Effects of Seed Mass and Seed Coat on Germination and Seedling Emergence of *Acacia ehrenbergiana* Hayne

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Abstract: This study focused on the effect of variations in seed mass and seed coat thickness of the natural forests of *Acacia ehrenbergiana* Hayne on seed germination percent (GP) and germination mean time (GMT), and seedling emergence indicators. The study was carried out in three locations in the Kingdom of Saudi Arabia (Al Madinah (24°89´N, 39°16´E), Aseer (17°55´N, 42°11´E) and Baha (19°13´N, 41°80´E). The seeds were pretreated by soaking for 60 minutes in H_2SO_4 (98%) and germinated on moist filter paper. Also some seeds were sown directly in soil (sand: clay: peat moss 2:2:1; v/v). Seed mass showed greater CV in the same population (27, 20 and 25%, respectively). Seeds were categorized into three non-overlapping size classes: small (0.14-0.15 mg), medium (0.16-0.22 mg) and large (>0.22 mg). Large seeds recorded significantly (P=0.0001) the highest GP as compared to medium and small seeds. However, small seeds emerged faster than large and medium seeds i.e. had the least GMT). Emergence indicators such as speed of germination (SOE), mean emergence date (MED) and emergence rate index (ERI) of seedlings were much better in seedlings originated from large seeds. A significant positive correlation between seed coat thickness and GMT was found.

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1. Introduction

Due to increased interest in environmental issues the need for the establishment of forests using indigenous tree species had increased (Araújo Neto et al., 2003). Thus, there has been an increasing demand for good quality seeds and seedlings for environmental protection projects (Bonner, 1992). The increasing demand for good quality seeds and seedlings had increased the research interest (Santos and Aguiar, 2000). In tropical forests the recorded deforestation rates have much exceeded afforestation and re-forestation (Grainger, 1993; Laurence, 1999). FAO (2007) estimated the worldwide deforestation at 13 million ha or 0.7% of the total forested area annually. Studies on seed biology are of utmost importance for the afforestation projects in these vulnerable areas (Kozlowski, 2000). To ensure raising vigorous seedlings that may survive under stress environments and to be transplanted successfully in the field, it is necessary to have knowledge on the effect of the variability of seed size and mass on germination parameters (Du and Huang, 2008). Forest plantations play important roles other than commercial production of wood (Birch et al. 2010). They protect the soil against erosion by reducing the energy of rain drops and accumulation of litter, reduction of runoff and increasing rain water infiltration (Benayas et al. 2009; Allen et al. 2010). It has been reported that, among other factors, successful establishment of the juvenile phase of plants relies greatly on seed mass (Grubb and Burslem, 1998; Meyer and Carlson, 2001). Studies on the effect of seed variability on seedlings survival and growth are crucial for prescribing sound management practices for tree species to secure their better restoration (Sa´ nchez-Vela´squez et al., 2004; Martı´nez-Garza et al., 2005). Variation of a plant seed size may reach up to ten folds (Hawke, 1989). Seed mass had greater effects on germination percentage and velocity and seedling growth and survival of plants (Walters and Reich, 2000; Paz and Martinez Ramos, 2003; Iortsuun et al., 2008). Large seeds produced the heaviest and tallest seedlings compared to smaller seeds (Khan, 2004). However, seedlings originated from small seeds had more RGR (Paz and Ramos, 2003). The rate of biomass allocation was significantly higher in seedlings from larger seeds than in those from small seeds (Yanlong et al., 2007). A positive link has been established between seed mass and seedling performance (Castro et al., 2006). Seed mass has been considered as an indicator of forest seeds quality (Moleele et al., 2005). Seed mass of *Acacia tortilis* varied with rainfall and soil organic matter (Moleele et al., 2005). Seed size has been considered as an indicator to the quantity of food reserves that will be availed to the embryo (Triphati and Khan, 1990; Westoby et al., 1992; Lloret et al., 1999). Several investigators expressed the advantages of large seeds as high percentage of seed germination (Wilcox, 1984; Triphati and Khan, 1990; Tremayne and Richards, 2000), improved seed emergence

(Winn, 1988; Seiwa, 2000), increase in seedling growth (Buckley, 1982; Stock et al., 1990; Triphati and Khan, 1990; Osunkoja et al., 1994;), reduction in mortality (Buckley, 1982; Triphati and Khan, 1990; Seiwa 2000) and increase in root/shoot ratio (Buckley, 1982; Lloret et al., 1999).

2. Materials and Methods

2.1. Seed collection

Seed size is a measure of seed volume (Cordazzo, 2002). However, there was great variation in seed shapes to measure their volumes. Therefore, seed mass was used as a size index (Wilson, 1983). Fresh pods of naturally growing *Acacia ehrenbergiana* Hayne were collected randomly from Al Madinah (24°89´N, 39°16´E), Aseer (17°55´N, 42°11´E) and Baha (19°13´N, 41°80´E) in the Kingdom of Saudi Arabia. Table 1 summarized some meteorological components of the study locations. The pods were collected randomly from twenty trees in each location then broke using a mortar and a pestle to release the seeds. Infested and broken seeds were discarded. Out of about 1000 seeds from each location, a sub-sample of 200 seeds was drawn randomly. Seeds were then air dried to approximately constant weights (Shaukat et al., 1999). Individual seeds were weighed using an electronic balance. Descriptive statistics of seed mass from the three locations were shown in table 2. Seeds were categorized into three non-overlapping size classes: small (0.14-0.15 mg), medium (0.16-0.22 mg) and large (>0.22 mg).

2.2. Seed coat thickness

Five seeds each from large, medium and small seeds from each location were randomly selected and soaked in distilled water for 48 hours to soften the hard seed coat. Using a scalpel and forceps each seed was separated into approximately two halves. Seed coat thickness was measured in mm using a vernier caliper.

2.3. Germination test

A total of 125 seeds from each seed mass class per location were soaked in H_2SO_4 (98%) for 60 minutes, rinsed thoroughly in tap water and dried on filter paper. Twenty five seeds were germinated in 9.0 cm Petri dishes containing two layers of Whitman filter paper No. 1 moistened with distilled water and replicated 5 times per seed mass from each location. The Petri dishes were kept randomly in a controlled environment chamber (Sanyo MLR-351H) maintained at 30°C with 14 h light (2000 Lux) and 10 h dark treatment (Aziz and Shaukat, 2010). Germination of seeds was recorded daily for 4 weeks. A seed was considered germinated when the radical emerged. The following germination parameters were calculated:

Germination percentage (GP): the number of germinated seeds as a percentage of the total number of tested seeds:

GP = (germinated seeds/total number of tested seeds) × 100 %

GMT (Germination mean time) calculated according to Scott et al. (1984) as:

GMT (days): = $\Sigma T_i N_i / S$

Where T_i is the number of days from the beginning of the experiment, Ni is the number of seeds germinated per day and S is the total number of seeds germinated.

2.4. Seedling emergence

Another set of seeds of *A. ehrenbergiana* were soaked in 5N H2SO4 (98%) for 60 minutes, rinsed thoroughly in tap water and dried on filter paper. A total of hundred seeds each from large, medium and small seeds were then sown in plastic pots (32 x 40 cm) containing sand, clay and peat moss (2:2:1 v/v). Five seeds were planted in each pot. This experiment was replicated 20 times for every seed mass from each location in a CRBD (completely randomized block design). The pots were kept in a glass house at 30 ± 2 °C (N 42° 24´ E 46° 44´, 600 m.a.s.l.). Seedling emergence was calculated by daily counting of the number of newly emerged 1mm long seedlings for 4 weeks. Seedling emergence parameters were assed as follows:

SOE (speed of emergence) was calculated according to (Tessier, 1988) as follows:

$$\text{SOE (plants per day)} = \frac{N_1 + N_2 + \cdots + N_n}{t_1 + t_2 + \cdots + t_n}$$

MED (mean emergence date days) was calculated using the formula of Bilbro and Wanjura, 1982) as follows:

$$\text{MED} = \frac{N1t1 + N2t2 + \cdots + Nntn}{t1 + t2 + \cdots + tn}$$

ERI (emergence rate index/day) was calculated according to the formula of Bilbro and Wanjura (1982) as follows:

$$\text{ERI} = \frac{N_1 + N_2 + \cdots + N_n}{\text{MED}}$$

where $N1, N2, \ldots, Nn$ are the number of newly emerged seedlings in time $t1, t2, \ldots, tn$ since the start of seedling emergence, respectively.

Statistical analysis

GP data were analyzed using 3 way ANOVA (seed mass) and means were separated by LSD (least significant difference) at P=0.05. GP data were transformed by arcsin Sqrt before analysis. Correlation analysis was carried out between seed coat thickness and germination mean time (GMT). All statistical analysis was carried out using SAS statistical package (SAS, 1997).

3. Results

The ranking of altitude in the study locations was Aseer > Baha > Al Madinah and the rainfall was much less in the latter (Table 1). Descriptive statistics were summarized in table (2). The calculation of the frequency distribution by skewness and kurtosis showed that seed populations from the three study locations were normally distributed.

3.1. Germination percent

Seed mass significantly (P=0.0001) affected GP (Table 3). The highest GP occurred in large seeds > medium > small seeds. In fact the GP was almost double in large seeds as compared to medium and small seeds (Table1). The only exception were the seeds from Al Madinah where the difference in GP was not significant between medium and small seeds.

3.2. Germination mean time

GMT was significantly (P=0.0001) influenced by the seed mass (Table 3). Although large seeds gave more GP, however the shortest GMT was recorded in small and medium seeds from all locations and the difference was not significant. Large seeds had the highest GMT i.e. they took more time to germinate (Table 3).

3.3. Effect of seed coat thickness on GMT

A positive significant correlation was found between seed coat thickness and GMT (Fig. 1-3). As the seed coat thickness increased GMT increased. That means that the thicker was the seed coat the more time was taken for germination.

3.4. Seedling emergence

3.4.1. Speed of seedling emergence

SOE was significantly (P=0.0001) affected by seed mass (Table 4). SOE ranking was large seeds > medium > small for seeds collected from Aseer and Baha, whereas it was large > medium = small for seeds collected from Al Madinah.

3.4.2. Mean emergence date (days)

Seed mass had significantly (P=0.0001) affected MED (Table 4). The trend was similar to SOE where the ranking was Large seeds > medium > small for seeds collected from Aseer and Baha, whereas it was large > medium = small for seeds collected from Al Madinah.

3.4.3. Emergence rate index

ERI was also significantly affected by the seed mass (P = 0.002, P = 0.0001 and P = 0.0001) for seeds collected from Aseer, Baha and Al Madinah, respectively (Table 4). The ranking of ERI was consistently large = medium >small.

4. Discussion

The results of the present study showed that seed mass of *A. ehrenbergiana* varied within the same population in the three study locations in Saudi Arabia. Seed size of flowering plants varies between and within the same species (Eriksson, 1999; Blade and Vallejo, 2008). The study locations varied in their maternal environment in terms of temperature, rainfall and altitude. Variation in seed size has been attributed to the prevailing environmental conditions during flowering and fruiting especially temperature and rainfall (Stratton, 1989; Wulff et al., 1999; Valencia-Diaz and Montata, 2005). This was further confirmed by Moleele et al. (2005) who attributed variations in seed mass of *A. tortilis* to rainfall and soil organic carbon. In this study seed mass significantly affected seed germination including both germination percent and germination mean time. Large seeds of *A. ehrenbergiana* gave the highest GP followed by medium seeds and small seeds had the least GP. In contrast, GMT was positively correlated with the seed coat thickness i.e. the thicker was the seed coat, the more time was elapsed for germination. Small and medium seeds had the least GMT as compared to large seeds i.e. they germinated faster. This might be attributed to the thicker seed coats of large sees. This is in line with the results of Norden et al. (2009) who studied the correlation between seed mass and GMT in the seeds of 1037 tropical tree species. Delayed germination of large seeds was attributed to underdevelopment of the embryo in some tropical trees such as *Minquartia guianensis* and *Virola* species (Camargo and Ferraz 2004; Piña Rodriguez and Figliolia 2005; Sautu et al., 2007). Also Vazquez-Yanes and Orozco-Segovia (1993) showed that longer GMT in large seeds might be explained in relation to water absorption. Prior to germination the embryo must reach full turgor for cell elongation. Small seeds absorb water faster than large seeds because they have a larger surface area to mass ratio (Kikuzawa and Koyama 1999). Also seedling emergence increased significantly with the increase in seed mass. Similar results were reported by Shaukat et al., (1999) where large seeds of *A. nilotica* gave higher GP and germination rates. Variation in seed size usually results in differences in stored food reserves and this affects germination and plant establishment (Wood et al., 1977). The germination rates of large seeds of *Ipomoea sindica* Stapf, *Cleome viscosa* L., and *Digera muricata* Forsk. were higher in large than in medium and small seeds (Aziz et al., 2010). Several investigators have attributed high germination and seedling establishment and survival to the more nutrient reserves contained in large as compared to small seeds of the same plant species (Khan and Shankar, 2001; Khan, 2004; Upadhaya et al., 2007 and Santos et al., 2009). Large seed size was considered as an adaptive advantage of tropical trees. The greater food reserves in large seed mass may prolong dormancy under unfavorable germination conditions and provide metabolic support to seedlings for better establishment. In addition, great seed reserves may provide other compounds for defense against pests and diseases (Foster, 1986).

Table 1. Climate in the study areas (2000 to 2012).

Location	Temperature (°C)			Rainfall (mm)		Elevation (m.a.s.l.)
	Monthly Mean Minimum	Monthly Mean Maximum	Mean Monthly	Monthly Mean Maximum	Mean Monthly	
Aseeer	31.2	7.6	19.2	115.2	11.8	2093.35
Baha	36.1	9.9	23.2	132.3	9.2	1651.88
Al Madinah	44.3	11.3	28.9	63.4	2.9	635.6

Source: National Metrological and Environmental Center, Presidency of Meteorology and Environmental Protection, Ministry of Defense and Aviation, Kingdom of Saudi Arabia.

Table 2. Descriptive statistics of seed mass of *A. ehrenbergiana* in the study locations

	Aseer	Baha	Al Madinah
Mean (mg)	0.190	0.178	0.193
S.D.	0.051	0.036	0.048
S.E.	0.013	0.009	0.012
Skewness	0.490	0.858	-0.028
Kurtosis	-1.09	0.004	-1.524
Variance	0.003	0.001	0.002
C.V.	27.16	20.42	24.8

Table 3. Effect of seed mass on germination of *A. ehrenbergiana* seeds (n=375)

Location	**Seed mass**	**Mean GP (%)**	**P (ANOVA)**	**LSD at P=0.05**	**R^2 (%)**
Aseer	Large	77.3 a*	0.0001		83
	Medium	39.3 b			
	Small	19.3 c		8.3	
Baha	Large	85.3 a	0.0001		87
	Medium	42.0 b			
	Small	21.3 c		7.7	
Al Madinah	Large	65.3 a	0.0001		71
	Medium	40.7 b			
	Small	37.0 b		6.1	
		Mean GMT (days)			
Aseer	Large	13.7 a	0.0001		52
	Medium	8.3 b			
	Small	7.8 b		1.9	
Baha	Large	14.2 a	0.0001		54
	Medium	9.0 b			
	Small	8.4 b		1.8	
Al Madinah	Large	12.5 a	0.0001		74
	Medium	7.5 b			
	Small	7.1 b		1.1	

*Means followed by the same letter in each study location are not significantly different at P=0.05

Table 4. Effect of seed mass on seedling emergence indicators (n=300)

Location	**Seed mass**	**SOE**	**P (ANOVA)**	**LSD at P=0.05**	**R^2 (%)**
Aseer	Large	0.27 a*	0.0001		83
	Medium	0.14 b			
	Small	0.05 c		0.03	
Baha	Large	0.29 a	0.0001		87
	Medium	0.14 b			
	Small	0.07 c		0.03	
Al Madinah	Large	0.22 a	0.0001		70
	Medium	0.14 b			
	Small	0.13 b		0.02	
		MED			
Aseer	Large	3.7 a	0.0001		81
	Medium	1.2 b			
	Small	0.5 c		0.5	
Baha	Large	4.2 a	0.0001		86
	Medium	1.3 b			
	Small	0.6 c		0.5	
Al Madinah	Large	2.9 a	0.0001		80
	Medium	1.1 b			
	Small	0.9 b		0.3	
		ERI			
Aseer	Large	3.8 a	0.002		55
	Medium	3.7 a			
	Small	2.2 b	0.0001	0.9	54
Baha	Large	3.4 a			
	Medium	3.1 a			
	Small	2.1 b		0.4	
Al Madinah	Large	4.2 a	0.0001		61
	Medium	4.0 a			
	Small	2.4 b		0.5	

*Means followed by the same letter in each study location are not significantly different at P=0.05

Fig. 1. Correlation between seed coat and GMT (Aseer)

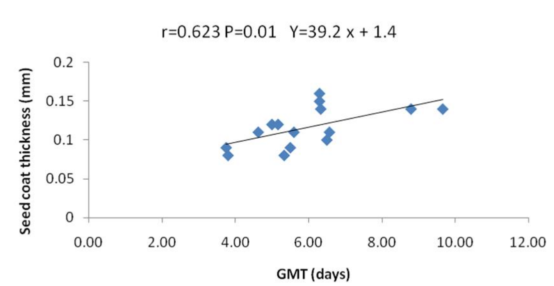
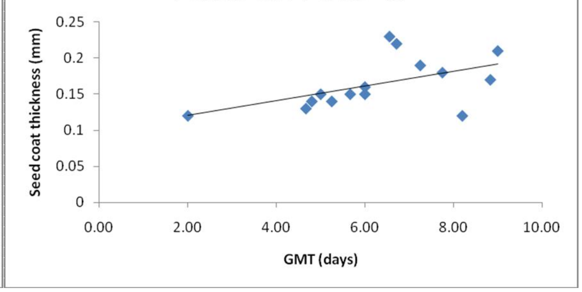
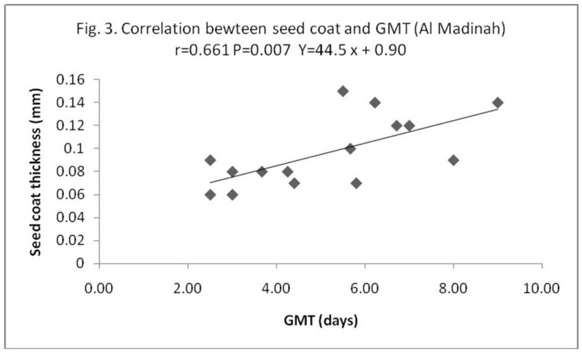


Fig. 2. Correlation between seed coat and GMT (Baha)





5. Conclusions

Seeds mass of *A. ehrenbergian* varied within population in the three study locations. Seed mass had significantly affected GP and GMT. Generally, the ultimate GP was greater in large seeds as compared to medium and small seeds. However, GMT was less in small seeds than in large seeds i.e. small seeds germinated before large seeds. In addition, seedlings originated from large seeds had the advantage of better SOE, MED and ERI. Therefore, seed mass is an important component for afforestation and re-forestation programs.

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