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Thermotolerance and Osmotic Potential Studies in Germinating Seeds and Growing Seedlings of Acacia sieberiana and Acacia tortilis

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ABSTRACT

The effects of temperature and osmotic potential on germination and radicle elongation of Acacia sieberiana and Acacia tortilis ssp. heteracantha seeds were studied. The first series of experiments examined percentage germination under continuous temperature treatments of 15, 25, 27, 30 and 35℃ in distilled water and / or PEG 6000 solutions adjusted to –0.1; -0.2 and –0.5 MPa. The thermal optimum for germination and radicle elongation for both species was found to be between 25 and 30℃. Both species showed progressive reduction in germination rates and radicle elongation in response to decreasing osmotic potential. A. tortilis was found to germinate under the widest range of temperature and osmotic potentials. A. tortilis also exhibited higher percent germination than A. sieberiana, but A. sieberiana showed greater radicle growth when compared with radicle elongation displaced by A. tortilis.

INTRODUCTION

The two species of trees chosen for this comparative study A. sieberiana DC. and

A. tortilis (Forssk.) Hayne ssp. heteracantha (Burch.) Brenan are representatives of one of the predominant Southern African plant subfamily Mimosoideae. In Zimbabwe, the trees are widespread in a variety of habitats. A. tortilis ssp. heteracantha is prominent in low altitude, high temperature and low rainfall areas while A. sieberana occurs in all regions of the country. A. siebeirana is adaptable to medium and higher altitude areas while A. tortilis is indigenous to lower altitude habitats characterized by arid and sodic soils and high temperatures.

The distribution of plant species is thought to be dependent amongst others on environmental effects on germination and seedling establishment (Thompson, 1971). The connection between distribution, germination and seedling survivability leaves open the possibility that other environmental factors that affect germination success particularly stressed environments may influence the distribution of the species (Chikono & Choinski, 1992). It is common that seeds from different plant species have varying requirements from temperature during germination. Plants growing in the field encounter a number of environmental stresses, for example moisture and temperature stress. The ability to withstand such stresses frequently becomes the limiting factor for plant growth, survival and geographical distribution. Moreover, study of the behavior of these species under stress is of practical importance since there is need to understand the seed physiology of these species as an essential element fundamental to the vigor and seedling establishment. The environmental conditions like extreme temperatures and moisture deficiency are characteristics of tropical regions in general and Zimbabwe in particular. In many cases this has resulted in poor or delayed germination.

Germination marks the end of the dormant period and the beginning of active growth for a plant. Once the radicle breaks the seed coat and emerges, it usually cannot return to a quiescent stage and the plant is literally betting its life (Jurado & Westoby, 1992). In arid environments, with unpredictable climate, these two species, A. sieberiana and A. tortilis should be grown at times which give the best chance for seedling establishment. It has been established that Acacia seed coats are impermeable to water and require pre – treatment to obtain maximum germination (Fagg & Greaves, 1990; Timberlake, et al, 1999). A few ecologically oriented studies with A. tortilis have shown a positive correlation between germination rates in the field and scarification caused by passage through herbivore digestive tracts (Choinski & Touhy, 1991; Timberlake, et al, 1999). It is closely associated with livestock and wildlife which disperse its seeds in their dung and therefore it is principally found in open habitats or disturbed areas in various woodland and bush-land types and can be invasive on old lands.

It was hypothesized that germination and seedling establishment were episodic events, occurring only during seasons of normal precipitation when enough salt could be leached from the soil to raise the osmotic potential to within limits necessary for germination and seedling survivability. Previous investigations with PEG (Choinski & Tuohy, 1991) aimed at determining the relationship between germination and differing concentrations of PEG showed that maximum germination percentage and radicle growth were affected by altering the osmotic potential with PEG of the imbibition medium.

The present investigation sought to:

(i) Examine the effect of temperature on the rate and total germination of each of these Acacia species.

(ii) Examine effect of moisture limitation on the rate and total germination of each of these Acacia species.

(iii) To determine the effect of temperature and moisture limitation on radicle elongation.

(iv) To find out how temperature and water stress interact to affect germination percentage and radicle growth.

The ultimate goal of this study was to determine if there are correlations between the habitat of these species and the ability of seeds to germinate at various temperatures and water stress conditions. This information is useful in identifying factors involved with the process of regeneration and also particularly in light of the rapid utilization of these species as sources of fuel wood and animal fodder (Fagg & Greaves, 1990). How and where these trees may most efficiently be replanted in reforestation programmes.

MATERIALS AND METHODS

The seeds used in this investigation were scarified using a sharp razor blade. The damaged seeds were discarded as Acacia seeds are known to show damage from infestations by bruchid beetles and other insects (Fagg & Greaves, 1990; Choinski & Tuohy, 1991). All seeds were surface sterilized for 3 minutes in 0.35% sodium hypochlorite containing a drop of detergent and then rinsed with distilled water 5 times prior to planting. 10 seeds per plate were then transferred to 9 cm petri – dish containing

10 ml of distilled water or solution of PEG 6000 and one sheet of filter paper. Five replications were used for each treatment combination.

Incubation temperatures

Germination trials determined total per cent germination and rate germination for each species at five constant temperatures (15, 25, 27, 30 and 35°C).

Incubation osmotic potentials

Seed incubation were carried out at five constant temperatures in 10 ml of distilled water and / or PEG 6000 solutions adjusted to –0.1; -0.2 and –0.5 MPa.

Scoring of germination

Germinability was expressed as a percentage of viable seeds that germinated after the incubation period. Germination was defined as emergence of an embryo through the seed coat. Germination was scored after every two days until constant readings were obtained and such a reading was assumed to be the maximum germination for that treatment. At the end of the incubation period, the germination percent and radicle length were scored for each treatment.

Analysis of germination

Starting from the time of imbibition began; the number of seeds germinated at each count was tabulated with corresponding accumulated time. After each germination trial was completed, total percent germination was determined. The rate of germination was calculated for each temperature regime – osmotic potential after Maguire (1962) where:

Rate of germination = $\sum i$ [gi-g(i-1)/i]

In which g is the total germination percentage on incubation period i minus the total germination percentage on the previous period (g(i-1) divided by the incubation period i.

While percent germination is frequently used as expression of germinability, speed and completeness of germination were used to determine germination index (GI). The germination index (GI) has been defined by Czabator (1962) as a measure of the ability of a seed to germinate and develop into a seedling. The germinability can be influenced by a number of exogenous and endogenous factors. Czabator (1962) also described GI as a measure of seed vigor and defined it as a function of total germination and mean germination rate as shown in the equation below.

GI = Total germination X Mean germination rate

RESULTS

Germination response to temperature and osmotic potential

Characterization of germination properties over temperatures of 15, 25, 27, 30 and 35°C indicated a differential response within the two Acacia species (Table 1). At all temperature regimes, the seeds of A. tortilis showed overall higher percent germination than the seeds of A. sieberiana. The optimum temperature for germination was found to be between 25 and 30ºC with minimum percent germination at 15 and 35ºC (Table 1). When incubated in distilled water, all species showed a maximum percent germination of between 80 to 100% and the incubation of seeds in stressed media resulted in reduced germination (Table 1). Higher germination percentages were obtained at optimum temperatures and lower values at 15 and 35ºC. Further reductions in germination percentages were obtained at more negative osmotic potentials (Table 2).

Table 1: The Effect of Osmotic Potential and Temperature on Germination Rate of A. tortilis and A. sieberiana

Species and / or Osmotic potential	Incubation temperature				
	15ºC	25ºC	27ºC	30ºC	35ºC
Distilled Water					
A. tortilis	98±2	100	100	100	94±2.4
A. sieberiana	94±2.4	78±5.8	94±4	80±10.5	10
-0.1Mpa PEG					
A. tortilis	88±2	92±3.7	78±10.2	32±8	12.5±2.2
A. sieberiana	20±6.3	52±3.7	78±3.7	18±3.7	10
-0.2Mpa PEG					
A. tortilis	76±2.4	92±4.9	68±2.2	31±2.4	14±2.4
A. sieberiana	10	13.3±2.6	28±1.8	8	0
-0.5Mpa PEG					
A. tortilis	13.3±2.6	24±7.5	44±6.8	26.7±3.6	0
A. sieberiana	0	12	16±2.4	10	0

The Effect of Temperature and Osmotic Potential on Radical Growth

The radicle length increased with increasing temperature and decreasing osmotic potentials (Tables 3 and 4 respectively). The seeds of both species had longer radicles when grown between 25 and 30°C (Table 2). A. sieberiana showed greater radicle growth at 25 and 30°C In distilled water and osmotic potential at –0.1MPa (Table 2) than A. tortilis at the same temperature and osmotic potential treatments.

Table 2: The Effect of Osmotic Potential and Temperature on Radical Length (mm) of A. tortilis and A. sieberiana

Species and / or Osmotic potential	Incubation temperature				
	15ºC	25ºC	27ºC	30ºC	35ºC
Distilled Water					
A. tortilis	9.3±1.1	15.2±1.1	18.4±1.3	18.1±1.1	7.5±1.2
A. sieberiana	4.3±1.5	17.9±1.2	24.3±1.5	23.9±1.4	2.3±0.4
-0.1Mpa PEG					
A. tortilis	5.4±2	8.0±1.4	13.6±0.8	13.0±1.0	3.4±1.1
A. sieberiana	6.1±1.2	11.3±1.0	16.1±.0.8	15.3±1.2	2.1
-0.2Mpa PEG					
A. tortilis	4.2±0.5	5.0±0.6	6.8±1.2	3.1±0.6	1.2±0.3
A. sieberana	4.9±0.6	8.2±0.9	2.8±1.8	1.8	0
-0.5Mpa PEG					
A. tortilis	1.8±0.5	2.5±0.6	3.2±0.4	3.3±0.6	0
A. sieberiana	0	4.2±0.7	5.7±0.5	5.2±0.7	0

Speeds of Germination and Germination Indices

Germination index increased with increasing temperature until the optimum temperatures of 25 to 30°C and germination index declined above 30°C (Table 3). Germination index decreased with a decline in osmotic potential (Table 3).

Table 3: The Effect of Osmotic Potential and Temperature on Germination Rate of A. tortilis and A. sieberiana

Species and / or Osmotic potential	Incubation temperature				
	15ºC	25ºC	27ºC	30ºC	35ºC
Distilled Water					
A. tortilis	13.1±0.2	34.7±1.7	43±1.0	43.7±1.0	43±1.8
A. sieberiana	9.7±0.4	21.7±1.3	27.5±2.6	25.9±2.5	2.5
-0.1Mpa PEG					
A. tortilis	11.6±0.6	29.1±1.3	29.7±3.5	15±3.2	5.6±0.6
A. sieberiana	2.4±0.6	15.5±2.2	21.3±1.2	7.7±1.4	2.5
-0.2Mpa PEG					
A. tortilis	9.8±0.3	26.2±1.6	28.1±1.2	13.1±0.6	4.5±1.5
A. sieberiana	11.1±0.2	3.1±0.4	2.8±0.2	1.8	0
-0.5Mpa PEG					
A. tortilis	1.3±0.3	4.5±1.4	10.3±1.6	4.4±0.4	0
A. sieberiana	0	2.3±0.2	2.7±0.4	1.7	0

Table 4: The Effect of Osmotic Potential and Temperature on Germination Index of A. tortilis and A. sieberiana

Species and / or Osmotic potential	Incubation temperature				
	15ºC	25ºC	27ºC	30ºC	35ºC
Distilled Water					
A. tortilis	1286±34.2	3468±168.1	4300±122.5	4366±97.2	4091±274.8
A. sieberiana	918.7±60.7	1691±172.4	2627±342.1	2170±442.8	25
-0.1Mpa PEG					
A. tortilis	1022±69	2696±209.5	2449±566.3	580±257.7	75±22.4
A. sieberiana	61±31.6	838.5±167.6	1680±171.9	157±56.1	25
-0.2Mpa PEG					
A. tortilis	746.5±39.8	2408±189.8	1911±81.6	406±18.6	75±34.5
A. sieberiana	11.1±2.2	44±15.1	78.4±5.6	14.4	0
-0.5Mpa PEG					
A. tortilis	20±7.7	148±82.5	493±130.0	122±21.5	0
A. sieberiana	0	23±1.9	47±12.2	17	0

DISCUSSION

A. tortilis attained higher germination and growth rates under the widest range of osmotic potentials and temperatures than A. sieberiana. These results correlate very well with the adaptations of these species in their natural habitats. The displayed high germination index of A. tortilis (Table 3) compared with low germination index of A. sieberiana suggest that the former has highly acceptable germination behavior and long term seedling survivability.

A larger seed (given similar shape) are expected to take longer to imbibe (and hence to germinate) than a smaller one due to smaller one due to smaller surface / volume ratio (Jurado & Westoby, 1992). This prediction held in so far as seed of A. sieberiana were slow germinators while seeds of A. tortilis were fast germinators (Table 3). A. sieberiana showed greater radicle growth at temperatures between 25 and

30ºC (Table 2). Such an observation indicates that greater length of the radicle is correlated with larger seed size of A. sieberiana as observed by Brown and Booysen (1969).

The results of this study have shown that A. sieberiana and A. tortilis were less able to germinate in the presence of PEG and presumably less well adapted to water stress or drought. The results also showed that little germination occurred at –0.5Mpa at the two extreme temperatures of 15 and 35ºC.These results are comparable to the findings of Choisnki and Tuohy (1991) on the same species where no germination occurred when A. tortilis seeds were incubated at –0.6Mpa.

Two or more factors (treatments) have been shown to interact for germination to take place and combined factors may be additive in their effects (Popay & Roberts, 1970). Suitable temperature and osmotic potential (moisture availability) should be considered as requisite treatments for complete germination. A faster germination rate and larger germination percentage over a wide regime of environmental stress may confer a greater chance for successful seedling establishment. From this study it can be deduced that low germination indices, indicating low germination rates may be a disadvantage on semi – arid sites or areas where precipitation is sporadic, for the establishment selected Acacia species without high seedling tolerance. The results suggest that seedling drought tolerance to water stress. This may be a necessary compliment to high germination rates for successful establishment of selected Acacia species on rangelands where the amounts and timing of precipitation are sporadic.

It must be noted that the seeds in the field would not necessarily behave as they did in these studies as storage and germination conditions might be different. For example, exposure of soil – stored seed to the strong daily temperature fluctuations in the soil surface has been shown to increase germinability in some species (Jurado & Westoby, 1992). Similarly, fluctuating temperatures during germination might have different effects from constant temperatures. However, the obtained results under simplified conditions reflect genuine differences between A. tortilis and A. sieberiana.

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