Response of *Balanites Aegyptiaca (L.) Del.* Seedlings from Varied Geographical Source to Imposed Drought Stress

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Abstract:

Seedlings of *Balanites aegyptiaca* (Hegleig) from eight geographical sources were exposed to a multiple series of drought cycles to test the effect of drought conditioning on seedlings survival. Three successive cycles were imposed to a four-month old seedlings. At the end of the cycles, water was withheld from both stressed and well-watered seedlings and mortality was counted for one month. Water stress significantly reduced seedlings mortality, suggesting that stress conditioning enhanced adaptive traits for drought tolerance. There is observed relationship between drought tolerance and original soil type of the sources. Seedlings exhibit highest mortality were from clay provenances, while those having the least were from sandy clay loam. Seedlings mortality was positively correlated with leaf area, leaf weight and specific leaf weight, and negatively correlated with specific leaf area, indicating that leaf traits are the most adaptive characters for drought tolerance for this species. The study showed that Hegleig tree is a drought tolerant species with significant differences among provenances. Selection of Id Elfrissan provenance for drought prone sites and Ed Dinder source in relatively favourable sites with clay soil, may enhance first year survival of this species.

Key words: Balanites aegyptiaca, geographical source, seedling survival, tolerance, drought, arid and semi-arid areas, breeding, water stress, soil properties, *Sudan*

Résumé

Des jeunes plants de *Balanites aegyptiaca* (Hegleig) de huit provenances géographiques ont été exposés à une série de plusieurs cycles de sécheresse pour examiner l'effet induit par la sécheresse sur la survie de jeunes plants. De jeunes plants de quatre mois ont été soumis à trois cycles successifs. A la fin des cycles, on a arrêté tout apport d'eau aux plants élevés dans des conditions de stress hydrique et à ceux qui étaient normalement arrosés, et puis leur mortalité a été ensuite suivie pour une période d'un mois. Le stress hydrique a réduit de manière significative la mortalité de jeunes plants, et ceci suggère que le traitement de stress hydrique a augmenté des propriétés d'adaptation et de tolérance à la sécheresse. On a observé une relation entre la tolérance à la sécheresse et le type de sol d'origine géographique de la provenance. Les jeunes plants chez lesquels on a observé la mortalité la plus élevée provenaient des milieux argileux, alors que ceux avec une faible mortalité étaient issus des sols gravilo-argilo-limoneux. La mortalité de jeunes plants a été positivement corrélée avec la surface foliaire, le poids de la feuille et le poids foliaire spécifique, et elle a été négativement corrélée avec le surface foliaire spécifique, suggérant que les traits de la feuille sont des caractères les plus adaptatifs pour la tolérance à la sécheresse chez cette espèce. L'étude a montré que l'arbre de Hegleig est une espèces tolérante à la sécheresse avec des niveaux significativement différents au sein des provenances. Le choix de la provenance de Id Elfrissan pour les sites enclins à la sécheresse et Ed Dinder pour les sites relativement humides avec un sol argileux, peut augmenter la survie de cette espèce au cours de la première année.

Mots-clés: *Balanites aegyptiaca,* provenance géographique, survie de jeunes plants, tolérance, sécheresse, régions arides et semi- arides, sélection, stress hydrique, propriétés du sol, Soudan

Introduction

Balanites aegyptiaca is one of the most widely distributed trees in the dry-lands of Africa and Sudan (Sands, 2001). Its main belt is within 400 to 800 mm/ annum rainfall isohyets through sub-Saharan Africa from Senegal to Sudan (Hall and Walker, 1991). It could be found north of this belt under rainfall as low as 250 mm/annum (Suliman and Jackson, 1959; Hall, 1992). Field observations showed that Balanites seedlings directly grown in the field had better survival than those raised under uniform nursery conditions and that was attributed to deep root system in the field (Von Maydell, 1986; Badi *et al.*, 1989; Schmidt and Joker, 2000). The tree is a drought resistant species and can not be damaged by grass fires. In Sudan, it is suitable for difficult sites where water is the main limiting factor

Plants in arid zones are usually subjected to a series of drought stresses where rainfall is erratic and multiple cycles of drought naturally occur. Survival of seedlings and trees is accomplished by drought avoidance and drought tolerance mechanisms (Kozlowski and Pallardy, 2002). Significant genotypic variations between tree species were reported to drought stresses (Abrams, 1994). Between tree species, and among and within provenances, variation in physiological and morphological traits related to drought tolerance were observed in many studies (Stewart *et al*., 1994; Cregg, 1994; Mayne *et al*., 1994; Ranney *et al*., 1991; Polley 1999; Devitt *et al*., 1997, Yin *et al*., 2005). Focusing on the deleterious impacts of stresses has tended to obscure the beneficial effects of environmental stresses on plants (Grierson *et al*., 1982). Gene regulation in response to drought conditioning can enhance drought tolerance (Mayne *et al*., 1994). Directly transplanted seedlings usually undergo a severe physiological shock because their capacity for water absorption is greatly reduced (Kozlowski and Pallardy, 2002), while seedlings previously exposed to water stress undergo less injury from drought and transplanting than the unexposed seedlings indicating tolerance for dehydration has been increased (Levitt, 1980; Kozlowski and Pallardy, 1997).

Hegleig tree is in the priority list of the Forest National Corporation for Afforestation and Re-afforestation and Conservation (Warag *et al*., 2002). Also, the integrated strategy for seed procurement and tree improvement of the National Tree Seed Centre was put hegleig in the top four species for improvement. The occurrence of the species over a wide range of climatic and soil conditions, may call for screening of the tree for different sites. Selecting provenances with high drought tolerance may enhance first-year plantation establishment for this species, a most limiting factor for plantation success in the arid lands of the Sudan.

The objective of this study was to investigate the effect of drought conditioning on seedlings survival and growth of eight geographical sources of *B. aegyptiaca.* Enhancing survival of seedlings and screening of provenances according to their ability to withstand drought, could be very essential in the first-year survival and conservation of the tree.

Material and Methods

Seed sources:

Eight geographical sources were identified to represent the natural range of *B. aegyptiaca* in Sudan (Table 1). Seeds were collected from more than 25 trees at least 150 meters apart in each source. Then seeds was bulk into one seed.

Nursery experiment:

Seeds from each geographical source were germinated in a vermiculite media in germination room. Immediately after the emergence of the radicle, seedlings were transplanted in polythene bags (27X30 cm when flat) filled with clay soil and then transferred to a greenhouse. They were arranged on a bench one meter above ground in a randomized complete block design with three replicates. Irrigation was carried out by sprinklers twice a day.

After four months, seedlings of similar heights were taken from each provenance and divided into two lots (L0 and L1). Six seedlings per source per treatment in each block were assigned. Lot L0 was watered every three days to field capacity and lot L1 was subjected to three drought stress cycles. In every stress cycle, water was withheld until some seedlings show sign of wilting, after which irrigation for a recovery period was applied. The first cycle was for 10 days and 5 days for recovery. Cycle two ended in 14 days and 5 days recovery, while cycle three took 17 days and 7 days for recovery. Seedlings from L0 and L1 were measured for shoot height, root collar diameter, number of leaves and number of branches immediately before and after the stress treatment. Leaf area and leaf weight were measured at the end of the stress cycles. Plotting paper was used for leaf area determination. Specific leaf area (SLA m^2 $100g^{-1}$) was calculated as leaf area divided by leaf weight. Specific leaf weight (SLW g cm^2) is a reciprocal of SLA which is weight divided by area.

By the end of the recovery at cycle three (end of drought cycles), water was withheld from both treatments for one month and Death of seedlings was evaluated every week. A seedling is considered dead when all the leaves were completely wilted. At the end of survival test, all the seedlings were harvested and every seedling was separated into shoot, root and leaves. Shoot length, root length, and number of branches were measured. Leaves, shoots and roots dry weight were recorded and weight ratios were calculated.

Data analysis:

Two way analysis of variance (ANOVA) was done using SAS statistical analysis to determine the significance of treatments and provenances and the interactions on the measured variables. Duncan multiple range test was used to separate between means. Correlation was analyzed to determine linear relationship between some parameters with seedlings mortality.

Results:

Drought cycles and geographical sources effects were significant on most of the measured variables, while the interactions were not significant for any of the variables. Leaves and shoot variables of seedlings subjected to drought cycles have lower values while root traits and survival were significantly higher than the regularly watered seedlings.

Shoot Parameters:

Provenances showed significant differences in shoot length, root collar diameter, number of branches and number of leaves before and after the drought cycles and at the final destructive harvest (Tables 2 and 3). In general, DD and RW have the highest values in most of the traits and GB and IF have the least (Table 2).

Leaves Parameters:

Leaf area, leaf weight, specific leaf area and specific leaf weight were highly significant between provenances (Table 3). Stress treatments reduced the values in most of the traits. DD and DM exhibit highest values in leaf area and weight, while GB and IF are the least. In contrast, SLA is higher in IF and GB (Table 3). SLW did respond to stress treatment in all the provenances (Table 3).

Biomass:

The difference in total weight, shoot weight, leaf weight, and root weight is very highly significant between the sources. Water stress reduced shoot and leaf weight and increased root weight and root to shoot ratio (Table 4).

Survival:

Water stress significantly reduced seedlings mortality (Fig. 1). Stressed seedlings start dying one week later than well-watered seedlings. IF and ZA have higher survival and DD, JZ and RW have the least. Positive correlation was observed between seedlings mortality with Specific leaf weight, soil type, leaf weight and leaf area, (r= 0.82 p= 0.01 and r= 0.79 p= 0.02, r= 0.63 p= 0.09, r= 0.54 p= 0.1 , respectively)(Table 5). Specific leaf area was negatively associated with seedlings mortality (r -0.64 p= 0.08) (Table 5).

Table 1: Locations and distribution of *B. aegyptiaca* geographical sources in Sudan used in the study.

Location	Code	Lat.°N	Long.°E	Soil	Rainfall mm	Elevation Masl	Seed zone[1]
Id Elfrisan	IF	11.483	24.350	Sandy clay loam	700	585	5.2
Abu Zabad	ZA	12.350	29.250	Sand	450	545	5.2
Abu Gubeiha	GB	11.450	31.233	Silty clay	700	543	4.1
Kassala	KL	15.467	36.400	Silty clay	400	519	2.4
Ar Rawashda	RW	14.200	35.583	Clay	600	605	3.1
Ed Dinder	DD	12.600	35.033	Clay	700	479	4.1
Ad Damazin	DM	11.767	34.350	Clay	700	493	5.1
Al Jaza'ir	JZ	13.067	33.967	Clay	550	406	4.1

[1]seed zones according to Aelbaek and Kananji (1995).

Table 2: Mean shoot length, root length, number of branches and root collar diameter of eight geographical sources in *B. aegyptiaca* under two irrigation levels.

Provenance	Shoot length (cm)		Root length (cm)		Number of branches		Root collar diameter (cm)		Number of Leaves	
	Well watered	Stressed	Well watered	Stressed	Well watered	Stressed	Well watered	Stressed	Well watered	Stressed
DD	30.6^{a}	29.4^{ab}	43.0^{ab}	48.3^{a}	43.0^{ab}	48.3^{a}	2.9^{a}	3.1^{ab}	79^{ab}	80^{ab}
DM	24.4^{ab}	23.0^{abc}	34.2^{ab}	46.8^{a}	34.2^{ab}	46.8^{a}	3.8^{a}	3.4^{ab}	708^{ab}	54^{b}
ZA	29.1^{ab}	30.2^{a}	47.4^{a}	50.3^{a}	47.4^{a}	50.3^{a}	3.0^{a}	3.2^{ab}	778^{ab}	90^{a}
JZ	29.3^{ab}	27.9^{ab}	46.6^{a}	41.3^{a}	46.6^{a}	41.3^{a}	2.4^{a}	2.1^{b}	67^{ab}	65^{ab}
KL	30.2^{a}	22.1^{bc}	30.6^{b}	51.6^{a}	30.6^{b}	51.6^{a}	2.8^{a}	2.4^{b}	72^{ab}	53^{b}
RW	32.2^{a}	22.7^{abc}	41.1^{ab}	51.7^{a}	41.1^{ab}	51.7^{a}	3.0^{a}	2.7^{ab}	83^{ab}	60^{b}
GB	24.7^{ab}	22.9^{abc}	45.7^{a}	34.8^{a}	45.7^{a}	34.8^{a}	3.7^{a}	3.8^{a}	86^{a}	69^{ab}
IF	20.5^{b}	18.4^{c}	37.1^{ab}	43.2^{a}	37.1^{ab}	43.2^{a}	3.2^{a}	3.0^{ab}	54^{b}	54^{b}
Treatment(p)	0.03		0.09		0.5		0.002		0.09	
Prov (p)	0.001		0.6		0.01		0.0006		0.01	
TRT*prov(p)	0.53		0.13		0.98		0.32		0.45	

Means with the same letter in the same column are not significantly different at P= 0.05 using Duncan multiple range test.

Table 3: Mean leaf area, leaf weight, Specific leaf area and specific leaf weight of eight geographical sources in *B. aegyptiaca* under two irrigation levels.

Provenance	Leaf area LA (cm^2)		Leaf weight LW(g)		Specific leaf area SLA m^2 $100g^-$		Specific leaf weight SLW cm^2 g^-	
	Well watered	Stressed	Well watered	Stressed	Well watered	Stressed	Well watered	Stressed
DD	3.99^{a}	2.35^{a}	0.06^{a}	0.039^{a}	0.69^{ab}	0.65^{ab}	0.015^{a}	0.015^{a}
DM	3.10^{b}	2.39^{a}	0.05^{ab}	0.037^{a}	0.63^{ab}	0.61^{b}	0.016^{a}	0.016^{ab}
ZA	2.12^{cd}	1.41^{c}	0.03^{cd}	0.021^{de}	0.67^{ab}	0.69^{ab}	0.015^{a}	0.014^{ab}
JZ	2.28^{bcd}	2.24^{ab}	0.04^{bc}	0.036^{abc}	0.60^{b}	0.62^{b}	0.017^{a}	0.016^{ab}
KL	2.95^{bc}	1.76^{abc}	0.04^{bc}	0.026^{cde}	0.83^{a}	0.68^{ab}	0.014^{a}	0.015^{ab}
RW	1.82^{d}	1.67^{bc}	0.03^{cd}	0.028^{bcd}	0.65^{ab}	0.64^{ab}	0.016^{a}	0.017^{a}
GB	1.55^{d}	1.25^{c}	0.03^{cd}	0.0170^{e}	0.64^{ab}	0.77^{a}	0.014^{a}	0.013^{b}
IF	1.37^{b}	1.23^{c}	0.02^{d}	0.0178^{de}	0.75^{ab}	0.72^{ab}	0.013^{a}	0.014^{ab}
Treatment(p)	0.0001		0.0001		0.8		0.8	
Prov (p)	0.0001		0.0001		0.09		0.8	
TRT*prov(p)	0.05		0.2		0.4		0.3	

Means with the same letter in the same column are not significantly different at P= 0.05 using Duncan multiple range test.

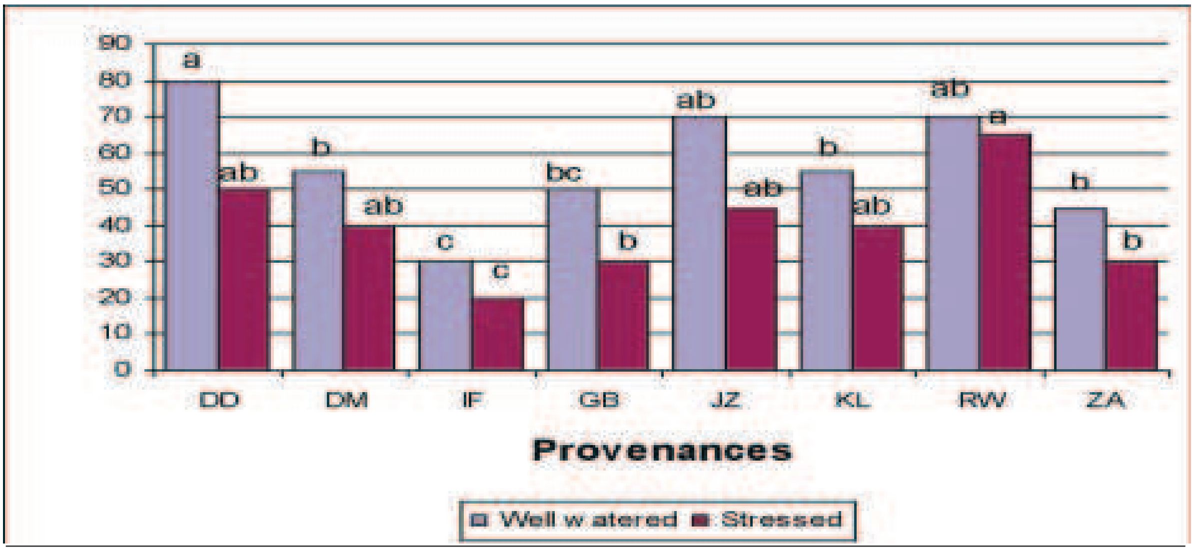
Table 4: Mean leaves weight, shoot weight, root weight, root : shoot ratio, leaves weight ratio of eight geographical sources in *B. aegyptiaca* under two irrigation levels.

Provenance	Leaves weight(g)		Shoot weight (g)		Root weight (g)		Root: Shoot ratio		leaves weight ratio	
	Well watered	Stressed	Well watered	Stressed	Well watered	Stressed	Well watered	Stressed	Well watered	Stressed
DD	1.8^{a}	1.7^{a}	1.6^{a}	1.3^{a}	6.1^{a}	6.0^{a}	4.3^{b}	5.8^{ab}	0.23^{a}	0.22^{a}
DM	1.2^{bc}	1.1^{b}	0.9^{bc}	0.9^{ab}	4.2^{ab}	4.5^{ab}	5.5^{ab}	5.2^{ab}	0.21^{a}	0.21^{a}
ZA	1.2^{bc}	1.1^{b}	1.0^{abc}	1.3^{a}	4.7^{ab}	4.6^{ab}	5.2^{ab}	4.9^{b}	0.22^{a}	0.19^{a}
JZ	1.2^{bc}	1.1^{b}	0.8^{bc}	0.9^{ab}	4.6^{ab}	4.3^{b}	5.7^{ab}	5.1^{ab}	0.25^{a}	0.21^{a}
KL	1.5^{ab}	1.0^{b}	1.3^{ab}	0.7^{b}	4.7^{ab}	4.5^{ab}	4.0^{b}	6.9^{ab}	0.25^{a}	0.20^{a}
RW	1.1^{bc}	0.8^{b}	0.8^{bc}	0.5^{b}	3.9^{ab}	4.0^{b}	5.1^{ab}	7.7^{a}	0.21^{a}	0.18^{a}
GB	0.9^{c}	0.9^{b}	0.7^{c}	0.8^{ab}	3.3^{b}	3.8^{b}	5.6^{ab}	4.9^{b}	0.21^{a}	0.19^{a}
IF	0.8^{c}	0.8^{b}	0.6^{c}	0.6^{b}	3.7^{b}	3.8^{b}	6.8^{a}	7.4^{ab}	0.19^{a}	0.17^{a}
Treatment(p)		0.09		0.3		0.9		0.09		0.03
Prov (p)		0.0001		0.0001		0.0008		0.02		0.3
TRT*prov(p)		0.8		0.2		0.9		0.07		0.93

Means with the same letter in the same column are not significantly different at P= 0.05 using Duncan multiple range test

Table 5: Correlations of stressed seedlings mortality with leaf parameters (LA, LW, SLA, SLW) and original soil of the sources.

Variables	LA	LW	SLA	SLW	Soil
R=	0.54	0.63	-0.64	0.82	0.79
p=	0.1	0.09	0.08	0.01	0.02



Means with the same letter in the same provenance are not significantly different
At P= 0.05 using Duncan multiple range

Fig. 1: Mortality percent between well watered and stressed seedlings of *B. aegyptiaca* in the eight geographical sources.

Discussion:

Seedlings of Balanites exposed to drought conditioning exhibit prolonged stress resistance and enhanced survival, suggesting that drought conditioning manipulated the adaptive traits in these seedlings. Similar findings were reported for other species (Stewart *et al*., 1994; Cregg, 1994; Mayne *et al*., 1994; Ranney *et al*., 1991; Polley, 1999; and Devitt *et al*., 1997).

The positive correlation of seedling mortality with leaf area and weight, reflect that these traits may be associated with drought tolerance. It is evident that small leaf is the most important factor in the survival of desert plants (Orshansky, cited in Kozlowski, 1976). This can further be explained by a marked reduction of leaf area and weight when Balanites seedlings were exposed to drought stress. Specific leaf area has negative relationship with seedlings mortality. Beadle (1995) stated that SLA is linearly related to relative growth rate, while Cregg (1994) observed that SLA is correlated to the climate of the source. Our data showed that SLA is related to the soil type, increasing in sandy and sandy clay loam soils and decreasing in clay soil. However, in general, provenances with higher SLA have lower rate of growth, but with higher survival.

Kozlowski (1976) cited that thickening of leaves by deposition of epicuticular waxes is one of the most important adaptive characters for drought avoidance. Our findings were in contrast with this. The study reported very high positive correlation of SLW with seedlings mortality. This means that seedlings with thick leaves have higher mortality. Our explanation to this contrasting result is that SLW may not have strongly responded to water stress. This can clearly be seen from approximately more or less the same values of SLW between well-watered and stressed seedlings in all provenances (Table 3).

There is a clear relationship between survival of the seed sources and original soil in their natural range. IF and ZA sources with higher survival are from sandy clay loam and sandy soil and RW and DD with least survival from clay soil. This indicates that provenances are genetically adapted to their respective sites, which may call for restriction in transfer of seeds between zones, especially of different soils. The significance of genetic control on drought tolerance occurred in many forest tree species (Abrams *et al*., 1990; Stoneman *et al*., 1994).

More than two-thirds of biomass was partitioned to the roots, indicating that this tree is well adapted to drought prone environments. The increased root to shoot ratio in stressed seedlings in most of the provenances may be related to the fact that under water stress conditions plants increase the allocation of photosynthate for root production to increase the surface area for absorption. The difference in adaptive response of Balanites seedlings to water stress among provenance indicate that establishment of Balanites under severe environments can be improved by selection of matching provenances in terms of their drought tolerance. There is strong evidence that (IF) provenance is well adapted to the drought. It has higher survival in both treatments, lowest leaf weight, smallest area, highest root to shoot ratio and germination data showed that it starts germination earlier, increasing very fast reaching peak and stopping (Elfeel, 2004). This will favour selection of this provenance for planting in sites with high drought stress.

Conclusion

Major part of Sudan is covered by arid and semi-arid lands. In these areas, afforestation and re-afforestation is very difficult, requiring best selection of well adapted species and sources. This study showed that Hegleig is a drought tolerance tree with high variation among provenances. The study suggests selection of Ed Dinder source for clay sites with relatively higher rainfall and Id Elfrissan source for drought prone sites.

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