Variation in Seed and Germination Traits of *Sclerocarya birrea* subsp. *birrea* Populations

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**ABSTRACT**

The extent of variation in seed and germination traits of *Sclerocarya birrea* subsp. *birrea* was evaluated at the population level. Seeds were collected from five natural populations in Sudan (Rashad, Alfaid, Alkhwi, Aldamazin and Baw). Five replicates of 25 seeds per population were studied to assess variability in seed morphometric traits, germination index (seed vigor) and total germination percentage, using 100 water-soaked seeds per population sown in a completely randomized block design under controlled environmental condition. Significant (P≤0.05) differences among populations were obtained in seed and germination traits. The analyses revealed that some of the seed traits had significant correlations with the geographical variables of the populations. Among seed traits, significant (P≤0.05) correlations were found between seed length, seed weight and seed width. Germination percentage had no significant correlation with seed traits. With the exception of seed germination percentage, 95%-97% of the total variation in seed traits was attributed to population effect. Variations in seed and germination traits could be a reference point, when considering seed collection of this species for conservation and species restoration.

**Key words:** *Sclerocarya birrea* subsp. *birrea*; seed traits; germination

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**INTRODUCTION**

*Sclerocarya birrea* (A. Rich.) Hochst. (Anacardiaceae, the mango family) is native to the semi-arid, deciduous savannas of much of sub-Saharan Africa. It has three subspecies (*Sclerocarya birrea* subsp. *birrea*, *Sclerocarya birrea* subsp. *caffra* and *Sclerocarya birrea* subsp. *multifoliolata*) distributed in about 29 African countries from southern Africa through East to West Africa and Madagascar (Fox and Norwood 1982). It is widely used by rural populations in most countries (Palmer and Pitman, 1972; Shone 1979 and Shackleton *et al*. 2000). *Sclerocarya birrea* is a constituent of low elevation (mostly < 1600 m) vegetation, subsp. *birrea* is found at around 1700m in Jebel Marra...
area, Sudan (Meihe 1988). The species received international attention and FAO panel of experts on forest gene resources (2001) listed it as an African priority species, with the need for population, provenance and progeny studies. Seed traits, including seed size and germination, are central components of plant life histories (Thompson 1987); it may highly influence the regeneration process of a population (Fenner 2000). Seed size is an important parameter of plant fitness as increasing seed size within species was correlated with an increase in seed germination percent (Vera 1997; Shaukat et al. 1999; Cordazzo 2002). The most commonly cited within species advantages of large seed size through greater reserves are drought resistance, early shade tolerance, and other direct effects of larger initial seedling size (Westoby et al. 1992). Seed size affects dispersal distance (Stevenson 2000), dormancy (Rees 1997), or timing of germination (Simons and Johnston 2000; Tremayne and Richards 2000). Quantification of variations in seed size (seed length, width, and seed weight) and germination traits, was documented in many tree species and these traits are widely used in studies of genetic variation and are believed to be under strong genetic control (Ibrahim et al., 1997; Jayasankar et al., 1999; Lauridsen 2004; Hodge and Dvorak 2004; Mamo et al., 2006; Loha et al., 2006).

In Sudan Sclerocarya birrea species has been proposed as an intermediate ranked plant for national protection, as it is facing the risk of extinction in the wild (Gorashi 2001). The natural regeneration of this species is very scarce due to damages of seed and seedling by domestic and wild animals (Daldoum et al. 2012). Practical conservation measures, which ensure conservation of maximum genetic diversity of the species, are urgently required. Significant differences between provenances of Sclerocarya birrea subsp. caffra was reported in seed mass and the smallest were half the size of the biggest (Dlamini 2011). Despite wide variation in the natural habitat, very little is known about variation among populations of Sclerocarya birrea subsp. birrea in Sudan. Such knowledge is essential for both its ex situ and in situ conservation and improvement strategies. The aim of this study was to investigate genetic variation in seed and germination among five populations of Sclerocarya birrea subsp. birrea in Sudan. Genetic variation in seed length, seed diameter, seed weight, germination rate and total germination percentage were investigated. In addition, correlations between seed traits and geo-climatic data of seed sources estimated as means of testing for presence of clinal or ecotypic patterns of variation also associations between seed characters were examined.

MATERIALS AND METHODS

Seed sources and collection

Five populations representing the natural distribution of the species in Sudan were selected based on the existence of the species. The geographic distribution and climatic
conditions of the location of the five populations are depicted in Table (1), which were Baw (BA), Alkhwi (KW), Aldamazin (DZ), Alfaid (FD) and Rashad (RD). Fruits were collected between April and June 2006 from 30 trees per population. A minimum distance of 100 m was maintained between mother trees for maximizing genetic variation within populations. Seeds were extracted manually from drupes by de-pulping, cleaning and then sun-drying. Seeds from individual trees were equally sampled by number, and bulked by population. Seed processing, measurement and germination experiments were conducted afterwards.

**Seed morphometric characteristics**

The measured seed characters were, seed length, seed width, and seed weight on a random sample of five replicates of 25 seeds per population. Seed length and width were measured using a micro-caliper and seed weight was quantified by sensitive balance.

**Germination test**

Germination test was carried out under controlled environment in a germination chamber in the National Tree Seed Centre, Soba, Sudan. Four replications with one hundred seeds in each population were randomly drawn from each. Seeds of each population were soaked in tap water for 24 h prior to sowing. Four replicates of each population were sown in a completely randomized block design. The photoperiod of the germination chamber was set for 12 h light, and 12 h dark and watering was done every 2 days. Germination was monitored every week for 10 weeks. The seeds were considered germinated upon the emergence of the radicle. Number of germinated seeds were counted weekly from day of planting for 10 weeks. Germination rate was determined by counting the germinated seeds at the time intervals after planting (2, 4, 6, 8 and 10 weeks) as adopted by the ISTA (1999). Germination percent (G%) was estimated as the proportion of total germinated seeds to that of sown seeds, expressed in percentage at the end of the test.

**Table (1).** Geographical location of the studied populations of *Sclerocarya birrea* subsp. *birrea* in Sudan.

<table>
<thead>
<tr>
<th>Population Name</th>
<th>State</th>
<th>Latitude (N)</th>
<th>Longitude (E)</th>
<th>Altitude (m)</th>
<th>Rainfall (mm)</th>
<th>Soil type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baw</td>
<td>BN</td>
<td>11° 20' 24&quot;</td>
<td>34° 05' 04&quot;</td>
<td>590</td>
<td>700</td>
<td>Clay</td>
</tr>
<tr>
<td>Alkhwi</td>
<td>NK</td>
<td>13° 6' 37&quot;</td>
<td>29° 07' 33&quot;</td>
<td>685</td>
<td>300</td>
<td>Sand</td>
</tr>
<tr>
<td>Aldamazin</td>
<td>Bn</td>
<td>11° 50' 50&quot;</td>
<td>34° 29' 22&quot;</td>
<td>493</td>
<td>600</td>
<td>Clay</td>
</tr>
<tr>
<td>Rashad</td>
<td>SK</td>
<td>11° 49' 28&quot;</td>
<td>31° 02' 50&quot;</td>
<td>840</td>
<td>600</td>
<td>Rocky</td>
</tr>
<tr>
<td>Alfaid</td>
<td>SK</td>
<td>11° 7' 33&quot;</td>
<td>30° 29' 22&quot;</td>
<td>615</td>
<td>700</td>
<td>Loam</td>
</tr>
</tbody>
</table>

BN, NK and SK refer to Blue Nile, North Kordofan and South Kordofan States, respectively.
Statistical analyses

To determine the significance of variation in seed and germination parameters among populations, analysis of Variance (ANOVA) was used and means were separated by Duncan’s Multiple Range Test at ($P=0.05$). Correlation was carried out to determine the linear relationship between seed traits and the geoclimatic variables of population’s geographical location. Total phenotypic variation for each trait was partitioned into components due to genetic (hereditary) and non-genetic (environmental) factors according to Johnson et al. (1955)

RESULTS

Variation in seed size

The seed size traits (Seed length, Seed diameter and seed weight) of *Sclerocarya birrea* subsp. *birrea* displayed significant variations among populations at ($P<0.001$) (Table 2). The KW population showed the highest value in seed length and diameter (2.12 ±0.02), (1.96±0.03) and, seed of diameter of KW was not significantly different from that of BA population (1.93±0.02), however, the two (KW and BA) populations had significantly higher seed length and diameter values than the other populations. Seed length and diameter of FD (1.92± 0.04), (1.66±0.03) and RD (1.91±0.02), (1.76±0.03) showed intermediate values while DZ population, which showed the lowest value (1.76±0.02),(1.59±0.02) of seed length and diameter.

The seed weight of the studied populations ranged from 2.81 to 1.85 g. Seeds from KW population were significantly heavier (2.81±0.09) than the other populations. On the other hand, seed weight of BA population (2.59±0.08) was significantly heavier than the seed of RD, FD and DZ populations. Seed weight of RD population had seed weight (2.05±0.05) was significantly higher than that of DZ (1.82±0.05).

Table (2). Seed traits of five natural populations of *Sclerocarya birrea* subsp. *birrea* in Sudan.

<table>
<thead>
<tr>
<th>Population</th>
<th>Seed Length (cm)</th>
<th>Seed Diameter (cm)</th>
<th>Seed weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baw</td>
<td>1.95 ± 0.02 b</td>
<td>1.93 ± 0.02 a</td>
<td>2.59 ± 0.08 b</td>
</tr>
<tr>
<td>Aldamazin</td>
<td>1.76 ± 0.02 d</td>
<td>1.59 ± 0.02 c</td>
<td>1.81 ± 0.05 c</td>
</tr>
<tr>
<td>Alkhwi</td>
<td>2.12 ± 0.02 a</td>
<td>1.96 ± 0.03 a</td>
<td>2.81 ± 0.09 a</td>
</tr>
<tr>
<td>Alfaid</td>
<td>1.92 ± 0.04 c</td>
<td>1.66 ± 0.03 b</td>
<td>1.73 ± 0.06 c</td>
</tr>
<tr>
<td>Rashad</td>
<td>1.91 ± 0.02bc</td>
<td>1.76 ± 0.03 b</td>
<td>2.05 ± 0.05 c</td>
</tr>
</tbody>
</table>

Values are means of five replicates± standard error. Means in the same columns with the same letters are not significantly different at $P=0.05$ according to Duncan’s Multiple Range Test.
Fig. (1) Seed germination percentage of the five *Sclerocarya birrea* subsp. birrea populations (Rashad (RD), Alfaid (FD), Alkhwi(KW), Aldamazin(DZ) and Baw (BA), (vertical bar represents mean ± standard error).
Fig. (2) Seed germination rates of the five populations of *Sclerocarya birrea* subsp. *birrea*. Points are means ± standard error.

**Seed germination**

Germination percent of the five population of *Sclerocarya birrea* subsp. *birrea* seeds was generally low, ranged from 63 ± 14.97 to 35 ± 6.61 %, and did not differ significantly (P <0.001) among populations (Fig (1). Germination rate of BA population (26 ± 8.08) in the first two weeks was significantly higher (P <0.001) than that of KW population. Since then no significant difference was observed (P <0.001) among the studied population till the end of week five. However, week six displayed differences among the population in germination percent, where FD population had significantly highest percent (42±3.46). Moreover, the germination percent of KW population (25±2.52) was significantly higher than DZ (9±2.52) and BA population (8 ± 2.83) but significantly not different from RD population (15± 6.40). Thereafter, from week eight till the end of the experiment there were no significant difference among the five populations (Fig. 2).

**Estimates of variance components**

Estimates of the variance components showed that phenotypic variances due to population was close to genotypic variance and both the phenotypic and genotypic variances were larger than that of error (environmental effect) (Table 3). For all seed traits and the contributions of population variance to the total variance were extremely large. The seed germination percentage showed relatively low percentage contribution of population variation to the total variation.

**Correlations between seed traits and geoclimatic variable**
Significant (P≤0.05) correlations were found between seed length and germination percentage with latitude, longitude, as well as, between seed weight and latitude. Rainfall had significant correlations (P≤0.05) with seed length, seed weight and germination % (Table 4). With regard to correlations between the studied traits, a strong significant positive correlation (P≤0.05) were observed between seed length with seed weight and seed width (Table 5). Also seed width had strong correlation with seed weight. However, the seed germination percentage had a weak correlation (P≤0.05) with seed traits.

**Table (3).** Estimates of genetic variables as determined from measurement of seed and germination traits for the *Sclerocarya birrea* subsp. *birrea* natural populations in Sudan.

<table>
<thead>
<tr>
<th>Trait</th>
<th>Overall mean</th>
<th>Total variance (Vt)</th>
<th>Phenotypic variance (Vp)</th>
<th>Genotypic variance (Vg)</th>
<th>Error variance (Ve)</th>
<th>Contribution (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seed length (cm)</td>
<td>1.932</td>
<td>0.416</td>
<td>0.398</td>
<td>0.379</td>
<td>0.018</td>
<td>95.67</td>
</tr>
<tr>
<td>Seed width (cm)</td>
<td>1.780</td>
<td>0.673</td>
<td>0.656</td>
<td>0.639</td>
<td>0.017</td>
<td>97.47</td>
</tr>
<tr>
<td>Seed weight (g)</td>
<td>2.228</td>
<td>5.132</td>
<td>5.018</td>
<td>4.904</td>
<td>0.114</td>
<td>97.78</td>
</tr>
<tr>
<td>Germination %</td>
<td>48.4</td>
<td>264.7</td>
<td>153.8</td>
<td>142.9</td>
<td>110.9</td>
<td>58.1</td>
</tr>
</tbody>
</table>

**Table (4) Correlations between geographical variables of collection sites with seed and germination traits of *Sclerocarya birrea* subsp. *birrea.*

<table>
<thead>
<tr>
<th>Trait</th>
<th>Latitude</th>
<th>Longitude</th>
<th>Altitude</th>
<th>Rainfall</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seed length (cm)</td>
<td>0.603*</td>
<td>0.695*</td>
<td>0.422</td>
<td>0.664*</td>
</tr>
<tr>
<td>Seed width (cm)</td>
<td>0.478</td>
<td>0.227</td>
<td>0.328</td>
<td>0.476</td>
</tr>
<tr>
<td>Seed weight (g)</td>
<td>0.592*</td>
<td>0.229</td>
<td>0.178</td>
<td>0.591*</td>
</tr>
<tr>
<td>Germination %</td>
<td>0.729*</td>
<td>0.475</td>
<td>0.615*</td>
<td>0.692*</td>
</tr>
</tbody>
</table>

Values are Pearson correlation coefficient, r

*Correlation is significant at the 0.05 level.
DISCUSSION

This study showed considerable variation in seed traits among the five populations that represent the natural range of subsp. birrea in Sudan. The variation could be attributed to isolations that in turn influence gene flow and also could be attributed both to parental origin and to fruits within individual trees as indicated by Andrew et al. (2000). Genetic control of seed size traits has been observed in several tree species (Ibrahim et al., 1997; Rehab et al., 2003; Loha et al., 2006; Loha et al., 2008; Zheng et al., 2009; Rawat and Bakshi 2011). The magnitude of genotypic variance of seed traits was higher than the error variance in the one hand, while the phenotypic and genotypic variances were close to each other on the other hand. This indicates that the genotypic component was the major contributor to the total variance for these traits; i.e., most of the variability observed in the phenotype for seed traits has more of a genetic than a non-genetic basis. This variability that resulted from genotypic variance indicates considerable scope for selection.

The insignificant correlations between geographic variables of seed origin and some of seed traits were, most likely due to few seed sources represented in this study. Nonetheless, some positive significant correlations were found between seed length versus latitude and longitude of seed origin. Such correlation is expected in a material from a population of trees which is known to have naturally regenerated (Loha et al., 2006). It should be noted that the stands from which seeds were collected for the present study were relics of the natural forests. Some studies dealing with different plant species reported that seed weight increases with altitude (Ayana and Bekele 2000; Boulli et al., 2001). This was not the case of Sclerocarya birrea subsp. birrea, in this study and similar results have been reported on Betula pendula, Ranunculus acris and Carex flacca (Holm 1994; Totland and Birks 1996; Pluess et al., 2005). The presence of significant positive correlations between rainfall and some of seed traits suggests that these traits exhibit clinal pattern of variation along moisture gradient; similar results were found by Loha et al. (2008) while negative correlation between seed traits and rainfall of the seed origins had been found in Cordia Africana (Loha et al., 2006) and in Trigonobalanus doichangensis (Zheng et al., 2009).

The intercharacter correlations between seed width, seed weight, and seed length were strong. Thus, reasonably good selection for these traits can be made simply based on phenotypes. Correlated quantitative traits are of a major interest in an improvement program, as the improvement of one character may cause simultaneous changes in the other character. Similar results have been reported for Faidherbia albida (Ibrahim et al., 1997), Tectona grandis (Jayasankar et al., 1999), Juniperus procera (Mamo et al., 2006) and Cordia africana (Loha et al., 2006). In the present study, seed morphometric traits could not be used to judge the seed germination capacity of Sclerocarya birrea subsp. birrea and these traits might be adapted to the seed dispersal and dormancy. The variation in seed germination percentage of the studied population was mostly genetic
and environmental. In comparison with seed traits, germination was more affected by non-genetic effects due to the relatively low percentage contribution of population variation to the total variation. Wulff (1995) and Gutterman (2000) reported that maternal factors, such as position of the seed in the fruit/tree and the age of the mother plant markedly influence the germination capacity of seeds.

Seed traits of *Sclerocarya birrea* subsp birrea showed significant differences among populations, confirming the hypothesis that *Sclerocarya birrea* subsp *birrea* possesses very strong genetic differentiation among its populations. The environment changes during the evolution of the species, habitat destruction and excessive exploitation might have led to a low gene flow, which in turn resulted in the differences in seed traits. Different soil types between the studied populations may also contributed in the differentiation among populations (Mandal and Gibson 1998). The study provides evidence for the existence of genetic variations in seed traits, which could be exploited for future breeding or selection works, further collection from large number of seed sources is recommend.

**Table 5.** Correlation matrix of seed and germination traits of *Sclerocarya birrea* subsp. *birrea* natural populations in Sudan.

<table>
<thead>
<tr>
<th>Trait</th>
<th>Seed length</th>
<th>Seed width</th>
<th>Seed weight</th>
<th>Germination %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seed length</td>
<td>1.0</td>
<td>0.845*</td>
<td>0.846*</td>
<td>0.196</td>
</tr>
<tr>
<td>Seed width</td>
<td>1.0</td>
<td>1.0</td>
<td>0.976*</td>
<td>0.051</td>
</tr>
<tr>
<td>Seed weight</td>
<td></td>
<td>1.0</td>
<td>0.068</td>
<td></td>
</tr>
<tr>
<td>Germination%</td>
<td></td>
<td></td>
<td></td>
<td>1.0</td>
</tr>
</tbody>
</table>

Values are Pearson correlation coefficient, r

*Correlation is significant at the 0.05 level.

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