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EVALUATION OF GROWTH AND GAS EXCHANGE RESPONSE OF *ACACIA EHRENBERGIANA* HAYNE SEEDLINGS TO VARIATIONS IN SEED MASS

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Abstract

The effect of seed mass of *Acacia ehrenbergiana* Hayne on seedling growth and gas exchange activity under glass house conditions was investigated. Seeds were collected from three locations (Al Madinah (24°89´N, 39°16´E), Aseer (17°55´N, 42°11´E) and Baha (19°13´N, 41°80´E) in Saudi Arabia. The seeds were categorized into large, medium and small on the basis of the seed mass. Seeds were sown in potted soil (sand, clay and peat moss, 2:2:1 v/v) for six months. Seedling growth parameters and gas exchange were measured. Seedlings emerged from large seeds exhibited significantly faster growth in terms of shoot and root length and more dry weight, higher RGR (relative growth rate) and greater net photosynthetic and transpiration rates and stomatal conductance than those emerged from medium and small seeds. Leaf number also increased with seed mass but inconsistently.

Key words: Gas exchange, Seed weight, Growth parameters, Germination.

Introduction

In tropical forests, the existing deforestation rates have exceeded afforestation and re-forestation (Grainger, 1993; Laurence, 1999). Forest trees are commonly used to combat desertification, ecological restoration and a wide range of ecological services (Cortina *et al.*, 2011; Oliet & Jacobs, 2012; Abbas *et al*., 2013). Studies on seed biology are of utmost importance for restoration programs (Salati & Nobre, 1991; Kozlowski, 2000). Seed size has been considered as the most important component of forests reproduction (Harper *et al*., 1970). The success of forest plantations has been greatly affected by morpho-functional traits, competitive ability of seedlings and tolerance to stresses (Navarro *et al*., 2006; del Campo *et al.*, 2010). Seed mass considerably affects germination and seedling performance. Larger seeds are correlated with the best seedling growth over small seeds (Cordazzo, 2002). The survival rate and resprouting capability of seedlings originated from large seeds were much higher under all light intensities tested as compared to those emerged from small seeds (Khan, 2004). Du & Huang (2008) reported that the relative growth rate (RGR) and seedling emergence were significantly greater for large seeded-seedlings than small-seeded seedlings. In contrast, Blade and Vallejo (2008) reported a negative correlation between seed mass and RGR, however this didn't compensate the initial large seedlings originated from large seeds which resulted in higher survival rates as compared to those emerged from small seeds. Although early and post-germination are significant for the establishment of a plant population but not necessarily to maturity (Susko & Doust, 2000). Seed mass was correlated with seedling size and vigor across species (Long and Jones, 1996; Milberg *et al.*, 1998; Walters & Reich, 2000) and within the same species (Reich *et al*., 1994; Castro, 1999; Vaughton & Ramsey, 2001; Halpern, 2005). In dry lands, although seed mass has been considered as an extremely important trait, nevertheless there is a lack of consensus regarding traits determining high quality woody seedlings (Cortina *et al.*, 2013). Although, numerous research was carried out on the effect of seed mass on the growth parameters of seedlings, however no studies have been conducted on the physiological performance of seedlings as affected by seed mass.

The present study was conducted to investigate the effect of seed mass of *Acacia ehrenbergiana* Hayne on seedling growth and physiological performance (gas exchange) under glass house conditions.

Materials and Methods

Seed collection: Fresh pods of *Acacia ehrenbergiana* Hayne were collected randomly form natural populations in Baha (19°13´N, 41°80´E), Aseer (17°55´N, 42°11´E) and Al Madinah (24°89´N, 39°16´E) in Saudi Arabia. Seeds were extracted from the air-dry pods and only intact and healthy seeds were obtained. Seeds were weighed and divided into small (0.12-0.17 mg), medium (0.18-0.25 mg) and large (>0.25 mg). All seeds were pre-treated by soaking in H_2SO_4 (98%) for one hour to break the hard seed coat. Seeds were then washed thoroughly with tap water and dried with filter paper.

Sowing of seeds: One hundred seeds each from large, medium and small seeds from each location were sown in plastic pots (30 x 40 cm) containing sand, clay and peat moss (2:2:1 v/v). Each pot contained 5 seeds. The pots were irrigated regularly every other day. A completely randomized block design (CRBD) was used and it was replicated 20 times for every seed mass from each location. The experiment was carried out in a glass house (N 42° 24´ E 46° 44´, 600 m.a.s.l.) maintained at 35 ± 2°C.

Measurement of seedling growth parameters: After 6 months seedling shoot length and diameter, root length, shoot and root dry weight, total dry weight, leaf number and the relative dry growth rate (RGR) of the whole seedling were measured for all seedlings emerged from large, medium and small seeds, respectively. RGR was calculated according to the formula of Hunt (1990) as follows:

$$\text{RGR (g/g/day)} = \frac{\text{Wt - W0}}{t_2\text{-}t_1}$$

where W0 is the initial plant mass, Wt is the final plant mass and t is the number of days between the initial and the final measurement (Hunt, 1990).

Gas exchange measurements: Photosynthetic gas exchange measurements were taken under light conditions (before noon) using a handheld photosynthesis system (CI–Handheld Photosynthesis system, CID, Inc., Camas, U.S.A). In each measurement, a fully expanded leaf in the middle of the seedling was selected for gas exchange measurement. The leaf was inserted inside the leaf chamber and then photosynthetic gas exchange parameters were measured i.e. net photosynthetic rate, transpiration rate and stomatal conductance. These measurements were replicated 5 times/ seedling/seed mass/location.

Statistical analysis: The data concerning seedling growth parameters and gas exchange were analyzed by 3 way (seed mass) analysis of variance (ANOVA) and means were separated with the Least Significant Difference (LSD) at P = 0.05 using SAS statistical package (Anon., 1997).

Results

Shoot, root and total seedling length: Seed mass had significantly affected shoot, root and total length. Seedlings emerged from large seeds produced higher shoot, root and total length compared to those emerged from medium and small seed mass (Tables 1-2).

Table 1. Effect of seed mass on shoot and root length of *Acacia ehrenbergiana* seedlings.

Locations	Seed mass	Parameter	F-value	P	LSD (p=0.05)	R^2 (%)
		Shoot length (cm)				
Aseer	Large	59.9 a	5.84	0.02	5.59	50
	Medium	52.4 b				
	Small	52.2 b				
Baha	Large	59.9 a	3.83	0.05	9.65	39
	Medium	56.6 ab				
	Small	48.0 b				
Al Madinah	Large	58.2 a	4.40	0.03	5.13	5.1342
	Medium	52.3 b				
	Small	51.9 b				
		Root length (cm)				
Aseer	Large	48.0 a	34.9	0.0001	4.01	85
	Medium	35.9 b				
	Small	33.7 b				
Baha	Large	41.5 a	4.4	0.04	4.9	42
	Medium	36.4 b				
	Small	35.3 b				
Al Madinah	Large	42.5 a	3.5	0.05	7.7	37
	Medium	34.5 b				
	Small	34.1 b				

Means of a parameter followed by the same letter are not significantly different at p=0.05

Table 2. Effect of seed mass on seedling diameter and total length of *Acacia ehrenbergiana*.

Locations	Seed mass	Mean seedling diameter (mm)	F-value	P	LSD (p=0.05)	R^2 (%)
Aseer	Large	4.9 a	10.5	0.002	0.60	64.0
	Medium	3.9 b				
	Small	3.7 b				
Baha	Large	4.6 a	7.8	0.01	0.57	57
	Medium	4.3 a				
	Small	3.6 b				
Al Madinah	Large	4.8 a	26.4	0.0001	0.32	81
	Medium	3.3 b				
	Small	3.8 b				
		Total seedling length (cm)				
Aseer	Large	108.0 a	28.8	0.0001	7.6	80
	Medium	88.4 b				
	Small	85.9 b				
Baha	Large	101.4 a	11.3	0.01	8.3	65
	Medium	93.0 b				
	Small	83.0 c				
Al Madinah	Large	100.6 a	5.7	0.01	10.6	49
	Medium	86.8 b				
	Small	86.0 b				

Means of a location followed by the same letter are not significantly different at p=0.05

Table 3. Effect of seed mass on shoot and root dry weight of *Acacia ehrenbergiana* seedlings.

Location	Seed mass	Parameter	F-value	P	LSD (P=0.05)	R^2 (%)
		Shoot dry weight (g)				
Aseer	Large	11.4 a	80.0	0.0001	0.95	93
	Medium	7.4 b				
	Small	6.1 c				
Baha	Large	10.3 a	61.2	0.0001	1.05	91
	Medium	7.1 b				
	Small	5.0 c				
Al Madinah	Large	8.4 a	45.8	0.0001	1.00	88
	Medium	6.3 b				
	Small	4.0 c				
		Root dry weight (g)				
Aseer	Large	5.9 a	7.7	0.01	1.09	56
	Medium	4.2 b				
	Small	4.2 b				
Baha	Large	6.4 a	50.2	0.0001	0.69	89
	Medium	4.5 b				
	Small	3.2 c				
Al Madinah	Large	5.9 a	13.2	0.001	1.45	69
	Medium	4.4 b				
	Small	2.5 c				

Means of a parameter followed by the same letter are not significantly different at p=0.05

Table 4. Effect of seed mass on leaf number and total dry weight of *Acacia ehrenbergiana* seedlings.

Location	Seed mass	Parameter	F-value	P	LSD (P=0.05)	R^2 (%)
		Leaf number				
Aseer	Large	50.0 a	1.02	>0.05	5.9	14
	Medium	48.6 a				
	Small	46.2 a				
Baha	Large	51.0 a	4.53	0.03	2.95	43
	Medium	47.0 ab				
	Small	44.2 b				
Al Madinah	Large	54.2 a	2.05	>0.05	6.27	25
	Medium	49.6 a				
	Small	48.8 a				
		Total dry weight (g)				
Aseer	Large	17.2 a	87.9	0.0001	1.21	94
	Medium	11.9 b				
	Small	10.3 c				
Baha	Large	16.4 a	187.5	0.0001	0.94	94
	Medium	11.5 b				
	Small	8.2 c				
Al Madinah	Large	14.0 a	50.7	0.0001	1.63	89
	Medium	10.7 b				
	Small	6.5 c				

Means of a parameter followed by the same letter are not significantly different at p=0.05

Seedling diameter: Similarly, large seeds produced seedlings with diameters higher than those from medium and small seeds and these differences were significant (Table 2). However, no significant differences were found between the diameters of seedlings emerged from medium and small seeds.

Seedling mass: Large seeds produced significantly heavier seedlings compared to medium and small seeds from all locations studied (Tables 3-4). Seedlings from large seeds produced double the shoot, root and total dry weight as compared to small seeds, whereas medium seeds recorded medium values i.e. smaller and larger than large and small seeds, respectively.

Leaf number: Seed mass had no significant effect on leaf number of seedlings emerged from seeds collected from 2 of the study locations (Aseer and Al Madinah), nevertheless seedlings emerged from large and medium seed mass collected from Baha produced significantly more leaves compared to small seeds on the one hand and differences between medium and small seeds were not significant on the other hand for seeds collected from Baha (Table 4).

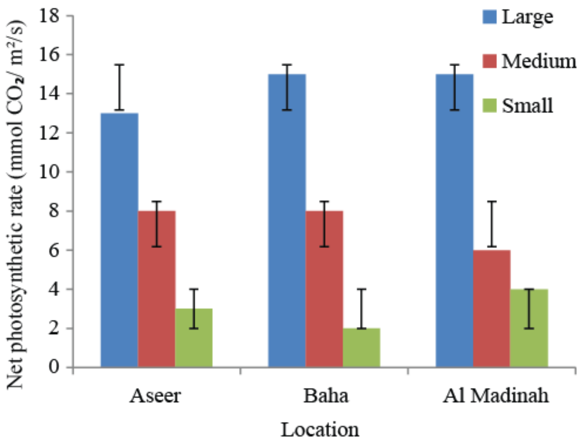

Fig. 1. Effect of seed mass on net photosynthetic rate of *Acacia ehrenbergiana* seedlings.

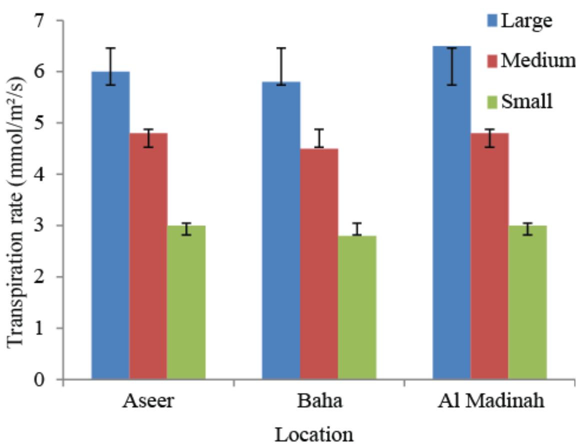

Fig. 2. Effect of seed mass on transpiration rate of *Acacia ehrenbergiana* seedlings.

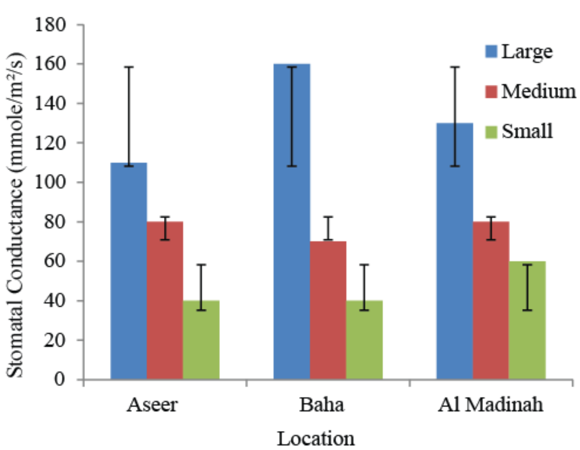

Fig. 3. Effect of seed mass on stomatal conductance of *Acacia ehrenbergiana* seedlings.

Relative growth rate (RGR): The RGR of seedlings was significantly (P=0.0001) affected by seed mass. RGR was consistently higher in seedlings emerged from large seeds as compared to those from medium and small seeds, respectively (Table 5). Indeed RGR was almost double in seedlings emerged from large seeds compared to those from small seeds.

Gas exchange: Seed mass significantly affected gas exchange parameters tested. Net photosynthetic and transpiration rates and stomatal conductance were significantly higher in seedlings originated from large seeds as compared to medium and small seeds and the ranking was large > medium > small seeds (Figs. 1-3).

Discussion

Seed mass variation has been reported in several tropical tree species (Foster, 1986; Khan *et al.*, 1999, 2002; Khan & Shankar, 2001). This was attributed to variations in temperature more than rainfall (Moles and Westoby 2003; Murray *et al.*, 2004). This variation significantly affected seedling performance and gas exchange. In this study, seedlings originated from large seeds were heavier, grew faster and recorded more efficient gas exchange in terms of net photosynthetic and transpiration rates and stomatal conductance than seedlings from medium and small seeds. These results were in line with several investigators (Reich *et al.*, 1994; Surles *et al.*, 1993; Cordazzo, 2002; Yanlong *et al.*, 2007; Upadhaya *et al.*, 2007; Blade & Vallejo 2008; Iortsuun *et al*., 2008; Santos *et al*., 2009). This might be attributed partly to the fact that large seeds had higher net photosynthetic rates and more efficient transpiration rate and stomatal conductance which imply the production of more food reserves necessary for seedling growth than small seeds. Kermode and Finch-Savage (2002) found that large seeds contain more water than small seeds due to the slow dehydration of large seeds. Even Daws *et al*. (2004) attributed the slow germination of small seeds to the variation in seed water content rather than to seed size. Large seeds were reported to contain more energy and food reserves for the embryo than small seeds (Escudero *et al*., 2000; Khurana & Singh; 2004; Mueller *et al.*, 2005). The duration of the effect of seed mass on seedling growth has been debatable. It was anticipated that seed mass effect on seedling size was temporary and lasted by the exhaustion of the cotyledon food reserves (Norgren, 1996; Castro, 1999; Lopez *et al*., 2003). However, other investigators claimed that the seed mass effect on seedlings growth of *Pinus sylvestris* continued for 5 years (Reich *et al*., 1994; Wennstrom *et al*., 2002) and even for 15 years in *Pinus elliottii* (Surles *et al*., 1993). The seed position on the mother plant during formation may play a role in seed size. Kromer (1984) reported that seeds of *Oenothera biennis* that were produced proximally on the inflorescence were heavier than distally produced seeds. In the present study, a positive association between seed mass and RGR of the emerging seedlings was recorded i.e. biomass allocation per time was much higher in seedlings originated from large seed mass. This was in agreement with the findings of Yanlong *et al*. (2007) and Du & Huang (2008). Such higher energy reserves provided a better chance for successful seedling establishment (Haig & Westoby, 1991; Khan, 2004). However, in some studies a negative correlation between RGR and seed mass was also reported (Milberg *et al*., 1998; Blade & Vallejo, 2008; Houghton *et al.*, 2013). In these studies, the positive correlation between small seeds and RGR had not compensated the heavier seedlings emerged from large seeds (Blade & Vallejo, 2008). Generally, the correlation between RGR and seed mass was debatable. Some investigators have reported a negative correlation (Meyer & Carlson 2001; Paz & Martínez-Ramos, 2003), or positive correlation (Meerts & Garnier 1996) and no correlation between seed mass and RGR was also reported (Reich *et al*., 1994; Tamet *et al*., 1996). However, most investigators have found a negative correlation between seed mass and RGR, especially between species (Reich *et al*., 1998, 2003; Wright & Westoby, 1999; Grotkopp *et al*., 2002; Poorter & Rose, 2005).

Table 5. Effect of seed mass on relative growth rate (RGR) of Acacia ehrenbergiana seedlings.

Location	Seed mass	RGR (g $g^{-1}day^{-1}$)	F-value	P	LSD (P=0.05)	R^2 (%)
Aseer	Large	0.37 a	65.7	0.0001	0.026	92
	Medium	0.29 b				
	Small	0.24 c				
Baha	Large	0.35 a	81.1	0.0001	0.024	93
	Medium	0.28 b				
	Small	0.21 c				
Al Madinah	Large	0.31 a	49.3	0.0001	0.032	89
	Medium	0.26 b				
	Small	0.16 c				

Means of a location followed by the same letter are not significantly different at p=0.05

Conclusions

For successful forest restoration and afforestation programmes it is more appropriate to use large seeds as much as possible. This is because they produce fast growing and more vigorous seedlings with more gas exchange activity as compared to small seeds.

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