Full Length Research Paper

Variation in Germination and Early Growth of *Sclerocarya birrea* subsp. caffra: A Provenance and Family Study

Cliff S. Dlamini

Department of Forest and Wood Science, Faculty of Agrisciences, University of Stellenbosch, Private bag X1, Matieland 7602, South Africa. E-mail: cliffsdlamini@yahoo.com. Tel: +268 76766612. Fax: +268 4041125.

Marula (*Sclerocarya birrea* subsp. caffra) forms an integral part of the diet, tradition and culture of rural communities in Swaziland. In addition contributes to economic, social and environmental stability. This study was an analysis of the variation between and within provenances of *Sclerocarya birrea* sub-species caffra (Marula) in germination and survival of seedlings, and height and root collar diameter increment within the first five and eight months in a randomized complete design. In germination percentage there were significant differences between provenances (p=0.0001) and between individual families (irrespective of provenances) (p=0.004) across provenances, yet no significant differences existed between families within provenances. Survival of seedlings was 100% for all families except one which had 80%. Significant differences occurred in height (p=0.01 and p=0.006) and root collar diameter (p=0.03 and p=0.006) at five and eight months. It was concluded that there was a strong positive relationship between height and root collar diameter.

Key words: Correlation, traits, increment, height, root collar diameter.

INTRODUCTION

Background of *Sclerocarya birrea*

S. birrea (Marula) is a common and widespread species throughout the semi- arid, deciduous savannas of much of sub-Saharan Africa (Peters, 1988). It is frequently a community dominant and hence is a keystone species in plant and animal community ecology and productivity. It is not only important as a dominant tree species in plant communities, but it is also widely used by rural popu-lations in most countries in which it is found (Palmer and Pitman, 1972; Shone, 1979; Walker, 1989; Shackleton et al., 2000) . It has multiple uses, including the fruits that are eaten fresh or fermented to make a beer, the kernels are eaten or the oil extracted, the leaves are browsed by livestock and have medicinal uses, as does the bark. The wood is carved into utilitarian items such as spoons and plates as well as decorative animal figures. Because of these multiple uses, and its significance in the landscape, several African cultures have specific beliefs and ceremonies associated with this species (Walker, 1989). A significant proportion of household nurture seedlings of *S. birrea* that germinate in the grounds of their homestead or arable fields, and maintain adult trees in

an agroforestry situation (High and Shackleton, 2000; Shackleton et al*.,* 2000). Others plant seedlings or propagate trees via stem cuttings.

Variation in germination and seedling growth

The differences in germination patterns and seedling growth rates may be due to climatic and geographic influences or, more importantly, even genetic differences (Weinert et al., 1990). More genetic variation studies were carried out in Marula in progress in Veld Products Research (Botswana) and Ben Gurion University, in Israel. Forest trees seedlings used for genetic testing are traditionally produced in outdoor nurseries. Most forest tree seedlings are characterized by slow growth rates. This means that the investigator must often wait several years before seedlings can be planted in test plantations and reliable genetic variation patterns discerned. Conse-quently, forest geneticists need methods that accelerate identification of superior genotypes and evaluation of

juvenile- mature correlations. The accelerated growth concept may be such a method. It is based upon the control of growth by the manipulation of one or more growth factors, such as light, temperature, mineral nutrients, water, carbon dioxide, growth regulating chemicals and container dimension (Wood and Hanover, 1981).

Applying the concept of accelerated growth to greenhouse production of containerized seedlings allows the investigator increased control over growth development and physiological status of seedlings. Thus, large seedlings can be produced in a few month rather than years. Outdoor nursery seed had shown no or very little provenance variation where accelerated seedlings of the same age have yielded highly significant differences. Results on the early genetic differentiation of *Acer saccharam* (sugar maple) by accelerated seedling growth revealed provenance differences in height, budbreak, nodes, and growth flushes to indicate existence of upper and lower Peninsula races in Michigan (Wood and Hanover, 1981). Accelerated growth techniques may have considerable potential for reducing the time required for genotype evaluation of sugar maple and possibly other tree species, but results must be substantiated by frequent field observations.

OBJECTIVES

General objective

The specific objective of the study is to study the genetic differences in germination and early seedling growth of provenances and families sampled in Botswana for *S. birrea* sub-species *caffra*.

Specific objective

The specific objective of the study is to study the differences between and within provenances, in germination and growth of seedlings for a year old seed.

MATERIALS AND METHODS

Seed source description

Seed from three provenances with four families per provenances were obtained from the Botswana National Tree Seed Centre, provenances were populations of trees that are at least 100 km apart and families were at least 100 m apart. The original seed collection sampled a diverse range of latitude, longitudes and longitude. The altitude ranged from 846 to 1070 m and the mean annual rainfall ranged from 400 to 500 mm.

Experimental design

In the nursery, a randomized complete block design was followed.

Three replications were used with each having three provenances. The twelve families were randomly mixed (with four families in a provenance). Randomization was carried out at two levels in the nursery. The first level was the random distribution of provenances within a whole plot and the second level was the random distribution of families in a provenances. There were three whole plots which all had similar treatment. This is a simple randomized block design with nested effects rather than a split plot which has different whole plots.

Laboratory and nursery work

The seeds were pre-treated by soaking in cold water for 24hours, to ensure uniform germination (Dlamini, 1996). The water was drained and the seeds were treated with Captain (a fungicide). The powder was applied at 2% of seed mass and sprinkled on wet seeds. Seeds were mixed up to ensure maximum distribution of the chemical, before sowing. Polythen10e bags (4.5 x 7.5 cm flat dimensions) were filled with soil. The soil was used was a loose sandy mix of rich soil mixed with sand (as the substrate) and was kept moist but not waterlogged by the computerized irrigation system in the University nursery. The computerized irrigation was set such that humidity is kept at the optimum. Soil was analysed only for pH which was 5.8. Two seeds were directly sown just below the surface in each pot.

Measurements

The following measurements were carried out: Germination percentage was recorded at 3 months (G3); survival rate was recorded at 5 months (S5); height measurements were taken at 5 and 8 months (H5, H8); root collar diameter was measured at 5 and 8 months (RC5, RC 8). The percentage height increment in relation to the height at first measurement was calculated, and the same was done for root collar diameter (HI%, RCI %).
The increment and the first measurement to get percentage increment were done using the following formulae:

$$H1\% = [H8-H5) / H5] \times 100$$

$$RC1\% = [RC8 - RC5/RC5] \times 100$$

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.

RESULTS AND DISCUSSION

Germination percentage at 3 months

The analysis of variance showed that there were significant between replications (p=0.03) (Table 1) and provenance (p=0.0001). The germination percentage summary shows that the highest germination percentage was from Tsetsebye provenance (79.1%), and the second highest was from Kgatleng (64.5%), and the

Table 1. ANOVA for provenance and family differences in germination percentage at 3 months.

(A) $Y_{ijk}=\mu+A_i+B_j+C_{k(j)}+\epsilon_{ijk}$			(B) $Y_{ijk}=\mu+A_i+B_j+\epsilon_{ijk}$		
SOV	Df	MS; p-value	SOV	Df	MS; p-value
Rep	2	979.9*; p=0.003	Rep	2	979.9*
Prov	2	3665.1**; p=0.0001			
Fam (Prov)	9	355.5NS	Fam	11	957.3**; p=0.004
Error	22	256.0	Error	22	256.0
Total	35		Total	35	

NS =not significant, *=significant at 0.05 probability and **=significant at 0.01 probability

Table 2. Germination % in the nursery at 3 months.

Provenance ID mean and germination %	Family code	Rep 1	Rep 2	Rep 3	Family Mean
	A20	50.0	83.3	83.3	72.2
Kgatleng (1)	B16	66.6	50.0	58.3	58.3
64.5	C15	83.3	66.6	50.0	66.6
	D17	58.3	41.6	83.3	45.8
	E5	33.3	25.0	33.3	30.5
Kweneng (2)	F13	50.0	58.3	83.3	63.8
43.7	G2	25.0	41.6	75.0	47.2
	H16	66.6	0 or no germination	33.3	33.3
	I6	66.6	66.6	83.3	72.2
Tetsebye (3)	J19	75.0	50.0	83.3	69.4
79.1	K10	83.3	75.0	91.6	83.3
	L16	83.3	83.3	100	88.8
MEAN		58.0b/	53.3	71.4	60.9a/

a/ indicates the trial mean, b/ indicates replication mean.

lowest was from Kwseneng (43.7%) (Table 2). No significant differences were obtained between families within provenances (Table 1).

There were highly significant differences between families when analyzed irrespective of provenances (p=0.004) (Table 1). This shows that the differences between families were simply due to the fact that they were from different localities (provenances), otherwise within one locality, the families did not differ significantly. The overall germination summary shows the variation to be so wide that the highest germination was 88.89% for family L16 of Tsetsebye provenance and the lowest as 30.56% for family E5 of Kweneng provenance (Table 2).

Germination percentage and survival

The slow and sporadic germination of seeds is attributed to the time of planting; seeds were germinated in February at Stellenbosch, in the Western Cape of South Africa. Previous research by Shone (1979), Goosen et al.

(1985), Taylor (1995) and Mateke (1996) revealed that the best time for germinating Marula seeds is Spring (October and November) when conditions are hot and wet *in situ*. The low germination percentage was entirely about low viability of seeds, probably due to poor storage conditions. The retarded growth rate of seedlings was attributed to the relatively low winter temperatures of the Western Cape Province of South Africa (mean minimum of 9°C and a mean maximum of 24°C. The provenance with the lowest germination percentage produced the tallest and thickest seedlings at 5 months. This might be due to lack of competition between seedlings, and Marunda (1994) reported a positive correlation between seed size and vigorous initial seedling growth.

Survival at 5 months

The survival rates were 100% for all families in all replications except for family 17 which had a survival rate of 80% in replication 3 for provenance one, however

Table 3. ANOVA for height at 5 and 8 months, percentage height increment at 8 months, root collar diameter at 5 and 8 months, percentage root collar diameter increment at 8 months (between provenances and between families within provenances).

SOV	Df	Ht 5	Ht 8	% IH	RC 5	RC 8	% IRC
				Mean squares			
Rep	2	19.07 [NS]	34.46 [NS]	1.97 [NS]	0.12 [NS]	0.17 [NS]	82.12[NS]
Prov	2	844.03**	1183.25**	8.41 [NS]	0.42**	1.03**	85.10 [NS]
Fam(Prov)	9	103.50 [NS]	137.80 [NS]	34.97 [NS]	0.18 [NS]	0.02 [NS]	55.63 [NS]
Error	21	156.29	183.40	87.48	0.11	0.15	76.60
Total				34			

NS =not significant, *=significant at 0.05 probability and **=significant at 0.01 probability.

Table 4. ANOVA for height at 5 and 8 months, % height increment at 8 months, root collar diameter at 5 and 8 months, % root collar diameter increment at 8 months (between individual families ignoring provenances).

SOV	Df	Ht 5	Ht 8	% IH	RC 5	RC 8	% IRC/
				Mean squares			
Rep	2	19.07 [NS]	34.46 [NS]	1.97 [NS]	0.12 [NS]	0.17 [NS]	82.10 [NS]
Prov	2	238.14 [NS]	327.88 [NS]	30.14 [NS]	0.23*	0.35*	60.98 [NS]
Fam(Prov)	9	156.29	183.40	87.48	0.11	0.15	76.60
Error	21						
Total				34			

NS =not significant and *=significant at 0.05 probability

Table 5. Mean height at 5 and 8 months.

Provenance code	Mean Ht 5 (mm) ±SE	Provenance code	Mean Ht 8 (mm) ±SE
Kweneng (2)	a[1/] 128.3±3.25	Kweneng (2)	a 158.89±2.94
Tsetsebye (3)	ba 118.0±2.94	Kgatleng (1)	b 144.7±2.98
Kgatleng (1)	b 111.1±2.67	Tsetsebye (3)	b 138.9±2.85

(1), (2), (3)=Provenance Codes. 1/ Means with same letter are not significantly different according to the Duncan's New Multiple Range Test

detailed results on the survival % are not presented here. This close similarity between the survival and the germination percentage made it unnecessary to perform in analysis of variance for survival rate. In another study Maghembe (1994) reported 71% seedling survival in the field in Malawi, which was lower than the survival rate in this study may be due to differences in the seed sources (including seed viability) and environmental conditions.

Height growth at 5 and 8 months

The analysis of variance revealed that there were no significant differences between replications (p=0.88) (Table 3). Provenances were significantly different (p=0.01) (Table 3). The Duncan's grouping indicated that the tallest seedlings were in Kweneng with a mean of 128.3 mm and the shortest were from Kgatleng with a mean of 111.1 mm (Table 5). While Kgatleng and Kweneng were significantly different from each other, they were no significantly different from Tsetsebye with a mean of 118.0 mm (Table 5). Families within pro-venances were not significantly different (p=0.73) (Table 3).

There were no significant differences between families analyzed when provenances were ignored (Table 4). At eight months replications, there were no significantly different (p=0.73), provenances were significantly different (p=0.006). The Duncan's grouping indicated that the Kweneng provenance was significantly taller than the rest with a mean height of 158 mm, while Tsetsebye and Kgatleng had means of 144.7 and 138.9 mm, res-pectively (Table 5). Families within provenances were not significantly different (p=0.66) (Table 3). There were no significant differences between families when provenances were ignored (p=0.12) (Table 4).

Table 6. Mean root collar diameter at 5 and 8 months.

Provenance code	Mean RC 5 (mm) ±SE	Provenance code	Mean RC 8 (mm) ±SE
Kweneng (2)	a[1/] 4.8.3±0.99	Kweneng (2)	a 0.0±0.15
Tsetsebye (3)	ba 4.5.0±0.07	Kgatleng (1)	b 5.5±0.09
Kgatleng (1)	b 4.5±0.08	Tsetsebye (3)	b 5.4±0.08

(1), (2), (3)=Provenance codes. 1/ Means with same letter are not significantly different according to the Duncan's new multiple range test

Percentage height increment

No significant differences existed between replications (p=0.97). Similarly, there were no significant differences between provenances (p=0.90). Kgatleng, Kweneng and Tsetsebye had means of 27.2, 26.9 and 25.6%, respectively. Families within provenances did not differ significantly (p=0.92) (see Table 3). Likewise, families analysed ignoring provenances did not differ (p=0.96) (see Table 4).

Root collar diameter at 5 and 8 months

Replications did not differ (p=0.33). Significant differences existed between provenances (p=0.03). Kweneng was significantly bigger than the others with a mean of 4.84 mm, while Tsetsebye and Kgatleng had means of 4.53 and 4.50 mm, respectively (Table 6). Families within provenances did not differ (p=0.15) (Table 3). Families ignoring provenances were significantly different (p=0.07) (Table 4). Family H16 of Kweneng provenance had the biggest mean root collar diameter with 5.24 mm, while family B16 of Kgatleng provenance had the smallest mean of 4.28 mm. At eight months, no significant differences were found between replications (p=0.35). Provenances were significantly different (p=0.006) (Table 3). Kweneng had the biggest diameter mean of 6.00 mm which was significantly different (p=0.005) (Table 3). Family H16 of Kgatleng had the biggest diameter mean of 6.47 mm and family J19 of Tsetsebye had the smallest diameter mean of 5.12 mm. Notably, the biggest at five months were still the biggest at eight months (Table 8).

Percentage root collar diameter increment

There were no significant differences between replica-tions (p=0.36) between provenances (p=0.34), between families within provenances (p=0.68) (see Table 3) and between individual families (p=0.64) (see Table 4). This indicates that the seedlings with biggest root collar diameter at five months were not fastest growing com-pared to those that had the smallest diameter, but the diameter differences were due to other factors. Perhaps with a bigger and more diverse sample (more provenances and families) or with a longer growth period we may see differences emerge.

Root collar diameter and height

The significant differences between provenances and lack of significant differences within provenances in both height and root collar diameter at 5 and 8 months imply that the families sampled per provenance were related, but the provenances were not related at all, as they may have been over 100 m apart and of unique population. The Duncan's Multiple Comparison revealed that Kweneng had a significantly higher mean for height at 5 months compared to Kgatleng with the lowest while Tsetsebye was median and not significantly different from either of the other two provenances. Tallest seedlings were from family 5 of Kweneng and the shortest were from family 17 of Kgatleng, and this was the same for 5 and 8 months, and the same occurred in root collar diameter. Consequently, there were no significant differences in height increment between 5 and 8 months. Striking opposite results in early seedling growth were reported by Salazar (1986) in *Gliricidia sepium*, Wood and Hanover (1981) in *Acer saccharam* and Faulkner et al. (1985) in open pollinated families of *Taxodium distichum*.

The scope of this study was not large enough to detect family variation as only twelve families were sampled while testing of families from a wider spectrum might have provided more within provenance variation. Another cause of the lack of within provenance variation (family variation) could be that seed was dispersed across the provenance by animals resulting of loss of within provenance variation and ultimately lack of degrees of freedom. This lack of family variation or within provenance variation points to a low potential for genetic gain in the growth of *S. birrea* through family selection.

On another note, rapid early seedling growth (height and root collar diameter) does not necessarily indicate that gains will continue through an entire rotation (Sheppered, 1981; Faulkner, 1985).

Rainfall patterns in the three provenances did not differ, but only slight differences were noted in altitude. This shows that family variation may not be due to rainfall and altitude but other factors. In this study, the parent tree where family 5 of Kweneng was collected was reportedly

Table 7. Pearson correlation coefficient for height and root collar diameter at 5 and 8 months.

	Root collar diameter at 5 months	Height at 5 months
Root collar diameter at 5 months	1.0 0.0	0.38 0.023*
Height at 5 months	0.38 0.023*	1.0 0.0

	Root collar diameter at 8 months	Height at 8 months
Root collar diameter at 8 months	1.0 0.0	0.30 0.07*
Height at 8 months	0.30 0.07*	1.0 0.0

*=significant at 0.05 probability

by many male trees, and this is the family that produced the tallest and biggest seedlings in the nursery.

There was a strong positive correlation between height and root collar diameter following a trend reported by Cotterill and Zed (1980) in *Pinus radiata* seedlings. This strong correlation between the traits suggests that selection for one trait would cause substantial positive gains in the other. The lack of significant differences in height and root collar diameter increment at 8 months indicate that the biggest seedlings at 5 months and 8 months were not necessarily the fastest growing compared to the rest. The big seedlings could come from big seeds with huge food reserves as described by Marunda (1993).

Correlation between traits (height and diameter) at 5 and 8 months

The Pearson correlation coefficients showed that there was a positive relationship between height and root collar diameter (p=0.02, R2=0.18). This means that for an increase in root collar diameter, there was an increase in height. At eight months: The Pearson correlation coefficients showed that there was a relationship between heights and root collar diameter, however this relation-ship was not as strong as that at five months (p=0.077). For an increase in root collar diameter there was an increase in height (see Table 7).

CONCLUSION AND RECOMMENDATIONS

The germination rate of *S. birrea* seeds evidently looks poor compared to recent studies from other countries in the region. This is attributed to omission of viability tests before sowing, sowing seeds at the wrong time of the year, poor storage conditions after collection and/or the nature of the seed (its geographic origin).

In the height and root collar diameter studies, it is clear that there is more provenance variation than within provenances variation. This indicates that families were of more or less the same population hence lack of within provenance diversity. A study with a wider representation of families within provenances is recommended to analyze for family variation at a broader spectrum. The number of observations per family could also be increased to lower the standard errors and improve the reliability of the results.

The height and root collar diameter of the seedlings varied even though environmental parameters were carefully controlled, so genetic diversity may be responsible. The genetic make-up of seeds was different because they were produced by different trees and flowers (flowers within trees were fertilized by pollen from different male trees) and developed under different envi-ronmental conditions. The combination of these different genotypes coupled with different environments (in the field) determined the early seedling growth, which varied even under uniform conditions like those in the nursery. Growth rates in terms of height and root collar was not significantly different regardless of the seed mass/size. However, seedlings from big seeds produced strong tall seedlings. Consequently a study to investigate the relation of seed size to germination and early seedling growth is recommended to verify whether the strong growing seedlings from the Kweneng provenance were like that because they indeed came from big seeds with a lot of food reserves or it was purely genetic. Ultimately variation in early seedling growth could be attributed to a combination of the following factors: Genetic differences between seedlings, environmental variation in the nursery, time of germination of seeds and size of the seed and amount of food reserves.

Additional research is necessary to determine whether the selection of seed from superior seed sources

(provenances) will eventually reduce variation in growth under controlled conditions, or whether seedlings which grow best under optimum conditions in the nursery will survive and grow well when planted in the field.

ACKNOWLEDGMENTS

REFERENCES

Cotterill PP, Zed PG (1980). Estimates of Genetic Parameters for Growth and Form Traits in Four *Pinus radiata* D. Don. Progeny Tests in South Australia. Aualian For. Res., 10(2): 155-67.

Dlamini CS (1996). Seed pre-treatment to facilitate germination and general morphology for *Sclerocarya Birrea* sub-species *caffra*. Unpublished B.Sc. Hons. Mini Project. University of Stellenbosh. Ecol. Food Nutr., 39: 225 - 245.

Faulkner P, Zeringae F, Toliver J (1985). Genetic variation among open-pollinated families of Balocypress seedling planted on two different sites. In Proc. 18th Southern Forest Tree Improvement Conf. Long Beach, Mississippi.

Goosen H, Holtzhausen LC, Van WP, Marneweck R (1985). The marula is tamed. South Afr. Panaroma, 30(2): 20-25

High C, Shackleton CM (2000). The comparative value of wild and domestic plants in home gardens of a South African rural village. Agrofor. Syst., 48: 141- 156.

Maghembe JA (1994). Achievements in the establishment of indigenous fruits trees of the miombo woodlands of southern Africa. In: Improvement of indigenous fruit trees of the miombo woodlands of southern Africa, eds. J.A. Maghembe, Y. Ntupanyama and P.W. Chirwa, Proceedings of a conference held on 23-27 January at Club Makokola, Mangochi, Malawi.

Marunda CT (1993). Genetic variation and physiological studies of *Acacia albida*. MSc Thesis, Australian National University, Canberra.

Mateke SM, Growing indigenous trees in Botswana. The case of marula *Sclerocrya birrea*. Botswana Natl. Conserv. News, 3(3): 1-8.

Palmer E, Pitman N (1972). Trees of Southern Africa. Volume 2. Struik, Cape Town.

Peters CR (1988). Notes on the disctribution and relative abundance of Sclerocarya birrea (A. Rich.) Hochst. (Anacardiaceae). Monograph in Systematic Botany of the Missouri Botanical Garden, 25: 403 – 410.

Saka JDK (1994). The nutrition value of edible indigenous fruits: present research status and future directions. In: Improvement of indigenous fruit trees of the miombo woodlands of southern Africa, eds. J.A. Maghembe, Y. Ntupanyama & P.W. Chirwa, Proceedings of a conference held on 23-27 January at Club Makokola, Mangochi, Malawi.

SAS Instit. Inc 1997. SAS/STAT. User's Guide, Verson 6-12, (4th edition). SAS Inst. Inc. Vol. 2, Cary, NC.

Shackleton CM, Dzeref OSCM, Shackleton SE, Mathabela FR (2000). The use and trade in indigenous edible fruits in the Bushbuckridge savanna region, South Africa.

Shapiro SS, Wilk MB (1965). An analysis of variance test for normality (complete samples). Biometrika, 52(3-4): 591-611.

Shepperd WD (1981). Variation in growth of Engelmann spruce seedlings under selected temperature environments. Research Note RM-404. USDA Forest Service.

Shone AK (1979). Notes on the marula. Bull. 58, Dept, of Forestry, Pretoria, RSA.

Walker N (1989). "King of foods": Marula economics in the Matabos. Afr. Wildlife, 43(6): 281-285.

Weinert IAG, Van Wyk PJ, Holtzhausen LC (1990). Marula. In Fruits of tropical and subtropical origin: Composition, properties and uses. (Eds) Nagy, S, Shaw, E.P. and Wardowski, W.F. Florida Science Source, Lake Alfred, Florida, pp. 88-115.

Wood BW, Hanover JW (1981). Early genetic differentiation of sugar maple by accelerated seedling growth. Can. J. Forest Res., 11: 287-290.