GLOBAL JOURNAL OF BIO-SCIENCE AND BIOTECHNOLOGY

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VARIATION IN SEED MORPHOMETRIC CHARACTERISTICS AND GERMINATION OF *ACACIA TORTILIS* SUBSPECIES *RADDIANA* AND SUBSPECIES *SPIROCARPA* AMONG THREE PROVENANCES IN SUDAN

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ABSTRACT
Seeds of two inter-specific subspecies of *A. tortilis* (mainly subspecies raddiana and spirocarpa) collected from three varied geographical sources (White Nile, Kassala and River Nile states) were evaluated in morphometric characters (weight, number of seed / kilogram, length, width) and germination behaviour. Different levels of concentrated sulphuric acid was used for seed pre-treatment; 0 (Untreated), 20, 30 and 40 minutes and germinated in the germination room under control environment. The study revealed highly significant differences between subspecies and among the three provenances in seed morphometric traits. Seeds of subspecies raddiana were longer, wider, heavier and hence less number of seeds/kg. In regard to provenances River Nile displayed heavier seeds followed by White Nile. In contrast to that Kassala seeds were relatively longer than the two other provenances, where the width showed no significant difference between provenances. The acid pre-treatment showed highly significant variation in seed germination percentage, whereas the three sulphuric acid durations improved the germination percentage over the control untreated seeds. 30 and 40 minutes acid durations showed highest germination across the subspecies and provenances and with no significant difference between them, indicating that 30 minutes acid pre-treatment will be the choice. The study revealed that there was great variation between the provenances, subspecies and treatments.

KEYWORD: *Acacia tortilis*, subspecies raddiana, subspecies spirocarpa, seed weight, number of seed/kg, thickness, width, treatment, germination percentage.

INTRODUCTION
A critical decision in forest management is the choice of seed sources for reforestation to ensure a successful crop. Seed zone and seed transfer guidelines are essential tools in assisting this decision and to quantify the distance of seed transfer and determine the size of seed zones to minimize the risk of planting of poorly adapted trees, (Hamann *et al.*, 2000; Russel, 1998). Plant species possess substantial genetic variation in several growth and adaptive traits and the use of selective genotypes can enhance site productivity and reduce the risk of maladaptation (Hamann *et al*., 2000).Variation in seed morphometric characteristics within species were reported for many forest tree species (Khalil and Siam, 2003). *Acacia karoo* displayed significant differences among geographical sources in morphometric seed characteristics *viz* seed weight, number of seed/kg, seed length, width and thickness (Abdelkhair *et al*., 2003). Spatial variation in seed weight of *Acacia tortilis* was primarily determined by rainfall and soil types (Moleele *et al*., 2005). Seed germination rate and performance and seed dormancy are physiological phenomena in the seed that are strongly related to prevailing environmental conditions (Mahgoub, 2002). The ability of the species to remain dormant is particularly associated with seeds of species from unpredictable environments and climate with variable rainfall trends (Khurana and Singh, 2001). Seed coat dormancy prevents the seed from germinating during isolated showers in the middle of a long dry season while permitting it during a sustainable rainy season (Willan, 1985 and Doran *et al.*, 1983). Seed of *Acacia tortilis* have a hard seeds coat impervious to water which causes the seed dormancy and germination may be extended over months and years. To ensure high germination percentage which is rapid and uniform, a pre-sowing treatment is necessary. In arid environments, with unpredictable climate, *Acacia tortilis* seed coat is hard and impermeable to water and required treatment to obtain maximum germination (Fogg and Greaves, 1990; Timberlake *et al*., 1999). Several studies on seed treatment indicate that maximum germination in the shortest time could be achieved by soaking seeds in concentrated sulphuric acid, the time of treatment varied from 20 to 60 minutes. The aim of this study was to investigate the inter-specific variation in seed morphometric characteristics and germination behaviour among and within three provenances of *Acacia tortilis* subspecies raddiana and subspecies spirocarpa in Sudan.

MATERIAL & METHODS
Seed source
The Study dealt with *Acacia tortilis* when the association of the two subspecies raddiana and spirocarpa were found together in their natural habitats in three sites. The seeds for both subspecies were collected during April - May 2005. The collection was done from three geographical locations.

TABLE 1. Seed sources of the three provenaces of Acacia tortilis subspecies raddiana and subspecies spirocarpa

Locations	Latitude	Longitude	Attitude (m)	Rainfall (mm)	Maximum (◦C)	Minimum (◦C)
White Nile	13˚ 30´N	32˚ 33´E	185	180	37.3	23.3
Kassala	15˚ 30´N	35˚ 58´E	458	318.6	37.9	21.7
River Nile	16˚ 20´N	32˚ 36´E	178	62.6	37.5	22

Modified from Ministry of Sciences and Technology with Meteorological Authority, Khartoum Airport station and Heavens-above.com, 2008

The three locations were White Nile state (Elgetiana area), Kassala state (Halfa Elgadida) and River Nile State (Shandi).The description of the three locations is shown in Table (1).

Mature and healthy fruits were collected from the tree crown by shaking with long hooked stick. Seed collection had been done from at least 30-40 trees 100-200 m apart from each other, for both the two subspecies in the three locations. The mature dry pods were kept loosely packed in plastic sacks and kept in open place to allow for adequate air circulation.

Seed extraction and cleaning

Seeds were extracted by beating up the pods with sticks and crushing by hands, then seeds were winnowed to separate the husk. Seeds were washed with water to remove empty seeds and debris, seeds were then soaked for 6 hours in water to remove insect damaged seeds which imbibed water and runoff with drainage water. Then the seeds were spread in thin layer on cemented platform and dry for one day under full shade.

Seed characterisation

Seed weight and number of seed /kg

Weight of 1000 seeds was determined according International Seed Testing Association (1993). In brief, 8 random replicates of 100 pure seed were counted for each subspecies and each locality; each replicate was weighed separately using a three decimal electronic balance. Means were calculated and the range of the weight was evaluated (Mahgoub and Jovall, 1994). Then number of seeds per kilogram was calculated.

Seed parameters (seed length, width and thickness)

Sample of 50 seed of each subspecies for each provenance were drawn and the three dimensions (length, width and thickness) were measured using vernier.

Germination behaviour

The germination potential of the two subspecies from the three provenances was assessed following the standard germination test (ISTA, 1993).The experiment was conducted in the National Tree Seed Centre at Soba, Khartoum, in the germination room under controlled environmental conditions (Temperature was 28-32 ˚C, 12hours light from florescent lamps). Four hundred seeds per subspecies per provenance per treatment by seed divider were drawn at random and divided into four replicates of 100 seeds. Conc. sulphuric acid was used for 0, 20, 30 and 40 minutes to break the seed dormancy. The seeds were well washed to avoid the sulphuric acid effects after the treatments. Seeds were sown in germination trays filled with sand and were kept moist by daily watering. Germination was evaluated weekly for period of a month. Total germination percentage was calculated by the equation.

Germination %= Total number of germinated seeds /total number of sown seeds×100.

Data analysis

Analysis of Variance (ANOVA) was used. Means were separated by Duncan's multiple range test. Statistical System (SAS), version 6.12 (SAS institute) was used for analysis.

RESULTS

Variation in seed morphometric characteristics

Seed weight and number /kilo gram

The provenance, subspecies and the provenance x subspecies interactions showed highly significant differences in seed weight and No/kg ($p \leq 0.0001$) Table (2).

TABLE 2. ANOVA results on the effects of provenance, subspecies and their interactions on *Acacia tortilis* subspecies raddiana and subspecies spirocarpa seed morphometric characteristics

Parameter		Weight	No/kg	Length	Thickness	Width
Source of variation	df	F. Value	F. Value	F. Value	F. Value	F. Value
Provenance	2	7.72***	13.39***	9.45***	15.46***	2.99*
Subspecies	1	953.78***	1231.70***	107.10***	74.90***	65.92***
Provenance*subspecies	2	3.23**	7.50***	-	-	-

***Significantly different at p<0.001 * significantly different at p<0.05

**Significantly different at P<0.01 ns not significantly different at P<0.05

For Subspecies raddiana, the heaviest seed weight revealed by River Nile followed by White Nile and lighter one by Kassala. The mean weight is range from (3.86 – 4.04 cm) Table (3). In the subspecies spirocarpa, White Nile scored the heaviest seed weight followed by the River Nile and Kassala scored the lightest seed weight and the mean weight is range from (2.693- 2.933 cm) Table (3). In same provenance, the subspecies raddiana in three provenances displayed the heaviest seeds than the subspecies spirocarpa, but subspecies spirocarpa displayed large number of the seeds Table (4).

Therefore Kassala was revealed larger number of seed/ kg for the two subspecies than the other provenances, but River Nile revealed the smallest number for subspecies raddiana and White Nile for subspecies spirocarpa. Whereas the provenance revealed the lightest seed weight revealed the largest seed number, Table (3).

TABLE 3. Variations of the seed characteristics of *Acacia tortilis* subspecies raddiana and subspecies spirocarpa from the three provenances

Species parameters	raddiana			spirocarpa		
	White Nile	Kassala	River Nile	White Nile	Kassala	River Nile
Seed weight/g	3.92[a]	3.86[a]	4.04[a]	2.923[a]	2.639[c]	2.782[b]
Seed number/kg	25555.3[a]	25931.9[a]	24716.3[a]	34726.9[c]	37915.8[a]	35976.5[b]
Seed length(cm)	0.600[ab]	0.657[a]	0.584[b]	0.5422[a]	0.522[a]	0.500[b]
Seed width(cm)	0.394[a]	0.382[a]	0.377[a]	0.351[a]	0.348[a]	0341[a]
Seed thickness(cm	0.224[b]	0.229[c]	0.256[a]	0.215a[b]	0.208[b]	0.226[a]

*Means with same letters in the same row for the same parameter for same subspecies are not significantly different at p=0.05 using Duncan new multiple range test

Seed length, width and thickness

There were highly significant differences between provenances and subspecies in seed length, thickness and width (P≤ 0.0001) and significant difference in seed width (p≤ 0.05) between the provenances for subspecies raddiana Table (2).

White Nile Subspecies raddiana revealed the highest seed length, width and the highest thickness revealed by River Nile, but Kassala revealed the smallest length and thickness Table (3). Therefore for the subspecies spirocarpa White Nile leading the other provenances in seed length, width and the smallest ones revealed by River Nile, but it revealed the thicker seed Table (3). In the same provenance subspecies raddiana showed the higher seed length, width and thickness than the subspecies spirocarpa Table (4). Figure 1 and 2 showed that the White Nile provenance leading the others provenances in seed length and width for both subspecies.

TABLE 4. Variations of the seed characteristics of *Acacia tortilis* subspecies raddiana and subspecies spirocarpa of the same provenance

Seed parameters	White Nile		Kassala		River Nile	
	raddiana	spirocarpa	raddiana	spirocarpa	raddiana	spirocarpa
Seed weight/g	3.92[a]	2.88[b]	3.86[a]	2.63[b]	4.04[a]	2.78[b]
Seed number/kg	25555.3[b]	34725.9[a]	25931.9[b]	37915.8[a]	24716.3[b]	35976.5[a]
Seed length(cm)	0.600[a]	0.542[b]	0.567[a]	0.522[b]	0.584[a]	0.500[b]
Seed width(cm)	0.394[a]	0.351[b]	0.382[a]	0.348[b]	0.377[a]	0.341[b]
Seed thickness(cm)	0.244[a]	0.215[b]	0.229[a]	0.208[b]	0.256[a]	0.224[b]

*Means with letter in same row for the same parameters for the same provenance are not significantly different at p= 0.05 using Duncan new multiple range test

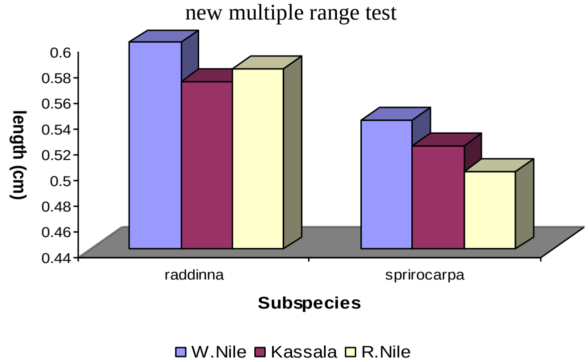

FIGURE 1: *A. tortilis* subspecies raddiana and spirocarpa seed length among and within provenance

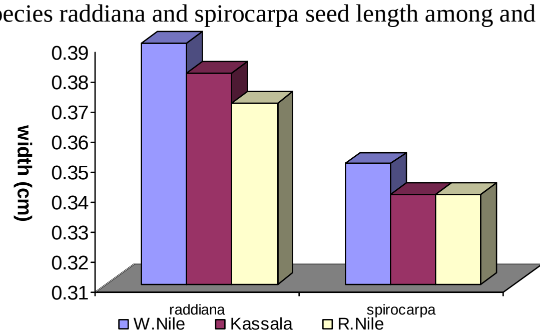

FIGURE 2: *A. tortilis* subspecies raddiana and spirocarpa seed width among and within provenance.

Seed germination

The result revealed highly significant variation between the subspecies and the treatment, but subspecies* treatment interaction showed significant variation, whereas no significant variation existed between provenance and provenance*subspecies interaction Table (5). All treatments duration of the sulphuric acid improved the germination over the control for both subspecies in the three provenances and led to level of germination significantly higher than untreated seeds Table (6). The two subspecies in the three provenances showed high seed viability and the sulphuric acid released theirs seed dormancy. However the control in the three provenances scored very low germination %, but White Nile showed better germination than the other two provenances for both subspecies Table (6). Figures (3 and 4) showed germination behaviour strategy of the two subspecies through five weeks.

TABLE 5. ANOVA on the effect of provenance, subspecies, treatment and their interactions on *Acacia tortilis* seed germination

Source of variation	df	ms	F. value
Provenance	2	63.16	11.44ns
Subspecies	1	2784.26	63.53***
Provenance*subspecies	2	31.29	0.71ns
Treatment	3	31129.31	710.25***
Subspecies*treatment	3	159.03	3.63**

*** Significantly different at p<0.001 * significantly different at p<0.05
* * Significantly different at P<0.01 ns not significantly different at P<0.05

TABLE 6. Cumulative germination percentatge as affected by different sulphuric acid treatments in *Acacia tortilis* subspecies raddiana and spirocarpa from the three provenances

Sulphuric acid	raddiana			spirocarpa		
duration	White Nile	Kassala	River Nile	White Nile	Kassala	River Nile
40 min	90.0^{a}	93.0^{a}	84.5^{a}	93.25^{a}	98.0^{a}	97.25^{a}
30 min	88.25^{a}	85.0^{a}	90.5^{a}	92.5^{a}	93.5^{a}	96.25^{a}
20 min	73.0^{b}	82.25^{a}	81.0^{a}	91.0^{a}	93.25^{a}	87.5^{b}
Control	14.25^{c}	5.5^{b}	7.7^{b}	22.0^{b}	32.75^{b}	23.75^{c}

*Means with letter in same column for same subspecies for same provenance are not significantly different at p=0.05 using Duncan new multiple range test.

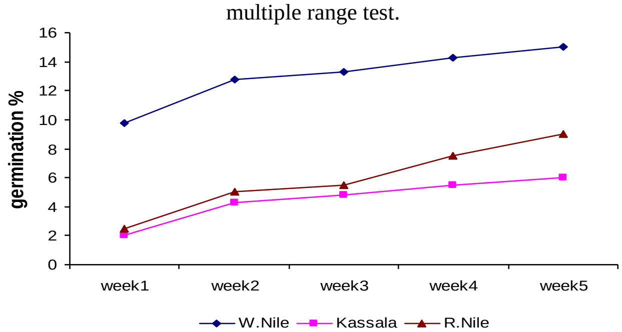

FIGURE 1: *A. tortilis* subspecies raddiana germination behaviour (control).

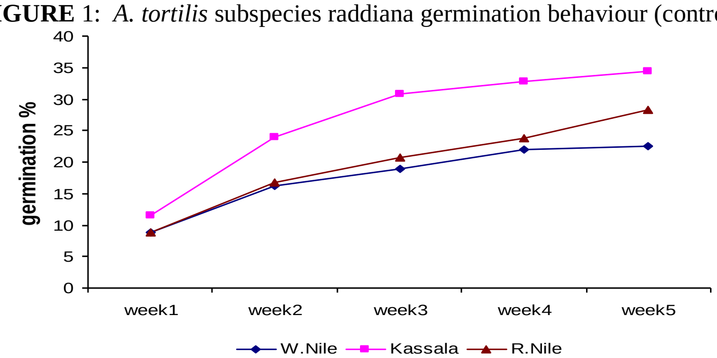

FIGURE 2: *A. tortilis* subspecies spirocarpa germination behaviour (control).

DISCUSSION

Observed phenotypic variation is generally assumed to reflect the inherent genotypic variation among and within the provenances grown under uniform conditions. The significant variation in seed morphometric characters among and within the provenances of *Acacia tortilis* may reflect the overriding impact of both environmental and genetic variation and this can be assumed to reflect true genetic variation and adaptation to different environmental conditions and soil types. The subspecies raddiana showed slight variations in seed weight, the River Nile displayed the heavier seeds followed by White Nile, but the subspecies spirocarpa displayed highly significant variation between the provenances in seed weight and this may be due to true inherent character of the two subspecies. The provenance displayed the heavy seed displayed lower number of seed /kg for both subspecies and that displayed the light seed scored the large number of seed /kg. The same finding by (Elfeel, 1996) and confirmed by (Raddad, 2007) who studied 8 provenances of *Acacia senegal* from clay and sandy soil, the provenance have smallest seed have higher seed number and that have large seed have lowest seed No/kg.

Variation in seed weight, length, width and thickness between or within plant species are due to evolutionary responses of plant to maximize the potential fitness by producing a larger number of seeds and increase the chance of establishment of resulting seedlings through great allocation of maternal resources to individual seeds (Zhang, 1998).The provenances and subspecies showed variation in seeds length, width and thickness and same finding with Dangasuk *et al*., 1997, the regional provenances showed consistent variation in seed length, width, thickness, weight and germination rate of 12 Africa provenances of *Faidherbia albida*. The same finding by Abdelkhair *et al*. (2003) who reported that *Acacia karoo* displayed significant differences among geographical sources in seeds characteristics seeds weight, number/kg, length, width and thickness. Phenotypic variation is determined by genotype and environment interaction and is assumed to express of genotypic variation when environmental conditions controlled (Dangasuk *et al*., 1997; Westoby *et al*., 2002; Raddad, 2007). Differences observed in *Acacia tortilis* provenances in seed morphology, reflected adaptation to the different environments. Spatial variation in seed weights of *Acacia tortilis* was primarily determined by rainfall and soil types. Acacia seeds quality is function of both genetic and environmental factors (Moleele *et al*., 2005). Tree species in which seeds germinate and survive in dry periods are expected to show variation in morphological and physiological traits that may arise due to evolution of certain potentiality and adaptive genetic correlation between germination and post-germination traits (Evans and Cobin, 1995; Raddad, 2007). Many Acacia species have a hard seed coat which makes it difficult to imbibe water unless some scarification pre-treatment is adopted (Teketay, 1996; Kulkarni *et al*., 2006).

Dormancy and germination comparing among the provenances for treated and untreated seeds, the treated seeds exhibited greater germination % than untreated seeds under the three different duration of the sulphuric acid for the two subspecies in the three provenances and all subspecies seeds responded positively to the treatment. This is because acacia seeds possess physical dormancy due to hard seed coat which is impermeable to water, any treatment that lead to scarification of the hard coat will render it permeable to water and shower give high germination (Bonner, 1974; Schmidt, 2002; Willan, 1985). This finding is consistent with (Walter *et al*., 2004) who studied the germination of burn and unburned seeds of *Acacia Karoo* and *Acacia nilotica*, burnt seeds achieved better germination than the unburnt seeds. The same finding was found by Kulkarni *et al*., (2006) when they use fire to break the dormancy of *A. hebelada*, *A. meansii* and *A. robusta* which exhibited greater germination % than untreated seeds. Sulphuric acid with the three durations used for the two subspecies in the three provenances released the seed dormancy and improved the germination over the control. Seed of *Acacia tortilis* have hard seed coat which is impervious to water which cause the seed dormancy and germination may be extend over months and years, to ensure high germination percentage which is rapid and uniform, pre-sowing treatment is necessary. The *Acacia tortilis* variability of seeds characteristics is similar to other findings for other species, seed traits of *Sesbania sesban* were significantely different among provenances (Odual, 1993). *Acacia mangium* seeds also showed considerable differences (Salazer, 1989). Populations of *Acacia tortilis* differ in seed characteristics in spite of great variation within- populations. This magnitude of inter-specific difference in seed weight can be considered substantial because overlaps in seed weight distribution are even commonly observed between species from different families or orders (Zhang, 1998; Westoby *et al.*, 1995). Variation in *Acacia tortilis* seed germination performance characters may is factor of site environmental conditions. Treated seeds of *Acacia tortilis* showed greater increase in germination percentage under the three different acid durations compared to the control untreated seeds.

CONCLUSION

Highly significant variation in seeds morphomteric characteristics among and within the provenances of *Acacia tortilis* may reflect the impact of both environmental and genetic variation. This can be assumed to reflect true genetic variation and adaptation to different environmental conditions and soil type. The seeds of three *Acacia tortilis* provenances responded positively to the three sulphuric acid treatments and improved germination % over control. The significant variation with the two subspecies in seed morphometic characteristics, germination %, may be due to true inherent and ecological requirement of the two subspecies. The study revealed that there is great variation between the provenances and subspecies in seed weight, No/kg, length, width and thickness and there is great variation between treatments.

ACKNOWLEDGEMENTS

The authors appreciate the technical assistances provided by Dr. Elfeel and finical support by Sudan University of Science and Technology.

REFERENCES

Abdelkheir, R.M., Ibrahim, A., and Khalil, A. (2003) Provenance variation in seed and germination characteristics of *Acacia karroo*, Sudan Silva 9 (2) pp 14-26. 2003.

Bonner, F.T. (1974) Presowing treatment of seed to speed germination, pp. 126-35 in 'Seeds of Woody Plants in the United States'. Agric. Handbook No. 450 Forest Service, Washington, D.C.

Doran, J., Turnbull, J.W., Boland, D.J. & Gunn, B.V. (1983) Handbook on seeds of dry –zone Acacias. A guide for collection, extraction, cleaning and storing the seed and for treatment to promote germination of dry- zone acacias. Division of Forest Research. CSIRO, Australia.

Dangasuk, O.G., Seuri, P. and Gudu, S. (1997) Genetic variation in seed and seedling traits in 12 African provenance of Faidherbia albida (Del) A. Che. at Lodwar Kenya, Agroforestry System : 133- 141, 1997. Kluwer Academic Publisher.

Elfeel, A.A. (1996) Provenance variation in seed characteristics, germination and early growth traits of

Acacia senegal (L) wild in Sudan. M.Sc thesis, University of Khartoum, Sudan.

Evans, A.S. and Cobin, R.J. (1995) Can Dormancy affects the Evolution of post germination traits? the case of *Lesquerlla fendlen*. Jostor, Ecology: Vol. 76, No. 2. Pp 334- 356.

Fogg, C. and Greaves, A. (1990) *Acacia tortilis* (1925-1988) Annotated Bibliography No. F41. CAB International, Oxford Forestry Institute.

Hamann, A., Koshy, M.P., Namkoong, G. and Ying, C.C. (2000) Genotype x environment interaction in *Alaus urbra*: developing seed zones and seed-transfer guidelines with spatial statistics and GIS, Forest Ecology and Management, volume 136, P, 107-119.

ISTA (1993) Seed Science and Technology 21- Supplement- Zurick, Switzerland.

Khurana, E. &Singh, J.S. (2001) Ecology of Seed and seedlings growth for conservation and restoration of tropical and dry forest: a review. Environmental conservation 28(1):39-52 © Foundation for Environmental conservation.

Kulkarni, M.G., Sparg, S.G. and Vansteden, J. (2006) Germination and post-germination response of Acacia seeds to smoke- water and butenlide, a smoke- derived compound. Journal of Arid Environments 69 (2007) 177-187.

Khalil, A.A. and Siam, A.M. (2003) Effect of geographic source of seed on morphometric characteristics of *Acacia senegal* (L.) Willd. Seeds. University of Khartoum. Journal of Agricultural Sciences. Sudan Silva.

Mahgoub, S. M. and Joval, A. (1994) Seed Laboratory Manual, National Tree Center Project, Soba, Sudan.

Mahgoub, S.M. (2002) Studies on the physiological, environmental and biochemical factors affecting the germinability of some forest tree species seeds.PhD thesis. Faculty of sciences, University of Khartoum.

Moleele, N.M. and Read, M.S., Motoma. L., and Seabe, O. (2005) Seed weight patterns of *Acacia tortliis* from seven seed provenance across Botswana, 2005, African Journal of ecology, Afr, J. Ecol- 43. 146- 149.

Odual, P.A. (1993) Genetic variation in seeds and seedlings of 65 *Sesbabia Sesban* (L) Mer. Half. sib provenances. Collorative Research on *Sesbania sesban* in East Africa. Nairobi, 103- 109 ITCA, Kenya.

Raddad, A.Y. (2007) Ecophosiological and genetic variation in seedling traits and in first-year field performance of eight Acacia Senegal provenances in Blue Nile, Sudan, Springer /New Forest. do, 11056- 007- 9049-4.

Russel, J.H. (1998) Genecolgy of *Chamaecyparis nootkalnsis*. In: laderman, A. D. (ED.), Coastally Resttricted Forest. Oxford University Press, Oxford, U K, pp. 44-59.

Salazar, R. (1989) Genetic variation of 16 provenances of *Acacia mangium* at nursery level in Furrialba, Cost Rica, Commonwealth Forestry Review 68:263- 272.

SAS (1996) SAS statistical analysis. Version 6. 12, SAS Institute Inc., Cary.

Schmidt, L. (2002) Guide to handling of tropical and subtropical forest seed. Danida Forest Seed Center, Hmelblak, Denmark.

Teketay, D. (1996) Germination ecology of twelve indigenous and eight exotic multipurpose leguminous species from Ethiopia. Forest Ecology and Management 80, 209- 223.

Timberlake, J.R, Fagg, C. and Bame, R., 1999. Field guide to Acacia of Zimbabwe. CBC. Publishing, Harare.

Walters, M., Midgleg, J.J. and Somer, M. J. (2004) Effect of fire and fire intensity on germination and establishment of Acacia Karroo, *Acacia nilotica*, *Acacia luederitzii* and *Dichrostachys cinerea* in the field, Bme Eco.v.4.

Westoby, M., Flaster, D.S., Moles, A.T., Vesk, P.A. and Wright, I. J. (2002) Plant ecological strategies: some leading dimensions of variation between species. Annu Rev Ecology syst 33: 125-159.

Willan, R. L. (1985) A guide to forest seed handling with special reference to the Tropic, Humlebach, Denmark: FAO Forestry Paper, DANIDA Seed Centre.

Zhang, J. (1998) Variation and allometry of seed weight in *Aeschynomence americana* Annals of Botany 82:843-847, USA. Ap.