysis of the 16S RNA gene are vital tools in clustering genetically related rhizobia. These have been frequently used in microbial taxonomy to determine inter and intra specific relationships (Vinuesa *et al*., 1998; Abaidoo *et al*., 2000; Doignon-Bourcier *et al*., 2000; Sarr *et al*., 2007). In these methods, the generated PCR fingerprints are unique to each isolate and are used to group them at strain level. Previous studies on the diversity of *Bradyrhizobia* from soyabean have used the PCR-RFLP analysis, a high-resolution genotypic fingerprinting technique based on the restriction of amplified fragments from total genomic DNA (Laguerre *et al.*, 1994). The PCR-RFLP technique was shown to provide an insight into the extent of genetic diversity of indigenous *Bradyrhizobium* isolates nodulating cowpea (Krasova-Wade *et al*., 2003).

Indigenous rhizobia associated with leguminous crops are diverse and usually exhibit this diversity in their genetic constitution as well as competiveness and effectiveness with and between hosts (Hansen, 1994). Previous studies have shown great diversity of strains isolated from nodules of the same legume in both

competiveness and effectiveness (Hansen, 1994). Genetic diversity of rhizobia nodulating promiscuous soyabean varieties has not been determined in Kenya. Determination of genetic diversity of indigenous *Bradyrhizobium* populations in Kenyan soils is a valuable step in the development of cost effective strategies to optimize biological nitrogen fixation and thus increase soyabean yields. The objectives of this experiment were to assess the genetic diversity and phylogeny of indigenous *Bradyrhizobium* strains nodulating introduced promiscuous soyabean varieties grown in contrasting sites in the Kenya and to determine genetic relatedness of the indigenous rhizobia with type and other strains in the GenBank.

MATERIALS AND METHODS

Isolation of *Bradyrhizobium* Strains from Nodules

Nodules were harvested at reproductive (R1) stage (Fehr *et al.,* 1971) for each variety. Ten nodules per treatment for each variety were used for analysis. Before analysis each nodule was surface sterilized with 96% ethanol for 30 seconds and rinsed with sterile water, then surface sterilized with 3.3% w/v Ca(OCl)2 for 3 minutes, and three times rinsed with sterile distilled water. From this stage the nodules were manipulated aseptically, in a lamina flow. Each nodule was crushed in 300 µl of sterile water with plastic micro pestles sterilized in 96% ethanol in a 1.5 ml eppendorf tube.

DNA Extraction

Total genomic DNA was extracted according to the procedure described by Krasova-Wade *et al*., 2003 with modifications made on centrifugation times. DNA from 10 nodules per treatment was extracted by adding 150 µl of 2X CTAB/PVPP buffer [0.2 M Tris HCL, pH 8.0; 0.04 EDTA pH 8.0; 2.8M NaCl; 4% CTAB and 2% (w/v) PVPP] to the nodule suspension and incubating the mixture in a water bath at 65°C for 60 minutes with intermittent shaking at 15 min intervals. This was followed by centrifuging for 15 minutes at 13000 rpm at room temperature and subsequently pipetting the supernatant into a sterile eppendorf tube. To this supernatant, 250 µl of phenol: chloroform: isoamylalcohol (25:24:1 v/v/v) was added and centrifuged at 13000 rpm at room temperature. The supernatant was pipetted and to it added 150 µl of chloroform:isoamyl alcohol (24:1 v/v) and centrifuged for 5 minutes at 13000 rpm at room temperature. The supernatant was pipetted into a sterile eppendorf tube and 100 µl of ice cold isopropanol added and placed at -20oC overnight for DNA precipitation. The mixture was centrifuged for 5-10 minutes at 13000 rpm at 4oC. The resulting DNA pellet was washed with 70% v/v ethanol by centrifugation for 10 minutes at 13000 rpm at room temperature, air dried and re-suspended in 50 µl of sterile double distilled water. Ten µl of RNase (40 µg/ml) were added to the DNA extract and incubated at 37°C for 30 min. DNA was also extracted from the reference strain USDA 110 using the same procedure.

PCR amplification of 16S-23S rDNA Region

The intergenic region between the 16S and 23S rDNA from 289 nodules was amplified by PCR with primers derived from the 3' end of the 16S rDNA (FGPS 1490-72; 5′-TGCGGCTGGATCCCCTCCTT-3′) corresponding to positions 4 (1521-1541) of *E. coli* gene (Navarro et al. 1992) and from the 5′ end of the 23S rDNA (FGPL 132-38; 5′-CCGGGTTTCCCCATTCGG-3′) corresponding to positions 6 (114-132) of *E. coli* gene (Ponsonnet and Nesme, 1994) within the rDNA operon close to the 16S-23S intergenic spacer. A PCR amplification was carried out in a 25 µl reaction volume containing 2µl of pure total DNA extract, freeze dried beads (Ready-to-Go PCR beads, Pharmacia Biotech, Uppsala, Sweden) containing 2.5 U of Taq DNA polymerase, 200 µM Tris-HCL, (pH 9 at RT), 50 mM KCL, 1.5 mM MgCl2, 200 µM of each dNTP and 1.0 µM of each primer. PCR amplification was performed in a Primus 96 plus thermal cycler (MWG AG BIOTECH) adjusted to the following program: initial denaturation for 5 minutes at 94°C, 35 cycles of denaturation (30 sec at 94°C), annealing (30 sec at 58°C) and extension (30 sec at 72°C) and a final extension (7 min at 72°C). PCR amplified DNA was visualized by electrophoresis of 3 µl of the amplified DNA on 1% (w/v) horizontal agarose gel in TBE buffer (1.1 w/v Tris-HCL; 0.1% w/v $Na_2EDTA\ 2H_2O$; 0.55% w/v Boric acid), pre-stained with 3.5µl of ethidium bromide (1µg/ml). The gel was photographed under UV illumination with Gel Doc (BIO-RAD) Software (USA).

Restriction of 16S-23S rDNA region

Aliquots (10 µl) of PCR products (46 nodules from lowland sites and 289 nodules from highland sites) were digested with 1 µl of the restriction endonuclease Msp I in 2 µl double distilled water and 2 µl of buffer in a total volume of 15 µl for 3 hours at 37°C. The restriction fragments were separated by horizontal gel electrophoresis in 1X TBE buffer on 3% (w/v) agarose (Sigma-Aldrich Chemie Gmbh, Steinheim, Germany) pre-stained with 3.5 µl of ethidium bromide (1µg/ml). The gels were run at 100 V for 3 hours and photographed under UV illumination with Gel Doc (BIO-RAD, USA) software.

Sequence Analysis of the 16S rRNA Gene

A sample of thirty nine 39 *Bradyrhizobium* isolates (29 from highland sites and 11 from lowland sites respectively) isolated from different varieties grown at different sites and treatments were selected for 16S rRNA gene sequencing at Macrogen in South Korea. The forward primer 27F (5′-AGA GTT TGA TCC TGG CTC AG-3′) corresponding to positions 27-46 of the E. coli of the 16S rRNA gene sequence and the reverse

primer 1492R (5′-GGTT TAC CTT GTT ACG ACT T-3′) corresponding to positions 1525-1506 of *E. coli* (Lane, 1991) were used to amplify the 16S rRNA gene. The 1500 bp PCR products were sequenced for the DNA region coding for the 16S rRNA gene in an ABI 377 (PE-Applied Biosystems sequence analyzer. The generated sequences were submitted to the national council for biological information (NCBI) GenBank database through basic local alignment search tool (BLAST) to search for significant 16S rRNA alignments.

A phylogenetic tree was constructed based on the partial 16S rRNA gene sequences of the TGx soybean nodule isolates and rhizobial reference strains from the GenBank. The sequences of the rhizobial strains were aligned pair wise and compared to strains in the GenBank database. A dendogram was inferred with Neigbour-Joining Algorithm (Saitou and Nei, 1987) using ClustalX software (Thompson *et al*., 1997) and the phylogenetic tree reconstructed with PHYLIP (Felsestein, 1993), package and a bootstrap analysis using 100 replications. Shannon's index of diversity (Ho) was estimated based on the number of strains belonging to each genus / species (Shannon and Weaver, 1949) using the formula:

Ho = $-\Sigma$ Pi ln Pi; where;
I = an index number for each strain (restriction profile) present in the sample
Pi = ni /N = the number of strains within a sample (ni) divided by the total number of strains (N) present in the entire sample with similar restriction profiles
ln = the natural log

RESULTS

Isolation of DNA

Whole genomic DNA was extracted from all the sampled nodules. It was of high molecular weight and was suitable for further processing through polymerase chain reaction (Figure 1).

PCR-RFLP of 16S-23S rDNA IGS region

Single IGS PCR products ranging from 930-1100 bp were obtained from all the analyzed nodules and one reference strain (USDA 110) (Figure 2). Each nodule presented a single profile in all the nodules analyzed in lowland and highland sites. The PCR amplicons generated from the nodules were further processed by restriction with Msp I enzyme. The recognition site for Msp I is C:CCG or GCC:C. Profiles generated revealed different IGS phenotypes (Figure 3).

In the highland sites, Bungoma and Mitunguu, strains were classified into 18 IGS groups (Figure 4.4). The five most predominant IGS groups were I, III, II, IV and VI which constituted, 43.9%, 24.6%, 8.3% 7.6% and 6.9% respectively of all the analyzed nodules from the two sites, while IGS groups VII, VIII, IX, X, XI, XII, XIV, XVI, XVII, XVIII each constituted 1% or less (Table 4.1). Both sites had relatively similar numbers of different indigenous *Bradyrhizobium* strains and IGS groups. Mitunguu had 141 strains disaggregated into 12 IGS groups while Bungoma had 148 strains disaggregated into 13 IGS groups. Some IGS groups were specific to sites and treatments but not varieties (Table 1). While five IGS groups (IX, XIII, XVI, XVII, and XVIII) were specific to Bungoma, six groups (V, VII, VIII, X, XII, XIV) were detected only in Mitunguu. The Shannon-Weaver (Ho) indices were higher at Bungoma (Ho = 1.9) compared to Mitunguu (Ho = 1.7) (Table 2).

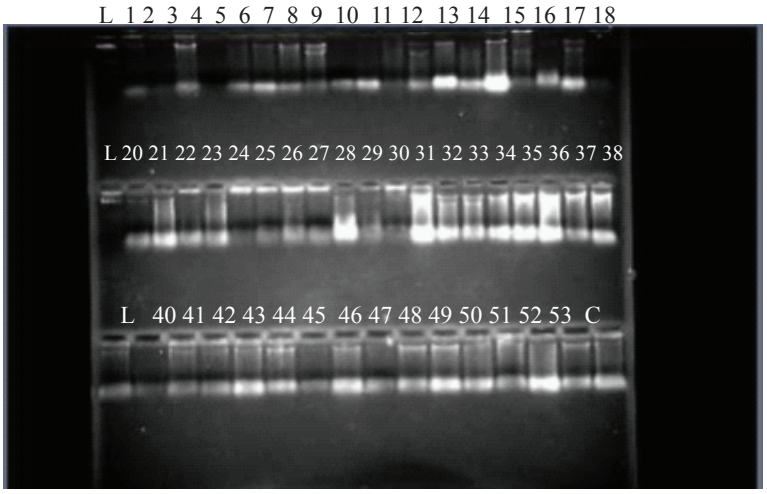

Figure 1: DNA extracted from *Bradyrhizobium* strains in nodules from varieties grown in different treatments. +Numbers represent the strains. L indicate the 100 base pair marker while C is the USDA 110 control

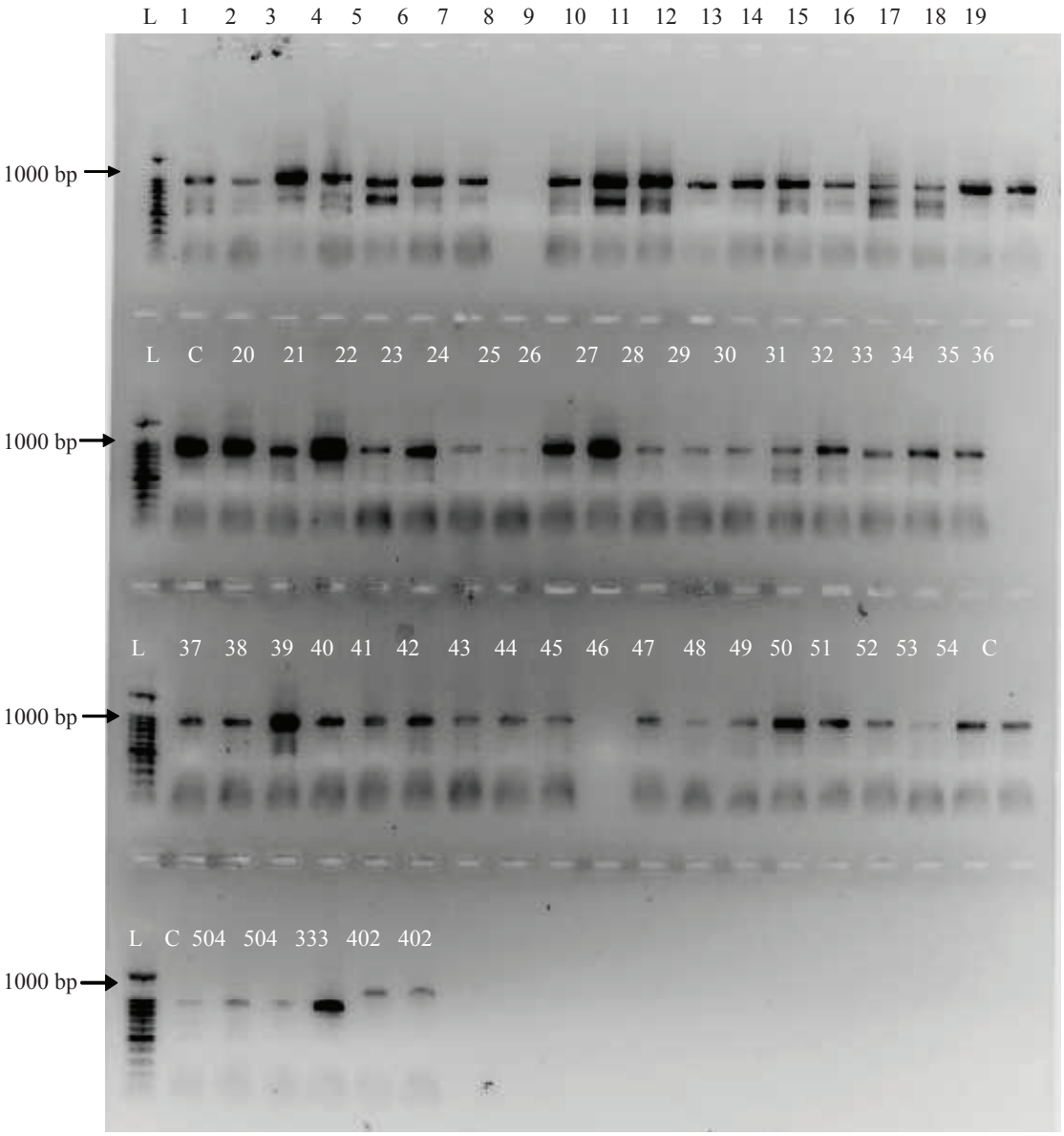

Figure 2: PCR amplified products of different rhizobia strains in soyabean nodules. Numbers are strain codes, C-USDA 110 and L-100bp ladder

In the lowland sites (Chonyi, Mtwapa and Msabaha), restriction with Msp I revealed 8 IGS groups while restriction with Hae III revealed 9 IGS groups (Figure 5). IGS groups A, E, B and C were the most predominant profiles representing 41.3%, 21.7%, 19.6%, 17.4%, and 10.9% respectively while IGS groups H, F and G were the least predominant constituting 4.3%, 2.2% and 2.2% of all nodules (Table 3). Chonyi site had higher ($P<0.05$) diversity (6 IGS groups) of indigenous rhizobia than Mtwapa site (2 IGS groups). IGS group A, B and D were specific to Msabaha, IGS groups A and C to Mtwapa, while IGS groups C, E, F, G and H were specific to Chonyi site (Table 3).

Sequence analysis of the 16S rRNA gene for Highland sites

All the selected 29 isolates produced a single PCR product of approximately 1500 bp. The partial sequences of the 16S rRNA gene of these selected isolates of indigenous *bradyrhizobia* were deposited in the GenBank and given accession numbers EU625518 to EU625546 (Table 4.4). Alignments of partial sequences of the TGx isolates

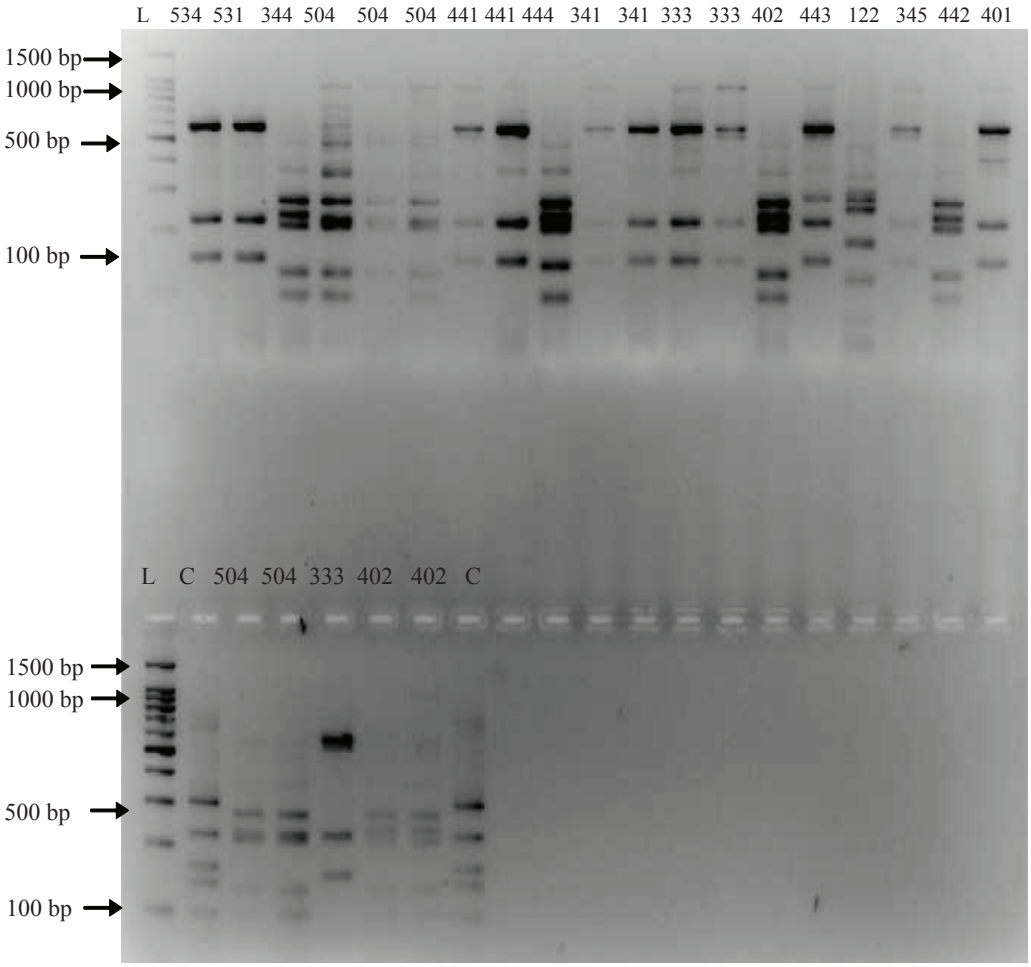

Figure 3: Restriction profiles of PCR-product of rhizobia strains from soyabean nodules digested with Msp I endonulease enzyme. Numbers are codes for strains isolated from nodules, C-USDA 110, L- 100 bp ladder

with related 16S rRNA gene sequences in GenBank database revealed that the 29 strains were all related to the *Bradyrhizobium* genus and were group in clusters A, B, C, D, E and F (Figure 4.6). *Bradyrhizobium elkanii, Bradyrhizobium* spp and *Bradyrhizobium japonicum* related strains were the most predominant and accounted for 37.9%, 34.5% and 20.7%, respectively, while *Bradyrhizobium yuanmigense* related strains accounted for 6.9% of all nodules analyzed. Eleven strains (TSBF 161, TSBF 402, TSBF 344, TSBF 444, TSBF 404, TSBF 442, TSBF 260A, TSBF 336A, TSBF 488, TSBF 137 and TSBF 530) were related to *Bradyrhizobium elkanii*, ten strains (TSBF 531, TSBF 523, TSBF 534, TSBF 331, TSBF 341, TSBF 333' TSBF 381, TSBF 504, TSBF 438 and TSBF 101A) to *Bradyrhizobium* spp, six strains (TSBF 345, TSBF 336, TSBF 131, TSBF 216, TSBF 101 and TSBF 102 to *Bradyrhizobium japonicum* while two strains (SBF-441 and TSBF-160) to *Bradyrhizobium yuanmingense* (Table 1). A phylogenetic tree derived from the partial sequences of the 16S rRNA gene by neighbor-joining analysis (Figure 6) confirmed the greater relationship of indigenous strains of *Bradyrhizobium* to reference strains of *B. elkanii, B. japonicum and Bradyrhizobium spp.* The *Rhizobium and Sinorhizobium* reference strains constituted an outside group in the phylogenetic tree.

The BLAST results of the partial 16S rDNA gene sequences from highland and lowland sites indicated that 11 isolates were related to *Bradyrhizobuim japonicum*; 15 isolates related to *Bradyrhizobium elkanii*; 3 isolates related to *Bradyrhizobium yuanmigense* while 10 isolates were related to *Bradyrhizobium* spp (Table 4). Across all five sites, the most predominant strains were *Bradyrhizobium elkanii, Bradyrhizobium japonicum, Bradyrhizobium* spp and *Bradyrhizobium yuanmigense* representing 37.5%, 30.0%, 25% and 7.5%

TABLE I-DISTRIBUTION OF *BRADYRHIZOBIUM* STRAINS AMONG DIFFERENT IGS GROUPS ACROSS VARIETIES IN HIGHLAND SITES BY MSP 1 ENZYME RESTRICTION

	Sites									
	Bungoma				Mitunguu					
Treatments	Control	P	P+Lime	Sub-total	Control	P	P+Lime	Sub-total	Total	% of total
IGS group										
I	18	17	16	51	28	17	31	76	127	43.9
II	6	8	4	18	1	2	3	6	24	8.3.
III	8	12	9	29	10	24	8	42	71	24.6
IV	6	6	6	18	3	1	0	4	22	7.6
V	0	1	2	3	0	0	0	0	3	1.0
VI	6	4	8	18	2	0	0	2	20	6.9
VII	0	0	1	1	0	0	0	0	1	0.3
VIII	1	2	0	3	0	0	0	0	3	1.0
IX	0	0	0	0	0	1	0	1	1	0.3
X	0	1	0	1	0	0	0	0	1	0.3
XI	1	0	0	1	0	0	1	1	2	0.7
XII	1	0	0	1	0	0	0	0	1	0.3
XIII	0	0	0	0	2	1	0	3	3	1.0
XIV	0	0	1	1	0	0	0	0	1	0.3
XV	2	0	1	3	1	2	0	3	6	2.1
XVI	0	0	0	0	0	1	0	1	1	0.3
XVII	0	0	0	0	1	0	0	1	1	0.3
XVIII	0	0	0	0	0	1	0	1	1	0.3
Total	49	51	48	148	48	50	43	141	289	

Values indicate the number of strains in each IGS groups for each treatment, n= 289. Treatments: Treatments were control (none), + P (40 kg/ha), + lime (1t/ha), + N (90 kg/ha, split applied + lime + P). No nodules formed in the + N treatment and is not reported in this table.

Key: M- 100bp ladder; C – Control strain (USDA 110)

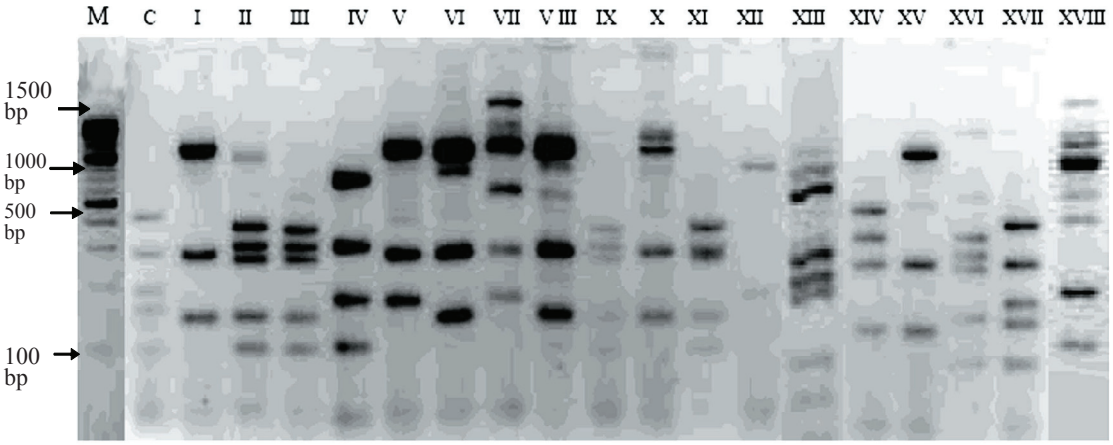

Figure 4: M- 100bp ladder; C – Control strain (USDA 110) I GS groups obtained from Msp I restricted products of indigenous *Bradyrhizobium* strains isolated from promiscuous soyabean varieties in Bungoma and Mitunguu sites in Kenya

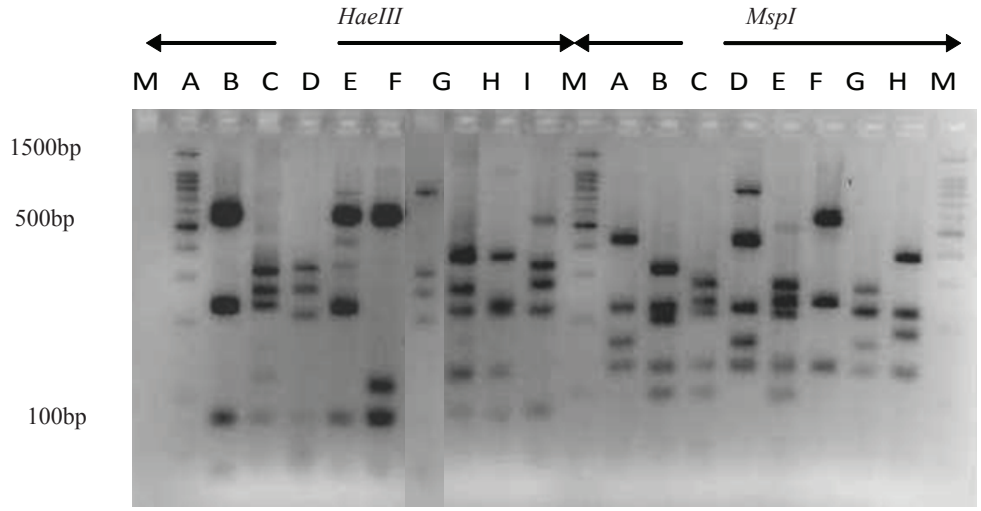

Figure 5: RFLP patterns obtained by restriction of different PCR-amplified 16S-23S rDNA IGS region using endonucleases Hae III and Msp I from crushed nodules isolated at the coastal lowland sites

TABLE II- SHANNON INDEX (HO) OF DIVERSITY OF *BRADYRHIZOBIUM* POPULATIONS FROM HIGHLAND SITES BASED ON 16S RRNA GENE SEQUENCING

Sites	Mitunguu	Bungoma
Number of individual species	16	13
Shannon index (Ho)	1.7	1.9
Evenness	0.31	0.49

TABLE III- DISTRIBUTION OF *BRADYRHIZOBIUM* STRAINS AMONG DIFFERENT IGS GROUPS ACROSS VARIETIES IN LOWLAND COASTAL SITES BY MSP 1 ENZYME RESTRICTION

IGS group	Chonyi	Msabaha	Mtwapa	Total	% of total
A	0	16	3	19	41.3
B	0	8	0	8	17.4
C	2	0	3	5	10.9
D	10	0	0	10	21.7
E	5	4	0	9	19.6
F	1	0	0	1	2.2
G	1	0	0	1	2.2
H	2	0	0	2	4.3
	12	28	6	46	

Values indicate the number of strains in each IGS group for each treatment, n= 46. All varieties received 9 kg /ha-1kg N and 23 kg/ ha/ P as basal application treatment.

respectively. However, site differences exist and certain strains predominate in certain sites and not others. *Bradyrhizobium* spp was most predominant in Mitunguu, *B. elkanii* in Bungoma and Mtwapa, *B. japonicum* in Msabaha while *B. elkanii* and *B. japonicum* equally prevalent in Chonyi (Table 5). A phylogenetic tree showed that the TGx soyabean isolates clustered together with *Bradyrhizobium* genus strains from the GenBank while the other genus Rhizobium, Mesorhizobia and Sinorhizobia, clustered away (Figure 6 and Figure 7). Majority of the test strains, however, clustered according to geographical region (Figure 7).

DISCUSSION

The TGx soyabean varieties developed by the breeding program at IITA for promiscuity nodulated with diverse indigenous rhizobia populations in both sites where they had been introduced for the first time. The presence of

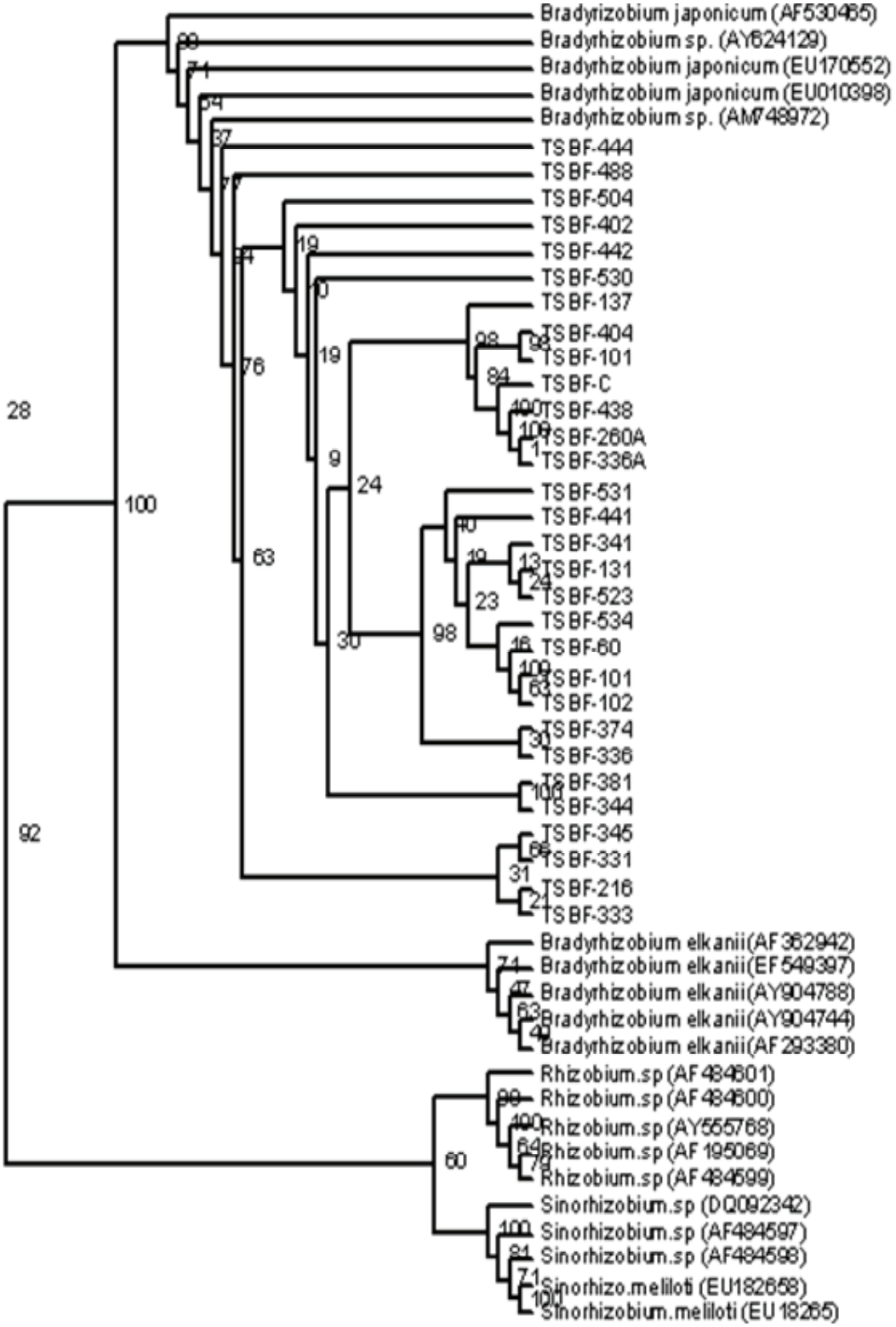

Figure 6: Phylogenetic relationship between highland strains and GenBank reference strains based on aligned sequences of 16S rRNA gene, constructed as unrooted tree using the nearest neighbour-joining method

TABLEIV- RELATIONSHIP BETWEEN THE PARTIAL 16S RNA GENE SEQUENCES OF GENBANK STRAINS AND ISOLATED STRAINS FROM BUNGOMA, MITUNGUU, MTWAPA, CHONYI AND MSABAHA SITES IN KENYA

Isolate ID.	Genbank Acc. No.	Site*	Altitude (m.a.s.l) **	Variety	Treatment	Sequence Length (bp)	Species affiliation	% Similarity
TSBF-438	EU625543	1	> 900 m	SB 20	P	1373	*Bradyrhizobium* spp KO3G	99
TSBF-666	AY649431	5	> 900 m	SB 17	DAP	794	*Bradyrhizobium elkanii* strain BR 3277	95
TSBF-701	EF549397	3	< 90 m	SB 8	DAP	774	*Bradyrhizobium elkanii* strain CCBAU 83387	94
TSBF-694	AY649431	3	< 90 m	SB 17	DAP	806	*Bradyrhizobium elkanii* strain BR 3277	94
TSBF-260A	EU625535	2	> 900 m	SB 9	P	1044	*Bradyrhizobium elkanii* isolate TSBF 694	93
TSBF-444	EU625531	2	> 900 m	SB 20	P+Lime	1125	*Bradyrhizobium elkanii*	93
TSBF 102	EU625546	2	> 900 m	SB 19	P	898	*Bradyrhizobium japonicum* isolate TSBF 734	92
TSBF-531	EU625518	1	> 900 m	SB 9	P+Lime	924	*Bradyrhizobium* spp	92
TSBF-523	EU625519	1	> 900 m	SB 9	P	898	*Bradyrhizobium* spp	91
TSBF-488	EU625540	1	> 900 m	SB 8	Control	1083	*Bradyrhizobium elkanii* strain USDA 61	91
TSBF-534	EU625522	1	> 900 m	SB 9	P+Lime	917	*Bradyrhizobium* spp	91
TSBF-336A	EU625536	1	> 900 m	SB 15	Control	902	*Bradyrhizobium elkanii* isolate TSBF 694	91
TSBF-331	EU625523	2	> 900 m	SB 15	Control	1024	*Bradyrhizobium* spp	91
TSBF 333	EU625526	1	> 900 m	SB 15	Control	996	*Bradyrhizobium* spp PAC 41	91
TSBF-402	EU625529	1	> 900 m	SB 19	P	815	*Bradyrhizobium elkanii* strain USDA61	89
TSBF-131	EU625527	2	> 900 m	SB 20	P	725	*Bradyrhizobium japonicum* isolate 734	88
TSBF-442	EU625533	2	> 900 m	SB 20	P+Lime	870	*Bradyrhizobium elkanii*	88
TSBF-60	EU625542	2	> 900 m	SB 15	P+Lime	809	*Bradyrhizobium yuanmingense* isolate TSBF 627	88
TSBF-336	EU625524	1	> 900 m	SB 15	Control	837	*B. japonicum strain* TSBF 734	88
TSBF-341	EU625525	2	> 900 m	SB 15	P	867	*Bradyrhizobium*. spp SjCL5 (MS 867)	88
TSBF-627	EU170554	4	< 90 m	SB 19	DAP	815	*Bradyrhizobium yuanmigense* strain CCBAU33230	88
TSBF-345	EU625520	1	> 900 m	SB 15	P	850	*Bradyrhizobium japonicum* isolate TSBF-607	88
TSBF-101	EU625541	2	> 900 m	SB 19	P	863	*Bradyrhizobium japonicum* isolate TSBF 734	87
TSBF-607	AF530466	4	> 900 m	SB 8	DAP	810	*Bradyrhizobium japonicum* isolate WC4	86
TSBF 216	EU625538	2	> 900 m	SB 8	Control	942	*Bradyrhizobium japonicum* isolate JZ 1	86
TSBF-374	EU625537	1	> 900 m	SB 17	P	942	*Bradyrhizobium japonicum* isolate JZ 1	86
TSBF-381	EU625528	1	> 900 m	SB 17	P+Lime	676	*Bradyrhizobium* spp MAF 210190	86
TSBF-734	AF530466	3	< 90 m	SB 19	DAP	815	*Bradyrhizobium japonicum* isolate W4	85
TSBF-441	EU625521	1	> 900 m	SB 20	P+Lime	592	*Bradyrhizobium yuanmingense* isolate TSBF-627	85
TSBF-344	EU625530	1	> 900 m	SB 15	P	680	*Bradyrhizobium elkanii*	84
TSBF-717	AF208512	3	<90 m	SB 15	DAP	813	*Bradyrhizobium elkanii* strain USDA 31	83
TSBF-695	EF394144	3	< 90 m	SB 17	DAP	809	*B. japonicum strain* CCBAU 53152	82
TSBF-640	AY624129	4	< 90 m	SB 20	DAP	788	*Bradyrhizobium* spp PAC 41	80
TSBF-404	EU625532	1	> 900 m	SB 19	P	295	*Bradyrhizobium elkanii* SEMIA 6425	80
TSBF-101A	EU625534	2	> 900 m	SB 19	P	289	*Bradyrhizobium* spp MAF 210190	78
TSBF-718	AF530466	3	< 90 m	SB 19	DAP	749	*Bradyrhizobium japonicum* isolate W4	78
TSBF-530	EU625545	1	> 900 m	SB 9	P	355	*Bradyrhizobium elkanii* isolate TSBF-734	77
TSBF-639	DQ26711	4	< 90 m	SB 20	DAP	780	*Bradyrhizobium* spp CCBAU 35186	76
TSBF-137	EU625544	2	> 900 m	SB 20	P	284	*Bradyrhizobium elkanii* isolate TSBF 717	73
TSBF 161	EU625539	2	> 900 m	SB 4	P	183	*Bradyrhizobium elkanii* isolate TSBF 717	69
Control	BA000040			-	-	760	*B. japonicum* strain USDA 110	67

*Sites notation: 1= Mitunguu, 2 = Bungoma, 3= Chonyi, 4= Msabaha, 5=Mtwapa ; ** m.a.s.l. - metres above sea level

Figure 7: Phylogenetic relationships among strains isolated from promiscuous soybean varieties grown in five sites in Kenya

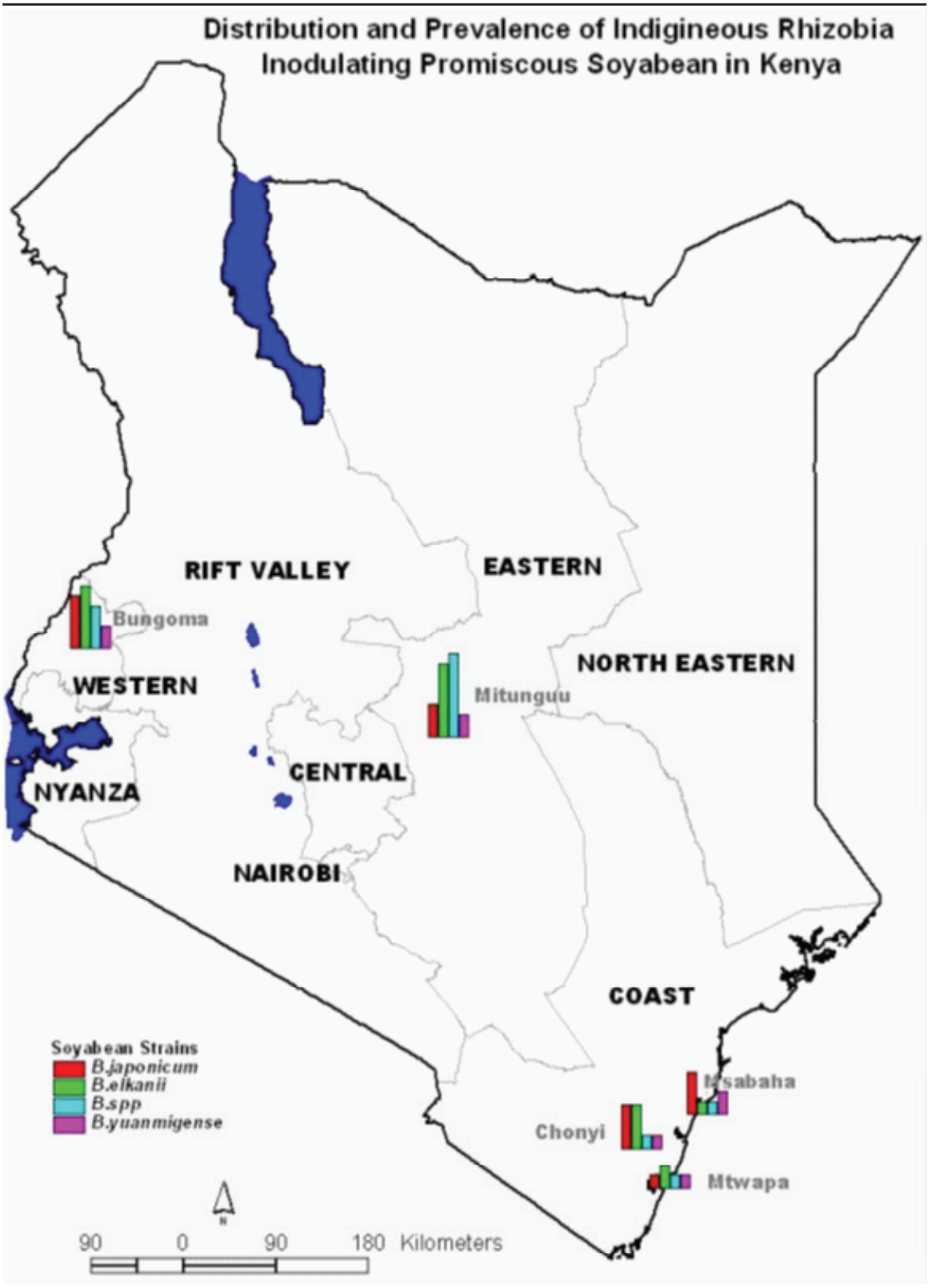

Figure 8: Distribution and prevalence of indigenous *Bradyrhizobium* strains nodulating promiscuous soyabean varieties in lowland and highland sites in Kenya

TABLE V- RELATIVE PROPORTION (%) OF STRAINS NODULATING SEVEN TGX SOYABEAN VARIETIES AT FIVE SITES IN KENYA

	Highland sites		Lowland sites			
	Mitunguu	Bungoma	Msabaha	Mtwapa	Chonyi	Overall
Strain	%	%	%	%	%	%
B. japonicum	12.50	30.77	75.00	0.00	50.00	30.00
B. elkanii	37.50	38.46	0.00	100.00	50.00	37.50
B. spp.	43.75	23.08	0.00	0.00	0.00	25.00
B. yuanmigense	6.25	7.69	25.00	0.00	0.00	7.50

these indigenous *Bradyrhizobium* in both Nigeria and Kenya soils separated by a tropical forest in central Africa suggests a common evolutionary path of bacteria influenced by comparable biotic and abiotic conditions. It could also be due to genetic exchange of nif genes through a variety and combination of events such as strain dispersion, genomic combination and horizontal gene transfer among indigenous *Bradyrhizobium* communities along the Nigerian and Kenya contiguous path through the Congo forest. In related studies, Mulongoy and Ayanaba (1986) reported the presence of *Bradyrhizobium japonicum* in some African soils even though soyabean was not commonly grown. Kasasa (1999) and Musiyiwa *et al*., (2005a) similarly reported the presence of indigenous rhizobia nodulating promiscuous soyabean varieties in many soils in Zimbabwe. Some of the isolates were as good or superior in N2 fixation effectiveness to commercial inoculant strains under greenhouse conditions.

The phylogenetic tree clearly shows that the Kenyan isolates form a distinct group. Thus the indigenous strains of *Bradyrhizobium* nodulating TGx varieties are distinct from *Bradyrhizobium* that nodulate North American soyabeans varieties. In Nigeria, similar results were obtained by Abaidoo *et al*., (2000) with the TGx varieties. This was not unexpected because the indigenous *Bradyrhizobium* from the two sites, with no previous history of soyabean cultivation and hence no introduction of exotic *Bradyrhizobium* strains, had been genetically isolated and consequently had evolved independently. The phylogenetic tree nevertheless shows that there is adequate genetic variation among the indigenous strains of *Bradyrhizobium*. This study showed that *Bradyrhizobium* strains nodulating promiscuous soyabean genotypes grown under lime and phosphorus application in two contrasting sites in Kenya were highly diverse. This diversity could be linked to the fact that *Bradyrhizobium* strains may have different capacities to utilize P in their metabolic activities which influence nodule initiation and effectiveness (Gunawardena, *et al*., 1993). There was a positive relationship between diversity assessed as number of IGS groups and abundance of bradyrhizobia strain population at the two sites. Bungoma had 13 IGS groups comprising 148 strains while Mitunguu had 12 IGS groups comprising 141 strains. The relatively higher diversity in Bungoma (Ho=1.9) compared to Mitunguu (Ho=1.7) could be attributed to a combination of factors such as the overall improved environmental soyabean growing conditions in Bungoma (humid) as compared to semi-humid conditions at Mitunguu. Elsewhere, several authors have reported similar genetic diversity indices of rhizobia nodulating soyabean (Sikora and Redzepovic, 2003; Chen *et al*., 2004; Giongo *et al.,* 2008) and Phaseolus vulgaris (Andrade *et al*., 2002) using molecular markers.

In this study, IGS groups were specific to sites and treatments but not varieties. This finding is in accordance with results described by Wei Tao Zang *et al.,* (2007) who showed that geographical location affects composition and biodiversity of indigenous rhizobia. This behavioural interaction between strains and geographical location is attributed to relative strain competitiveness or saprophytic competence under the conditions existing at specific sites (Wei Tao Zang *et al.,* 2007). Lime application has previously been reported to increase diversity of IGS groups in Phaseolus nodulating rhizobia in Brazil (Andrade *et al*., 2002). Strains restricted to a geographical location generally develop special phenotypic and genotypic characteristics (Xu *et al*., 1995; Vinuesa *et. al*., 1998). In contrast, Chen *et al*., (2004) and Thiao *et al*., (2004) found no relationship between IGS groups and geographical location.

In Kenya, few studies have investigated the genetic diversity of indigenous rhizobia nodulating legumes (Anyango *et al*., 1995; Odee *et al*., 2002). The preponderance of *Bradyrhizobium* spp related strains in Mitunguu and *B. elkanii* related strains in Bungoma sites may be attributed to their saprophytic competence at the respective sites (Anyango *et al*., 1995, Batista *et al*., 2006). Results corroborate those of Abaidoo *et al.,* (2002) which showed that TGx varieties in Nigeria were nodulated by *Bradyrhizobium* spp. They also suggest that *Bradyrhizobium* spp, *Bradyrhizobium elkanii* and *Bradyrhizobium japonicum* required for effective nodulation and cultivation of soyabean in Africa are endemic in eastern, western and coastal sites in Kenya.

CONCLUSION

This study has revealed genetic diversity among *Bradyrhizobium* nodulating seven promiscuous soyabean varieties grown at two contrasting sites. Results show that the tested promiscuous soyabean varieties in the two sites are nodulated by populations of *Bradyrhizobium* strains which are genetically diverse and are closely related to *B. japonicum, B. elkanii, Bradyrhizobium spp* and *B. yuanmingense*. The results also showed that the strains were restricted to geographical location and confirm the observation that strains generally develop special phenotypic and genotypic characteristics to adapt to local conditions. This implies that selection of strains for use under certain soil conditions should be isolated from soils with similar characteristics. However, these results need to be confirmed by analysis of a larger sample of strains from more sites in order to fully assess the diversity inherent in Kenyan soils and to select more competitive, efficient and adapted strain(s) at each site for potential use as inoculants to optimize biological nitrogen fixation.

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FORAGE SEED MARKETING IN THE SEMI-ARID KENYA

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ABSTRACT

Inadequate quantity and quality of feed is a major constraint to livestock production in the semi-arid region of Kenya. Feed deficit is mainly attributed to limitations in increasing the area under pastures, lack of good forage varieties, poor management and limited availability of quality seeds thus need to educate farmers on developing their pastures and create awareness on existence of more productive species in order to increase demand. Marketing of forage seed was studied among 71 stockists located in 15 urban and trading centres in the semi-arid region of eastern and southern Kenya between February and April 2011. The objective was to assess the role of stockists in forage seed marketing and future of the industry in the semi-arid region of Kenya. Data collection was through interviews, informal discussion and review of records. Of the 71 stockists interviewed, only about a quarter (26.8%) traded on forage seeds. The proportion of stockists who traded in forage seed was less than 6% in any of the urban or rural trading centres and in five urban centres there were no stockists who marketed forage seeds. Only a paltry 2% had been trained on seed quality aspects implying that their knowledge on seed was poor. Slightly over 80% of the stockists sourced their seeds directly from the seed producer while the rest; had seeds delivered to their business premises by appointed agents who were also stockists. Rhodes grass (*Chloris gayana L.*) was the only grass seed marketed with cv. Boma being the most widely sold. Generally seed sales were low with highest amount sold in October and November with average of 5 kg for each stockist primarily because farmers did not establish pastures. Majority of stockists ranked low seed sale (46.2%) as the most important constraint while a similar percentage ranked high price of seeds as the second most important constraint. The fact that stockists sold small quantities of seed and depended on one grass species in the market is a major constraint to growth of the forage seed industry. For the industry to grow, it is recommended that stakeholders and stockists in particular should market other grass species that are more drought tolerant and productive in the region. There is also need to educate farmers on need to develop their pastures and create awareness on existence of more productive species in order to increase demand.

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INTRODUCTION

Livestock are a major component of Kenya's economy and contribute about 42% of the agricultural Gross Domestic Product (GDP) and 12% of Kenya's total GDP. In the semi-arid region livestock particularly ruminants, are of great importance due to frequent crop failure. They are a source of income and provide milk, meat, manure and draught power for crop production for many communities. Inadequate quantity and quality of feed is a major constraint to livestock production in the semi-arid region of Kenya (Njarui *et al.*, 2011a). Feed deficit is mainly attributed to limitations in the area under pastures, lack of good forage varieties, poor management and limited availability of quality seeds. Past studies have shown that farmers in semi-arid areas have made little effort to improve their pastures (Njarui *et al*., 2011a) and generally establish small acreage for their livestock in about 8% of their total land holding. Further, less than 40% reported having purchased seeds of improved forage varieties (Njarui and Gatheru, 2011). Lack of and the high cost of forage seed are among the major constraints to pasture establishment in the region. Inadequate supply of quality seeds of high yielding forage and fodder has also been cited as a major reason for lack of adoption of pasture grass re-seeding among pastoral communities in southern rangeland of Kenya (Mnene, 2006).

Stockists who operate in major urban and trading centres within the semi-arid areas, are important suppliers of seeds and link farmers with seed producers. They are popularly known as agro-vet because they also trade in other farm inputs. The number of stockists operating in the region has continued to increase after liberalization of the seed sector and abolition of price control of agricultural inputs in 1989 (Muhammad *et al*., 2003). The importance of stockists in marketing of food crops seeds in semi-arid regions is well documented by Muhammad *et. al*. (2003). However, their level of involvement in forage seed trade is not understood. Further, there are limited grass varieties sold in the formal market and there is need to establish the reason underlining this. The objective of this study was therefore to assess the role of stockists in forage seed marketing and make recommendations on the future of the industry in the

eastern and southern semi-arid region of Kenya.

MATERIALS AND METHODS

Study area

The study was carried out across 15 urban and rural trading centres located in the semi-arid region of eastern and southern Kenya. The Arid and Semi-Arid Lands (ASALs) covered in this study stretched from latitude 0°56'S in Mwingi to 3°53'S in Mwatate. The region receives bi-modal rainfall which is poorly distributed ranging from 500 - 900 mm (Jaetzold *et al.*, 2006). Inter-seasonal rainfall variation is large with coefficient of variation ranging between 45-58% (KARI-NDFRC, 1995). Annual evaporation exceeds the amount of rainfall and ranges from 1650 - 2300 mm (KARI, 2001). Mean monthly temperature ranges between 17 - 30°C. Because of low rainfall and high evaporation the potential for crop production in ASALs is marginal but high for livestock production. The altitude ranges from 550 m on the southern region in Voi to 1620 m above sea level on the northern region in Kangundo.

Crop-livestock agriculture is highly integrated in this region with cereals and grain legumes being the major food crops. Different livestock species are kept and among the ruminants are cattle, goats, sheep and donkeys (Njarui and Mureithi, 2006). The major urban centres located in the region such as Machakos, Kitui, Mwingi, Voi, Wote and Wundanyi are important for commerce and are the local headquarters of government ministries and departments. Traders in these urban centres supply most of the agricultural inputs required by farmers. The urban centres are linked by a network of mainly earth roads which are in poor state of repair (Muhammad *et al*., 2003).

Sampling method and data collection

Background information was collected from secondary sources and key informants. To determine a sampling frame, reconnaissance surveys were conducted to establish the number of licensed stockists in different urban and trading centres. The surveys targeted seed stockists since they are known for distribution and marketing of seeds. The stockists interviewed were selected using a proportional random sampling technique.

The sample size was derived using the following formula for finite population size:

$$N*(Z_{\alpha/2} / (2*E))^2 / [N - 1 + (Z_{\alpha/2} / (2*E))^2] <= n$$

Where;
-N is the size of population of stockists in urban and trading centres
-Zα/2 is the critical value, the positive z value that is at the vertical boundary for the area of α/2 in the right tail of the standard normal distribution (Zα/2=1.96 for 95% confidence)
-n is the required sample size
-E is the error margin

The population of stockists in the study area was 102. Using the above formula and an error margin of 5%, a sample of 81 stockists was required for the survey. However, because the survey covered only the stockists who had been in operation for at least 12 months, only 71 stockists were interviewed. A single visit was employed to collect information using a structured questionnaire. Information was collected through direct interviews using memory recall, discussion and review of kept records. Key information collected included; demographic characteristics of the traders, source and type of seeds sold, distribution and marketing. The survey was conducted between February and April, 2011.

Data analysis

Data was coded and stored in a spreadsheet and analysis was carried out using the Statistical Procedures for Social Sciences (SPSS) version 12 for Windows (SPSS, 2002). The results are presented using descriptive statistics, tables and graphs.

RESULTS AND DISCUSSION

Distribution of forage seed stockists

Of the 71 stockists interviewed, about a quarter (26.8%) were involved in trading on forage seeds (Table 1). The proportion was slightly less than that reported by Wanyama *et al*., (2012) in western Kenya. In a survey of 42 stockists, they found out that 33% traded in forage seeds. As shown in Table 1, the proportion of stockists who traded in forage seed were less than 6% in any of the urban or rural trading centres. There were no stockists who marketed forage seeds in five centres. This clearly shows that only a few stockists marketed forage seeds in the region and there is therefore limited accessibility of seeds by farmers. In Ethiopia forage seed marketing is non existent and seed supply mechanism is free, contractual basis or seed revolving mechanism (Dejene *et al*., 2011). The situation is similar in Eritrea, where all the forage seeds are imported and distributed mainly by government public extension services (Njarui *et al*., 2011b). Asked why they did not trade in forage seed, the majority (64.5%) reported that it was because there was little or no demand (figure 1). Low and variability in forage seeds demand was also reported by Hackers and Loch (1997) in Australia. Those who reported that seeds were not available or were either not aware of forage seeds accounted for less than 10%.

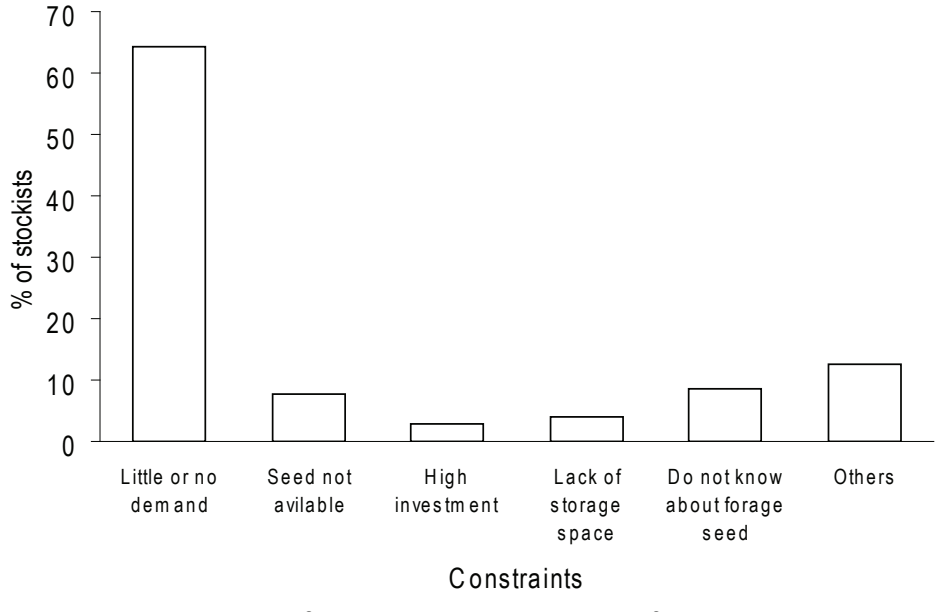

Figure 1. Reasons for stockists not trading in forage seeds in semi-arid region of eastern and southern Kenya

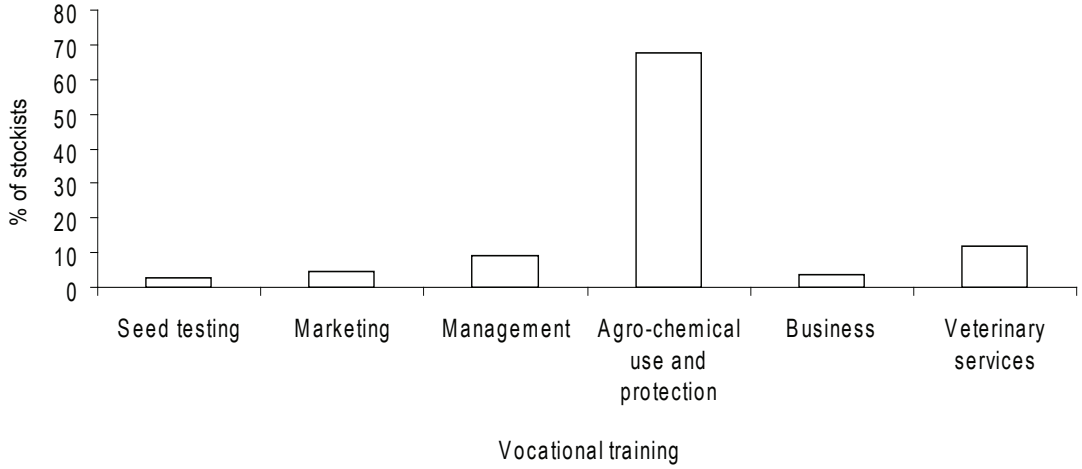

Figure 2. Proportion of seed stocking that have received vocational training

General characteristic of seed stockists

Most of the businesses that traded in forage seed were male owned (83.8%) with only 16.2% being owned by females. Only a few (11.1%) owned the business premises while the rest (89.9%) had rented the premises. All the stockists were literate with majority having attained post secondary education (86.5%), 10.8% secondary and 2.7% adult education. In addition to selling seeds, slightly more than half (53%) were involved in other formal business and 29.4% were engaged in farming. A similar proportion (29.4%) was employed in public or private sector implying that the seed trade was not a full time occupation.

Only 50% had received some vocational training with majority (68%) having been trained on usage of agro-chemicals and protection. Only a paltry 2% had been trained on seed testing implying their knowledge on seed was poor. Likewise only a few had received training in business management implying that their knowledge and skills in marketing of forage was low.

TABLE I- DISTRIBUTION OF SAMPLED STOCKISTS IN MAJOR URBAN AND TRADING CENTRES IN SEMI-ARID REGION OF EASTERN AND SOUTHERN KENYA.

Urban and rural trading centres	County	Latitude	Longitude	Stockists interviewed		Proportion of stockists trading on forage seeds	
				Frequency (n)	%	Frequency (n)	%
Emali	Makueni	2°04'S	37°27'E	5	7	2	2.8
Kangundo	Machakos	1°18'S	37°20'E	4	5.6	1	1.4
Kibwezi	Makueni	2°24'S	37°58'E	3	4.2	0	0
Kitui	Kitui	1°28'S	37°00'E	7	9.9	2	2.8
Machakos	Machakos	1°34'S	37°14'E	9	12.7	2	2.8
Masii	Machakos	1°29'S	37°25'E	4	5.6	2	2.8
Matuu	Machakos	1°08'S	37°32'E	6	8.5	2	2.8
Mwatate	Taita Taveta	3°53'S	38°22'E	1	1.4	0	0
Mwingi	Mwingi	0°56'S	38°03'E	10	14.1	4	5.6
Sultan Hamud	Makueni	2°01'S	37°22'E	3	4.2	0	0
Tala	Machakos	1°17'S	37°18'E	4	5.6	0	0
Voi	Taita Taveta	3°23'S	38°33'E	6	8.5	1	1.4
Wamunyu	Machakos	1°24'S	37°33'E	1	1.4	0	0
Wote	Makueni	1°46'S	37°37'E5	7	2	2.8	
Wundanyi	Taita Taveta	3°03'S	38°31'E	3	4.2	1	1.4
Total				71	100	19	26.8

The stockists operated their business at different levels. About 72% sold forage seed on retail basis while 28% were involved in both wholesale and retail. The stockists were not specialized and had diversified in marketing of other agricultural inputs. Their core business was sale of crop seeds, fertilizer, veterinary drugs, farm implements and agro-chemicals as forage seed accounted for insignificant income of their total sales because of low demand. Further, seeds sale is normally seasonal with most of the sales made mainly during the wet seasons. Lack of specialization has also been reported by Wanyama *et al*., (2012) for stockists in western Kenya. The stockists broadly dealt in similar farm inputs across the region. All (100%) the stockists who traded on forage seeds sold veterinary drugs and agro-chemicals (Figure 3) while close to 70% sold crop seeds and over 80% sold animal feeds. In the semi-arid regions, livestock are virtually kept by all households and thus demand for veterinary drugs is high. A relatively few stockists (5%) traded in pharmaceuticals drugs mainly because sale of pharmaceuticals requires special training and license from Ministry of Health.

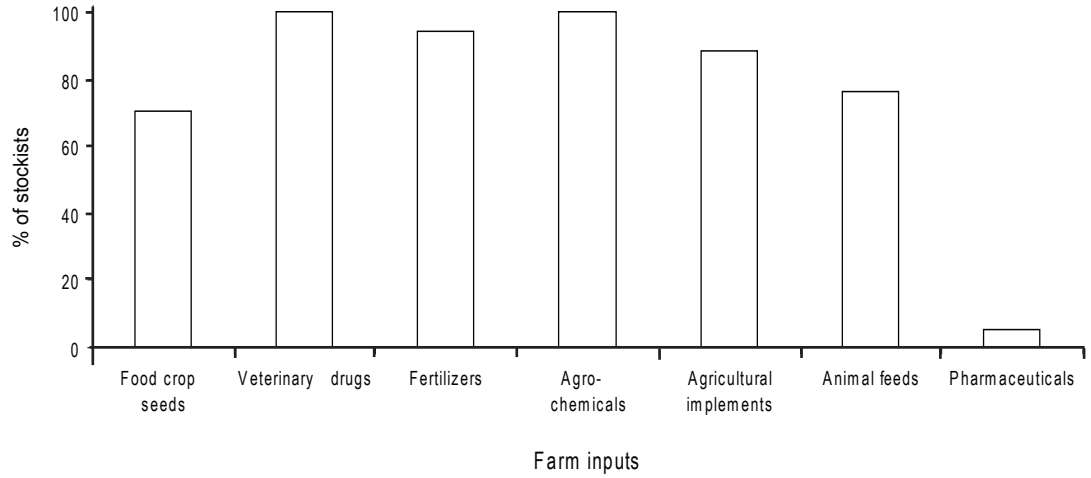

Figure 3. Other farm input sold by forage seed stockists

Experience of seed stockists

Most of the stockists were relatively young in business with majority having operated for less than 9 years. Figure 4, shows that only 2 (11%) stockists were operating between 1976 and 1980. During this period seed trade was controlled and seeds were sold through government appointed agents. The Kenya Farmers Association (KFA) had the monopoly and the main distributor of seed in the country with branches located in major urban centres. From 1996 onwards, more stockists ventured in forage seed marketing following liberalization of the agricultural farm inputs sector in the country in 1989. The government abolished price and other controls on trade in farm inputs. It has also been documented that the large number of seed stockist to trade on seed was as a result of liberisation of the agricultural sector (GOK, 2004). Between 2001 and 2005 a relatively large number of stockists (32.5%) joined forage seed trade and this was attributed to the consistent growth of the agriculture sector during the period. Since then, more stockists have ventured in forage seed marketing.

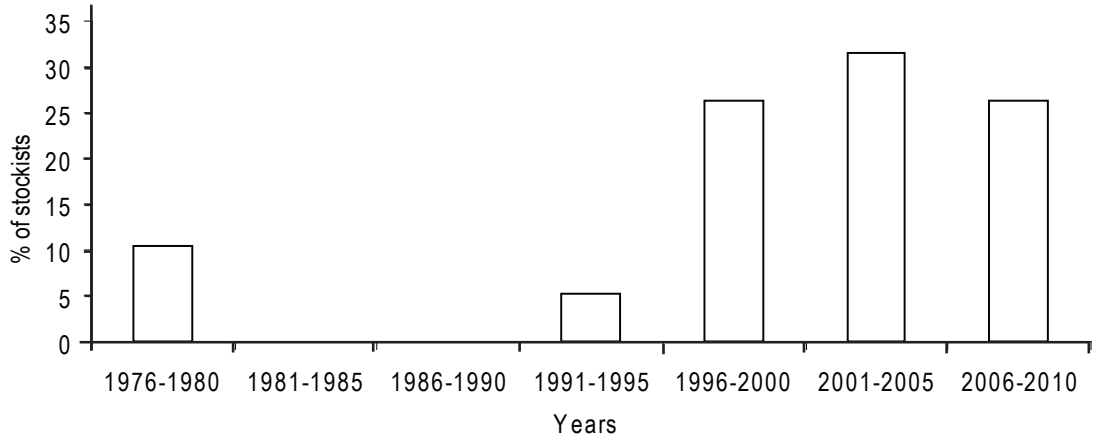

Figure 4. Period the stockists commenced trading in forage seed.

Forage Seed supply

Kenya Seed Company (KSC) is the only major seed producer and sole supplier of forage seed to stockists. Slightly over 80% of the stockists sourced their seeds directly from the seed company while the rest had seed delivered to their business premises by company appointed agencies who were also stockists. There was a large disparity on the quantity of seed sourced from the KSC or agents. The average quantity was 36 kg (range 2-200 kg).

The large quantity was mainly ordered by the larger stockists who operated wholesale and retail basis. Lucerne (*Medicago sativa, L.*) was the only legume traded in the region and was only sold by one stockist who acquired about 2.25 kg from the company although not on regular basis.

The stockists encountered several constraints while acquiring the seed from their suppliers. Majority (31%) pointed out that high price of seed was the major drawback followed by long distance to source the seed (22%). Normally the price of 1 kg of Rhodes seed was sold to them at between KES 400-500 compared to less than KES. 100 for price of the maize. Depending on the location of the stockists the distance ranged from 65 to 350 km away and was therefore a major constraint. However, although there were appointed agencies of KSC, they were not efficient in delivering the seed to other stockists.

Distribution and marketing of forage seed

Although farmers can purchase seed directly from the company warehouses in Nairobi, the stockists were the link between the producers and the farmers. The KSC markets its seeds through a network of stockists operating in urban centres and in the local trading centres. None of the stockists re-package the seed as it is illegal and re-packaging would undermine customer confidence. Further, the seed sold was usually available in 1 kg packet which was convenient for farmers.

Rhodes grass seed was the only grass seed being marketed with cv. Boma and Elmba being the only varieties marketed. Approximately 70.6% sold cv. Boma only, 5.9% cv. Elmba while 23.5% traded on both cultivars. Only one (5.3%) stockists traded on Lucerne and sales were restricted in Taita Taveta where it is wetter. These finding differ from those of Wanyama *et al.* (2011) who found that in the Rift valley region, stockists sell several different species of forage seed. Lucerne is not suitable

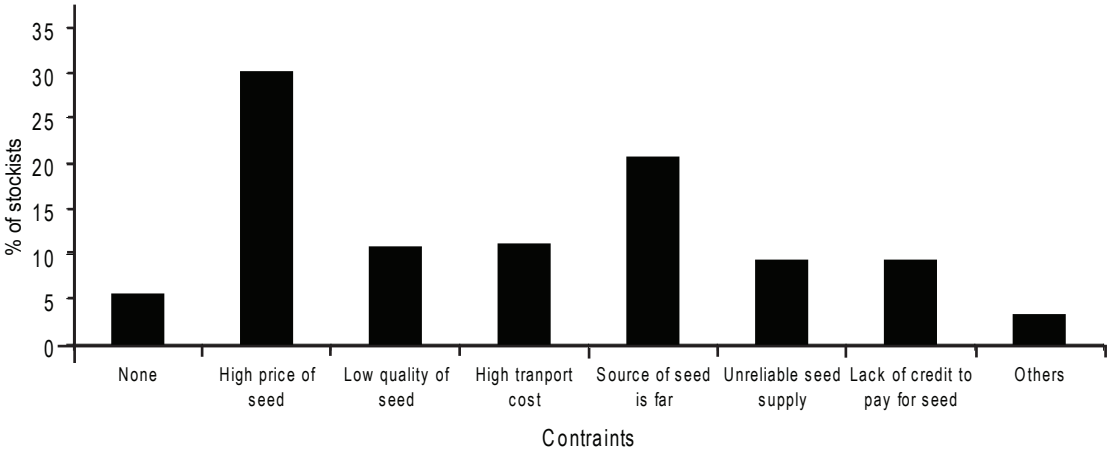

Figure 5. Constraints encountered by stockists in acquisition of seed

for the semi-arid region unless under irrigation thus sales were confined in hilly masses. Individual farmers were the major buyers of forage seed and absorbed slightly over 86% of the forage seed sold by stockists (Figure 6).

The demand for seeds was low and consequently the volume of seed sold by individual stockists was low. However, the quantity sold was highly influenced by the rainfall pattern with more sales recorded during the short rains (Oct/Nov) than in the long rains (Mar/Apr). In October and November, on average each stockist sold about 5 kg compared to less than 1 kg in March and April. In the semi-arid areas, the short rains are more reliable for pasture establishment than the long rains. For obvious reasons no seed were sold during the dry season.

Only a few farmers establish a limited acreage of pasture for their livestock thus purchase little quantity of seeds. Livestock in the region generally depend on the indigenous pastures. Surprisingly, about a third (35.3%) of stockists reported that in most seasons they did not meet the demand of their customers. Consequently, there is need to improve the availability of seeds. When asked whether they were aware of the most suitable grass for their region, 55.6% indicated that they knew while 47.1% did not. Nevertheless, Rhodes grass, the only grass sold by stockists is not suitable in Lower Midland 5 agro-ecological zone where some of the stockists were located. Rhodes grass thrives best in areas receiving over 800 mm of rainfall with a relatively short dry period.

The price of seed fluctuated between seasons and it was usually highest in wet and lowest in the dry seasons. During the LR wet season (March and April) and in SR (Oct/Nov) seasons the price usually averaged KES 650 / kg of Rhodes grass but declined to KES. 500 during

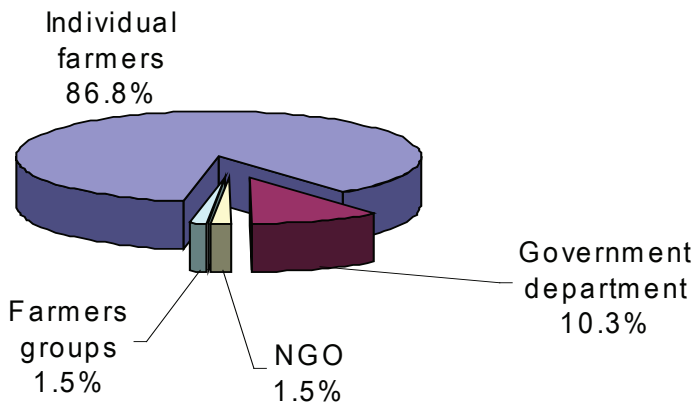

Figure 6. Market outlet for forage seed

the other months. About 71% of stockists sold the seeds according to the recommended price while the rest (29%) adjusted the price depending on marketing costs.

Policy and Government regulation

The largest proportions (94.4%) of stockists were aware of government regulation on seed marketing. Some of these government policy regulations included the need to indicate the expiry date on seed packets, it is illegal to re-package the seed, requirement to sell only certified seed and have a valid license to stock and trade on seeds. Nevertheless, a relatively high proportion (66.7%) indicated that the enforcement agent inspected their seed for truthful labeling.

Seed regulation is meant to protect consumers and to promote a responsible industry (Tripp, 2000). The Kenya Plant Health Inspectorate Services (KEPHIS) which is a government agent is mandated to regulate the quality of seed being sold in the country. The agent also licenses seed traders interested in production of seeds. There is an act for regulation and control of production, processing and marketing of released varieties (Anon, 2002). They set seed quality standards in order to ensure production, packaging and sale of high quality for released varieties. However, in some instances enforcement has been weak because of limited staff and distance. Some of the stockists indicated that they were not aware of the regulation laws implying that KEPHIS does not carry out enforcement in all the areas.

Challenges and future of the forage seed industry

Stockists faced several challenges in marketing of seed (Table II). Majority of stockists ranked low seed sale (46.2%) as the most important constraints while a similar percent ranked high price of seeds as the second most important constraints. As a result the profit margins were very low. In western Kenya, low seed sales and seasonality in demand were the first and second major drawbacks in growth of the industry (Wanyama *et al*., 2012). Despite these constraints, over 77.8% of stockists reported increased sales between 2006 and 2010 compared with 5.6% who reported that sales were declining. About 11% of the respondents reported that

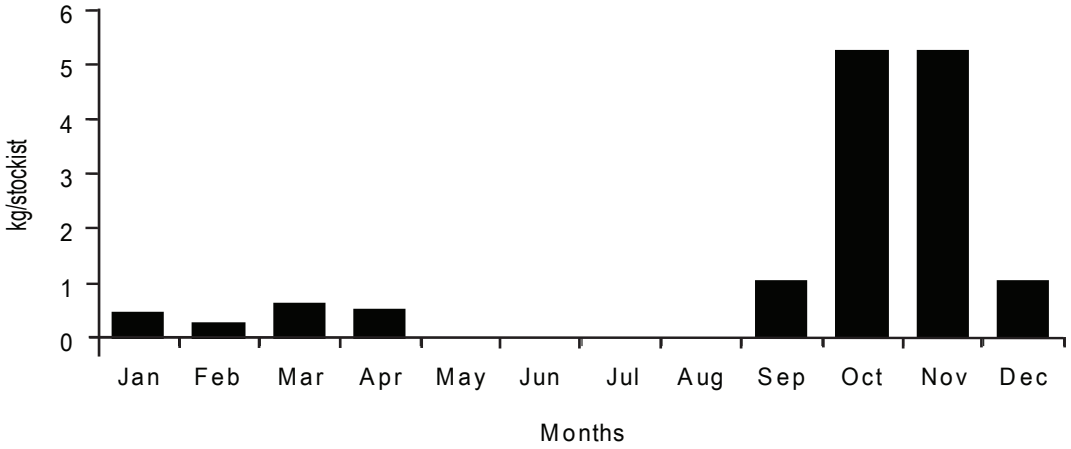

Figure 7. Average quantity of grass seed sold by stockist by month in the semi-arid region of eastern and southern Kenya

TABLE II- CONSTRAINTS IN FORAGE SEED MARKETING BY STOCKISTS IN SEMI-ARID REGION OF EASTERN AND SOUTHERN KENYA

Constraints	% of farmers for the ranks				Overall rank	Total farmers responded
	1	2	3	4		
Competition from others	36.4	45.5	9.1	9.1	4	11
Low sale of seed	46.2	23.1	23.1	7.7	1	13
Seasonality in demand	12.5	18.8	43.8	25.0	3	16
Low viability of seed	25.0	0	50.0	25.0	6	4
Inappropriate packaging	10	10	20	60	5	10
High price of seed	30.8	46.2	15.4	7.7	2	13

sales fluctuated within the years and 5.6% reported that seed sales did not change within the last five years.

Asked what was needed to stimulate the growth of the seed industry, 48% of the stockists reported that there was need to provide information on performance of the various forages species to the stockists which they could in turn pass to farmers. About 13% stated that credit facilities would help their business to grow while close to 20% reported that reducing price of seed was important. Muhammad *et al*.(2003) found out that 92% of seed stockists passed on information on suitable seed varieties to their customers. The price of seed was high and there is need to reduce the cost of seeds and improve supply. The fact that stockists sold small quantities of seed and only one grass species was available is a major constraint to development of the forage seed industry. Further Rhodes grass is not well adapted to semi-arid condition that receives low rainfall. There is need to identify and develop other grass species that are more drought tolerant and productive. The Kenya Agricultural Research Institute which has the mandate of carrying out pasture research in the country should embark on breeding work to identify more adapted and productive forages for the region. Nevertheless, a few ecotypes from the genus Cenchrus, Eragrostis and Panicum have been identified for semi-arid areas but no cultivar has been released for commercial production. There is also need to create awareness and educate farmers on existence of better and productive forages species and on importance of developing their pastures using improved grass varieties so that demand for seed could be met.

CONCLUSION

The study indicated that stockists are an important link between farmers and seed producers. Seed sales were generally low and were limited only to one grass species. To strengthen the growth of seed industry there is need to select and release drought tolerant forages suitable for the semi-arid region for commercial production. There is also need to educate farmers on the need to improve their pastures in order to create more demand for seed.

ACKNOWLEDGEMENT

The authors are grateful to the stockists who participated in the survey and the staff of Kenya Agricultural Research Institute (KARI), Katumani for administering the questionnaires. They also extend their gratitude to the Director, KARI for the support. This study was funded by the Association for Strengthening Agriculture in Eastern and Central Africa (ASARECA). The views expressed in this report are not necessary those of ASARECA.

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EFFECT OF DIFFERENT CONSERVATION TREATMENTS ON SURVIVAL OF POTATO (*SOLUNUM TUBEROSUM*)

Journal Brief

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ABSTRACT

Limited supplies of disease free nuclear seed stocks for further multiplication is a serious obstacle to further development of the potato industry in Kenya. A nucleus set of disease free and true to type cultivars must therefore be conserved to ensure regular supply of disease free planting material to the industry. The objective of this study was to establish an *in vitro* germplasm collection of potato using slow growth *in vitro* techniques. The conservation media consisted of Murashige and Skoog salts with normal vitamins, 8 g/l agar supplemented with 40 g/l mannitol, 40 g/l sorbital with and without GA3, incubated at 20 ± 20C under a 16 h photoperiod with 3,000 lux. Ten potato accessions were assessed for the survival rate of meristems, slow growth media and *in vitro* viability after 14 months of conservation. The results showed that 60-70 % of the meristems per genotype survived to form plantlets. Mannitol and sorbital at 40g/l with 0.001g GA3 provided the best plantlet survival rate of 80-90% after 14 months of conservation with high regeneration rates. Conservation media consisting of mannitol and sorbital at 40g/l but without GA3 had plantlets with a survival rate of 55-60% after 14 months of conservation with high regeneration rates and can be used for slow growth *in vitro* potato conservation.

Key words: *In-vitro* conservation, disease free nuclear seed stocks, potato

INTRODUCTION

A fledgling potato industry such as the one in Kenya requires ready availability of varieties upon demand. Since the cultivated potato (*Solanum tuberosum L.*) is highly heterozygous, potato varieties are generally maintained through vegetative propagation instead of true (botanical) seeds because sexual reproduction leads to segregation of the variety. Conservation and maintenance of potato germplasm in field repositories is, however, cumbersome since the germplasm must be grown continuously or replanted frequently. Accessions in field repositories are also exposed to hazards such as outbreaks of pests, diseases, drought and other natural calamities (Espinoza *et al.*, 1989). The virus load increases inside the tubers after every generation in the field, which results into yield reduction (Djurdjina *et al.*, 1997, Reza *et al*., 2007).

In vitro conservation of germplasm is considered an efficient way to conserve potato germplasm (Espinoza *et al*., 1989; Gopal *et al*., 2009), since the plant materials maintained are homogeneous, and their pathogen states are tested, thereby facilitating safer distribution. Further, the cultures are not subjected to environmental disturbances (Withers and Engelmann, 1997). Reduction in the frequency of sub-culturing is achieved by growing the plantlets on MS (Murashige and Skoog, 1962) medium supplemented with growth retardants or osmotic stress inducing polyols and incubating them under low temperature, low light intensity, and varied photoperiod (Lopez-Delgado *et al.*, 1998; Gopal *et al*., 2002).

At the Kenya Agricultural Research Institute-Tigoni, more than 60 advanced potato lines and 40 potato varieties are maintained under field conditions. Potato tubers are usually stored at 2 to 5 ºC and a relative humidity (RH) around 70% in the dark for up to 6 months. Some of these materials are routinely maintained under tissue culture conditions on Murashige and Skoog (1962) media supplemented with glycine 0. 2 g /L, nicotinic acid 0.05 g/ L, pyridoxine 0.05 g/L, inositol 10 g/l, thiamine 0.01 g /L, gibberellic acid 0.001 g/L, and sucrose 30 g /L with sub-culturing performed every 3-4 weeks. There is, however, little information on how various varieties respond to different in vitro conservation media. The objective of this study was to establish an *in vitro* germplasm collection of potato using slow growth *in vitro* techniques based on the osmoticums (mannitol and sorbital).

MATERIALS AND METHODS

The study was carried out in the plant tissue culture laboratory at KARI-Tigoni between January 2009 and November 2010.

Stage 1: Meristem culture-Virus elimination

Virus infected tubers of ten potato cultivars (Kenya Karibu, Arka, Asante, Tana Kimande, Dutch Robyjn, Kenya Sifa, Kenya Baraka, Anett, Sterling and Tigoni)

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were first established under screen-house conditions. Upon attaining a plant height of 15-20 cm, the plants were subjected to thermotherapy (37ºC for 16 h and 30ºC for 8 h, light intensity of 5000 lux and a RH of 80 ± 5%). After four weeks, the developed upper ends were cut off and taken to the laboratory where they were cut into nodal segments (0.4-0.5 cm each). These nodes were washed using 70% sodium hypochlorite for 5 minutes followed by four washings with sterile distilled water to remove excess hypochlorite. A microscope was placed under the laminar hood, where the nodes were cut and the meristem excised and transferred into 20 mm universal bottles containing 5 ml medium (MS salts, glycine 0.2 g /L, nicotinic acid 0.05 g/ L, pyridoxine 0.05 g/L, thiamine 0.01g mg /L, gibberellic acid 0.001 mg/L, kinetin 0.001 mg /L, Inositol 10g/l and sucrose 2.5 g /L) .The pH of the media was adjusted to 5.8 ± 0.1before the addition of 0.8% agar, which was then covered using aluminium foil and autoclaved at 121^{0}C for 15 min. From each potato cultivar, 20 meristem cultures each measuring 0.01-0.03 mm were obtained. The meristem cultures were then transferred to a growth chamber at 22 ± 2ºC and 3000 lux illumination. After 4 months, the cultures were transferred to larger jars containing MS media containing normal vitamins, 30 g sucrose and 8 g agar for further growth.

Stage 2. Potato germplasm conservation

Treatments consisted of ten varieties (Kenya Karibu, Arka, Asante, Tana Kimande, Dutch Robyjn, Kenya Sifa, Kenya Baraka, Annet, Sterling and Tigoni), and four conservation media (MS + mannitol + GA3, MS+ mannitol without GA3, MS + sorbitol + GA3 and MS + sorbitol without GA3). The treatments were laid out in a factorial randomized complete block design replicated four times. Ten universal bottles per experimental unit were set up. Agar (8%) was used as the solidifying agent for all media. Each universal bottle consisted of 10 ml of conservation media (pH 5.8). After 14 months of conservation, the cultures that had withstood slow growth were transferred to the propagation medium described below.

Stage 3. *In vitro* viability after 14 months of conservation

Nodes obtained after 14 months of storage were cultured on Murashige and Skoog media with normal vitamins supplemented with 0.001 GA3 and 0.001 Kinetin, 30 g sucrose 8 g, PH adjusted to 5.8 and 8 g agar incubated in a growth chamber at 22^{O}c under 16hrs photoperiod provided by fluorescent white light of 3000 lux for four weeks and data was taken on the percentage of survival.

Data collection and analysis

Data collected included survival of meristems (%), survival of *in vitro* plantlets after 14 months of conservation (%), and viability of conserved plantlets after 14 months of conservation. Data were subjected to analysis of variance using Genstat 11.1 software. Differences among the treatments means for all data were compared using Fischer's protected least significant differences (LSD) test at $P<0.05$.

RESULTS AND DISCUSSION

Survival of meristems

The protocol in stage 1 permitted the survival and growth of all 10 potato cultivars after 4 months of incubation. Table I shows that 50-70% of the meristems were able to regenerate shoots which were ready for transfer in the single node cuttings for further multiplication. Varieties Tigoni, Asante and Sterling had the highest % regeneration rate among the 10 potato cultivars evaluated (Table I). Kenya Karibu, Kenya Baraka and Kenya Sifa had the highest number of cultures that formed callus having a percentage mean value of 23.7, 23.3 and 26.0 %, respectively. Minimal contamination was observed among all potato cultivars ranging from 1-3 culture.

TABLE I - PERCENTAGE REGENERATION 3 MONTHS AFTER MERISTEM EXCISION OF 10 POTATO CULTIVARS.

Varieties	Total no of cultures	Survival No. of cultures	%
Kenya Karibu	20	13.7	68.3
Arka	20	13.3	66.7
Asante	20	14.3	71.7
Tana Kimande	20	12.7	63.3
Dutch Robyjn	20	13.7	68.3
Kenya Sifa	20	12.3	61.0
Kenya Baraka	20	12.1	60.0
Annet	20	12.7	63.3
Sterling	20	14.0	70.0
Tigoni	20	15.3	76.7
Grand mean		12.1	66.9
LSD(p=0.05)		2.8	13.5
C.V%	10.9	12.1	11.7

The results in Table II reveal that the stored potato cultures remained healthy without any serious signs of senescence during storage period. The highest survival rate (86.7%) was observed on two varieties (Annet and Kenya Karibu) when the explants were cultured in Sorbital with GA3, while the lowest plantlet survival (60.0%) was observed on variety Tigoni cultured in Mannitol alone. Sorbital+GA3 and Mannitol +GA3 had the highest % mean value of 82.78 and 81.67 respectively but not significantly different from each other, but they were significantly different from Mannitol and sorbital. Mannitol and Sorbital were not significantly different

from each other having a percentage mean value of 67.33 and 67.44 %.

Table III shows that it is possible to store *in-vitro* potato plantlets for 14 months with a high survival percentage after recovery, regardless of the storage treatments. The highest percentage of survival cultures were regenerated from conservation treatments Sorbital+GA3 and Mannitol +GA3 having a percentage mean value of 60.2 and 61.5%., whereas, Mannitol and Sorbital conservation agents were not significantly different from each other and the percentage mean value was 40.3 and 42.7%.

The observations are in agreement with earlier works by Desamero, 1990; Acedo, 1993; and Guo *et. al*.1995, who reported that sweet potato in 10-40 g/l of mannitol or sorbital effectively extended the interval between subcultures to one year at 22°C and the cultures resumed growth when placed in culture medium without osmoticums. In addition, *in vitro* shoot cultures of asparagus survived for 20 months when stored on medium containing 3% sucrose and 4% Sorbitol (Fletcher, 1994).

TABLE II - EFFECT OF DIFFERENT CONSERVATION TREATMENTS ON SURVIVAL (%) OF 10 POTATO CULTIVARS UNDER CONSERVATION

	Conservation treatments			
Potato cultivars	Sorbital	Mannitol	Sorbital+GA3	Mannitol+GA3
Kenya Karibu	61.1	66.7	86.7	83.3
Arka	65.6	65.6	80.1	81.1
Asante	67.8	65.6	84.4	81.1
Tana Kimande	70.1	73.3	81.1	82.2
Dutch Robyjn	68.9	67.8	86.2	82.2
Kenya Sifa	71.1	64.4	82.2	80.0
Kenya Baraka	74.5	74.4	82.2	84.4
Annet	67.8	68.9	86.7	81.1
Sterling	61.1	67.8	82.2	81.1
Tigoni	67.8	60.0	81.1	80.0
Mean	67.3	67.4	82.8	81.7
C.V %	7.2	6.6	3.0	3.1
L.S.D P=0.05	8.3	7.6	4.3	4.3

CONCLUSION AND RECOMMENDATION

It can be concluded that the medium used for potato germplasm conservation is an efficient method since viability of the mother plant was maintained for up to

CONCLUSION AND RECOMMENDATION

TABLE III - EFFECT OF THE CONSERVATION TREATMENTS ON % SURVIVED CULTURES OF THE 10 POTATO CULTIVARS AFTER RECOVERY

	Conservation treatments			
Potato cultivars	Sorbital +GA3	Mannitol+GA3	Sorbital	Mannitol
Kenya Karibu	70.0	73.3	56.7	53.3
Arka	80.0	63.3	40.0	56.7
Asante	70.0	90.0	66.7	43.3
Tana Kimande	56.7	76.7	60.0	46.7
Dutch Robyjn	59.3	45.7	45.0	21.3
Kenya Sifa	55.0	46.3	25.3	45.3
Kenya Baraka	52.7	52.3	24.5	38.3
Annet	52.3	55.3	34.7	26.0
Sterling	63.3	62.3	52.3	34.3
Tigoni	42.7	49.3	21.7	37.3
Mean	60.2	61.5	42.7	40.3
L.S.D p=0.05	41.7	31.4	29.3	30.0
CV%	22	29.7	25	18

16 months. However, agar and phytagel are commonly used gelling agents while sucrose is the carbon source used in potato micro-propagation and they are expensive therefore, further research is required to explore the low cost alternatives to reduce the cost of micro-propagation with extension to *in vitro* conservation protocol.

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