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Original Article

DETERMINATION OF WATERING REGIMES AND MYCORRHIZAE INOCULATION REQUIRED FOR ESTABLISHMENT OF SEEDLINGS OF *Acacia senegal* (L.) WILD IN THE NURSERY

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ABSTRACT

The influence of watering regimes and inoculation of mycorrhizae through the measurement of seedling heights, root length, leaf number, stomatal conductance and chlorophyll content, on growth performance of *Acacia senegal* seedlings were elucidated in this study. Generally, inoculated seedlings recorded higher values of the parameters measured than the uninoculated. Also seedlings that were watered biweekly and weekly recorded higher values than those watered daily. Endomycorrhiza inoculation and biweekly watering produced significantly high leaf number (23), root length (13.06cm), stomatal conductance (147.55mmol m^{-2s-1}) and chlorophyll content (27.5mg/l). Seedling death was recorded under weekly watering regime as from 12 weeks after planting (WAP) ; both with ectomycorrhiza and endomycorrhiza treatments. Biweekly watering and employment of ectomycorrhiza are recommended as they enhanced the best morphological and physiological performance of *Acacia senegal*.

Key words: Mycorrhiza inoculation, Watering regime, Stomata conductance, Chlorophyll content

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INTRODUCTION

Water availability is a significant factor in dry land forest nursery and it is critical to tree growth and development in the Tropics (Awodola and Nwoboshi, 1993). Water is required by plants for the manufacture of carbohydrates and as a medium for transportation of foods and mineral elements. Various vital processes in plants such as cell division, cell elongation, stem as well as leaf enlargement and chlorophyll formation depends on plant water availability (Price *et al*., 1986). As noted by Levy and Krikum (1983), insufficient water in plants below a critical level is usually demonstrated by changes in all structures leading to the death of the plants. According to Kabir and Abubakar (2012), for each ton of vegetative growth, hundreds of tons of water may be consumed by the growing plant particularly in dry climates. As observed by Awodola (1984), the reduction in relative water contents affects physiological processes and hence growth. Similarly, too much water, in excess of plant need may retard the physiological processes. In particular, stomata conductance which is a numerical measure of the maximum rate of passage of either water vapour or carbondioxide through the stomata and the xylem pressure potential which is the component of water potential due to hydrostatic pressure that is exerted on water in a cell are influenced by the soil water balance (Komer *et al*., 1979). Mycorrhiza fungi have been found to increase the drought-tolerance of the host plants (Osunubi and Mulongoy, 1991; Pandel *et al*., 2000,). Nevertheless, the effect of mycorrhiza on growth and drought tolerance of *A. senegal* is yet to be assessed (Komer *et al*., 1979; Huang *et al*., 1985) reported that root to shoot ratio can be 3.5 times higher in water stressed plants. Since poor growth performance of this species on the field is a major constraint to high yield in the semi-arid region, identifying efficient nursery management practice through adequate supply of water is very crucial. The objective of this study therefore was is to investigate the influence of watering regime and mycorrhizal inoculation on the stomatal conductance and xylem pressure potential of *A. senegal* in the nursery of the semi-arid region of Nigeria.

MATERIALS AND METHODS

Experimental Site

This investigation was carried out in a screen house of the Botanical Garden of Usmanu Danfodiyo University, Sokoto. Sokoto is located between latitudes 11^0 30^0 and 13^0 50^0N and longitude 4^0 - 6^0E (Ogigirigi, 1993). The city lies in the North – Western Nigeria in the Sudan Savannah vegetation of the country.

Experimentation

Standard plastic pots (25 x 15cm) with drainage holes at the bottom were filled with 4.0kg of sterilized sandy-loan soil, which were irrigated with distilled water just before planting (Awodola, 1984). Thereafter, three viable seeds of *A. senegal* were sown in each pot. The polythene bags were then distributed into nine experimental treatments and labeled as:

NMD: No mycorrhiza with daily watering
NMB: No mycorrhiza with biweekly watering
NMW: No mycorrhiza with weekly watering
MECD: Ecto-mycorrhiza with daily watering
MECB: Ectomycorrhiza with biweekly watering
MECW: Ectomycorrhiza with weekly watering
MEND: Endomycorrhiza with daily watering
MENB: Endomycorrhiza with biweekly watering
MENW: Endomycorrhiza with weekly watering

Ten grammes (10g) each of the ecto- and endo-mycorrhiza was applied in a ring of 1cm deep under the seeds of *A. senegal* sown in the respective pots. The experiment was laid out in a completely randomized design with five replicates per treatment. The pots were watered daily for two weeks for the germinated seedlings to establish well. The watering regimes (daily, biweekly, and weekly watering) of the seedlings actually began after two weeks of growth. The seedlings were later thinned to one seedling per pot. The seedlings were grown for sixteen weeks. The seedlings of the respective treatments were assessed after 4,8,12 and 16 weeks, for morphological and physiological parameters. The plant height was measured with meter rule, leaf number by physical count and chlorophyll content was determined using spectrophotometer method (Chang and Troughton, 1971).

The stomata conductance was assessed at 12noon using AP_4 poromater while xylem pressure potential (xpp) was measured using a pressure Bomb with Nitrogen gas. Readings were taken at the emergence of water bubbles (Turner, 1982).

Statistical analysis

Data obtained from all the variables in the investigation were subjected to analysis of variance (ANOVA) using SARS statistical package. Significant difference in the treatments was further subjected to Duncan Multiple Range Test (DMRT) for the separation of treatment means.

RESULTS

The combined effects of watering regime and mycorrhiza inoculation on plant height and number of leaf measured at different periods of growth is shown in Tables 1 and 2, respectively. At 4, 8, 12 and 16 weeks after planting (WAP), the plant height

and number of leaves vary in response to watering regime and mycorrhiza inoculation. The plant height and number of leaves of seedlings inoculated with mycorrhizae were higher than the uninoculated seedlings (control). The endomycorrhiza inoculated seedlings recorded higher plant height (32.0cm) and number of leaves (23 leaves/plant) than the seedlings inoculated with ectomycorrhiza (22 leaves/plant), respectively. Seedlings that were watered twice weekly had higher plant height (32cm) and number of leaves (22 leaves/plant) than those that were watered daily (16) and once weekly. At 16 WAP, the seedlings that were watered once weekly wilted irrespective of whether inoculated or not. At 16 WAP, significant variation ($p < 0.05$) was recorded in the root length of the seedlings in response to watering and inoculation regimes (Table 1). The seedlings that were watered twice a week had higher seedling root length for both ectomycorrhiza (12.65cm) and endomycorrhiza (13.06cm) inoculation treatments than those that were watered daily which were 9.96cm and 10.77cm for ectomycorrhiza and endomycorrhiza treatments respectively. The chlorophyll content, stomata conductance and xylem pressure potential of *A. senegal* seedlings in response to watering regimes and mycorrhiza inoculations at different periods of growth are shown in Table 2, 3 and 4 respectively. At 4, 8, 12 and 16 WAP, the chlorophyll of the seedlings varied in response to watering regimes and mycorrhiza inoculation (Table 4). Accordingly, the chlorophyll content of seedlings inoculated with mycorrhizae was greater than the uninoculated seedlings (control). The endomycorrhiza inoculated seedlings gave a higher chlorophyll content (27.5mg/l) and number of leaves (23) than the ectomycorrhiza inoculated seedlings. Seedlings that were watered twice weekly had higher chlorophyll content (27.5mg/l) than the daily and weekly watered seedlings (26mg/l).

Table 1: Seedling height (cm) of *A. senegal* in response to watering regimes and mycorrhizae inoculation at different periods after planting.

Watering Regime	Mycorrhizae Treatment	Seedling Height(cm) 4 WAP	8 WAP	12 WAP
Daily	No Mycorrhiza	14.0g	22.0^{e}	22.5^{e}
Bi weekly	No Mycorrhiza	14.5g	22.5^{e}	23.5^{e}
Weekly	No Mycorrhiza	14.0g	22.5^{e}	23.0^{e}
Daily	Ectomycorrhiza	16.0^{f}	24.0^{e}	26.0^{d}
Bi-weekly	Ectomycorrhiza	17.0^{f}	30.0^{c}	32.0^{b}
Weekly	Ectomycorrhiza	17.0^{f}	26.0^{d}	27.0^{d}
Daily	Endomycorrhiza	17.0^{f}	26.0^{d}	26.5^{d}
Bi-weekly	Endomycorrhiza	17.5^{f}	31.0^{c}	32.0^{b}
Weekly	Endomycorrhiza	18.0^{f}	32.0^{b}	37^{a}

Means in a column followed by the same letter (s) are not significantly different using DMRT at 5% level.
WAP = weeks after planting

Table 2:Leaf number of *A. senegal* seedlings in response to watering regimes and mycorrhizae inoculation at different period after planting

Watering Regime	Mycorrhizae Treatment	Mean Leaf number/plant 4 WAP	8 WAP	12 WAP
Daily	No Mycorrhiza	9.0g	11.5^{f}	12.5^{e}
Bi-weekly	No Mycorrhiza	9.5g	13.0^{e}	13.0^{e}
Weekly	No Mycorrhiza	9.5g	12.0^{e}	13.0^{e}
Daily	Ectomycorrhiza	11.0^{f}	13.0^{e}	16.0^{d}
Bi-weekly	Ectomycorrhiza	13.0^{e}	14.0^{e}	22.0^{b}
Weekly	Ectomycorrhiza	13.0^{e}	18.0^{c}	16.0^{d}
Daily	Endomycorrhiza	11.3^{f}	14.0^{e}	16.0^{d}
Bi-weekly	Ecndomycorrhiza	13.0^{e}	19.0^{c}	23.0^{a}
Weekly	Endomycorrhiza	13.0^{e}	19.0^{c}	22.0^{b}

Means in a column followed by the same letters are not significantly different using DMRT at 5% level.
WAP = weeks after planting.

Table 3:Root length (cm) of *A. senegal* seedlings in response to watering regime and mycorrhizae inoculation at harvest (16 WAP)

Watering regime	Mycorrhizae treatment	Root length (cm)
Daily	No Mycorrhiza	8.57f
Biweekly	No mycorrhiza	9.17e
Daily	Ectomycorrhiza	9.96d
Biweekly	Ectomycorrhiza	12.65b
Daily	Endomycorrhiza	10.77c
Biweekly	Endomycorrhiza	13.06a
S.E		3.27

Means in a column followed by the same letter(s) are not significantly different using DMRT at 5% levels.

WAP = weeks after planting

Table 4: Stomatal conductance (mmol m^{-2} s^{-1}) of *A. senegal* seedlings at 12 noon in response to watering regime and mycorrhizae inoculations at different periods of growth.

Watering Regime	Mycorrhizae Treatment	Stomatal conductance ($mmolm^{-2}s^{-1}$)			
		4 WAP	8 WAP	12 WAP	16 WAP
Daily	No Mycorrhiza	33.51k	35.22k	37.39k	39.18k
Biweekly	No Mycorrhiza	48.63k	51.75j	58.20j	62.83i
Weekly	No Mycorrhiza	64.32i	68.53i	72.46h	SD
Daily	Ectomycorrhiza	74.32h	78.14h	82.76g	84.37g
Biweekly	Ectomycorrhiza	116.22d	122.15d	130.50c	132.54c
Weekly	Ectomycorrhiza	93.69f	101.43e	103.33e	SD
Daily	Endomycorrhiza	92.89f	98.72f	103.21e	105.49e
Biweekly	Ecdomycorrhiza	135.84c	143.20b	145.72b	147.55b
Weekly	Ecdomycorrhiza	148.11b	155.10a	158.51a	SD
S.E		4.33	3.53	2.46	1.84

Means in a column followed by the same letter(s) are not significantly different using DMRT at 5% level.
WAP = Weeks After Planting Sd = Seedling Death

The stomata conductance of the seedlings inoculated with mycorrhiza were higher ($P < 0.05$) than the uninoculated seedlings at the different periods of growth assessment (4, 8, 12 and 16 WAP). At 12WAP, the stomata conductance of the seedlings that were watered twice weekly (58.2 $mmol^{-2s-1}$) which also was higher ($P < 0.05$) than those watered daily (37.39$mmol^{-2s-1}$) for the uninoculated seedlings. For the seedlings inoculated with ectomycorrhiza, the stomata conductance of the seedlings that were watered twice weekly (130.50$mmol^{-2s-1}$) was higher than those that were watered weekly (103.33$mmol^{-2s-1}$). For the endomycorrhiza inoculated seedlings, those watered weekly had higher stomata conductance (158$mmol^{-2s-1}$) than the ones watered twice weekly. For both ecto– and endo–mycorrhizae inoculated seedlings the stomata conductance of the daily watered ones was lowest throughout the period of plant growth. At 16 WAP, the seedlings that were watered weekly wilted irrespective of whether inoculated or not.

The xylem pressure potential of the seedlings also varied significantly ($P < 0.05$) in response to watering regime and mycorrhizae inoculation. As show in Table 3, the batch of seedlings that was watered twice weekly had higher xylem pressure potential than either the daily or weekly waterings without mycorrhizae inoculation, while those watered once per week had the lowest. Throughout the period of the growth trial, the seedlings without mycorrhiza inoculation had lower xylem pressure potential than the mycorrhizae

inoculated seedlings irrespective of the watering regime. The seedlings inoculated with endomycorrhiza recorded significantly lower xylem pressure potential than the ectomycorrhiza inoculated seedlings. The seedlings inoculated with mycorrhiza had improved growth, more than the uninoculated seedlings. It was also observed that seedlings that were watered twice weekly (slightly stressed) had enhanced height growth, and produced more leaves than those watered daily and weekly. Also slightly water-stressed mycorrhizae treated seedlings twice weekly watering had higxher mean growth parameters than non–mycorrhiza (control) and highly water-stressed (weekly watering) mycorrhizae treated seedlings. The results showed significant difference in plant height between water – stressed (weekly watering) plants inoculated with either of the mycorrhizae types (ectomycorrhiza or endomycorrhiza).

It was also observed that the total chlorophyll content of all the seedlings gradually increased with the age of the seedlings within the same treatment. As observed in his study, the root length of seedlings increased with water stress. Thus, those seedlings that were watered twice weekly had higher root length than those watered daily. Similar findings were reported by Fisches (1980).

Table 5: Chlorophyll content (mg/l) in the leaves of *A. senegal* seedlings in response to watering regimes and mycorrhizae inoculation at different period after planting

Watering Regime	Mycorrhizae Treatment	Chorophyll content(mg/l)		
		4 WAP	8 WAP	12 WAP
Daily	No Mycorrhiza	24.0[b]	22.0[c]	19.5[d]
Bi weekly	No Mycorrhiza	23.0[c]	23.0[c]	22.0[c]
Weekly	No Mycorrhiza	24.5[b]	23.1[c]	17.5[e]
Daily	Ectomycorrhiza	25.1[b]	25.2[b]	26.1[b]
Bi weekly	Ectomycorrhiza	24.8[b]	26.0[b]	26.5b
Weekly	Ectomycorrhiza	25.2[b]	25.6[b]	26.0[b]
Daily	Endomycorrhiza	24.5[c]	25.5[b]	26.0[b]
Bi weekly	Endomycorrhiza	26.0[b]	27.0[a]	27.5[a]
Weekly	Endomycorrhiza	24.4	27.5[a]	SD
S.E		2.15	3.10	2.29

Means in a column followed by the same letter(s) are not significantly different using DMRT at 5% levels.
WAP = weeks after planting

Table 6: Xylem pressure potential (mg) of *A .senegal* seedlings in response to watering regime and mycorrhizae inoculation at different period of growth

Watering Regime	Mycorrhizae Treatment	Xylem Pressure Potential (mg)			
		4 WAP	8 WAP	12 WAP	16 WAP
Daily	No Mycorrhiza	-2.07a	-2.27a	-2.31a	-2.38a
Bi weekly	No Mycorrhiza	-1.17c	-1.28b	-1.37b	-1.42b
Weekly	No Mycorrhiza	-1.35b	-2.21a	-2.97a	SD
Daily	Ectomycorrhiza	-0.98d	-1.23b	-1.29b	-1.38b
Bi weekly	Ectomycorrhiza	-0.98d	-1.22b	-1.27b	-1.29b
Weekly	Ectomycorrhiza	-0.98d	-0.99e	-0.96d	SD
Daily	Endomycorrhiza	-1.15c	-1.38b	-1.61b	-1.84b
Bi weekly	Endomycorrhiza	-0.88d	-1.19c	-1.23b	-1.53b
Weekly	Endomycorrhiza	-0.36e	-0.57d	1.12c	SD
S.E		3.38	1.79	2.54	1.95

Means in a column followed by the same letter(s) are not significantly different using DMRT at 5% level.
WAP = weeks after planting,
SD = Seedling Death

DISCUSSION

The growth response of *A. senegal* was better with endomycorrhiza than with ectomycorrhiza. These findings support the earlier observations by Shinkafi (2000) that mycorrhiza inoculation increase soil water extraction and root hydraulic conductivity. He also noted higher growth attributes from endomycorrhiza treated seedlings of *Acacia albida* than the ectomycorrhiza treated ones. Under serious water stress, the seedlings stopped growing; leaves turned yellow, wilted and eventually died. Abubakar (2002) also observed that *A. senegal* showed high sensitivity to water stress at seedling stage and indicated that most of the growth parameters studied performed better in unstressed conditions.

Mycorrhizae inoculations whether ecto- or endo-mycorrhiza combined with different watering regimes were found to have significant influence on the growth of *A. senegal*. This result supported the observation by Awotoye *et al*. (1992) who noted that slightly stressed mycorrhizae-inoculated seedlings had higher mean growth values than the non-mycorrhiza (Control) and water stressed *Leuceana leucocephala.* In this present study, *A. senegal* had enhanced leaf number, increased collar diameter, higher shoot height, increased stomata conductance and xylem pressure potential largely due to the presence of mycorrhiza. This, therefore, suggests that the ecological range of *A. senegal* could be extended to marginal lands characterized by low soil moisture with endomycorrhiza and ectomycorrhiza improving growth.

This is because the association between the seedlings and the mycorrhizae will enhance plant growth and good seedling vigour and improve the plant physiological activities like stomata conductance and xylem pressure potential which are directly related to soil-water balance and growth determinant in water-stressed environment. This is evident from the increase in plant height, collar diameter and leaf number of inoculated seedlings more than the plants not inoculated which were both subjected to weekly watering (highly stressed). Levy and Krikum (1983) and Read and Boyd (1986) observed higher soil water extraction and root hydraulic conductivity in mycorrhiza inoculated plants. Partially stressed *A. Senegal* seedlings that were watered twice a week were significantly better in terms of growth performance than uninoculated plants receiving the same watering frequency.

In general, the interaction between mycorrhiza and drought was found to be significant as a result of positive mycorrhiza effect on young seedlings of *A. senegal.* No growth depression was observed, rather the growth of the inculated seedlings was significantly enhanced. The interaction between mycorrhiza and water-stress resulted in a positive growth performance of all the plant parameters measured, indicating that both endo- and ecto-mycorrhizae were not parasitic on *A. senegal.*

Total chlorophyll content of the leaves of *A. senegal* seedlings inoculated with mycorrhiza, particularly those inoculated with endomycorrhiza and watered twice weekly was significantly higher than what was observed in other treatments. These findings agreed with the work of Bello (2005) who in a similar study discovered that mycorrhiza inoculation enhanced total chlorophyll content of *A. senegal* and *Acacia nilotica*. The enhanced root growth in response to stress condition maybe due to high osmotic adjustment in the root cells which may likely result to rapid elongation of the root meristem.

CONCLUSION

It is evident from the results of this study that mycorrhiza inoculation (particularly endomycorrhiza) is required for healthy and adequate growth of the seedlings of *A. senegal* in the nursery. From the study, it can be concluded that twice weekly watering is most adequate for tending the seedling of *A. senegal* in the nursery. This is evident because daily watering produced fragile seedlings that may not be able to withstand the harsh drought condition in the field while the weekly watering regime resulted to stress condition that wilted the seedlings even in the nursery before transplanting into the field. It is also noticeable from this study that mycorrhizae inoculation (particularly endomycorrhiza) enhanced growth performance and seedling survival of *A. senegal* in the nursery.

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