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Full Length Research Paper

A study on the variation between provenances of *Sclerocarya birrea* sub-species caffra and seed mass of dried fresh seed

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Sclerocarya birrea **(A. Rich.) Hochst. subsp. caffra (Sond.) Kokwaro (Marula) is one of the traditionally important indigenous multi-purpose fruits trees in Africa, which has been commercialized as its fruits and processed nuts/kennels have entered local, regional and international trade. This study was an analysis of the variation between and within provenances of S. birrea sub-species caffra with respect to seed mass of dried fresh seed, total soluble solids (total sugars) content of fresh fruits, and the vitamin C content of fresh fruits as well as family heritabilities of fruit parameters and seed mass. A nested sampling procedure was followed for where there were four areas (provenances), eight to ten families (parent trees) and fifty fruits per family. There were significant differences in provenance and family in seed mass (p=0.06 and p=0.0001) and vitamin C (p=0.001 and p=0.0001), total soluble solids lacked provenance variation (p=0.35) but had wide family variation (p=0.0001). There was no correlation between parameters except a significant correlation between rainfall intensity and total soluble solids content; lower the rainfall higher the sugar content and the higher the rainfall the lower the sugar content (p=0.06). The most variable parameter was vitamin C with the highest family heritability, however all traits had relatively high family heritabilities (above 0.5). It was concluded that the results of fruit composition and seed mass could be of remarkable importance in the identification of superior genotypes, making use of the wide family variation that exists. A study to capture a wider range of provenances and families including studying the environmental parameters of soils, rainfall and altitude for more precision.**

Key words: Traits, parameters, correlation, variance.

INTRODUCTION

Sclerocarya birrea subspecies caffra (Marula) is a widely distributed species found throughout the semi- rid, deciduous savannas of much of southern and East Africa (Peters, 1988; Dlamini, 1998; Dlamini, 2010). The fruit, seeds, leaves, wood and bark of this species have been used extensively by rural communities for many hundreds of years in most countries in which it is found. Probably one of the most important uses of this multi-purpose tree has been, and still is, the production of an alcoholic beverage (commonly known as wine or beer) from its fruit. Evidence indicates that this brew was made as long ago as 9000 BC after piles of marula nuts were found in caves in the Matobos Hills in Zimbabwe (Walker, 1989). Other important uses include the consumption of fresh fruit and kernels, the extraction of oil from the kernels for a variety of purposes from cooking to cosmetic (although the oil is not used in the study area - Bushbuckridge), the harvesting of leaves and bark for traditional medicine, and the use of wood for fuel, fencing and carving (Dlamini, 1998; Shackleton et al., 2002; Dlamini and Geldenhuys, 2009; Dlamini, 2010).

Variation in seed mass

A positive relationship between seed weight and amount of food stored in the seed storage tissue has been demonstrated in numerous plant species. A large seed normally indicates a large endorsperm, or a large embryo and cotyledons. In albuminous seed, the endosperm is the reservoir of energy necessary to facilitate the germination process and in exalbuminous seeds such as those of Fidherbia albida (Marunda, 1993; Dlamini, 1998; Dlamini, 2010). In most tree species need size has been found to be strongly correlated with germination rate and seedling size, however, the advantage could be short-lived depending on the species. Marunda (1993) reported that for Acacia nilotica, F. albida and Azandirachta indica, the ease of germination did not vary with seed weight, but early seedling growth did, more in particular height increment. Similarly, Shivkumar and Benerjee (1986) and Dlamini (1998) revealed the provenances of Acacia nilotica with bag seeds produced plants which were strong growers (tall and thick) while smaller seed germinated with difficulty and germination and early seedling growth were slower.

Seed mass is thought commonly to be an important focus of selection in life histories of plants (Harper et al., 1970; Janzen, 1977; Dlamini, 1998), because the likelihood of dispersal (Howe and Vande Kerckhove, 1980), germination (Putievsky, 1980), and survival (Black, 1957; Schaal, 1980) can all depend on seed mass or size. As a consequence, demographic patterns within plant populations may result partly from the distribution of seed masses in the seed pool. Nonetheless, little is known about variation in seed mass within and among individuals within natural plant population. Studies of seed mass have usually been based on variation between lots of a given number of seeds (often 100) rather than on individual seed masses (Cawers and Harper, 1966; Twamely, 1967; McWilliams at al., 1968; Maun and Cavers, 1971; Baker, 1972; Schimpf, 1977; Bentley et al., 1980; Thompson, 1981; Dlamini, 1998). Such analysis can be used in identifying differences in means between treatments in an experiment and between populations. The few studies of variation in individual seed mass based on large samples in natural population have shown variation up to 5.6 fold (Janzen, 1977; Janzen, 1978; Schaal, 1980; Dlamini, 1998). These values for variation provide an estimate of the range of plasticity in seed mass within a population, but are for plants with varied (uncontrolled) histories. There appears to be no estimated, however, of the magnitude and partitioning of variation in individual seed mass within and among total seed crops of uncultivated plants grown from seedlings under similar conditions, which would provide a baseline for evaluating variability in seed masses in natural plant population. A study by Thompson (1984) on the variation among individual seed masses in Lamatium grayi (Umbeliferae) under controlled conditions show much more variability in individual seed masses than indicated by the common ecological impression that seed masses tend to be fairly constant.

Chemical composition of fruit chemical composition

Information on the chemical composition of indigenous fruits in eastern and southern Africa has been scarce and limited until recently (Wehmeyer, 1966; FAO, 1983; Saka, 1994; Dlamini, 1998). A study of the chemical composition of edible indigenous fruits in Malawi was initiated by Saka (1994). The vitamin C of 28 fruits tree species was analyzed and some results were published (Saka, 1994). From these findings it was concluded that the pulp of the fruit and the seed kernel of some species contain vitamin C levels comparable to those of exotic and domesticated fruits (Williamson, 1975; Wills et al., 1983). Among the fruits analyzed Adansonia digitata, Diospyros usamberensis, Bauhinia thonningii and Vitex payos contain about 70 mg per 100 g fresh weight and are thus excellent source of this vitamin. D usamberensis had the highest levels of vitamin C at 337 mg per 100 g. The concentration of ascorbic acid (Vitamin C) is higher when the fruit is harvested in June. Saka (1994) produces a summary of the vitamin C content of 28 different species where a wide variation in vitamin C content between species of the same genus was seen. The concentration of other important vitamins such as A and B has not been analyzed; however, a study was recently started at the University of Malawi by Saka (1994). The seed kernels or nuts of numerous fruits species in Southern Africa are edible and from an important role in the diet of moral people (Shone, 1979; A-Ogle, 1990; Taylor et al., 1995; Dlamini, 1998; Dlamini and Geldehuys, 2009; Dlamini, 2010). For example the edible nuts of such species as A. digitata, Telifania pedata, Terminalia catappa, Treculia Africana, Parkia filicoredea and Parinari curatellifolia are a good source of vegetable oil and are rich in protein. The seed kernel of A. digitata has been analysed for proximate composition by Saka (1994). Mwamba (1989, 1994) studied variation in fruit composition of Uapaca Kirkiana and effects of in situ silvicultural treatments. The parameters studies were fruit colour, fruit size and yield, skin %, seed %, pulp weight and total soluble solids concentration. The findings revealed that all other parameters are physiologically controlled and can be silviculturally altered except fruit colour.

Objectives

1. To study genetic variation in seed mass and fruit composition between and within provenances of *Sclerocarya birrea* sub-species caffra in four areas in Swaziland.
2. To study genetic variation, between and within provenances, in fruit composition;
3. To study genetic variation in seed mass between and within provenances;
4. To study correlation between traits (height and root collar diameter);
5. To study correlation between mean annual rainfall and the total soluble solids and vitamin C content;
6. To study correlation between seed mass and altitude;
7. To correlation between mass and vitamin C content;
8. To study the variance components for formulation of family heritabilities in sugars, vitamin C and seed mass.

Table 1. ANOVA for Total Soluble Solids (TSS), Seed Mass (SM) and Vitamin C (VTC).

SOV	Mean Squares (MS)			
	Df	TSS	SM	VTC
Provenance	3	97.5 NS	93.1*	1200465.8**
Fam (Provenance)	33	86.2***	35.6***	184867.7***
Error	1812	1.39867	0.49100	7225.60
Total	1848			

NS=not significant, *=significant at 0.05 probability, **=significant at 0.001, ***=significant at 0.0001.

MATERIALS AND METHODS

Study area description

Fruits of four different provenances that were at least 100 km apart were harvested from Swaziland. Swaziland is one of the smallest countries in Africa (17 364 km²), yet it has a remarkable geographic variability with six clearly defined physiographic zones, each with distinct vegetation and climatic features. In this study the fruit collection sampled a wide range of altitude and latitude. The altitude ranged from 340 to 500 m and the mean annual rainfall ranged from 500 to 750 mm (Dlamini, 1998).

Experimental design

The design of the experiment was a double nested sampling with trees used as repetitions for area, and fruit/seed used as repetitions for trees. This implies that parent trees were nested in provenances and individual fruits were nested in parent trees. There were four provenances from which eight to ten parent trees were identified, and in turn 49 to 51 fruits were selected per tree and analyzed for Total soluble solids and seed mass. In the case of vitamin C analysis instead of having 50 fruits analyzed there were only three composite fruits samples analyses per tree (Dlamini, 1998).

The field sampling procedure had two levels of randomization. The first level was random selection of trees in the provenances and the second level was random selection of fruits form trees. The same procedure was followed in the laboratory analysis (Dlamini, 1998).

Field and laboratory work

In each provenance eight to ten trees that were at least 150 m apart were identified and ripe fruits were picked from each. Specially made 4 m wattle rods were used to shake branches of the selected Marula trees to shed fruits on to the ground. Shaking was done all around the tree to ensure maximum coverage to obtain a relatively representative sample of the whole tree. A random sample of 360 fruits was picked from the ground out of which only a further random sample of 50 fruits was picked as a working sample (Dlamini, 1998).

Fruit collection was followed by cleaning of fruits before storage in the fridge for a least a week before analysis. Fruits were then analyzed for their composition specifically in total soluble solids (mainly sugars) and ascorbic acid (vitamin C).

Determination of total soluble solids was done through a hand refractometer (Type A.S.T. Japan 0-32).

Vitamin C was determined by the AOAC Method no. 967.21 from the Association of Analytical Chemists, as described by Kirk and Sauyer (1990). Seed mass was measured seed by seed using the “Mattler pm 300”.

Statistical analysis

The analysis of variance was performed using SAS statistical software version 6.12 (SAS Institute INC, 1997). The Shapiro-Wilk statistic was performed to test for normality of the data (Shapiro and Wilk, 1965). The data was considered normally distributed as W > 0.95 and p > 0.05. All significance tests were computed at the 5% level of probability. Duncan’s Multiple Range test was used to compare treatment means. Correlation analyses were undertaken following Pearson Correlation Coefficients to study the relationship between the parameters: seed mass, vitamin C content, total soluble solids, altitude and rainfall (p=0.05).

Variance components estimates

The variance components, following the analysis done for a two factor experiment (n observations per cell) with factor B nested in factor A, were used to estimate heritability for fruit parameters as described by Ott (1993)

Analysis of variance components for the differences between provenances and between families within provenances, assumed fixed effects for provenances and random effects for families within provenance, and used the mixed model.

RESULTS AND DISCUSSION

Total soluble solids

The analysis of variance indicated no significant differences in the total soluble solids content between provenances (p=0.35). Similar results were found by Mwamba (1989, 1994) in a variation in the fruits of Uapaka kirkiana. Families within provenances were significantly different (p=0.0001) (Table 1). Significant differences existed between individual families ignoring provenances (p=0.0001), in total soluble solids (Table 2). Some families within provenances were more variable in the total percentage sugar content compared to others, those of Dvokolwako showing the highest variance. Family D3 of Dvokolwako had the highest mean of 14.11% and family D6 of Dvokolwako had the lowest at 7.65%. This implies that the differences between families were not due to the localities. The average annual rainfall of the area of origin of the parent trees appeared to have had some influence on the percentage sugar content of the fruits: this may mean only that the water in the fruit may be more and the amount of sugar per fruit may be

Table 2. ANOVA for TSS, SM and VTC for 37 families ignoring provenances.

SOV	Df	MS TSS	MS SM	SOV	Df	MS VTC
Family	36	87.1***	40.4***	Family	36	269500.0***
Error	1812	1.3	0.4	Error	74	7225.6
Total	1848			Total	110	

***=significant at 0.0001 probability.

Table 3. Provenance mean Vitamin C and seed mass.

Provenance code	Mean SM (g) ±Std Error	Provenance code	Mean VTC (mg/100 ml juice) ±Std Error
Lungungu (4)	4.1±0.06 ^{a}	Kalanga (1)	694.2±48.33 ^{a}
Kalanga (1)	3.9±0.04 ^{ba}	Lungungu (4)	625.3±56.24 ^{a}
Dvokolwako (2)	3.9±0.05 ^{ba}	Dvokolwako (2)	539.2±53.60 ^{a}
eBuhleni (3)	3.1±0.04 ^{ba}	eBuhleni (3)	224.6±19.41 ^{b}

NB. Means with same letter are not significantly different according to the Duncan's new multiple range test.

less with less rain. The provenance with the lowest rainfall (Kalanga) yielded the highest total sugar content, while the area with the highest rainfall (Lungungu) yielded the lowest total sugar content. There was no clear trend between the altitude of origin of parent trees and the total sugar content, for example, in this study areas lying on the same altitude gave different total sugar contents; Kalanga and Dvokolwako are 340 m above sea level, yet they yielded different total sugar content.

Total soluble solids (sugars)

There were significant differences between provenances in the sugar content of the fruits sampled, with the highest with 11.6% and the lowest with 10.6%. However, there were significant differences (p=0.0001) between families. The highest family had 14.11%, which was highly significant from the lowest family which had 7.65%, and these coincidentally come from the same prove-nance (Dvokolwako). Similar results were found by Mwamba (1994) in Uapaca kirkiana. According to Barker and Taylor (1982) total sugar contents in a sample of Marula fruits ranged between 10.4% and 16.0%. Mwamba (1994) reported total sugar contents ranging from 8.8% to 21.1% in the fruits of Uapaca kirkiana. Wills, Schriven and Greenfield (1983) reported that rich vege-tables and fruits should have between 8% and 18% total sugars, and this study falls within this range. As a result, *Sclerocarya birrea* may then be classified under rich fruits and vegetables. The wide variation in total sugars between families within provenances can be utilized to propagate more trees with high sugar levels within a short space of time, which would greatly benefit Marula jam producing companies.

The results show a very low coefficient of variation of 10.7% which implies that more provenances need to be sampled to increase provenances variation. Though results are not statistically different, the provenance with the highest mean was the hottest and driest while the provenance with the lowest mean was the coolest and wettest. This could mean available water and sunlight have an effect on the sugar content of the fruits. However, the "sugar levels" are a % and therefore it is a ratio and may not mean that the amount of sugar per fruit (g) is different. A future study to determine the g sugar per fruit can be used to check if it is not simply the water increases per fruit that reduces the % sugar content.

Vitamin C content (ascorbic acid)

The analysis of variance shows that there was a statistically significant differences between areas in the vitamin C content of fruits analyzed (p=0.001). Kalanga had a mean of 694.2 mg/100 ml juice, Lungungu had 625.3 mg/100 ml juice, Dvokolwako had 539.2 mg/100 ml juice all of which were significantly different from eBuhleni with 224.6 mg/100 ml juice (Table 3). Differences between families within areas were highly significant (p=0.0001) (Table 1). The vitamin C content for families within areas was the most variable parameter. Further analysis of variance to compare families on individual bases revealed highly significant statistical differences between families (p=0.0001) (Table 2). Family K8 of Kalanga had the highest vitamin C content while family E6 of eBuhleni had the lowest. The driest area had the highest vitamin C content, and it appears the solar radiation has an effect on the vitamin C content in Marula trees.

Shone (1979) and Dlamini (1998) reported an average Vitamin C of 237.6 mg/100 g and 195 mg/100 g in the Marula fruit skin and flesh, respectively.

Vitamin C content

There were significant differences between provenances in vitamin C contents of fruits (p=0.001). However, the Duncan's multiple comparisons indicated no significant differences between Kalanga, Lungungu and Dvokolwako, while eBuhleni recorded about half the amount of vitamin C content of the other three provenances. Research has shown that vitamin C content differ drastically even among fruits of the same species reported Saka (1994) on vitamin C contents of numerous indigenous tree species that were analyzed. Variation discovered in this study could be of great importance, particularly in the preliminary selection of superior trees for vitamin C. Progeny trials may be necessary to prove whether this superiority is indeed genetic or it is just due to conducive environmental parameters like sufficient rainfall, optimum temperatures and rich fertile soils. The co-efficient of variation is low at 14.09% between provenances and 16.1% between families. Though the sunlight intensity was not critically studied, the Kalanga provenance is known to be the hottest amongst the four. According to Fourie (1996) the important environmental factor in the determination of level of vitamin C is sunlight; the greater the amount of sunlight received during growth the higher the vitamin C content, and results of this study show that the highest vitamin C came from the provenance with highest sunlight intensity.

Seed size (seed mass)

The ANOVA for seed mass indicated statistically significant difference between provenances in seed mass (p=0.06, Table 3). A similar trend like in total soluble solids occurred here, Lungungu had a mean of 4.10 g, Kalanga had 3.93 g, Dvokolwako had 3.9 g, all of which were higher than eBuhleni with a mean of 3.10 g .Highly significant differences existed between families within provenances (p=0.0001). Likewise highly significant differences were found between families analyzed on individual basis (p=0.0). Family L6 of Kalanga had the highest mean of 6.50 g while family E1 of eBuhleni had the lowest of 2.35 g. This means that provenances had weak effect on the family variation. Mean annual rainfall of the provenances seems to have no influence on the seed mass, since provenances like eBuhleni and Dvokolwako with the same rainfall regime of 650 mm per annum yielded completely different seed masses. Although the provenance with the highest rainfall had the biggest seeds, amazingly the driest provenance came second.

Seed mass

Significant differences in seed mass between provenances were noted. Multiple comparison indicated that the provenances with the highest seed mass (4.10 g) was significantly higher than the provenance with the lowest seed mass (3.10 g). Kalanga and Dvokolwako had a mean of 3.9 apiece (not significantly different from neither the highest nor the lowest). Rainfall and altitude did not have much effect on seed mass, since the provenance with the highest altitude of 500 m and the highest annual rainfall of 750 mm produced the biggest and heaviest seeds, but the provenance with the lowest annual rainfall of 500 mm and the lowest altitude of 340 m did not produce the smallest but produced the second biggest/heaviest seeds. These results are different from those in Salazar (1986) in *Gliricidia sepium*, where driest provenances produced the biggest seeds and wet provenances produced smallest seeds. Variability may reflect differences in pollen that fertilized the ovules of the different flowers. What is clear at this point is that within provenance, among families, there is more variability in seed mass than would be expected from usual ecological impression that seed masses that seed masses are fairly constant (Obeid, 1967; Dlamini, 1998). The observation that the smallest seeds are almost half the size of the largest seeds in study are similar to findings made by Maun and Cavers (1971) with seeds of Silene alba, where seeds were divided into three classes using a soil sieve, and indicated that the masses of seeds with largest class are two times that of seeds in smallest class. Marunda (1993) reported that bigger seeds are the best for selection for early seedling growth and development. Consequently seeds for Lungungu provenance can be selected as root stocks and for other traits (vitamin C and sugars) then other provenances may be chosen and used as scions in the vegetative propagation of superior genotypes in tree improvement programmes.

Correlation between parameters

There was no correlation between fruit and seed parameters, between these parameters and rainfall and altitude in all cases, except for total soluble solids and rainfall, p=0.06, see Table 4. The driest area produced the highest total soluble solids levels and the wettest had the lowest total soluble solids.

Variance components estimates

The range of the family heritabilities of the traits was comparable to that found by Cotterill and Zed (1980), which ranged from 0.40 to 0.97, in family heritabilities of Pinus radiata progeny tests in South Australia. Vitamin C had the highest family heritabilities at 0.89 and total soluble solids and seed mass had 0.54 and 0.58, respectively (see Table 5). Generally one needs more than 100 families to get stable estimates of heritabilities, especially if the heritability is low (Dlamini, 1998). Analysis of variance components for total soluble solids %, seed mass and vitamin C is presented in Table 6.

Table 4. Pearson Correlation Coefficients for fruit and environmental parameters.

Variable	Vitamin C	Total Soluble Solids	Altitude	Rainfall
Seed mass	0.14035 0.4074 NS	-0.21153 0.2088 NS	0.13137 0.4383 NS	0.02805 0.8691 NS
Vitamin C	-	0.04706 0.7821 NS	0.06372 0.7079 NS	-0.14994 0.3758 NS
Total Soluble Solids	-	-	-0.24029 0.1520 NS	-0.30426 0.0671*
Altitude	-	-	-	0.80996 0.0001***
Rainfall	-	-	-	-

NS= not significant, *= significant at 0.05 probability and ***= significant at 0.0001

Table 5. Family heritabilities for TSS, VTC and SM.

Trait	Family heritability
TSS	0.54
VTC	0.89
SM	0.58

Table 6. Analysis of Variance Components Estimates (VCE) for Total Soluble Solids (TSS), Seed Mass (SM) and Vitamin C (VTC).

SOV	DF	VCE		SOV	DF	VCE
		TSS	SM			VTC
Areas	3			Areas	3	
Families (Areas)	33	1.66	0.71	Families (Areas)	33	59214.0
Error	1812	1.39	0.49	Error	74	7225.6
Total	1848			Total	110	

NB. VCE are only calculated for random effects not fixed effects, as a result there are none for areas.

Family heritabilities

Family heritabilities in the context of this paper can be used to determine expected responses from selection of superior trees according to the performance of their progeny. The range of family heritabilities of the traits (sugars, vitamin C and seed mass) was comparable to that found by Cotterill and Zed (1980) in family heritabilities of Pinus radiata progeny trials in South Australia and those of Foster (1986) on Pinus taeda. From family heritabilities found in sugar content, seed mass and vitamin C content it was clear that significant genetic gains can be expected from family trials for selection for any of these traits. The total sugars of 0.54 in Marula fruits in this study is almost the same with that of 0.50 in peaches reported by Janick and Moore (1996), Dlamini (1998).

CONCLUSION AND RECOMMENDATIONS

The results of the fruit composition studies showed little provenances variation (only one provenance was significantly different from the rest), but has wide family variation which suggests a family study could be of great value in identifying superior genotypes. Furthermore the family variation needs to be tested through the establishment of progeny trials of the superior phenotypes to see whether the superiority is genetic or due to conducive environmental conditions. Alternatively *in situ* silvicultural treatments may be established in the wild population to determine the response of these families to the treatments. Such treatments may be re-spacing, thinning, no thinning and so forth.

There were significant differences between provenances in seed mass and the smallest were almost half the size of the biggest, but rainfall and altitude did not have much effect on this and biological basis for the variation in *Sclerocarya birrea* in not known at present. Variability may be due to differences in pollen sources that fertilized the ovules of the different flowers.

This current study considered general soil type, the average annual rainfall and the altitude. It thus recommended that a detailed research study in the same provenances is carried out to do in-depth soil analysis for macro and micro elements and minerals for each parent tree (family), the minimum and maximum daily temperatures, the previous season's rainfall distribution (and not the annual rainfall which is an estimate) for each provenance. This will seek to find out whether or not variation is environmental or genetic.

Family heritabilities for sugar content, vitamin C and seed mass were high, and it is concluded that genetic improvement of these traits can be easy and faster through establishment of family trials.

Eventually the study has shown that provenance means alone may often be insufficient in the understanding of variability in seed mass and fruit parameters in *Sclerocarya birrea*. Ultimately families within provenances provide a good basis for studying between populations of trees. In this case had it not be family variation the results would have indicated that there was no variation at all.

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