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Study of the watering frequency of young seedlings of *Acacia nilotica* (L.) Willd. ex Del. (Mimosaceae) in the nursery on recovery after field transplanting in Maroua, Cameroon

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ABSTRACT

The environmental and social impact of deforestation can be partly offset by the planting of local woody species such as *Acacia nilotica*, but unless sown directly into their final positions, sooner or later, young seedlings have to be set out in the soil. This a crucial stage when the plants are vulnerable to drought stress. Therefore, this work aims at studying the effect of the induction of water stress in the nursery on the behavior of *Acacia nilotica* seedling after transplanting. The experimental design was a one-factor randomized block (watering frequencies). The blocks (03) constitute the repetitions. The five (05) watering frequency levels was F0 (control), F1, F2, F3, F4 and F5 respectively for twice-daily watering at field capacity after 12h, 24h, 48h, 72h, 96h and 120h. The stress applications took place seven (07) days after germination for ninety (90) days and transplant into the natural environment. After transplanting (60 days), the rate of increased in height and collar diameter as well as the survival rate increase with the stress level of the seedlings in the nursery. It emerges from this work that the more the level of stress increased, the more the aerial growth of the seedlings was reduced, unlike the root part. The aerial biomass went from 10.23g (F0) to 9.22g (F3) and to 8.67g for F5, ie a respective reduction rate of about 10 and 16%. On the other hand, the root biomass increased from 3.07g (F0) to 4.31g (F3) and 5.56g for F5, that is to say an increase rate of about 40 and 81% respectively. Biochemical analysis revealed that with the exception of chlorophylls (Chl T: 7.27 mg/g FM for F0 to 3.38 mg/g FM for F5), the other measured elements (Proline: 5.27% (F0) to 27.84% to F5; Polyphenol: 6.25% (F0) to 7.47% to F5, Soluble sugar: 15.17% (F0) to 36.66% to F5 and total Proteins: 24.84% (F0) to 28.64% to F5) increase with the level of stress but decrease at the highest level. Subjecting young seedlings to water stress in the nursery allowed them to develop mechanisms for acclimatization to water stress. These results could be useful to the various reforestation programs for the production of vigorous seedlings.

Keywords: *Acacia nilotica*, water stress, transplanting stress, watering frequency, biochemical parameters, dendrometric parameters.

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INTRODUCTION

A. nilotica is a multipurpose shrub very popular among people in semi-arid tropical areas in general and those in Cameroon in particular (Ginwal and Mandal, 2004). Indeed, *A. nilotica* produces good firewood and lumber (Egeru and al., 2014) and good aerial fodder for small ruminants (Ouattara and Louppe, 2000). It is also highly sought after for its therapeutic virtues (Sereme et al., 2008) and especially for its pods and bark which are very rich in tannins used in the leather industry (Alhaji et al., 2020). *A. nilotica* is also a good specie for restoring soil fertility (Abdou et al., 2013) because like any legume, it is able to fix atmospheric nitrogen and return it to the soil through decomposition litter.

In Northern Cameroon, *A. nilotica* grows naturally. However, strong anthropogenic actions recorded in recent decades have led to the scarcity of its natural populations like that of a good number of species (Seignobos and Iyébi-Mandjek, 2005). Given its socio-economic and ecological importance, *A. nilotica* is very much in demand in reforestation programs, but the very high tegumentary dormancy of its seeds leads to the production of seedlings in nurseries. Conclusive results have been obtained by several reforestation programs, but some programs have ended in failure since they have come up against serious problems, including the short life of the seedlings. Indeed, the quality of the seedlings greatly conditions the success and the start of the plantations (M'Sadak et al., 2013) because the quality of a seedling is the result of the integration of numerous physiological and morphological characteristics which control the possibilities of further development and growth of seedlings. In addition, Wahbi et al. (2013) already concluded their work by mentioning that cultural practices in the nursery can influence the potential for recovery at planting. It is therefore becoming urgent to seek to develop reliable and operational physiological criteria which will make it possible to obtain vigorous seedlings, able to adapt to the dry environments of semi-arid zones in general and those of the Far North of Cameroon especially.

Drought is one of the constraints that strongly limit the yield of grain legumes, particularly when seedling growth depends on symbiotic nitrogen fixation (Khadriet al., 2001). The differential effect of water stress on the physiology of African seedlings and Cameroon in particular remains little known. Work had been carried out with this in mind, in the case of Mahadeo et al. (2014) on the genetic selection of provenances resistant to abiotic stresses, those of Wahbi et al. (2013) on the effect of water and saline constraints on the germination of African Acacia seeds. However, very few studies have focused on obtaining vigorous seedlings that can withstand transplant stress in a contrasting medium but no such study has been done and not at all on *A. nilotica*. It is in this perspective that this study aimed at studing the effect of an induction of water stress in the nursery on the transplant stress of young seedlings of *A. nilotica* in the Far Northern Region of Cameroon.

MATERIALS AND METHODS

Presentation of the study area

This work was carried out in the city of Maroua, capital city of Diamare Division, regional capital of the Far North Region, Cameroon. It extends between the 10th and 13th degree of North latitude and the 13th and 15th degree of East longitude. The Far North Region is governed by a climate of the Sahelo-Sudanian type characterized by a very long dry season and lower rainfall. The average temperature in this Region is 28°C and the annual rainfall is 726.2 mm (Olivry and Noah, 2000). The average humidity is between 30 and 35%.

Plant material and seed pretreatment

The seeds of *A. nilotica* constitute the plant material of this study. They were harvested manually in 2020 in Madjema in Maroua I Sub-Division. This work was carried out in 3 stages: cultivation in the nursery, biochemical analysis and cultivation (transplanting) in the field. Seed collection, soil sampling and experimentation were carried out respectively in Douggoi, Madjema and

Djarengol- Delegate (Figure 1). After obtaining the seeds, they were pretreated by scalding in water at 100°C for 20 minutes (Wahbi et al. (2013). This operation made it possible to remove the integumentary inhibition because the seeds of *A. nilotica* have a very hard integument.

Methods

Setting up the experimental desing

The seeds were sown about three (03) cm deep in pots containing humus soil. Water stress was stimulated by watering frequencies. The experimental design was a randomized block with a factor including watering frequencies. The blocks (03) constituted the repetitions. The six (06) watering frequency levels were F0, F1, F2, F3, F4 and F5

Before transplanting (90 days in nurseries) and 60 days later, dendrometric (seedling height, leaf area, number of leaves and collar diameter) and physiological parameters (chlorophyll, proteins, proline and polyphenol contents) were measured and performance indices were calculated. The total height of the seedling and the neck diameter were measured using respectively a graduated ruler and an IPX54 digital caliper. The leaf area

The total chlorophyll content was measured on fresh leaves by the method of Arnon (1949) and calculated according to the equations of Lichtenthaler and Wellburn (1983):

$Chl(a) = 12.21Do663 - 2.81.Do646$

$Chl(b) = 20.13Do645 - 5.03.Do663$

$Chl(a+b) = Chl(a) + Chl(b)$

The total protein content was determined by the method of Bradford (1976) and the soluble sugar content by the method of Cooper et al. (1970) after extraction by the method of Conroy and al. (1988). The proline content was estimated according to the method of Ringel et al. (2003) and the polyphenol content was assayed with the Folin-Ciocalteu reagent which in an alkaline medium which is reduced to tungsten and molybdenum oxide giving a blue color in the presence of polyphenols (Dewanto et al., 2002). The

respectively for twice-daily watering at field capacity after 12h, 24h, 48h, 72h, 96h and 120 h. Seedlings watered every 12 hours (morning and evening) were considered as controls. The different levels of watering frequency were chosen to approximate the methodology of Wahbi et al. (2013). A total of 54 pots were placed, that's 3 blocks were repeated 3 times and 6 levels of watering frequency (3 x 3 x 6). The stress applications took place seven (07) days after germination for ninety (90) days. Subsequently, the seedlings were transplanted into the natural environment, so the characteristics of the soil are recorded in Table 1.

Measurement of dendrometric parameters

was obtained after scanning the complete leaves, and the images analyzed by the Win-Rhizo software (2002c Régent Instruments INC. Canada). The number of leaves was counted manually. Aerial (AB) and root (RB) biomasses were evaluated after drying in the oven for 72 hours at 45°C and AB/RB ratio was calculated.

Measurement of biochemical parameters

increase rate (Ir) of the height and diameter of the collar and the survival rate (Sr) were calculated using their respective below.

$Ir\ (\%) = ((Vf-Vi)/Vi) \times 100$

$Sr\ (\%) = 100 \times (Ni - Nf) / Ni$

Where Vf and Vi representing the final and initial values respectively, Ni and Nf representing the initial and final number of plants respectively.

Statistical analysis

Statiscal analysis were performed on the data after a normality test. Using a one-way ANOVA, following by Scheffe's mean comparison test at 5% (if ANOVA was significant), we compared among water stress levels for each dendrometric and biochemical parameters. These tests were conducted through software Stratigraphic 5.0plus.

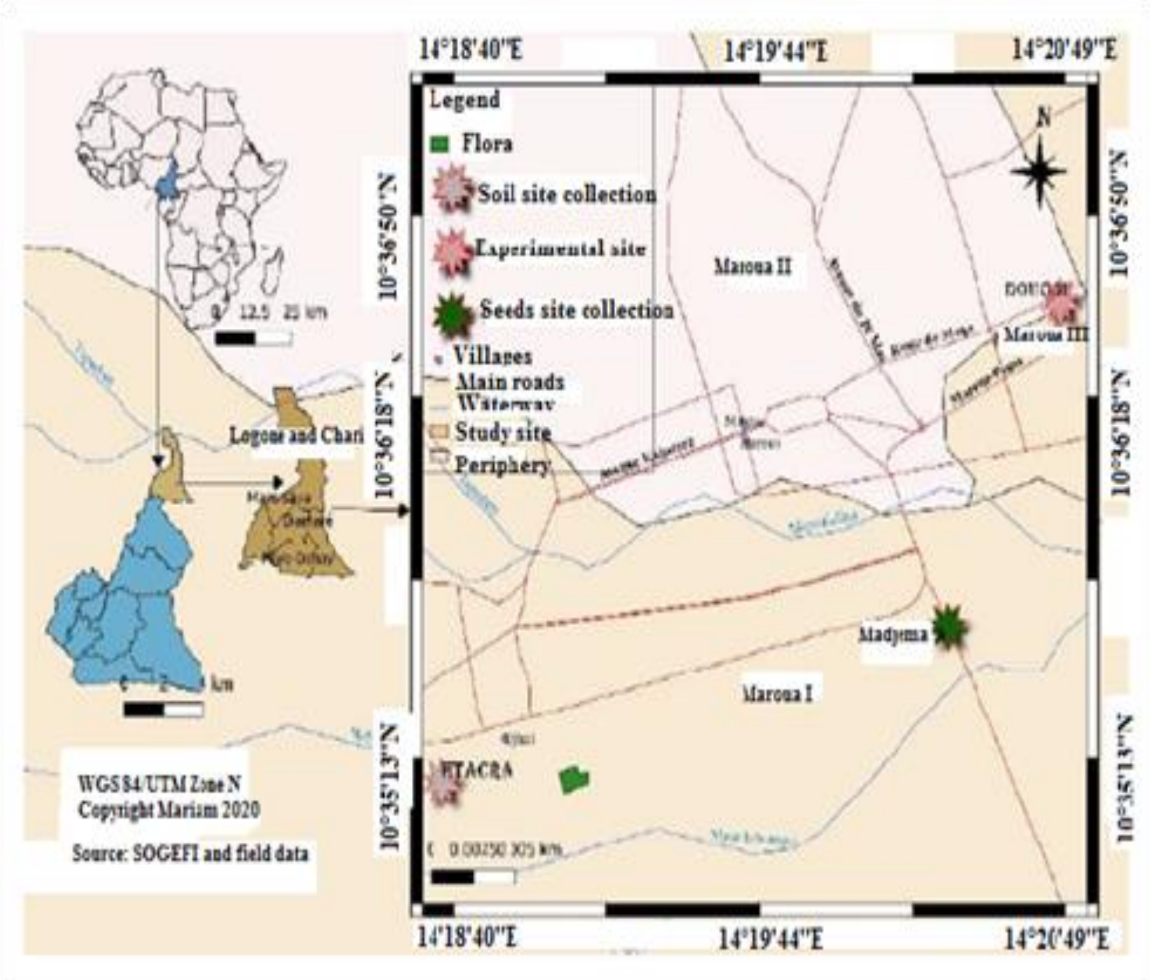

Figure 1: Map of location of the study site.

Table 1: Chemical characterization of nursery soils and the transplanting environment.

Elements of soil characterization	Soils of nursery cultivation	Soils of transplanting
pH_{water}	6.98	5.22
pH_{KCl}	5.47	4.40
C/N	15.06	14.06
CEC (Cmol. kg^{-1})	2.38	3.17
Organique Matter (%)	3.5	4.21

C/N : Carbon to Nitrogen ration ; CEC : Cation Exchange Capacity.

RESULTS

Variation of dendrometric parameters according to water stress

In general, all the dendrometric parameters except root biomass are significantly (P = 0.001) and negatively influenced by the frequency of watering young seedlings in the nursery (Table 2). Indeed, the values of these parameters are higher in control seedlings and decrease as the level of stress increases. By calculating the reduction rate compared to the values of the control seedlings, it emerges that the reduction rate increases from 11.70% for F1 (19.17cm) to 42.42% for F5 (12.50 cm) for the height parameter. The same observations were made for the collar diameter, so the reduction rate goes from 20.47% for F1 (2.02 cm) to 43.70% for F5 (1.43 cm). The leaf area also decreases with the severity of the stress because the reduction rate goes from 19.05% for F1 (0.68 cm^2) to 41.67% for F5 (0.49 cm). The reduction rate is less than 20% for the highest stress level (F5) for leaf number (19.76%; 15.84 LN) and aboveground biomass (15.25%; 8.68 LN).

Root biomass, unlike aerial biomass, increases with the level of stress (Table 3). This value is the lowest in the control (3.00±0.02 g) and the highest in the treatment with high water deficit or highest level of stress (F5). The consequence of this increase in root biomass is the increase in the RB/ABratio compared to control seedlings. This ratio varies significantly (P<0.01) from 0.3 with F0 to 0.64 for F5.

Variation of biochemical parameters according to water stress level

Plant biochemical metabolism was also significantly influenced (P<0,001) by the different water stress level. Indeed, from Table 4, it emerged that the chlorophyll contents (a and b) decrease as the level of stress increases. They varied from 2.21 to 4.86 mg/g and from 1.17 to 2.41 mg/g for chlorophyll a (chla) and chlorophyll b (chlb) respectively. The highest values were observed at F0 and F1 and the lowest at F5 and F4, the intermediate values at F2 and F3 for the both chlorophylls. However, proline and soluble sugar contents increase with the level of stress. They varied from 5.27 to 27.84% and from 15.17 to 36.66% respectively for proline and soluble sugar. The proline content found in F0 (5.27) was tripled in F2 and multiplied by about 5 for F5 plants. For the soluble sugar content, it was about double in F5 compared to F0. As for the Polyphenol and total Protein contents, they increase with the severity of the stress but beyond F4 (Poyphenol: 7.71%; Total Protein: 29.30%), they decreased at F5 (Poyphenol: 7.47%; Total Protein: 28.64%). Indeed, similarly, the total protein content goes from 24.84% at F0 to 28.64% at F5.

Variation of dendrometric parameters according to water stress level after transplanting

After ninety (90) days in the nursery, the seedlings were transplanted into the fieldland and after sixty (60) days in the field, the total height of the plants and their collar diameter were measured. The results obtained showed that, whatever the type of stress level, the increase in the height and diameter of the collar of the seedlings was significant compared to the control (Figures 2a and 2b). Indeed, seedlings subjected to water stress in the nursery, after 60 days in the field showed a higher rate of increase than the control plants (35.64% for height and 25.84% for collar diameter). These rates increased up to F4 and then decrease at the highest stress level F5 but however remained higher than those of F0. The exploitation of data on the survival rate shows that after 60 days in the field, the level of stress positively and significantly influenced this rate (Figure 2c). The survival rate of plants subjected to water stress in the nursery was higher than that of the control (26.56%) for all water stress level, but the highest value was obtained from treatment F2 (84.20-29.30% at F4 then drops to 28.64% at F5.

Table 2: Influence of different water stress level on the seedling growth in the nursery.

F arr	H (cm)	CD (cm)	LA (cm²)	LN
F0	21.71±0.77e	2.54±0.43e	0.84±0.03e	19.74±0.16f
F1	19.17±1.14d	2.02±0.44d	0.68±0.05d	17.06±0.09e
F2	18.12±0.26cd	1.94±0.42cd	0.63±0.02cd	16.89±0.09d
F3	17.40±2.12bc	1.66±0.19bc	0.56±0.03bc	16.56±0.11c
F4	15.94±3.41b	1.53±0.29a	0.53±0.13ab	16.20±0.14b
F5	12.50±0.77a	1.43±0.18a	0.49±0.07a	15.84±0.21a
F	27.94***	12.77***	32.74***	1301.80***

Total plant height (H), Collar diameter (CD), Leaf area (LA) and leaf Number (LN). Different letters in a column indicate the means are significantly different, ***: P<0.001.

Table 3: Influence of water stress levels on the seedling biomass in the nursery.

F_{arr}	AB (g)	RB (g)	RA/AB
F0	10.23±0.21d	3.07±0.02a	0.30±0.001a
F1	9.51±0.36c	3.78±0.02b	0.39±0.001b
F2	9.39±0.03c	4.29±0.00c	0.46±0.003c
F3	9.22±0.08b	4.31±0.02c	0.47±0.001c
F4	8.69±0.07a	4.83±0.12d	0.56±0.004d
F5	8.67±0.02a	5.56±0.06e	0.64±0.002e
F	96.70***	85.95***	72.24***

Watering frequency (Farr.) ; Above-ground biomass (AB) and Root biomass (RB). Watering frequency respectively for twice-daily watering at field capacity after 12h, 24h, 48h, 72h, 96h and 120h (F0, F1, F2, F3, F4 and F5). Fisher's coefficient (F).Different letters in a column indicate the means are significantly different, ***: P<0.001.

Table 4: Influence of different stresses on the metabolism of seedlings in the nursery.

F arr	Chla (mg/g MF)	Chlb (mg/g MF)	ChlT (mg/g MF)	Polyphénol (%)	TProt (%)	Proline (%)	Sucre so. (%)
F0	4.86±0.12d	2.41±0.03e	7.27±0.14e	6.25±0.22a	24.84±1.94a	5.27±0.15a	15.17±0.63a
F1	4.79±0.15d	2.29±0.04d	7.08±0.19d	6.31±0.20a	25.20±1.51a	9.86±0.60b	16.94±2.08b
F2	4.55±0.07c	2.23±0.02c	6.78±0.13c	7.03±0.60b	25.52±0.36a	15.43±0.30c	17.32±2.55b
F3	4.56±0.10c	1.81±0.02bc	6.31±0.09c	7.19±0.49bc	29.00±2.05b	18.64±0.44d	24.98±0.41c
F4	3.37±0.05b	1.69±0.02ab	5.06±0.07b	7.71±0.55cd	29.30±1.81b	23.02±0.71e	33.40±1.12d
F5	2.21±0.06a	1.17±0.01a	3.38±0.08a	7.47±0.30d	28.64±1.79b	27.84±0.57f	36.66±0.46e
F	52.76***	114.83***	59.80***	17.90***	14.06***	2485.26***	354.35***

Watering frequency (Farr.), Chlorophyll a (Chla), Chlorophyll b (Chl b), Total protein (TProt) and Soluble sugar (Sucreso). Watering frequency respectively for twice-daily watering at field capacity after 12h, 24h, 48h, 72h, 96h and 120h (F0, F1, F2, F3, F4 and F5). Fisher's coefficient (F). Different letters in a column indicate the means are significantly different, ***: P<0.001.

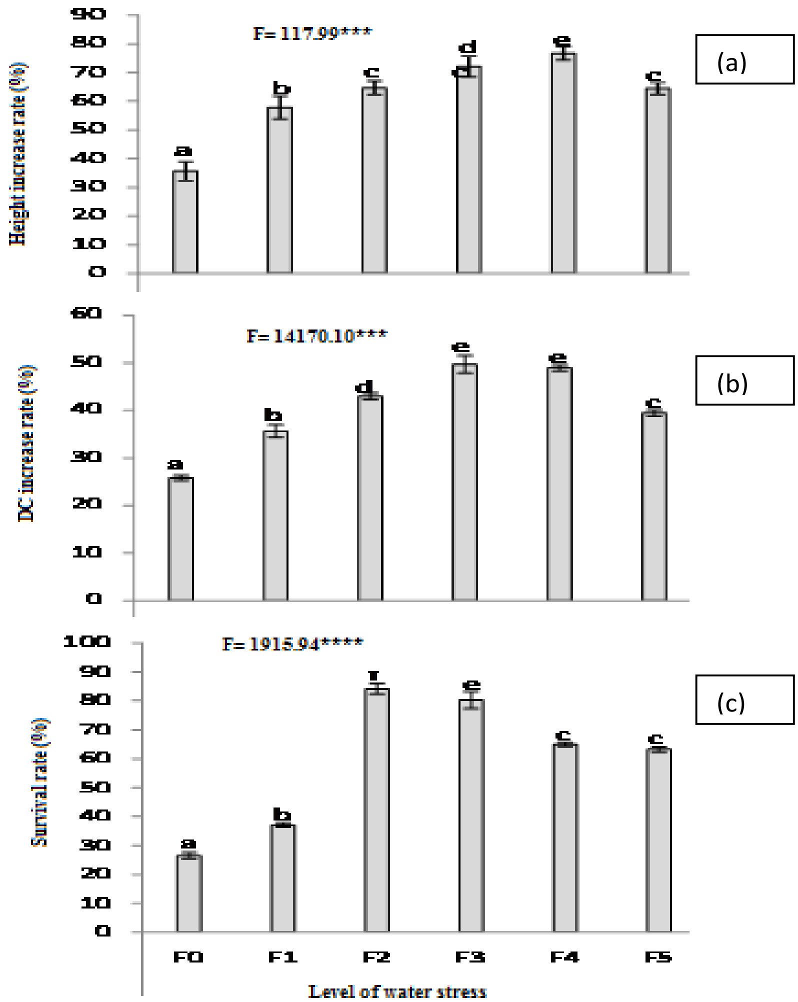

Figure 2: Seedlings performance, as height (a), DC increase (b) and survival rate (c) after transplanting in the fiel.

DISCUSSION

The results obtained showed that the reduction in the frequency of watering induced the drop in aerial growth contrary to the root growth of young seedlings of *A. nilotica*. According to Dione et al. (2002), the sustained growth of the root system under water stress conditions would be a factor of resistance to water stress. Indeed, according to Clavel et al (2007), the plant under water deficit frequently used the allocations of dry matter to develop the root part in order to explore the maximum layer of the soil in search of water. These results are similar to those of Kagambega et al. (2019) on the water deficit tolerance of five (05) reforestation species in Burkina-Faso, Ferradous and al. (2013) on the effect of different irrigation regimes on the production of argan seedlings in nurseries in Algeria and Nguinambaye and al. (2020) on ground lentil (*Macrotyloma geocarpum*). This decrease could be explained by the decrease in photosynthetic activity. Indeed, the results obtained revealed a decrease in the chlorophyll content (a, b and total) as the level of stress increases. This could be related to the attempt to reduce water consumption through the limitation of the evaporation surface which is an important determinism of transpiration. Results confirmed this hypothesis because the leaf area and the number of leaves were negatively affected by water stress. These results are in agreement with those obtained by Toudou et al. (2017) in Niger, Meftah (2012) and Benkrinah and al. (2018) in Algeria respectively on cowpea and durum wheat. This would be explained according to Mouellef (2010) by the fact that to limit water losses by evaporation, saving water resulted in relative turgidity leading to a dilution of chlorophyll. Moreover, according to Toudou et al. (2017), the reduction in chlorophyll content in stressed seedlings can be explained by the fact that chlorophylls are more degraded than synthesized in the latter, whereas in control seedlings these pigments are synthesized progressively as they are degraded.

Unlike chlorophyll levels, polyphenol, total protein, proline and soluble sugar levels increase with the severity of stress. The results thus obtained are similar to those of Chen and Dickman (2005) and Goyal and al. (2005) who mention that in the medium term, the seedling adapts to the water deficit by an "active" osmotic adjustment following the accumulation of osmolytes such as proline, polyamines, organic acids, sugars. Additionally, Meftah (2012) and Benkrinah et al. (2018), mention that soluble sugars ware considered good osmoregulators that could play an important role in osmotic adjustment and plant adaptation to water deficit. Macheix et al. (2005) on their part specify that the stimulation of the synthesis of total polyphenols made it possible to resist constraints and participate effectively in the tolerance of plants to various stresses.

After transplanting the seedlings in the fields, it emerged that the stressed seedlings in the nursery had a survival rate and a rate of increase in growth (height and diameter of the collar) higher than those of the unstressed seedlings. The results thus found were similar to those found by Nasrallah and Lakhdar (2010) on the recovery of stressed seedlings. This good recovery of stressed seedlings in the field would be due to the accumulation of sugars during the stress period. Indeed, according to Al-Humaid and Moflash (2007), the application of a limitation of watering before transplanting seems to promote the storage of carbohydrates in the roots, carbohydrates which will be available for root regeneration which is a guarantee for a recovery of seedlings in the field.

Conclusion

The aim of this study was therefore to investigate the effect of inducing water stress in the nursery on the transplant stress of young *A. nilotica* seedlings in the Far North, Cameroon. The results of this study showed that in nurseries, the more the stress level increased, the more the aerial growth of the seedlings is reduced, unlike the root part. Biochemical analyzes revealed that with the exception of chlorophylls (a and b), the other biochemical compounds (proline, polyphenol, soluble sugar and total proteins) increased with the level of stress but decrease at the highest stress level. After transplanting, the rate of increase in dendrometrics parameters and the survival rate increased with the level of stress of the seedlings in the nursery. These results could be useful to the various reforestation programs for the production of vigorous seedlings.

COMPETING INTERESTS

The authors declare that there is no competing interests.

AUTHORS' CONTRIBUTIONS

CFA designed the study, analyzed and interpreted the data, and wrote the original draft. ITNY participated in the study design, provided guidance during data collection, and edited the manuscripts. KS participated in the study design and was a major contributor in the writing of the manuscript. AI contributed in the study design. All authors read and approved the final manuscript.

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