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Effect of drought and salt stress on *Balanites aegyptiaca* and *Populus nigra* seedlings

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Dedication

I dedicate this work to my sister (Salma), my daughter (Areeg), my son (Sami) and my husband (Tarig) for their love, support and encouragement.

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Abstract

The objective of this study was to investigate drought and salinity response and resistance in *Balanites aegyptiaca* and *Populus nigra* seedlings. Four greenhouse experiments were carried out at BOKU (Vienna) and AIT (Seibersdorf), Austria.

In the first experiment the seedlings of *Balanites aegyptiaca* were subjected to NaCl at concentrations of 0, 75, 150 and 300 mM. The maximum quantum yield of fluorescence (F_v/F_m), electron transport rate (ETR) and plant dry weight were significantly reduced with increased NaCl.

In the second experiment seedlings of *Balanites* from two provenances were fertilized with different levels of potassium (0, 220 and 2200 mg/L) and nitrogen (65 and 2580 mg/L) and subjected to gradually increasing drought over four weeks with maximum drought maintained for another three weeks. Drought reduced stomata conductance (g_s), maximum quantum yield of chlorophyll fluorescence and plant water potential. Drought and low nitrogen reduced plant total dry matter. The difference between the two provenances in their response to drought stress indicates the higher dehydration tolerance of Um Abdalla plants and the more efficient mechanisms of drought avoidance in Damazin plants according to the parameters measured. *Balanites* showed various drought responses such as increased root weight and root/shoot ratio, and appears to be a suitable plant to be grown in areas affected by drought stress.

In the third experiment, *Balanites* seedlings of two provenances were treated with saline solution ($NaCl: CaCl_2 : MgSO_4$, 2:1:1) in concentrations of 0, 4, 8 and 16 mS/cm. Surprisingly, salt significantly increased F_v/F_m and by the end of the experiment net photosynthesis was higher in plants under salt treatment. Stomata conductance (g_s) and intercellular CO_2 (C_i) were high in plants under salt treatment except for the last two measurement days, when they were decreased. Plants responded to salt stress by developing thicker leaves with higher water volume and increased root/shoot ratio, but leaves were dropped at the highest salt concentrations. *Balanites* appears suitable to be grown in areas affected by moderate salt stress. The two provenances differed significantly and should be selected according to the condition of the site relative to the strategy of each provenance.

In the fourth experiment, cuttings of three clones of *Populus nigra* were exposed to salt solutions ($NaCl : CaCl_2 : MgSO_4$ 5:1:1) of concentrations corresponding to 0, 4 and 8 mS/cm. The highest concentration significantly decreased chlorophyll content, g_s, several parameters of chlorophyll fluorescence as well as total plant dry matter and root/shoot. Salt of 4 mS/cm significantly increased net photosynthesis, stomata conductance and intercellular CO_2 after three weeks of application. Clones differed significantly in chlorophyll content,

photosynthesis, $g_{s,}$ fluorescence ($F_{0,}$), performance index (PI) and plant dry matter, where clone EF was more sensitive to salt. The leachate water of landfills of 8 mS/cm is not suitable for irrigation of *Populus nigra* unless treated and diluted, and clones LO14 and LO50 appear more suitable than clone EF to be cultivated on landfills sites irrigated with saline eluate.

Zusammenfassung

Ziel dieser Studie war es, den Effekt von Trockenheit und Salzgehalt auf Keimlinge von *Balanites aegyptiaca* und *Populus nigra* zu untersuchen. Dazu wurden vier Versuche im Glashaus der Universität für Bodenkultur, Wien und am Austrian Institute of Technology, Seibersdorf durchgeführt.

Im **ersten** Experiment wurden Kelimlinge von *Balanites aegyptiaca* Natriumchlorid in vier verschiedenen Konzentrationen ausgesetzt (0, 75, 150 und 300 mM). Zunehmender Salzgehalt verringerte den quantum yield (F_v/F_m), die Elektronentransportrate (ETR) und das Trockengewicht signifikant.

Im **zweiten** Experiment wurden Keimlinge von *Balanites* aus zwei verschiedenen Provenienzen mit unterschiedlichen Mengen an Kalium (0, 220 und 2200 mg/L) und Stickstoff (65 und 2580 mg/L) gedüngt und steigendem Trockenstress ausgesetzt. Die Bewässerung wurde dabei innerhalb von vier Wochen stufenweise von 100g Wasserdefizit / Topf auf 400g Wasserdefizit verringert, dann wurde das Gießen für drei Wochen ganz eingestellt. Trockenheit reduzierte die stomatäre Leitfähigkeit (g_s), F_v/F_m und das Wasserpotential der Pflanzen. Trockenheit und geringe N-Versorgung reduzierten die Trockenmasse der Pflanzen. Die Pflanzen aus den unterschiedlichen Provenienzen reagierten dabei unterschiedlich. Allgemein reagierte *Balanites* auf Trockenstress mit zunehmender Wurzelmasse und Wurzel/Spross-Verhältnis.

Im **dritten** Experiment wurden *Balanites* Keimlinge zweier Provenienzen mit einer Salzmischung ($NaCl : CaCl_2 : MgSo_4$ 2 : 1 : 1) in den Konzentrationen von 0, 4, 8 und 16 mS/cm behandelt. Salz steigerte signifikant den F_v/F_m in der dritten und vierten Woche. Am Ende des Experimentes war die Photosynthese in salzbehandelten Pflanzen höher. Auch die stomatäre Leitfähigkeit und intercelluläres CO_2 waren in salzgestressten Pflanzen im Allgemeinen höher. Unter Salzstress entwickelten die Pflanzen dickere, sukkulente Blätter und höheres Wurzel/Spross-Verhältnis. Allerdings verloren die Pflanzen unter der höchsten Salzkonzentration die alten Blätter. *Balanites* erscheint durchaus geeignet für Böden mit moderatem Salzstress. Die zwei Provenienzen unterschieden sich auch in ihrer Reaktion auf Salzstress.

Im **vierten** Experiment wurden Stecklinge von drei *Populus nigra* Klonen mit Salzlösungen ($NaCl : CaCl_2 : MgSO_4$, 5 : 1 : 1) von 0, 4 und 8 mS/cm gegossen. In der höchste Konzentration waren Chlorophyllgehalt, g_s, verschiedene Parameter der Chlorophyllfluoreszenz sowie die Gesamttrockenmasse und das Wurzel/Spross-Verhältnis signifikant reduziert. Bei 4 mS/cm dagegen waren nach drei Wochen die Nettophotosynthese, gs und der interzelluläre CO_2–Gehalt höher als bei der Kontrolle. Die

Klone unterscheiden sich signifikant in Chlorophyll Gehalt, Phyotosynthese, g_s, Grundfluoreszenz (F_0), performance index (PI) und Trockensubstanz, wobei der Klon EF empfindlicher auf Salz reagierte. Sickerwasser von Mülldeponien mit einem Salzgehalt von 8 mS/cm erscheint nicht geeignet für die Bewässerung von *Populus nigra,* sofern es nicht behandelt und verdünnt ist. Die Klone LO14 und LO 50 dürften sich für Mülldeponien, die mit salzhaltigem Eluat bewässert werden, besser eignen als der Klon EF.

1 Introduction

1. 1 Drought

Water constitutes 80-90% of the fresh weight of herbaceous plants and over 50% of fresh weight of woody plants, and it plays an important role as solvent in which gases, minerals and other solutes enter plant cells and move from cell to cell and from organ to organ. It is important as a reactant substrate in many processes such as photosynthesis and in the maintenance of turgidity in plants, which is essential for cell enlargement and growth, and in the opening of stomata (Kramer, 1983). Water is essential for plant growth starting from seed germination to seedling growth and development (Salakari *et al*., 1989). Water movement from soil to plant and via transpiration to the atmosphere is determined by the water potential gradient between the soil solution and water vapour in the atmosphere. Well-watered plants have a high water potential (-0.5 to -1.2 MPa) while plants under saline and arid climates often have low water potential (less than -2.5 MPa) that enable plants to extract water from the soil solution (González and Roger, 1995).

Plant water deficit is generally understood as a reduction in plant water potential and turgor that interfere with the normal functioning of plants (Kramer, 1983). This situation is the result of transpiration when water loss exceeds water uptake. In the field, water deficit mostly results from the combination of low precipitation and high evaporation. In arid regions drought is of regular and prolonged occurrence. Low water potential alters the composition of cell wall proteins and enzymes leading to changed growth pattern during drought (Smirnoff, 1995).

The most sensitive processes to water deficit are the decrease in turgor and slowing down of growth processes particularly elongation, reduction in protein metabolism and synthesis of amino acids (Larcher, 1995, Lambers *et al*., 1998). Water deficit decreases the size of leaves as a result of reduced cell expansion and cell division and reduces the proportion of epidermal cell that form stomata and increases number of epidermal trichomes (Kozlowski and Pallardy, 1997). Water deficit often reduces shoot growth before root growth, resulting in increased root-shoot ratios in water-stressed plants (Kramer and Boyer, 1995). Some enzyme and hormone synthesis is enhanced under water deficit such as the abscisic acid (ABA) that is involved in the alteration of cell wall extensibility. For example root cell walls may become more extensible (more root growth), while leaf cell walls become less extensible, preventing leaf expansion. ABA is also involved in stomata closure that reduces transpiration under water deficit (Smirnoff, 1995; Larcher 1995). Water deficit reduces photosynthesis due to limited CO_2 diffusion to the intercellular spaces of the leaf as a consequence of reduced stomata conductance, and by direct inhibition of biochemical processes caused by ionic, osmotic or other conditions induced by the loss of cellular water, all of which reduces the efficiency of carbon fixation (Kramer 1983).

1. 2 Tolerance of plants to water deficit

Some plants have the ability to avoid or tolerate drought by using different mechanisms. The first reaction of plants to water deficit is the closure of stomata to reduce water loss through transpiration. Some plants such as pineapple and cacti close stomata during the daytime and fix the CO_2 in darkness such as crassulacean acid metabolism (CAM), that results in comparatively high water-use efficiency but lower growth rates than those of C_3 and C_4 plants. The highly compartmentalized leaf anatomy and the special CO_2 pathway are the important characteristics of C_4 plants that increase the water-use efficiency and nitrogen-use efficiency in many grasses. Photorespiration, which constitutes a major loss of carbon of plants in dry areas, results from the closure of stomata and is highly reduced in CAM and C_4 plants (Kramer and Boyer 1995, Kramer 1983). One of the mechanisms of drought tolerance in plants is the reduction of leaf area by which plants reduce water loss through transpiration as found in *Eucalyptus camaldulensis* (Gibson *et al*., 1991). Also, dehydration can be postponed by developing deep roots such as in upland rice (Kramer and Boyer 1995). Some plants accumulate large stores of water in fleshy tissues such as cacti and the baobab trees (*Adansonia digitata*), which store water in trunks (Kramer and Boyer 1995, Kramer 1983). Plants tolerate water stress by osmotic adjustment in which solutes such as inorganic ions, carbohydrates and organic acids are accumulated in plants tissues to maintain turgor and turgor-dependant processes to lower water potential (Kramer 1983, Jones1992). Employing these and other mechanisms, many desert species are drought tolerant by increasing productivity through maximizing assimilation in relation to the amount of available water (Jones, 1992).

Examples of woody plants that have been studied and survive drought by using different tolerance mechanisms in arid and semi arid areas are *Acacia senegal*, *Acacia tortilis subsp raddiana, Leptadenia pyrotechnica* and *Eucalyptus microtheca*. Susiluoto and Berninger (2007) reported that *Eucalyptus microtheca* developed deep root system and increased root:shoot ratio under drought stress, which are the primary reactions that initiate a series of compensatory reactions that mitigate the effects of drought stress. Elfeel and Al-Namo (2011) reported that *Acacia tortilis subsp raddiana* tolerated drought stress by increasing root :shoot ratio. Some *Acacia* species in the arid and semi-arid areas in Africa developed different strategies to avoid water stress during germination, for example all viable seeds of *Acacia senegal* germinate rapidly in order to be installed as soon as possible based on the seed reserves, while *Acacia seyal* with small seeds and hard coats react by inducing dormancy under severe water stress (Kassa *et al*., 2010).

1. 3 Salinity

Soil salinity refers to the total amount of soluble salts in the soil solution. Generally, to assess soil salinity, two measuring procedures or means are applied. The most common way is by measuring the electrical conductivity (EC) in dS/m or mS/cm in a saturated paste of soil and water, or measuring EC with a conductivity meter in a soil-water extract based on a fixed soil/ solution ratio (e.g.1:2 or 1:5) (Richards, 1954) . The other way is by measuring the soluble salt concentration or how much salt in a solution of soil or water, this measurement is called total dissolved solids (TDS) or total dissolved ions (TDI). It is measured in units of mg/l, g/m^3 (grams of salt per cubic meter of water). The units of mg/l, or g/m^3 are equivalent numerically and equal to ppm (parts per million). Salinization of soil which is defined as the accumulation of excess salts in the root zone resulting in partial or complete loss of soil productivity and eventual disappearance of the vegetation is a major problem in irrigated arid and semi-arid lands where evapo-transpiration exceeds precipitation and is one of the main problems in land degradation.

According to various estimates, the extent of salt-affected soils in the world differs considerably. This is due to the lack of systematic surveys and the continuous change in the extent of salinization and differences between countries' approaches for detecting and classifying salt-affected soils (Mashali, 2009). As general figure about 7% of the total soil surface of the world is covered by salt-affected soils, which are defined as soils that have been adversely modified for the growth of most crop plants by the presence of soluble salts, exchangeable sodium or both (Mashali *et al*., 1999). For many crops, the yield starts declining from an EC value of 2 dS/m onwards, and already shows a depression of 20- 25 % at an EC value of 4 dS/m (Van Hoorn and Van Alphen, 1994).

Salinity often upsets the nutritional balance of plants by one or more mechanisms including the osmotic effect of salts, competitive interactions among ions in the substrate, and effects on membrane selectivity (Kozlowski, 1997). Most plants are non-halophytes, with a relatively low salt tolerance and their growth is inhibited at low salinity levels.

EC, (dS/m)	Classification	Crop yields
0 – 2	Non-saline	Not affected
2 – 4	Slightly saline	Sensitive crops affected
4 – 8	Saline	Many crops affected
8 – 16	Strongly saline	Only tolerant crops
> 16	Extremely saline	A few very tolerant crops

Table 1. 1: Soil salinity classification from (Van Hoorn and Van Alphen, 1994)

The reduction in growth of plants caused by high salinity is probably related to the accumulation of salt in the plant as to reduced availability of water in the substrate (Kramer 1983). Salinity reduces shoot growth and total photosynthesis as a result of inhibition of leaf formation and expansion as well as early leaf abscission (Kozlowski and Pallardy, 1997, Ziska *et al*., 1990). Salinity reduces plant growth by changing the allocation of assimilates, ion relation, water status and other biochemical and physiological processes and conditions, or by a combination of such factors (Kalaji and Nalborczyk, 1991).

Salt stress reduces photosynthesis, transpiration rate and stomatal conductance. The effect on photosynthesis can be due to reduced stomatal conductance and/or reduction of the chloroplast activity (Chaves, 1991).

1. 4 Tolerance of plants to salinity

Salt tolerance by plants depends on the mechanisms by which high salt concentration in the soil can be tolerated without injuring the protoplasm. Some plants maintain low salt concentration in leaves by restricting the entry of salts into roots and transport in the xylem and/or retaining most of the salts entering in old leaves such as in *Acacia nilotica* and *Acacia ampliceps* (Ashraf *et al*., 2006). Many halophytic plants accumulate salts in the cell sap to lower their osmotic potential therefore developing osmotic gradient by which water can move from the soil into plants and a high concentration of salt ions accumulate in the vacuoles. With a very negative potential in vacuoles they maintain osmotic equilibrium between the cytoplasm and the vacuole by compatible non-toxic organic compounds such as amino acids and sugars. Some plants develop salt succulence to dilute the high concentrations of salt such as in some coastal plants, mangroves and xerohalophytes of dry regions. Others have salt glands or special trichomes which excrete salt from the epidermal surface of leaves as in Tamarix species (Larcher 1995, Lambers *et al*., 1998).

1. 5 Municipal solid waste landfills

With the global increase of population and development of industrialization, the amount of wastes increases. Accumulation of wastes has a negative impact on the environment, therefore, waste management is very important to reduce, dispose and remove the wastes. Different methods are used in waste management such recycling, incineration and landfilling.

Municipal Solid Waste (MSW) landfill is the common method used for the disposal and burying of solid waste. Though in developed countries the MSW landfills are mostly well-designed and managed, it causes some problems to the environment, including the production of CO_2 and methane through the aerobic and anaerobic breakdown of organic waste. Landfills are the second largest anthropogenic source of methane emissions (Kreileman and Bouwman, 1994).

Other problematic by-products are landfill leachates. These could be used in irrigation, especially of the vegetation covering the landfill. This reduces the amount of leachate through evapotranspiration and adds nutrients to the landfill soil. Cultivation of the top cover of the landfill is important for the following reasons: sustainable nutrient supply by the roots; optimisation of water balance and water carrying capacity; protection of the sealing element; prevention of direct contact between harmful substances and environment; avoidance of erosion and reduction of gas emission especially of methane as was cited by Watzinger, 2004. Growing vegetation on the top cover of landfills can improve the air capacity of less permeable soil materials through forming pores and absorbing soil water by spreading roots, therefore, enhancing methane oxidation rate by at least 50% (Bohn and Jager, 2009). Watzinger *et al*. (2005) reported that irrigation with a limited small amount (<150 mm per year) of landfill leachate promoted microbial methane oxidation due to good plant performance, low soil moisture content and hence increased aeration.

However, the major limitations for using leachate for irrigation are often high concentrations of heavy metals and salts. In Austria, the quality of landfill leachate varies strongly and some leachates are rich in heavy metals and salts especially NaCl (Watzinger, 2004). Table 1 shows the quality and composition of one of the landfill leachates in Austria. Revegetation of landfill sites in Austria is helped because the soil solution is diluted by rainfall and leaching of salts from soil is expected due to low evapotranspiration in winter (Watzinger, 2004). *Poulus nigra* and some *Salix* species are used in some landfills areas and are irrigated by landfill leachate as in Rautenweg, the municipal landfill in Vienna, where their tolerance of saline soil water is an important trait in their usefulness in covering landfills.

Table 1. 2: Landfills leachate quality and load of the municipal solid waste landfill site Rautenweg in Vienna (Watzinger, 2004).

Parameter	**Values [mg l^{-1}] ^{1}**	**Loads [g m^{-2}]**
EC ^{2} [mS cm^{-1}]	9.9	
pH	6.85	
TOC ^{3}	71* ^{4}	17
Cl	1800 *	420
Cl	1300	310
Na	1100 *	260
Na	700	160
K	300 *	71
Ca	190 *	45
Mg	210 *	49
P total	0.07 *	0.02
NO_3-N	4.52 *	1.06
NH_4-N	0.13 ^{2}	0.03

1: except EC and pH; 2: Electric conductivity; 3: Total organic carbon; 4: marked values (*) were supplied by the MA 48, Vienna.

1. 6 Species studied

Balanites aegyptiaca and *Populus nigra* were the tree species that have been studied in this work. Although they are grown under different climates, these trees face similar physiological stress through drought and salts.

1. 6. 1 *Populus nigra* L.

Black poplar (*Populus nigra* L. Salicaceae) is a deciduous pioneer tree of riparian ecosystems. It is a medium-size to large size tree reaching up to 30 m. In Europe, black poplar has a wide range of distribution from the Mediterranean borders in the south to latitude 64° in the North and from the British Isles to Western Asia (Zsuffa, 1974). Generally poplar is fast growing and easily propagates through cuttings. Its wood is lightweight, resistant to fire and has excellent shock absorbing qualities. As a result, it has a long history of use for clogs, fruit baskets, furniture, flooring sheep hurdles and wagons (Cottrell *et al.*, 2005). *Populus nigra* and related species used for crossbreeding are among the tree species best-characterized genetically and physiologically. Because of its high growth potential and varieties known to be relatively tolerant to salt and heavy metals, black poplar is one of the trees used for recultivation of waste deposits in Austria.

1. 6. 2 *Balanites aegyptiaca* (L.) Del.

Balanites aegyptiaca (L.) Del. is a small to medium sized semi-deciduous tree reaching up to 15 m in height and 50 cm in diameter (Thirakul, 1984).The seedling-stage of *Balanites* is vulnerable, but once successfully established, it is very resistant to all damages and injuries such as fires and drought (Von Maydell, 1986). *Balanites* is a multi-purpose tree found in most arid to sub-humid tropical savannas of Africa, all over the Sahel and on many sites of Sudanese savannah, extending from the Atlantic coastline of Senegal to the Red Sea and Indian Ocean and the Arabian Peninsula (Von Maydell, 1986). Figure 1 showed the distribution of *Balanites* according to (Hall and Walker, 1991).

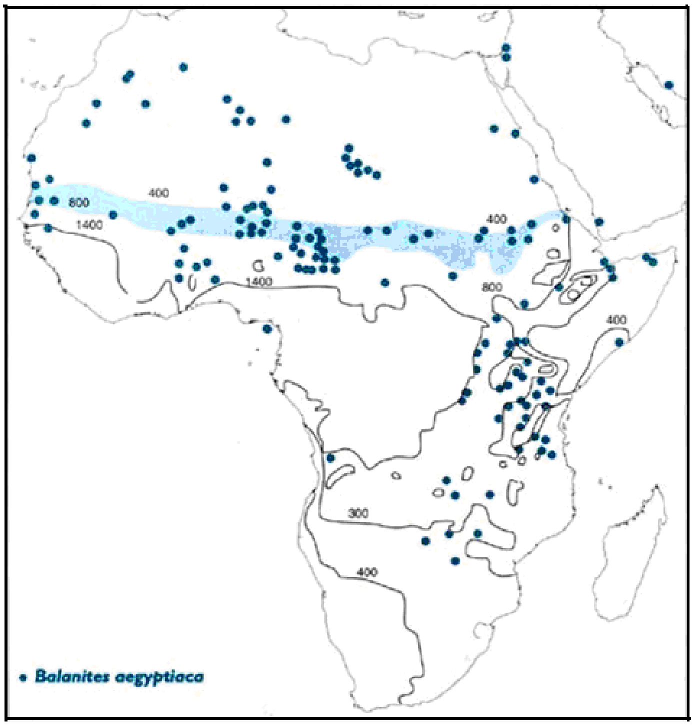

Fig. 1. 1: Distribution of *Balanites aegyptiaca* in relation to mean annual rainfall. The 400,800 and 1400 mm isohyets are shown. The hatched area is the „main range'. (Hall and Walker, 1991, downloaded from: http://www.fao.org/docrep/005/x3940e/X3940E02.htm9

In Sudan it is common on dark clays of central Sudan, often associated with *Acacia seyal* on short grass savannah (Elamin, 1990). It also occurs on hard surfaced sandy clays and slopes at the foot of rocky hills, sporadic on the Kordofan sands, in Wadis along the Red Sea coast up to the northern frontier of the Sudan, on the drier fringes of the ironstone country (Badi *et al*., 1989). *Balanites* has very deep root system that enables the tree to survive in arid areas (Elfeel, 2010, Badi *et al*., 1989, Von Maydell, 1986). In Sudan, the species makes up to one third of the total tree population in the country’s central provinces; Blue Nile Province alone is estimated to have a million trees (NRC, 2008). The fruits are high in edible oil and are used as feed for human consumption. The gummy, yellow-to-red pulp of the fruit contains about 40 percent sugar. It is sometimes eaten raw, but is more commonly converted into drinks, cooked foods, and medicines (NRC, 2008). Many parts of the tree are used in folk medicine in Africa. Leaves of *Balanites* are rich in proteins and are used as feed and for mulching, fruit and bark extracts are used in medicine and as pesticide, and wood for firewood and the production of charcoal (Ndoye *et al*., 2004). Fruits and seeds kernels of *Balanites* contain steroidal saponins primarily with diosgenins and sapogenins that are used in medicine such as treatment of heart diseases and as sex hormones (Hardman and Sofowora, 1972; Mohamed *et al*., 2002). Five new steroidal glycosides were isolated from the roots of *Balanites* (Farid *et al*., 2002). *Balanites* is actively managed and planted in agroforestry and as a boundary marker. Intensive and uncontrolled exploitation of *Balanites* fruits, combined with low rate of natural regeneration has led to the drastic depletion of this species (Ndoye *et al*., 2004). In Sudan, *Balanites* is severely affected by mechanized cropping, overgrazing, and selective felling for furniture (Elfeel, 2004). Cosstaler *et al* (1991) and Badi *et al*. (1989) reported a serious deterioration in the natural regeneration of *Balanites* and its disappearance from some parts of its habitat.

In Sudan, which is the source of *Balanites aegyptiaca* seeds studied, salt-affected soils occur mainly in the desert and semi-desert climatic zones, e.g. the high terrace of the River Nile and its tributaries, and in the arid semi-arid regions, e.g. central clay plain-north Gezira (Elsheikh, 1998).

1. 7 Objectives of the study

With the increase of saline areas in the world and the prevailing of the dry weather especially in arid and semi-arid regions, there is a vital need to study the physiological and ecological performance of different trees that can play a vital role to combat different environmental and human problems. Though *Balanites aegyptiaca* and *Populus nigra* are trees from very different climatic zones, both are subjected to drought and salinity stress in their natural (*Balanites*) or anthropogenic (*Populus*) habitat. The objective of this thesis was therefore to study drought and salinity response and resistance in both species, specifically:

1- To investigate the effect of drought stress on *Balanites aegyptiaca* (L.) seedlings under different nutrient supply, testing different physiological and morphological growth parameters, and to find out if there are differences between provenances.

2- To investigate the effect of salt on seedlings of *Balanites aegyptiaca* (L.), by measuring different morphological and physiological parameters and to find whether provenances perform differently.

3- As salt in the seepage water is an important factor limiting plant growth in landfills, the physiological and morphological performance of *Populus nigra* L. was studied under salt stress and to understand its effect to identify potentially stress-resistant, tolerant or sensitive clones.

2 Material and Methods

Seeds of *Balanites aegyptiaca* were brought from Forest Tree Seed Centre Soba-Sudan (FTSCS), from two provenances: Damazin, which is located in latitude 11° 46’ N and longitude 34° 21’ E, and Um Abdalla, which is located in latitude 11˚40'N, 30˚30'E. The two provenances belong to two different seed zones (5.1 and 6.2 seed zones for Damazin and Um Abdalla, respectively) according to tree seed zones of Sudan by Aelbaek and Kananji (1995).

2. 1 Pre-experiment with *Balanites aegyptiaca*

2.1.1 Experimental design

A pre-experiment was conducted on a smaller scale with younger plants of *Balanites* in the greenhouse of the Institute of Botany at University of BOKU, Vienna, Austria, from October 19 2006 to February 8 2007, to test germination conditions and response to salinity.

Seeds of *Balanites aegyptiaca* were soaked in tap water at room temperature for 24 hours and were sown on trays filled with sand and placed in the greenhouse, where the climate was not completely controlled. After one month, seedlings were transferred to pots of 9 × 9 × 10 cm filled with perlite. Six pots were placed in a tray of 16 × 60 cm filled with 3 litre water with 1 g / l of nutrient fertilizer. Plants were subjected to salinity stress on Dec. 20, with four concentrations of NaCl (0, 75, 150 and 300 mM). To avoid an uncontrolled increase of salt concentrations in the substrate due to evapotranspiration, water was replenished every 2-3 days and the salt solutions were changed every week with newly prepared ones.

2.1. 2 Measurements

Leaves were counted and shoot height was measured before and after the application of salt. Rapid light curves were measured twice in Jan 31and Feb 8 using the portable Mini PAM Photosynthesis Yield Analyzer (Walz, Effeltrich, Germany) in the afternoon using the internal light source with PAR increasing in eight consecutive illumination steps from 0 to 1200 µmol $m^{-2}s^{-1}$. This technique provides a fast assessment of photosynthesis including light response of the quantum yield and electron transport rate (ETR) = yield × PAR × 0.5 × 0.84, the standard factor 0.84 corresponds to the fraction of incident light absorbed by leaf (Rascher *et al*., 2000, Scarano *et al*., 2005). Maintaining each light level for 1 min is generally sufficient for the light reaction to adjust to the new light level, although photosynthetic CO_2 uptake does not reach steady state that fast because stomata react slower.

At the end of the experiment after 7 weeks of salt treatment, plants were harvested, oven dried at 90°C for 3 days, and whole plant, shoot, root and leaves weights were obtained for each plant and the root : shoot ratios were calculated.

2.2 Effect of drought and nutrient interactions on *Balanites aegyptiaca*

2.2.1 Experimental design

This experiment was carried out in the greenhouse of the Institute of Botany at University of BOKU, Vienna, Austria, starting in February 2007, in order to investigate the effect of nutrient supply and drought stress on *Balanites aegyptiaca* seedlings and if fertilization with nitrogen and potassium interacts with drought stress.

Seeds of *Balanites* from Damazin and Um Abdalla were pre-treated by soaking in tap water at room temperature for two days and on February 9 were sawn in sand in trays of 30x60 cm. The condition in the greenhouse was not completely controlled. Germination started after one week and after one month (March 7) seedlings were transferred to pots of 9 × 9 × 10 cm in a mixture of sand and white peat (2:1 v/v). Seedlings were transferred to larger pots of 12 × 12 × 20 cm when plants were two months old (April 4). Plants were irrigated every other day with tap water to field capacity. After April 4, a fertilizer nutrient solution including micro- and macronutrients was added once a week (Table 2. 2. 1), 200 ml in the first week and 100 ml afterwards.

Table 2. 2. 1: Composition of the nutrients stock solution (fertilizer).

Nutrient	**mg/l**
$MgSO_4$ x 7 H_2O	175
NH_4NO_3	8
$CaSO_4$	120
$NH_4H_2PO_4$	57
Minors:	
H_3BO_3	2.5
$MnCl_2$ x 4 H_2O	1.7
Zn-EDTA	0.45
Cu-EDTA	0.75
Na_2MoO_4 x H_2O	0.025
Fe-EDTA	40

The stock solution contained little N (65 mg/l) and no K (Table 2. 2. 1). Two nitrogen solutions were prepared, low nitrogen solution was found in the fertilizer solution ($NH_4H_2PO_4$ and NH_4NO_3), in total concentrations of 65 mg/l and the high nitrogen solution was prepared from NH_4NO_3 in concentrations of 2580 mg /l (N1 and N2 respectively) and three potassium solutions were prepared from K_2SO_4 in concentrations of 0, 220 and 2200 mg K/l (K0, K1 and K2 respectively). The nutrient treatment thus consisted of three K and two N treatments and each N-K combination was applied to eight plants from each provenance. 20 ml of each nutrient (N and K) solution was added in the first week and 10 ml for the following weeks for a total of 10 weeks. On June 20 the plants from each provenance were divided into two groups, the control group was irrigated with tap water and the other group was subjected to water stress. At first, pots were irrigated to field capacity and each full pot was weighed. Later, pots were irrigated to a target weight, starting with minus 100 g of saturation weight, after 10 days minus 200g, after 18 days minus 300, and reaching minus 400g after 26 days (Table 2. 2. 2). Plants were kept under this final regime for three weeks after which watering was completely stopped for three weeks for the stressed plants while it continued for the control. Apart from the complete stop of irrigation, all plants were watered twice a week. The experiment ended on Aug 26.

2. 2. 2 Measurements

The number of leaves and branches and stem height were measured in April, May and August. At the end on August 26 plants were harvested, washed carefully and each plant was separated into roots and shoot, which were oven-dried at 90 C° for two days. Root dry weight, shoot dry weight, whole plant dry weight and root:shoot ratio were measured.

Plant water potential was measured with a Scholander pressure chamber on branches with 7 to 10 leaves on August 1 and 25 between 10:00 and 16:00 h. Chlorophyll fluorescence was measured on two mature leaves for each plant using the Mini PAM Photosynthesis Yield Analyzer (Walz, Effeltrich, Germany). The maximum efficiency of photosystem II under ambient light (F_v'/F_m') with PAR ranging between 30 – 300 µmol m^{-2} s^{-1} in May and 70 – 760 µmol m^{-2} s^{-1} in August, was measured between 9:00 and 12:00 h. The maximum efficiency of photosystem II in dark adapted leaves (F_v/F_m) was measured in June and July. Stomata conductance (g_s) in mol $m^{-}2$ s^{-1} was measured on two mature leaves per plant in June, July and August using the Licor-1600 Steady State Porometer (LI-COR, Lincoln, Nebraska, USA) between 10:00 and 14:00 h.

Table 2. 2. 2: Different treatments of the experiment by date.

Treatment	Date
Sowing of seeds	09/02/2007
Seedlings transferred to small pots	07/03/2007
Seedlings transferred to large pots	04/04/2007
Start application of nutrient stock solution	28/03/2007
Start application of K& N solution	04/04/2007
End of application of K& N solution	19/06/2007
Start water stress	20/06/2007
-100	20/06/2007
-200	30/06/2007
-300	08/07/2007
-400	16/07/2007
Watering stopped completely from stressed plants	04/08/2007
Harvest of plants and end of the experiment	26/08/2007

2.3 Effect of salt in *Balanites aegyptiaca*

2.3.1 Experimental design

This experiment was started in the greenhouse of the Institute of Botany at BOKU in January 2008. Seedlings of *Balanites aegyptiaca* from two provenances (Damazin and Um Abdalla) were germinated as described above (chapter #2) and after one year cuttings were produced from the seedlings and grown in small pots of 9 cm in diameter in a mixture of Foragrad TKS 2 instant plus soil, sand and perlite (2:1:1). In the beginning of May plants were transferred to the greenhouse at the Austrian Institute of Technology (AIT) at Seibersdorf, where the experiment was carried out. The condition in the greenhouse was not completely controlled, during the salt treatment day temperature ranged between 22-37 °C and relative humidity between 23-42 %. In the mid of May plants were transferred to pots of 1.5 L with the same medium and were watered every other day with tap water.

On August 21 the salt experiment was started and plants were treated with three saline solutions of 0 (control), 4 (low) and 8 (high) mS cm^{-1} electrical conductivity (EC). De-ionized water was used to irrigate the control plants. The saline solution was prepared from a mixture of NaCl, $CaCl_2$ and $MgSO_4$ (2:1:1). The dominant cations in saline water are Na^+, Ca^{2+} and Mg^{2+} and in water of low to moderate salinity the ratio of $Na^+/(Na^+ + Ca^{2+})$ ranges between 0.1 and 0.7 (Grattan, 1999). Treatments were two provenances, three saline solutions and eight replicates. Plants were watered extensively every second day and excess water in the trays under the pots was removed and the trays were rinsed each time before irrigation with the saline solution to avoid that salts accumulate in the substrate. On Oct. 23 the salt concentration was doubled, 4 mS/cm was increased to 8 mS/cm and 8 mS/cm to 16 mS/cm until the end of the experiment. In July nutrients (3.5 g of Blue Corn per plant) were added and from the beginning of September to the end of the experiment Hoagland solution (full strength) was used once a week.

2.3.2 Measurements

Stem height and the number of leaves were measured twice (in September and November). One leaf from each plant was collected and leaf fresh weight (FW, g) was obtained. Leaf area (LA, cm^2) was measured with a photoelectric leaf area meter (LI-COR 3000, Lambda Instruments Corporation; Lincoln, Nebraska, USA). Dry weight (DW, g) was obtained after oven-drying at 80°C for 2 days. Specific leaf area SLA (LA/ LDW in cm^2/ g) and leaf water/ area ([FW-DW]/LA in g/m^2) were calculated.

Chlorophyll content was measured using the SPAD Chlorophyll meter 502 (Minolta, Japan). One leaf between the 7th-12th leaf from the branch tip was measured in each plant.

Chlorophyll fluorescence was measured using the Handy PEA (Hansatech Instruments King's Lynn, Norfolk England). One young (the 4th or 5th leaf from the tip) and one old (fully expanded mature leaf between the 7th-12th leaf from the branch tip) leaf from each plant were measured. Before each measurement leaves were dark adapted for 15 min with a leaf clip. The light intensity was set to 3000 µmol m^{-2} s^{-1}. Measured parameters were the initial fluorescence that occur at time 0 (F_0), the maximum quantum efficiency of photosystem II (F_v/F_m), the time at which the maximum fluorescence value (F_m) was reached (Tf_m) and performance index (PI) is essentially an indicator of sample vitality, it combines the three main functional steps (light energy absorption, excitation energy trapping, and conversion of excitation energy to electron transport) of photosynthetic activity by PSII reaction centre complex into a single multiparametric expression that expressed as internal forces (Van Heerden *et al*., 2004).

Photosynthesis (A_{sat}), stomata conductance (g_s) and intercellular CO_2 (C_i) were measured using the LI-COR 6400 (LI-COR, Lincoln, Nebraska, USA) portable photosynthesis system. The gas flow rate was set to 500 µmol s^{-1}; CO_2 mixture was adjusted to 380 µmol mol^{-1} and radiation to 1500 µmol m^{-2} s^{-1} using an artificial light source (6400-02B LED). In each plant one leaf between the 7th-12th leaf from the branch tip was marked and each time the same leaf was measured.

2. 4 Effect of salt in *Populus nigra*

2.4.1 Experimental design

In this experiment plant materials were collected from young trees of black poplar, grown at the Austrian institute of technology (AIT) at Seibersdorf. These young trees were produced from reference trees from two locations in Austria, Lobau (LO) in Vienna and Efferding (EF) in Upper Austria.

The experiment was started in the greenhouse at the Austrian Institute of Technology (AIT) in Seibersdorf. Branches of three clones, EF50, LO50 and LO14 of *Populus nigra* were cut in February 2009 and stored at 4 - 6 C° for two months. At the end of April, cuttings of 20 cm were made, treated with Seradix red auxin powder, which is plant hormone that stimulates roots initiation in cuttings and were raised in a hydroponic culture. Each cutting was put in a plastic pot of 0.5 L filled with perlite and pots were placed in trays of 20 L filled with 3.5 L of water. Flory 3 (trademark nutrient) was added to the water in concentration of 2 g/L. Cuttings were kept in a climate chamber at a temperature of (20/18 C°) day/night, 70 % relative humidity and artificial light with fluorescence lamp for 14 hours day length. Water was changed weekly. On June 26 plants were transferred to a cabin in the greenhouse under natural light, temperature of 24/22 C° (day/night). Relative humidity was not controlled. On July 20 plants were transferred to the greenhouse at BOKU University where conditions in the greenhouse were not fully controlled, temperature ranged between 22-36°C with natural light.

On July 28 six plants each of the three clones (EF50, LO50 and LO50) were exposed to three saline solutions except clone LO50 which was replicated five times because there were not enough plants from this clone. Six trays were used; two for each salt concentration and three pots from each clone were placed in each tray. Three concentrations of salt solution with an electrical conductivity (EC) of 0 (control), 4 (low salt) and 8 mS cm^{-1} (high salt) were prepared from a mixture of NaCl, $CaCl_2$ and $MgSO_4$, (5 + 1 + 1, is a composition similar to the leachate of the landfills (Table 1.1). Half-strength nutrient solution was added to the salt solutions and solutions were changed twice a week.

2.4. 2 Measurements

Stem height was measured for each plant on July 30 and August 31. At the end of the experiment, all plants were harvested, carefully washed and all the remains of perlite were removed. Shoot and roots were separated, oven dried at 80°C for 2 days and shoot weight, root weight, root:shoot ratio and total plant weight were obtained. Leaves were not measured as many leaves in the salt treatments had been lost by the end of the experiment

Physiological measurements were carried out between 9:30 and 13:00. The 5th leaf from the tip of each plant was marked and each time the same leaf was measured.

Chlorophyll content was measured using SPAD Chlorophyll meter 502 (Minolta, place of company), results are relative units of SPAD values.

Chlorophyll fluorescence was measured using the Handy PEA (Hansatech Instruments, King's Lynn, Norfolk, England). Before each measurement leaves were dark adapted for 15 min using the leaf clip. The light intensity was set to 3000 µmol m^{-2} s^{-1}. Parameters taken for the comparison were, F_0 which is the initial fluorescence that occur at time base 0, F_v/F_m which is the maximum quantum efficiency of photosystem II, Tf_m which indicates the time at which the maximum fluorescence value (F_m) was reached and PI (performance index) which combines the three main functional steps (light energy absorption, excitation energy trapping, and conversion of excitation energy to electron transport) of photosynthetic activity by PSII reaction centre complex into a single multiparametric expression that expressed as internal forces (Van Heerden *et al.*, 2004).

Rate of net photosynthesis under saturating light (A_{sat}), stomata conductance (g_s) and intercellular CO_2 concentration (C_i) were measured with the LI-COR 6400 (LI-COR, Lincoln, Nebraska, USA) portable photosynthesis system. Flow rate was set to 500 µmol s^{-1}; CO_2 mixture to 400 µmol mol^{-1} and radiation to 1500 µmol m^{-2} s^{-1} using an artificial light source (6400-02B LED).

2.5 Statistical analysis

Statistical analysis was done using the SAS programme and analysis of variance (ANOVA) was used to determine the effect of treatments. For experiments with several factors, ANOVAs were limited to two-way interactions of factors. Difference considered significant at $P<0.05$. The Duncan post-hoc test was used to test differences pairs of treatments groups.

3 Results

3.1 Pre-experiment of *Balanites*

Germination in sand started after one week, with 25 % of seeds germinating within one month. Salt had no significant effects ($P > 0.05$) on plants shoot height and number of leaves also shoot and leaf dry weight and root/ shoot ratio (Table 3. 1. 1), but significantly ($P = 0.023$) affected root dry weight which was higher in control and 75 mM plants (Fig. 3. 1. 1). Electron transport rate (ETR) increases with light (Fig. 3. 1. 2). In January, ETR was saturated at 600 - 650 µmol $m^{-2}s^{-1}$ in control plants and at 400- 550 µmol $m^{-2}s^{-1}$ in salt treated plants. The maximum ETR strongly declined ($P = 0.0001$) with salt concentration and reached 84, 55, 33 and 18 µmol $m^{-2}s^{-1}$ for control, 75, 150 and 300 mM respectively. In February ETR was saturated at 600- 650 µmol $m^{-2}s^{-1}$ for the control and between 200-400 µmol $m^{-2}s^{-1}$ for the three salt treatments. ETR decreased still more strongly ($P < 0.0001$) with increasing salt concentration (Fig. 3. 1. 2). The maximum quantum yield of the photosystem II (F_v/F_m) decreased with the increased salt concentration ($P = 0.0071$ and $P < 0.0001$ in January and February respectively) and also decreased with the increase of light ($P < 0.0001$) in January and February (Fig. 2. 1. 3).

Plants exposed to the highest salt concentration (300 mM) started to die towards the end of the experiment.

Table 3. 1. 1: Effect of salt stress on the dry weight (g) of shoot, root and leaf and root: shoot ratio of *Balanites aegyptiaca* seedlings, N = 6.

	Salt treatment	**Mean ±SD**
Shoot	0	0.759 ± 0.227
P = 0.622	75	0.645 ± 0.069
	150	0.623 ± 0.227
	300	0.636 ± 0.223
Root	0	0.312 0.134±
P = 0.023	75	0.318 ± 0.173
	150	0.134 ± 0.038
	300	0.170 ± 0.074
Total weight	0	1.070 ± 0.288
P = 0.151	75	0.963 ± 0.162
	150	0.756 ± 0.244
	300	0.806 ± 0.292
Root:shoot	0	0.422 ± 0.173
P = 0.065	75	0.506 ± 0.295
	150	0.241 ± 0.135
	300	0.265 ± 0.043

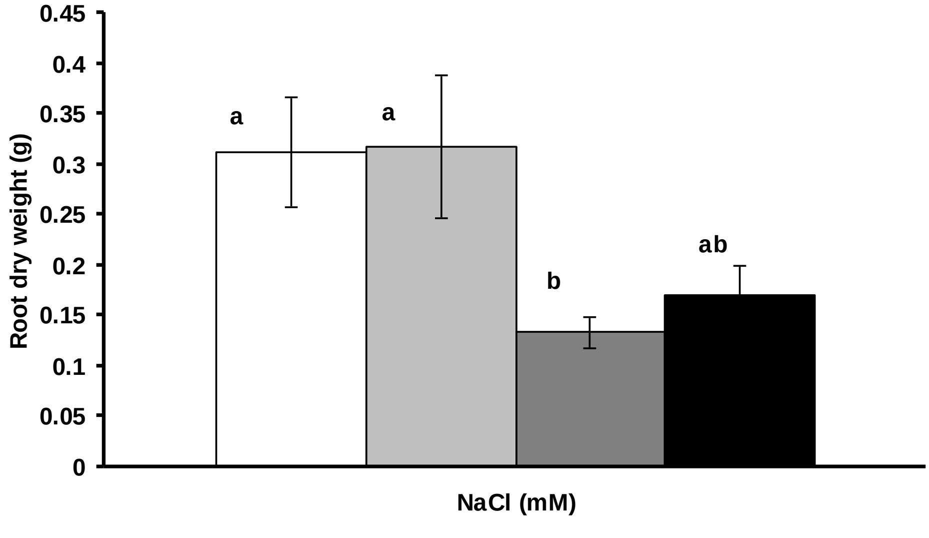

Fig. 3. 1. 1: Effect of NaCl treatment; 0 (white), 75 (light gray), 150 (dark grey) and 300 (black) mM on root dry weight (g) of *Balanites aegyptiaca* seedlings. Error bars indicate SE.

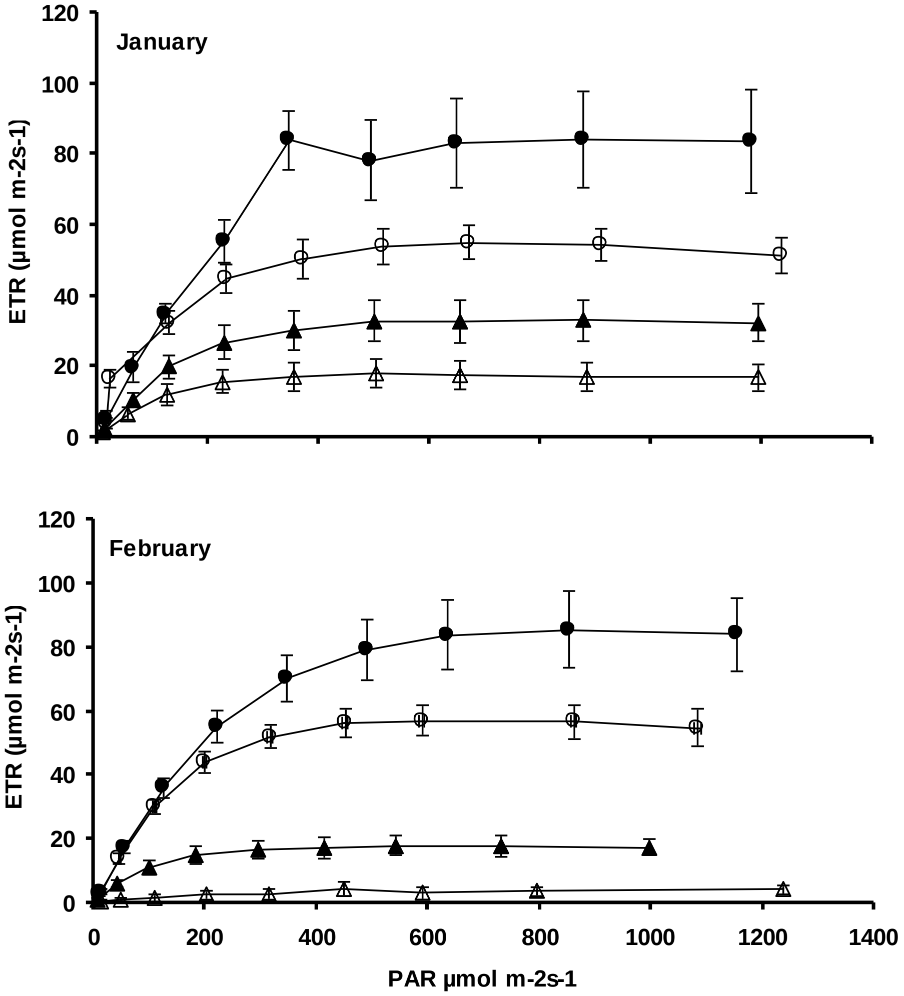

Fig. 3. 1. 2: Electron transport rate (ETR) measured with rapid light curves of leaves of *Balanites aegyptiaca* seedlings treated with four NaCl concentrations (control (●), 75 mM (O), 150 mM (▲) and 300 Mm (Δ)) on Jan 31and Feb 8.

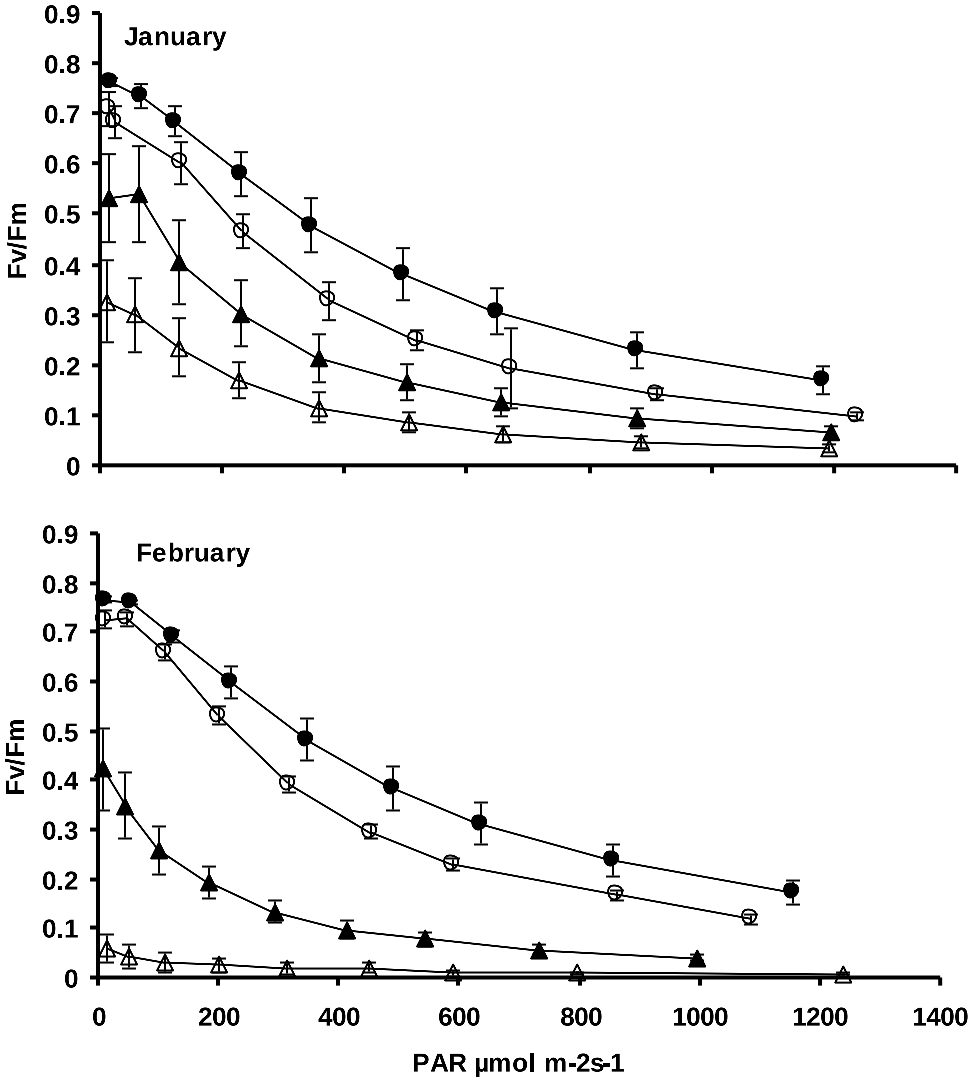

Fig. 3. 1. 3: Quantum yield of fluorescence (F_v/F_m) under variable PAR flux (0 -1200 µmol $m^{-2}s^{-1}$) of seedlings of *Balanites aegyptiaca* treated with four NaCl concentrations (control (●), 75 mM (O), 150 mM (▲) and 300 Mm (Δ)) in Jan 31 and Feb 8.

3. 2 Effect of nutrient and drought interaction on *Balanites aegyptiaca*

3.2.1 Biometry

With time plants showed visible symptoms of drought stress (Fig. 3. 2. 1) and physiological effects that appeared in the different physiological parameters measured as shown below. This experiment evaluated several potentially interacting factors with a potentially high number of interactions. Since complex interactions are often difficult to interpret, the ANOVA was limited to two-way interactions.

The number of leaves differed significantly between provenances (Table 3. 2. 1), where plants from Damazin had the higher number of leaves (Fig. 3. 2. 2). At the beginning of the nutrient treatment, the high nitrogen (2580 mg/l) plants had a significantly lower number of leaves (P = 0.050), but with time nitrogen had no effect on the number of leaves. Addition of potassium had no effect on the number of leaves. At the end of the experiment, drought stressed plants had significantly (P = 0.0006) fewer leaves (Fig. 3. 2. 3), which was mostly the result of leaf shedding rather than growing fewer new leaves. While shedding old leaves, drought-stressed plants also developed smaller curled new leaves than control plants. Damazin plants had significantly more branches (P = <.0001, P = 0.0124 and P = 0.002 in April, May and August, respectively) compared to plants from Um Abdalla (Fig. 3. 2. 4), but drought had no significant effect on the number of branches. At the end of the drought treatment, plants previously treated with high nitrogen had more branches than low nitrogen plants (P = 0.0331).

Fig. 3. 2. 1: Effect of drought stress on *Balanites agyptiaca* seedlings from two provenances (top Damazin and bottom Um Abdalla).

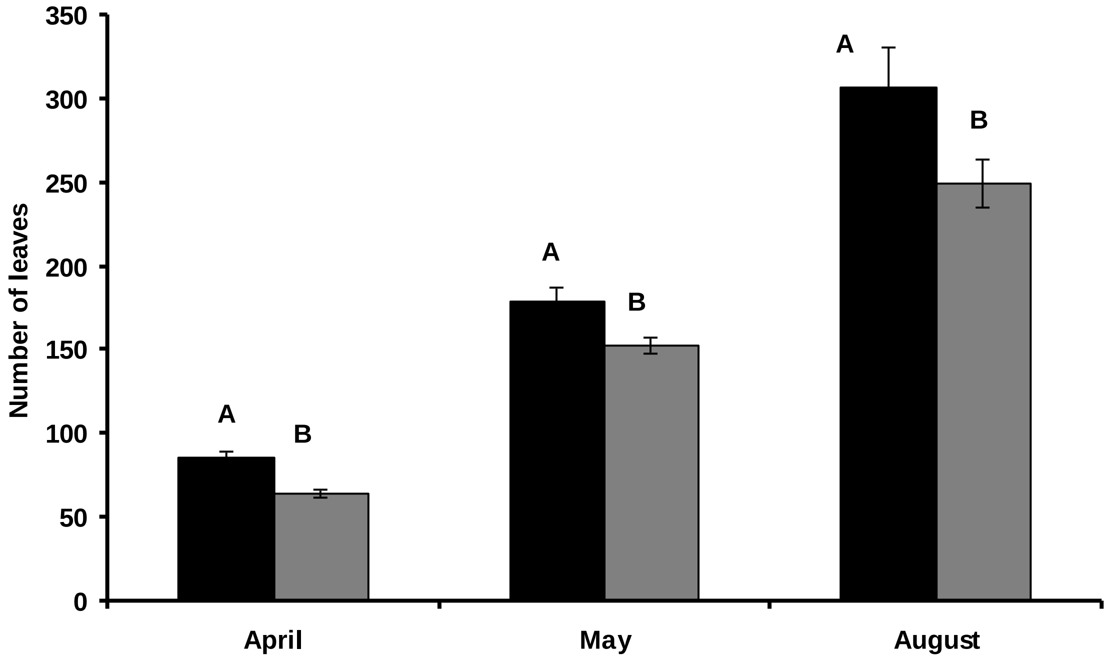

Fig. 3. 2. 2: Development of the number of leaves of *Balanites. aegyptiaca* seedlings from two provenances (black for Damazin and grey for Um Abdalla). Plants were subjected to different nitrogen and potassium supply for 66 days until June19 and then subjected to increasing drought stress until August 26. Error bars indicate SE. Letters indicate the Duncan's Multiple Range Test; means with the same letters are not significantly different.

Table 3. 2. 1: Significant effects (P < 0.05) of provenance, nitrogen, potassium and drought on number of leaves (n.s not significant, n.a. not applicable, before drought stress).

Number of leaves	April	May	August
Provenance	< 0.0001	0.007	0.023
N	0.0504	n.s	n.s
K	n.s	n.s	n.s
Drought	n.a	n.a	< 0.0001
N x K	0.06	n.s	n.s
Drought x N	n.a	n.a	0.029

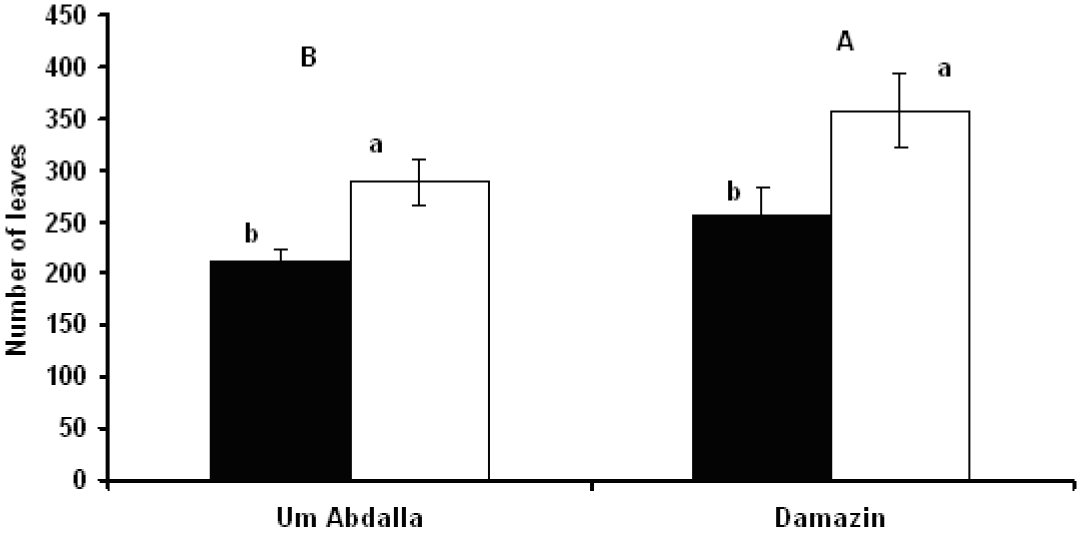

Fig. 3. 2. 3: Effect of drought stress on the number of leaves of *Balanites agyptiaca* seedlings from two provenances (Damazin and Um Abdalla), black for drought stress and white for control. Error bars indicated SE. Letters indicate the Duncan's Multiple Range Test; means with the same letters (capital letters for provenances and lower case letters for drought stress) are not significantly different.

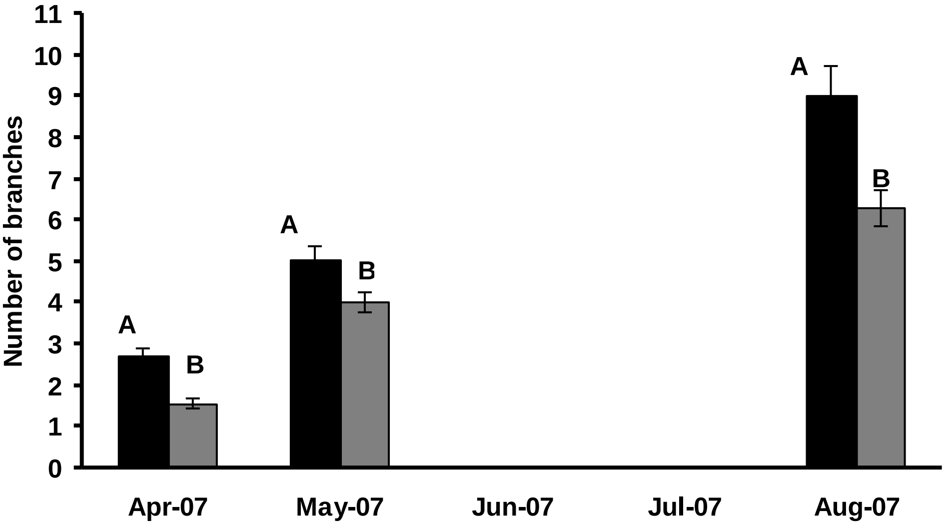

Fig. 3. 2. 4: Development of the number of branches of *Balanites aegyptiaca* seedlings from two provenances (black for Damazin and grey for Um Abdalla).Plants were subjected to different nitrogen and potassium supply for 66 days until June19 and then subjected to increasing drought stress until August 26. Error bars indicate SE. Letters indicate the Duncan's Multiple Range Test; means with the same letters are not significantly different.

Drought stress significantly ($P < 0.0001$) reduced plant total dry weight (Fig. 3. 2. 6, Table 3. 2. 2). High nitrogen significantly increased plant total dry weight ($P = 0.011$) and root dry weight ($P = 0.036$, Fig. 3. 2. 5). Low or high potassium had no effect on plant dry matter.

Drought significantly (P = 0.0001) decreased root dry weight (Fig. 3. 2. 6, Table 3. 2. 3). Um Abdalla plants had significantly higher root dry weight (P = 0.0076; Fig. 3. 2. 6). While under drought stress plants form Um Abdalla showed a reduction of 34% in root dry weight and 38% in total dry weight compared to control, reduction root dry weight and total dry weight in plants from Damazin plants was only 11% and 21%, respectively. Drought reduced shoot dry weight (P <.0001, Fig. 3. 2. 6). Drought significantly increased root: shoot ratio (P = 0.021), provenances significantly differed in root: shoot ratio (P = 0.0013), with Um Abdalla plants showed the higher root :shoot ratio (Fig. 3. 2. 6, Table 3. 2. 4).

Table 3. 2. 2: Significant effects (P < 0.05) of provenance, nitrogen, potassium and drought on plant total dry weight.

	DF	Anova SS	Mean square	F value	Pr > F
Provenance	1	15.36	15.36	1.29	0.260
N	1	81.44	81.44	6.82	0.011
K	2	39.13	19.56	1.64	0.201
Drought	1	612.36	612.36	51.32	< 0.0001
Provenance*N	1	9.44	9.44	0.79	0.377
Provenance*K	2	6.65	3.32	0.28	0.758
Provenance*Drought	1	79.75	79.75	6.68	0.012

Table 3. 2. 3: Significant effects (P < 0.05) of provenance, nitrogen, potassium and drought on root dry weight.

	DF	Anova SS	Mean square	F value	Pr > F
Provenance	1	48.96	48.96	7.54	0.0076
N	1	29.48	29.48	4.54	0.0365
K	2	18.81	9.41	1.45	0.2416
Drought	1	104.83	104.83	16.15	0.0001
Provenance*N	1	1.09	1.09	0.17	0.6829
Provenance*K	2	8.48	4.24	0.65	0.5237
Provenance*Drought	1	40.20	40.20	6.19	0.0152

Table 3. 2. 4: Significant effects (P < 0.05) of provenance, nitrogen, potassium and drought on root:shoot.

	DF	Anova SS	Mean square	F value	Pr > F
Provenance	1	2.38	2.38	11.49	0.001
N	1	0.02	0.02	0.09	0.766
K	2	0.55	0.28	1.34	0.269
Drought	1	1.15	1.15	5.54	0.021
Provenance*N	1	0.17	0.17	0.82	0.368
Provenance*K	2	0.75	0.37	1.81	0.172
Provenance*Drought	1	0.00	0.00	0.01	0.926

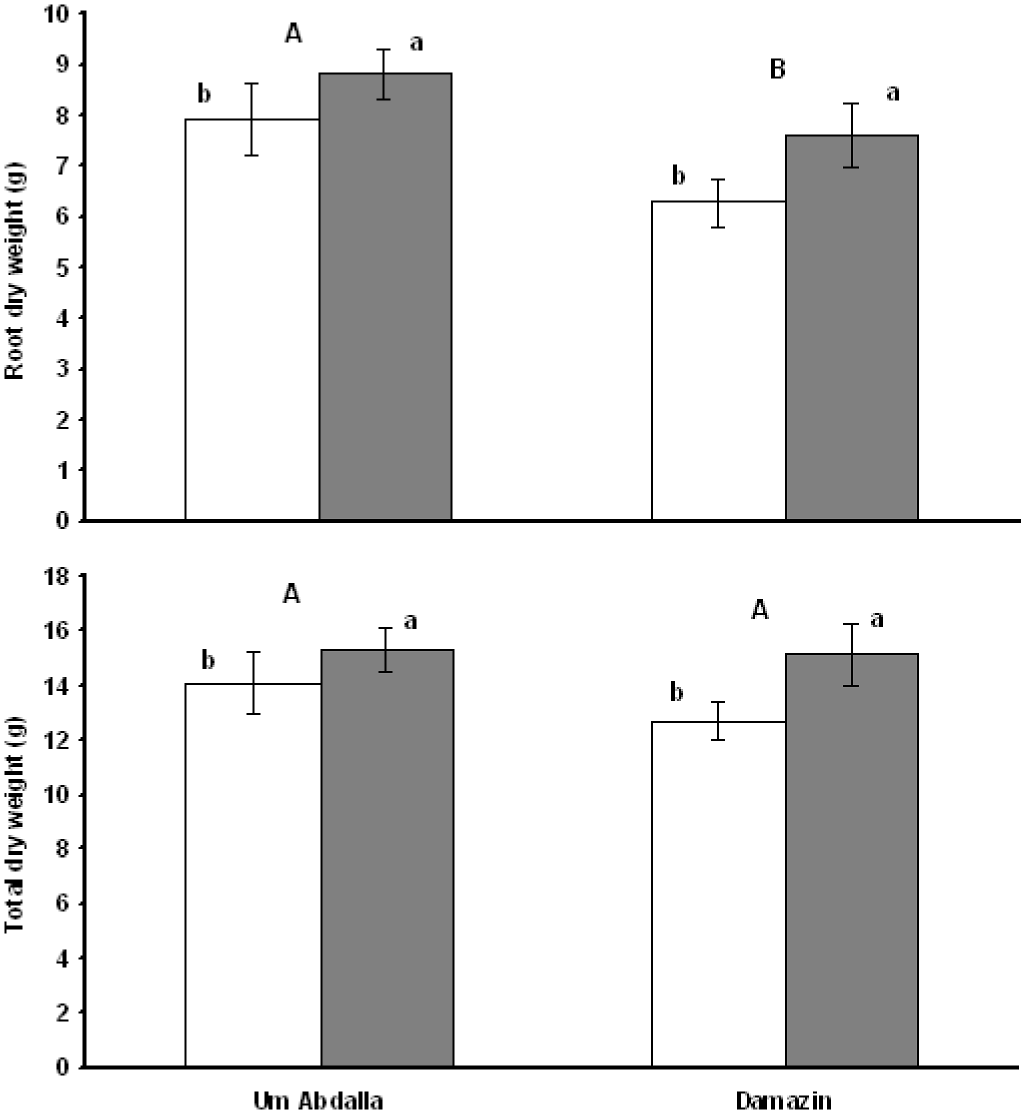

Fig. 3. 2. 5: Effect of nitrogen (white for low N concentration and gray for high N concentration) on root and total dry weight of seedlings of two provenances of *Balanites agyptiaca* (Damazin and Um Abdalla), error bars indicated SE. Letters indicate the Duncan's Multiple Range Test, means with the same letters are not significantly different; capital letters for provenances and lower case letters for nitrogen treatments.

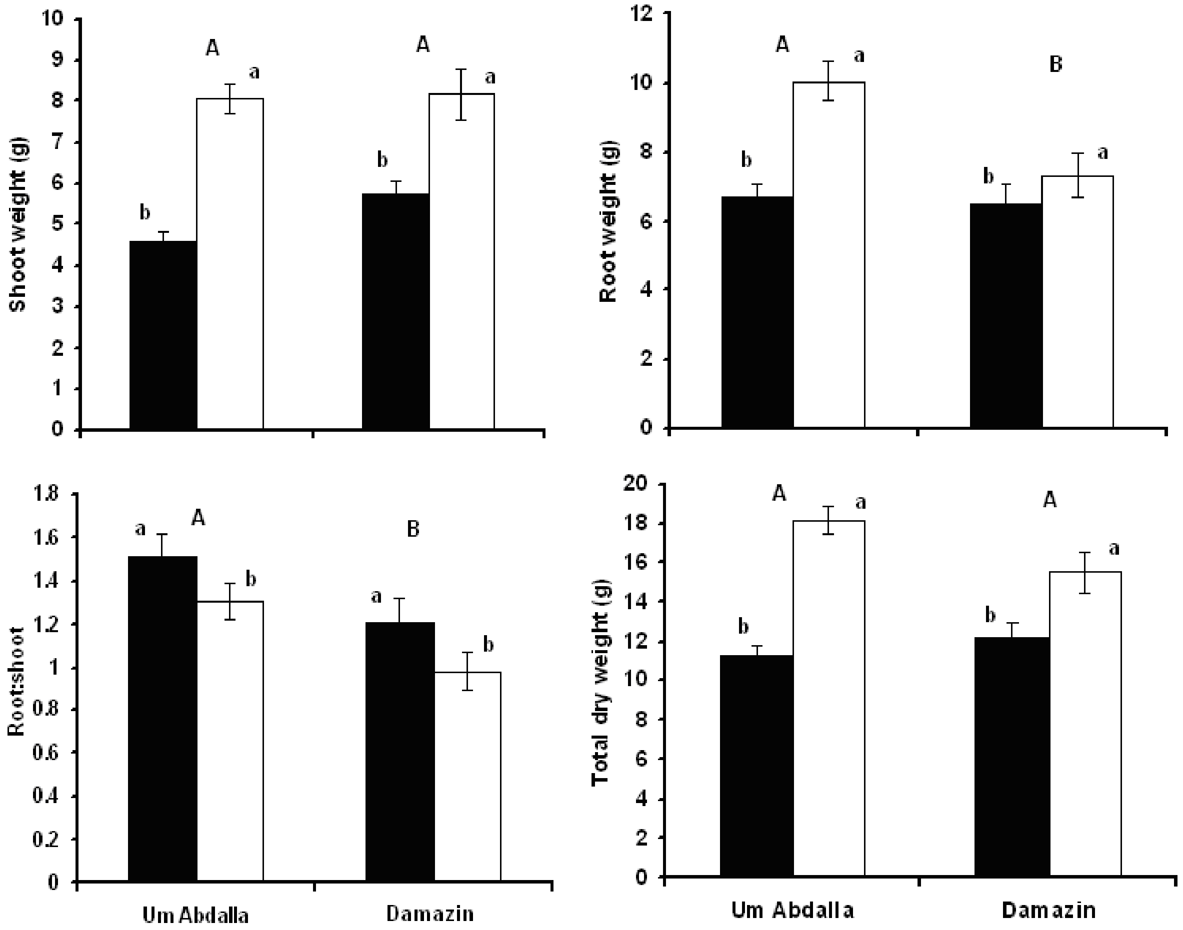

Fig. 3. 2. 6: Effect of drought stress on shoot, root and total dry weight and root:shoot ratio of seedlings of two provenances of *Balanites aegyptiaca* (Damazin and Um Abdalla), black for drought stress and white for control. Error bars indicated SE. Letters indicate the Duncan's Multiple Range Test, means with the same letters are not significantly different; capital letters for provenances and lower case letters for drought stress.

3. 2. 2 Plant physiology

3. 2. 2. 1 Water potential

Plant water potential (Ψ, in MPa), measured on August 1 (between 12:00 and 16:00) at a soil water deficit of -400 g/pot showed significant difference (P < 0.0001) between drought stressed and control plants where Ψ ranged between -1.33 MPa and –2.47 MPa for control plants and drought stress plants, respectively, while provenances were not significantly different. At the end of the experiment, after water was withheld for three weeks, plants showed significant difference (P < 0.0001) in water potential that ranged between -1.66 MPa and -4.23 MPa for control and drought stress plants, respectively. Also, provenances differed significantly with Um Abdalla plants under drought stress having more negative water potential (-4.71 MPa) than plants from Damazin (-3.74 MPa). Water potential of plants under drought stress treatment became more negative with the increased duration of the drought stress, while control plants did not change significantly (Fig. 3. 2. 7).

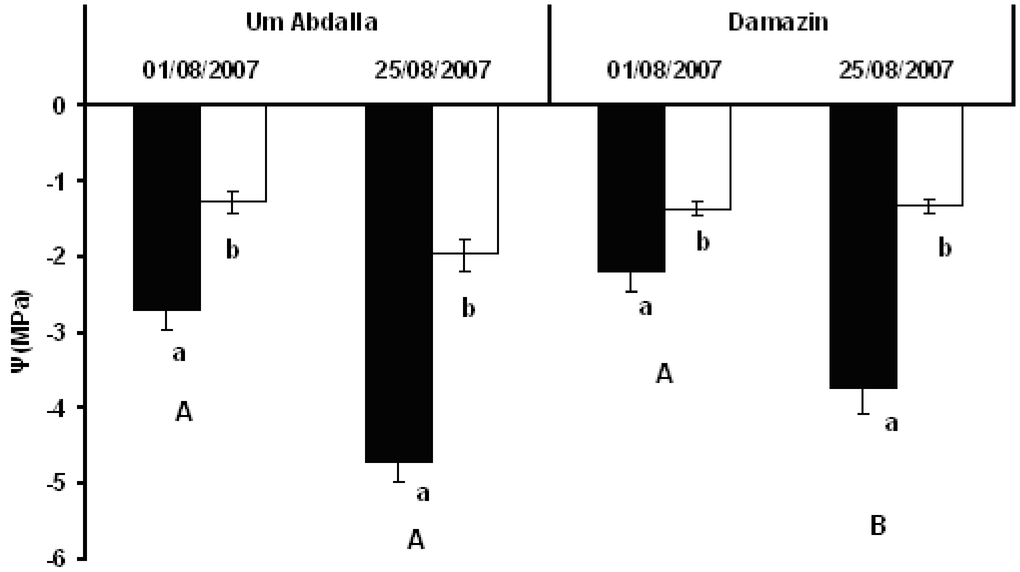

Fig. 3. 2. 7: Effect of drought stress (black for drought stress and white for control) on plant water potential of two provenances of *Balanites aegyptiaca* seedlings Damazin and Um Abdalla. Error bars indicate SE. Letters indicate the Duncan's Multiple Range Test, means with the same letters are not significantly different; capital letters for provenances and lower case letters for drought stress.

3. 2. 2. 2 Chlorophyll fluorescence

On May 15, the yield of chlorophyll fluorescence (F_v'/F_m') under ambient light ranging between 35 – 300 µmol $m^{-2}s^{-1}$ was significantly higher ($P = 0.017$) in plants from Damazin than from Um Abdalla (Table 3. 2. 5). Fertilization with nitrogen significantly increased photosynthetic yield under low light on May 15 ($P = 0.015$). The interaction between provenances and nitrogen treatment in May showed that low nitrogen significantly reduced the yield of fluorescence more in Um Abdalla plants although in August 22 low nitrogen plants of Um Abdalla had the highest yield. Drought was highly significantly ($P < .0001$) reduced yield in August 22 at PAR ranging between 85 – 759 µmol $m^{-2}s^{-1}$ (Table 3. 6, Fig. 3. 2. 10). In August 22 plants showed different response to K where the K0 treatment showed higher yield of fluorescence in drought stressed plants while increased K concentration increased yield of fluorescence in control plants (Table 3. 2. 7). In August 22 Damazin plants at high nitrogen had the higher F_v'/F_m' while Um Abdalla plants at high nitrogen had the lower F_v'/F_m'.

Fertilization with nitrogen significantly increased photosynthetic yield in dark-adapted leaves (F_v/F_m) measured on June 6 ($P < 0.0001$) (Fig. 3. 2. 8). Drought significantly reduced yield of fluorescence ($P = 0.006$) in July 21 and ($P < 0.0001$) in July 27 (Table 3. 2. 5, Fig. 3. 2. 9). In July 27 Um Abdalla plants had higher yield compared to Damazin. K did not affect the yield of fluorescence on any measured date, but there was a significant potassium x drought interaction in July 27 where the yield of fluorescence increased with the increase of K concentration in drought stressed plants while it decreased with the increased K concentration in control plants (Table 3. 2. 6).

Table 3. 2. 5: Significant effects ($P < 0.05$) of provenance, nitrogen, potassium and drought on chlorophyll fluorescence yield (F_v/F_m) (n.s not significant, n.a. not applicable, before drought stress). (* indicates F_v'/F_m' measured under ambient light ranging between 30 – 300 µmol m^{-2} s^{-1} in May and 70 – 760 µmol m^{-2} s^{-1} in August.

	15-May*	**06-Jun**	**21-Jul**	**27-Jul**	**22-Aug***
Provenance	0.017	n.s	n.s	0.002	n.s
Nitrogen	0.015	< 0.0001	n.s	n.s	n.s
Potassium	n.s	n.s	n.s	n.s	n.s
Drought	n.a	n.a	0.006	< 0.0001	< 0.0001
Provenance*Nitrogen	n.s	n.s	n.s	n.s	0.0430
Provenance*Drought	n.a	n.a	n.s	n.s	0.0345
Drought*Potassium	n.a	n.a	n.s	0.0170	0.0014

Table 3. 2. 6: F_v/F_m interaction between drought and potassium in July 27 “N = 32“

	Stressed	**Control**
Potassium	Mean ±SD	Mean ±SD
0	0.721 ± 0.069	0.757 ± 0.038
220 mg/l	0.717 ± 0.078	0.758 ± 0.036
2200 mg/l	0.679 ± 0.146	0.778 ± 0.024

Table 3. 2. 7: F_v'/F_m' interaction between drought and potassium in August 22 " N = 32 except for the high N treatment where N = 31 because one plant was lost."

	Stressed	Control
Potassium	Mean ± SD	Mean ± SD
0	0.375 ± 0.177	0.389 ± 0.144
220 mg/l	0.261 ± 0.155	0.437 ± 0.158
2200 mg/l	0.277 ± 0.138	0.453 ± 0.122

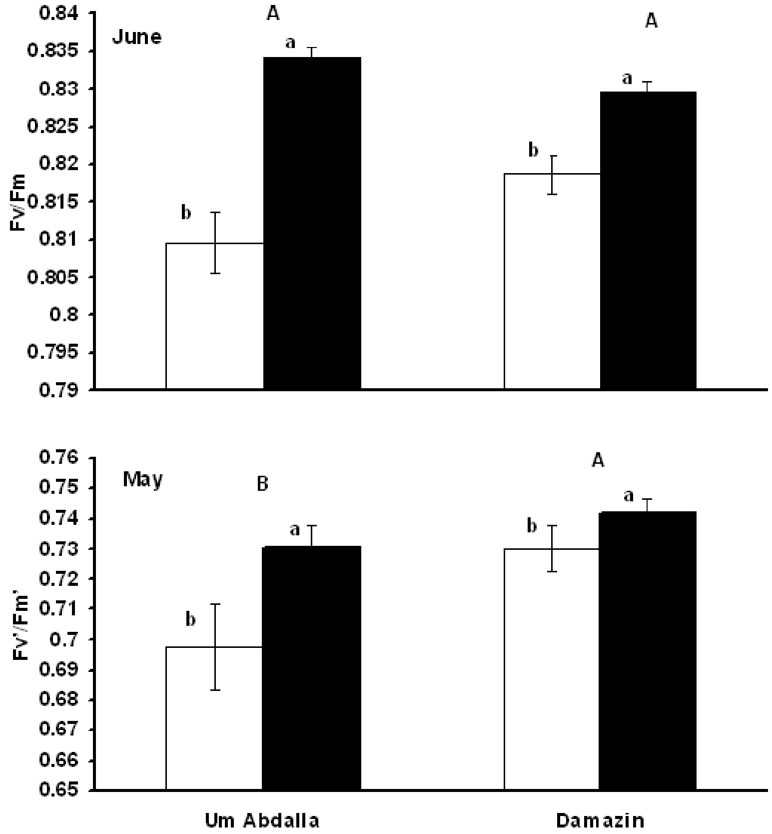

Fig. 3. 2. 8: Effect of nitrogen (low N concentration white; high N concentration black) on F_v'/F_m' on seedlings of two provenances of *Balanites aegyptiaca* measured in dark-adapted leaves (June, top) or under ambient light (May, bottom) before drought treatments started. Error bars represented SE (n = 24). Letters indicate the Duncan's Multiple Range Test, means with the same letters are not significantly different; capital letters for provenances and lower case letters for nitrogen treatments.

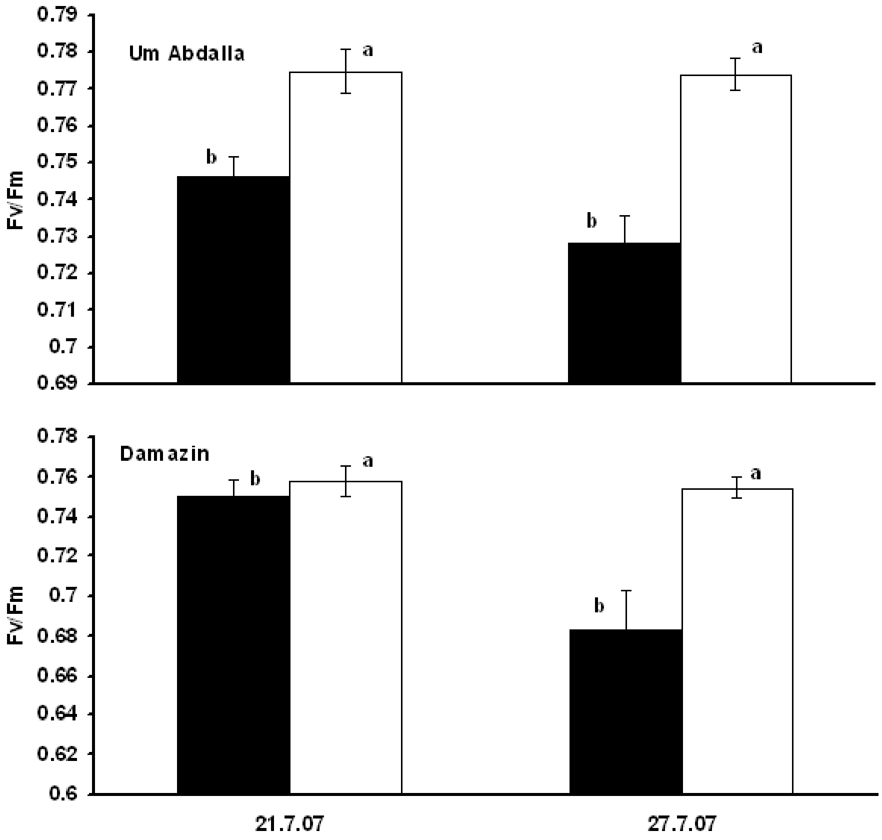

Fig. 3. 2. 9: Effect of drought stress (black for drought and white for control) on F_v/F_m (on dark) on seedlings of two provenances of *Balanites aegyptiaca* Damazin (white) and Um Abdalla (black). Error bars represent SE (n = 24), drought treatment started from the 2nd measurement.

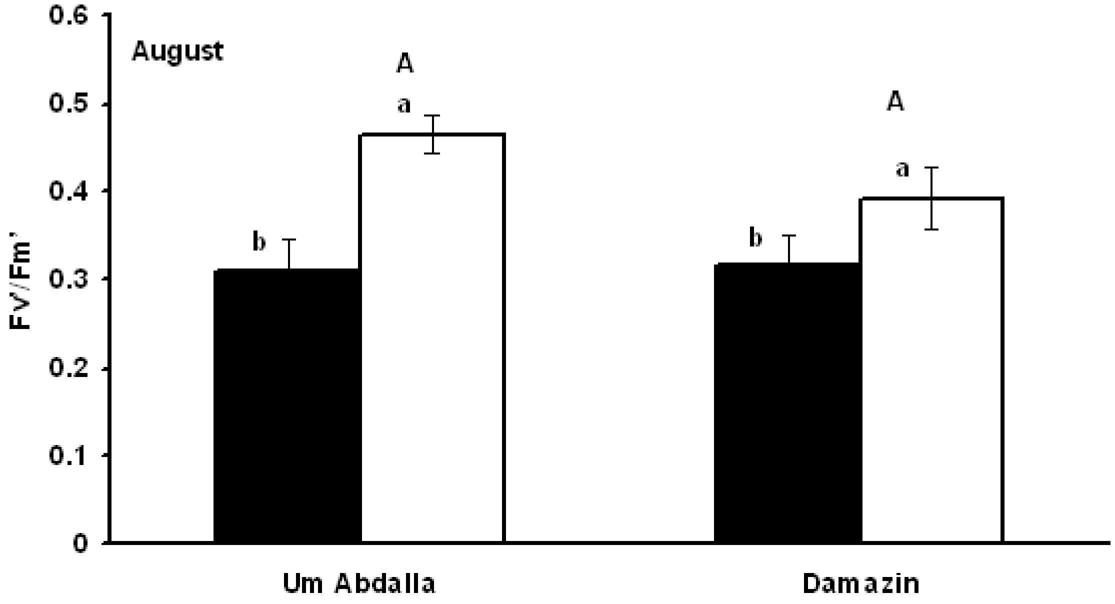

Fig. 3. 2. 10: Effect of drought stress (black for drought, white for control) on F_v'/F_m' of seedlings of two provenances of *Balanites aegyptiaca* (Um Abdalla and Damazin). Error bars represent SE, N = 24. Letters indicate the Duncan's Multiple Range Test, means with the same letters are not significantly different; capital letters for provenances and lower case letters for drought stress.

3. 2. 2. 3 Stomata conductance

Stomatal conductance (g_s) is not only affected by drought stress but also by light, tempeature and other factors. Because climate was not controlled in the greenhouse, g_s fluctuates throughout the experiment (Fig. 3. 2. 11 top). The effect of drought on g_s relative to control is shown in (Fig. 3. 2. 11 bottom), where g_s decreased as drought increased. Stomatal conductance decreased continuously in both provenances, except for the last measurement, when g_s decreased more in drought-stressed plants from Damazin (Fig. 3. 2. 11), even though, these had a less negative water potential (Fig. 3. 2. 7). Under severe drought stress at the end of the experiment, g_s was reduced to 52% and 38% of control plants for Um Abdalla and Damazin respectively (Fig. 3. 2. 11 bottom).

Plants from Damazin had highest stomata conductance before the drought treatment (Fig. 3. 2. 11 top). Three days after reaching a water deficit of -300 g/pot, plants from Um Abdalla had significantly higher stomata conductance (Fig. 3. 2. 11). Six days after the start of drought treatment and three days after reaching -300 g/pot, plants treated with high nitrogen showed significantly higher stomata conductance compared to plants with low nitrogen (Fig. 3. 2.12). Potassium only once had a marginally significant effect on stomata conductance (Table 3. 2. 8) with stomata conductance higher with increased potassium concentration. At drought regime of -400 g/pot, plants under drought stress showed significant reduction on stomata conductance (Table 3. 2. 8) and after watering completely ceased the reduction on stomata conductance was highly significant compared to control (Fig. 3. 2. 11). In August 22, the interaction between provenances and drought showed that Damazin plants had the higher stomata conductance in control plants while Um Abdalla had the higher g_s in drought stressed plants (Fig. 3. 2. 11).

Table 3. 2. 8: Significant effects ($P < 0.05$) of provenance, nitrogen, potassium and drought on stomata conductance (n.s not significant, n.a. not applicable, before drought stress).

	15-Jun	**26-Jun**	**05-Jul**	**11-Jul**	**17-Jul**	**25-Jul**	**22-Aug**
Provenance	0.005	n.s	n.s	0.01	n.s	n.s	n.s
Nitrogen	n.s	< 0.0001	n.s	0.018	n.s	n.s	n.s
Potassium	n.s	n.s	0.05	n.s	n.s	n.s	n.s
Drought	n.a.	n.s	n.s	n.s	0.001	< 0.0001	< 0.0001
Provenance*Nitrogen	n.s	0.002	n.s.	n.s	n.s	n.s	n.s
Nitrogen*Drought	n.a.	n.s	n.s	n.s	0.052	n.s	n.s
Provenance*Drought	n.a.	n.s	n.s	n.s	n.s	n.s	0.001
Provenance*Potassium	n.a.	n.s	0.01	n.s	n.s	n.s	0.001
Potassium*Drought	n.a	n.s	n.s	n.s	n.s	n.s	n.s

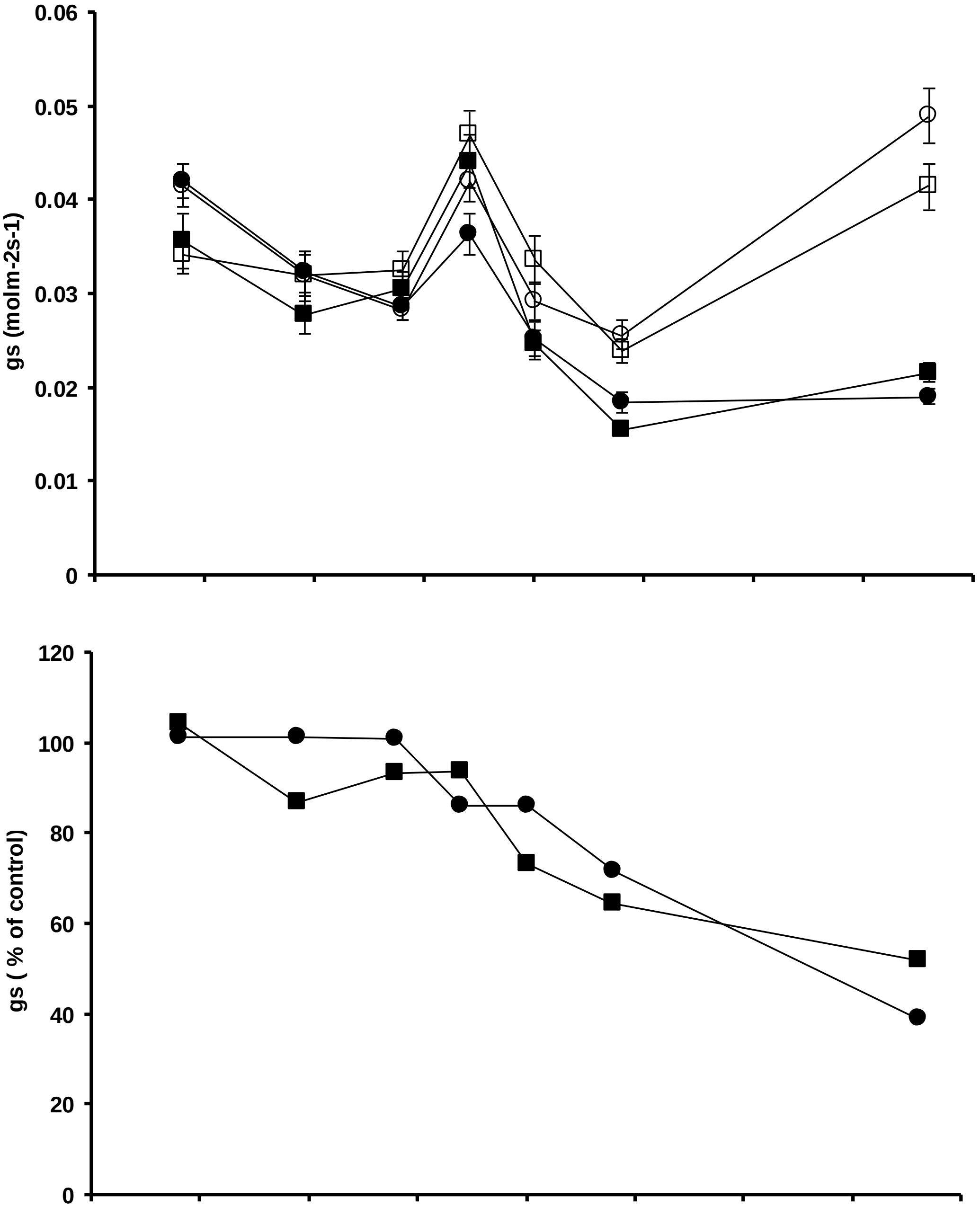

Fig. 3. 2. 11: Effect of drought stress (open symbols for control and black symbols for drought stress) on stomata conductance (g_s) of seedlings of tow provenances (Damazin: O and Um Abdalla: □) of *Balanites aegyptiaca.* Error bars represented SE (n = 24); drought treatment started after the 1st measurement (top); reduction on stomata conductance (g_s) in percentage of control of seedlings of the tow provenances under drought stress (bottom).

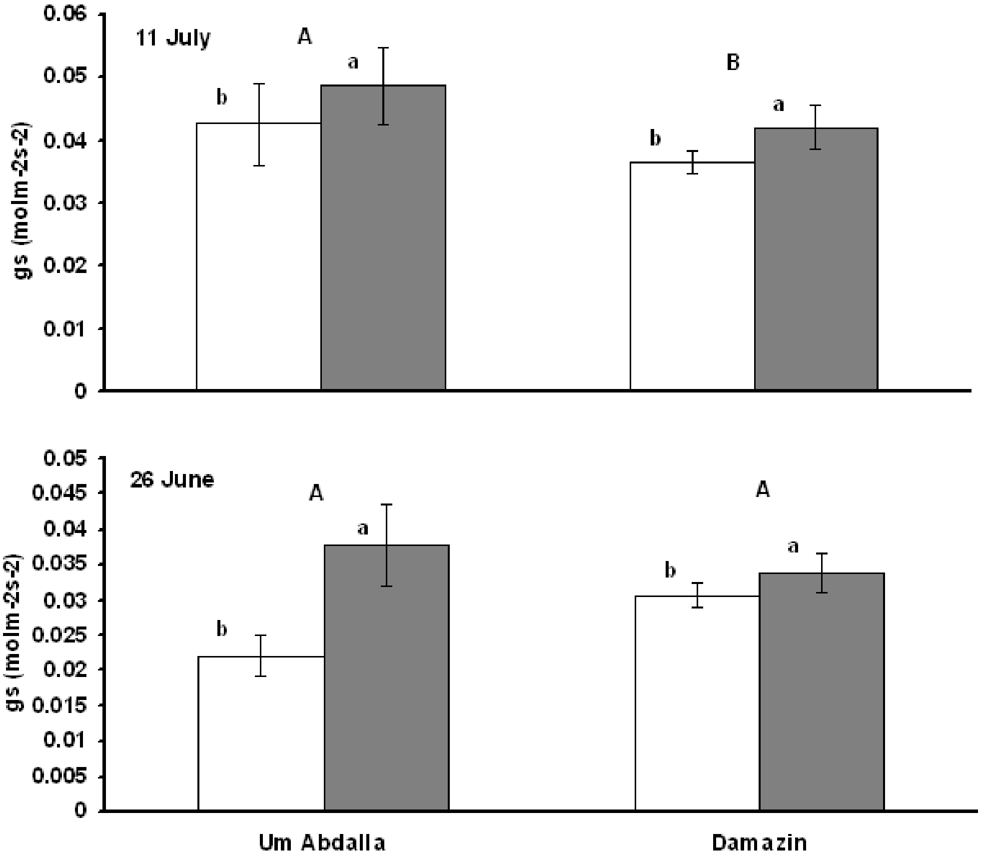

Fig. 3. 2. 12: Effect of nitrogen (white for low concentration and gray for high concentration) on stomata conductance (g_s) of seedlings of *Balanites aegyptiaca* from tow provenances (Damazin and Um Abdalla). Error bars represented SE. Letters indicate the Duncan's Multiple Range Test, means with the same letters are not significantly different; capital letters for provenances and lower case letters for nitrogen treatments.

3.3 Effect of salt stress on *Balanites aegyptiaca*

3.3.1 Biometry

There was a visible effect of salt on *Balanites aegyptiaca* treated with low and high salt concentration, where plants developed thick, succulent leaves with high water per leaf area and somewhat lower specific leaf area (Fig. 3. 3. 1). Plants also developed small hairs on the leaf surface. The thicker leaves in salt treated plants had higher leaf water per area ($P < 0.0001$) and lower SLA ($P = 0.026$ and $P = 0.011$ in November and December respectively) compared to control plants (Fig. 3. 3. 1).

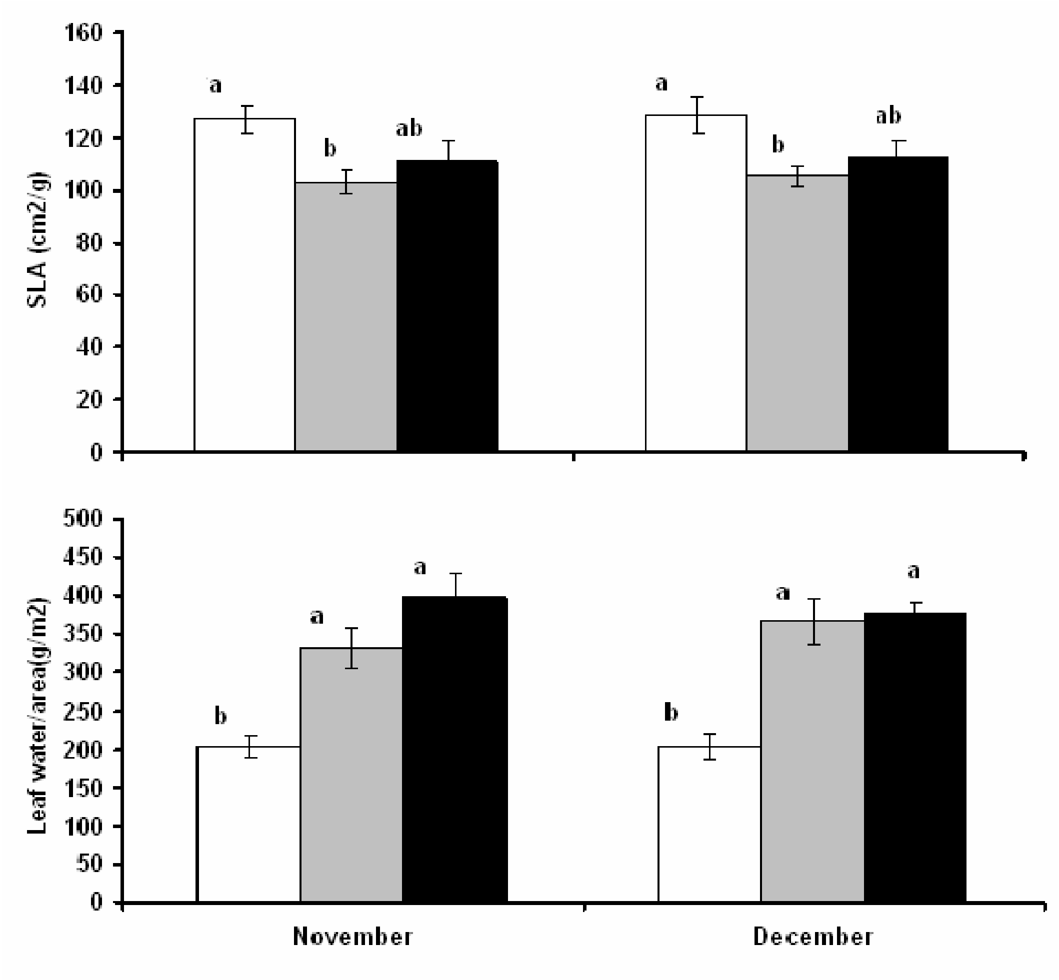

Fig. 3. 3. 1; Effect of salt treatment (white: control, gray: low, black: high) on leaf water per area (top) and specific leaf area (bottom) of *Balanites aegyptiaca* in November and December 2008. Error bars indicate SE. Treatments with the same letters are not significantly different (Duncan post hoc test).

There was no significant effect of salt on the number of leaves, with salt treated plants having a higher number of leaves compared to control (Fig. 3. 3. 3). There was loss of leaves after three month of application of salt, especially in plants at high salt concentration (Fig. 3. 3. 2). Salt significantly (P = 0.005) increased the number of branches and significantly affected stem height (P = 0.034) with plants under salt treatment having taller stems and more branches (Table 3. 3. 1, Fig. 3. 3. 3 & 3. 3. 4). Damazin plants developed more leaves and longer stems (Fig. 3. 3. 3) and more branches (Fig. 3. 3. 4). With time some plants of low salt and most plants of high salt concentration shed their leaves.

Fig. 3. 3. 2: Effect of salt (control, low and high salt concentration) on *Balanites aegyptiaca* seedlings (after nearly three month) Photos were taken on 14 Nov.

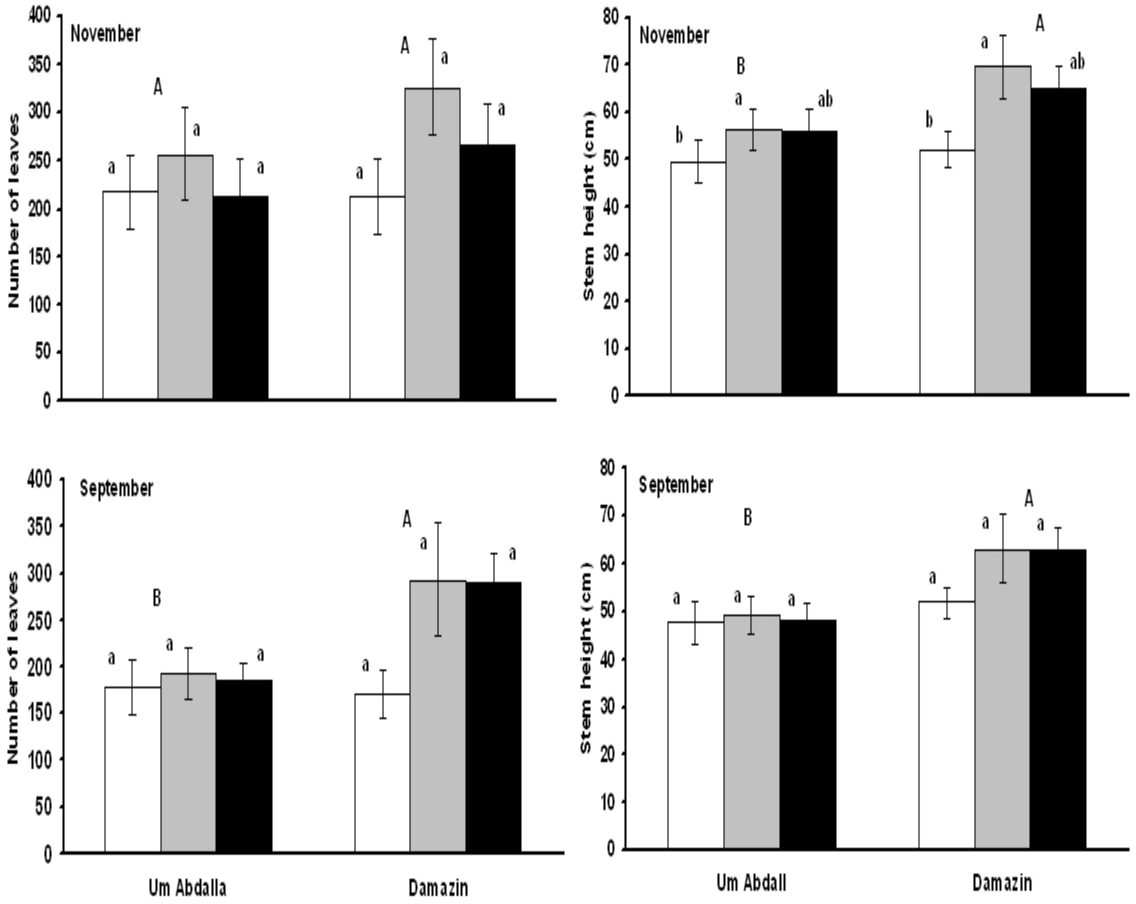

Fig. 3. 3. 3: Effect of salt treatment (control: white, low salt concentration: gray, high: black) on the number of leaves and stem height (cm)of *Balanites aegyptiaca* seedlings from two provenances (Um Abdalla and Damazin). Error bars indicate SE. Treatments with the same letters are not significantly different (Duncan post hoc test) capital letters for provenances and lower case letters for salt.

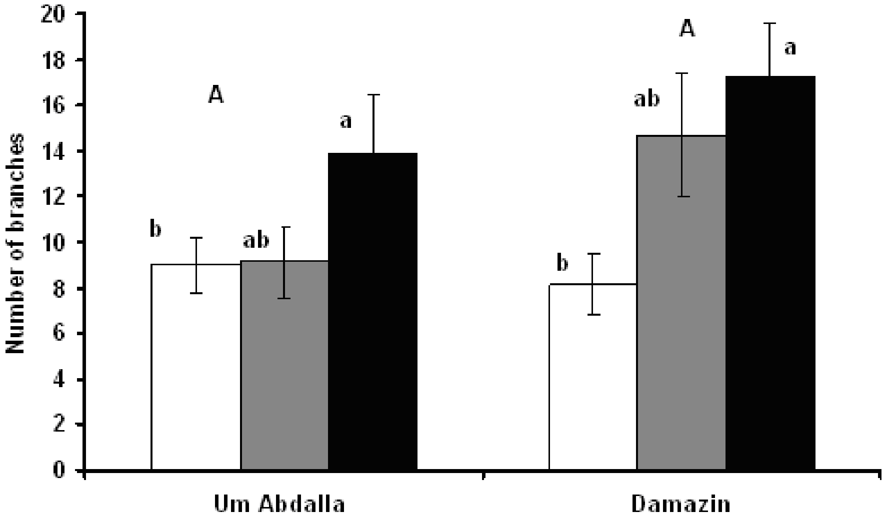

Fig. 3. 3. 4: Effect of salt treatment (control: white, low salt concentration: gray, high: black) on number of branches of *Balanites aegyptiaca* seedlings of two provenances (Um Abdalla and Damazin) in November. Error bars indicate SE. Treatments with the same letters are not significantly different (Duncan post hoc test), capital letters for provenances and lower case letters for salt.

Table 3. 3. 1: Significant effects (only $p < 0.05$ shown) of salt treatment and provenance on stem height, number of leaves and number of branches (n.s = not significant, -: no measurements).

	Sept.	**Nov.**
Stem		
Provenance	0.0049	0.0373
Salt	n.s	0.0371
Provenance*Salt	n.s	n.s
Leaves		
Provenance	0.0246	n.s
Salt	n.s	n.s
Provenance*Salt	n.s	n.s
Branches		
Provenance	-	n.s
Salt	-	0.0047
Provenance*Salt	-	n.s

3.3.2 Plant physiology

3. 3. 2. 1 Chlorophyll content

Chlorophyll content increased during growth, but salt treatment had no significant effect on chlorophyll content (Fig. 3. 3. 5). Um Abdalla plants at high salt concentration had the highest chlorophyll content till the mid of the experiment where Damazin plants at high salt concentration had the highest chlorophyll content to the end of the experiment.

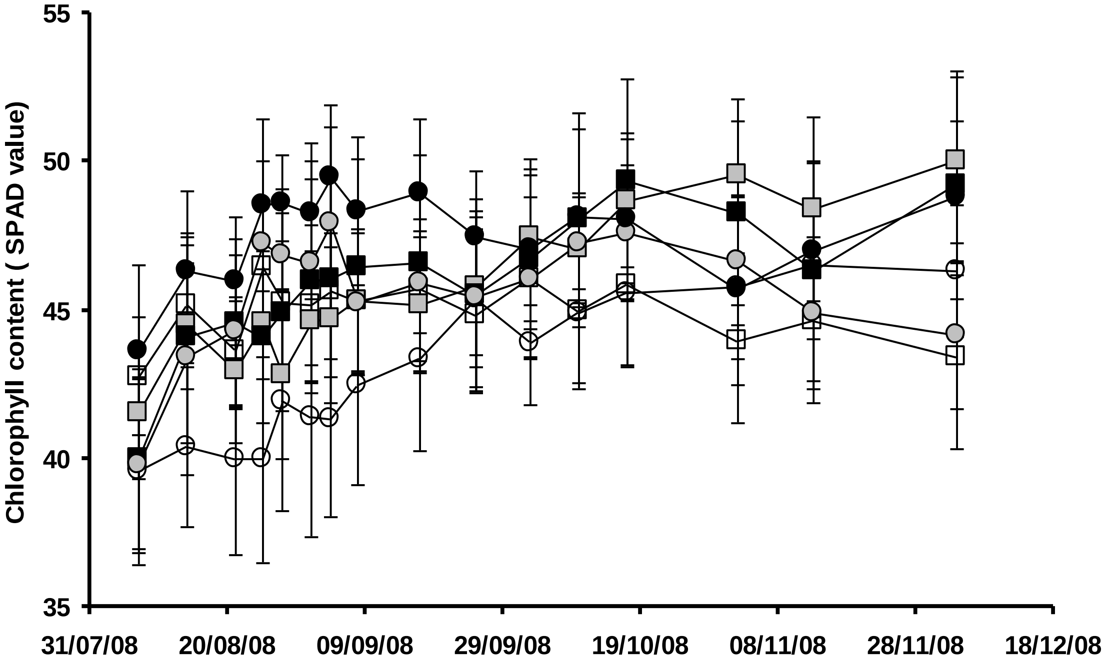

Fig. 3. 3. 5: Effect of salt treatment (control: white, low salt concentration: gray, high :black) on chlorophyll content of seedlings of tow provenances of *Balanites aegyptiaca* Damazin (O) and Um Abdalla (□). Error bars indicate SE.

3. 3. 2. 2 Chlorophyll Fluorescence

Salt showed effect on F_0 only in the September 8 (Table 3. 3. 2); when control plants had the highest F_0. Provenances showed significant difference (P = 0.005) only after five weeks from the start of salt treatment, when F_0 was higher in plants from Damazin (Fig. 3. 3. 7). Young leaves had significantly higher F_0 before the start of the salt treatment. Old leaves had higher mean of F_0 in the fourth and fifth week from the start of salt treatment and young leaves had the highest mean F_0 for other measurements, with no difference in the last two measurements.

Two, four and five weeks after the start of the salt treatment, maximum quantum efficiency of photosystem II (F_v/F_m) was higher in salt-treated than in control plants (Fig. 3. 3. 6 & 3. 3. 7).

Provenances showed significant difference one and three weeks after doubling the salt concentration where Damazin provenance had the highest F_v/F_m (Fig. 3. 3. 7). Old leaves had higher F_v/F_m on several days, in September, when F_v/F_m in all treatments was > 0.8 and in October when F_v/F_m in all treatments was < 0.8.

Leaf age affected Tf_m where young leaves reached the maximum fluorescence faster than old leaves. On September 17 and November 14 plants under high salt reached the maximum fluorescence faster than control plants. Provenances significantly differed in PI on October 3 and 17 and on December 4 where Damazin had the highest PI compared to Um Abdalla provenance (Fig. 3. 3. 6 & 3. 3. 7).

Salt had significant effect on PI on September 17 and September 25, where plants under salt treatment had the highest PI and on November 14 where plants of low salt concentration and control had the highest PI. Young leaves had the highest PI on September 25 while old leaves had the highest PI on the rest of measurements.

Table 3. 3. 2: Significant effects (only $p < 0.05$ shown) of salt treatment (S), provenance (P) and leaf age (L) on chlorophyll fluorescence parameters **F_0, F_v/F_m, Tf_m** and **PI** of *Balanites aegyptiaca* (**-**: not significant).

	14-Aug	**21-Aug**	**25-Aug**	**01-Sep**	**04-Sep**	**08-Sep**	**11-Sep**	**17-Sep**	**25-Sep**	**03-Oct**	**10-Oct**	**17-Oct**	**31-Oct**	**06-Nov**	**14-Nov**	**04-Dec**
F_0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
P	-	-	-	-	-	-	-	-	0.005	-	-	-	-	-	-	-
L	0.035	0.065	-		-	-	-	0.012	0.012	0.027	-	0.029	<0.0001	0.036	-	-
S	-	-	-	-	-	0.021	-	-	-	-	-	-	-	-	-	-
P*L	-	-	-	-	-	-	-	-	-	-	-	0.031	0.003	0.024	-	0.003
P*S	-	-	-	0.034	0.008	-	-	-	-	-	0.01	-	-	-	0.001	0.011
F_v/F_m	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
P	-	-	-	-	-	-	-	-	-	-	-	-	0.001	0.052	-	-
L	-	-	-	-	-	-	-	0.02	0.001	-	-	0.025	0.003	-	-	-
S	-	-	-	-	-	0.007	-	0.002	0.036	-	-	-	-	-	0.008	-
P*L	-	-	-	-	-	-	-	-	-	-	-	-	0.002	0.035	0.021	-
P*S	-	-	-	-	-	-	-	-	-	-	-	-	0.044	-	-	-
Tf_m	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
P	-	-	-	-	-	-	-	-	-	-	-	-	-	0.011	-	-
L	< 0.0001	< 0.0001	< 0.0001	0.003	< 0.0001	0.016	0.059	-	0.001	-	0.002	0.003	0.001	0.001	0.002	< 0.0001
S	-	-	-	-	-	-	-	0.04	-	-	-	-	-	-	-	0.022
P*L	-	-	0.028	-	-	-	-	-	-	-	-	-	-	-	-	-
P*S	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
L*S	-	-	-	-	-	-	-	-	-	0.039	-	-	-	-	-	0.026
PI	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
P	-	-	-	-	-	-	-	-	-	0.005	-	0.012	-	-	-	0.012
L	-	-	-	-	-	-	-	-	0.033	0.048	-	0.001	< 0.0001	0.001	0.001	0.04
S	-	-	-	-	-	-	-	0.027	0.009	-	-	-	-	-	0.006	-
P*L	-	-	0.037	-	-	-	-	-	-	-	-	-	-	0.028	-	-
P*S	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.037	-

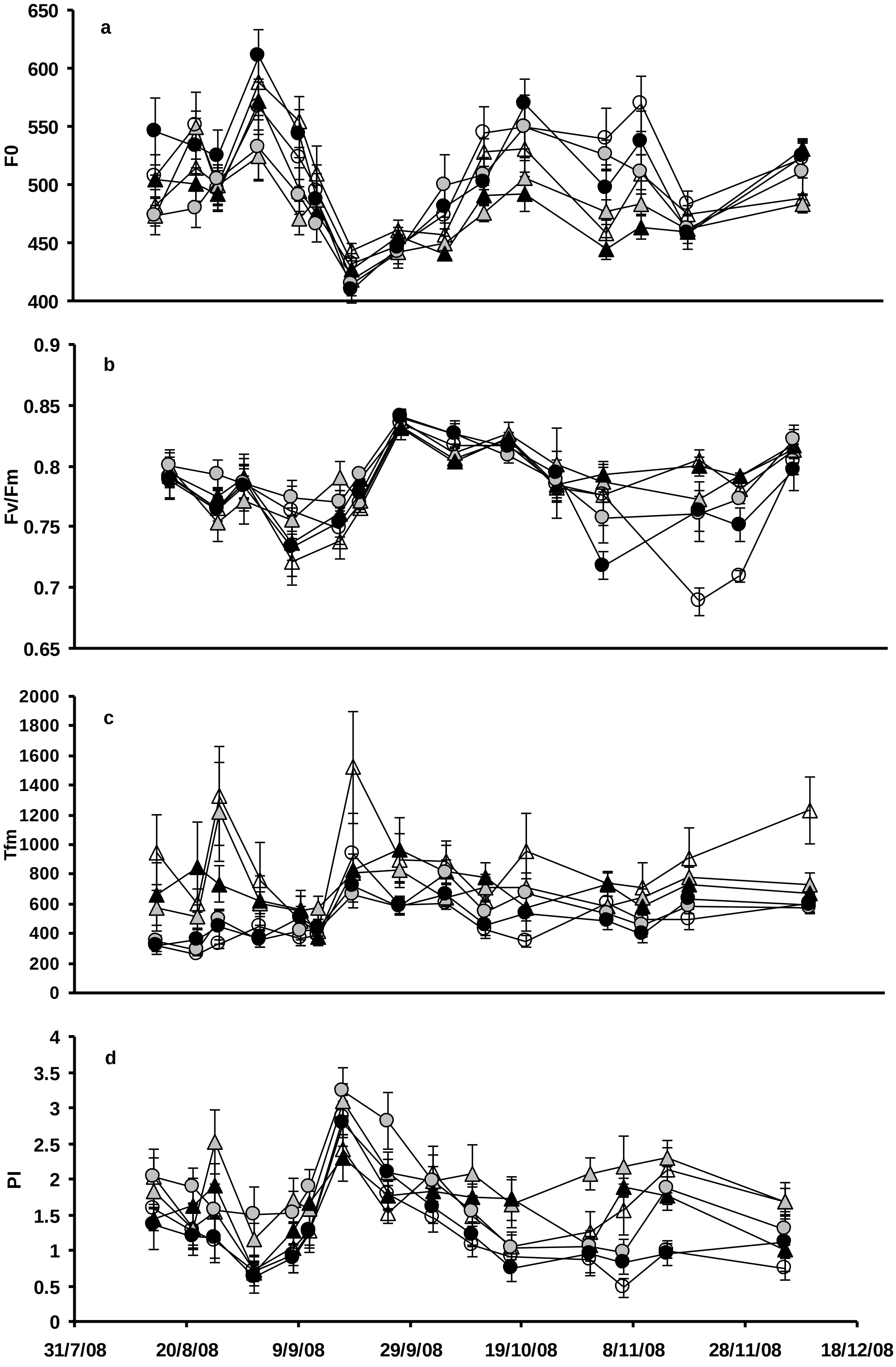

Fig. 3. 3. 6: Effect of salt treatment (control: white, low salt concentration: gray, high :black) on F_0, F_v/F_m, Tf_m and PI from chlorophyll fluorescence measurements (refer to material and methods for the abbreviations) on old (Δ) and young (O) leaves of *Balanites aegyptiaca* (Um Abdalla provenance). Error bars indicate SE.

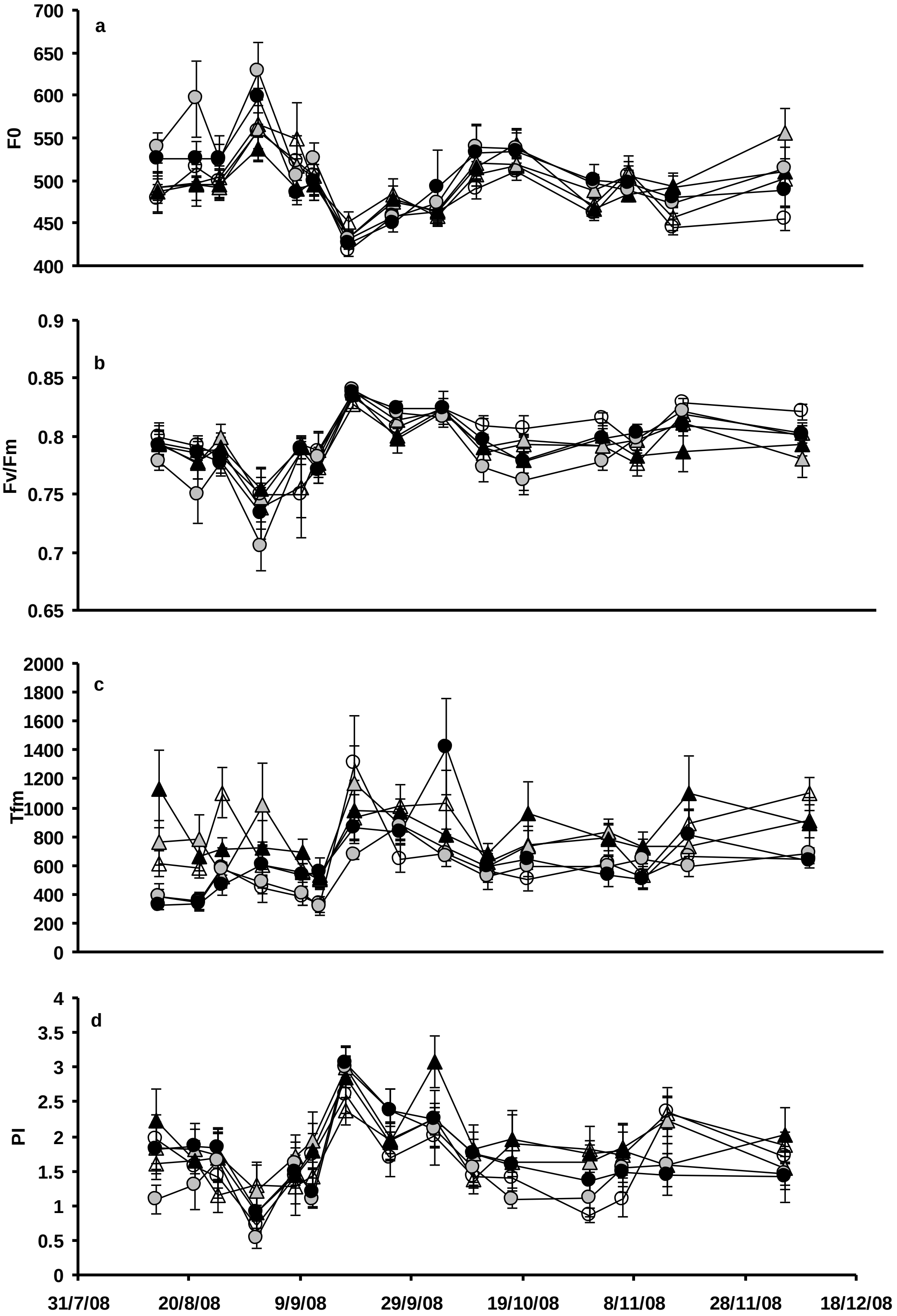

Fig. 3. 3. 7: Effect of salt treatment (control: white, low salt concentration: gray, high :black) on F_0 (a), F_v/F_m (b), Tf_m (c) and PI (d) from chlorophyll fluorescence measurements on old (Δ) and young (O) leaves of *Balanites aegyptiaca* (Damazin provenance). Error bars indicate SE.

3. 3. 2. 3 Gas exchange

Net photosynthesis and stomata conductance declined nearly continuously during the experiment from July to December while leaf internal CO_2 concentration increased somewhat. Salt had a significant effect on net photosynthesis only at the last measurement (December 4), where plants under salt treatment had a higher net photosynthesis than control plants (Fig. 3. 3. 8 a). On November 14 and December 4 salt significantly affected the stomata conductance (Table 3. 3. 3), where plants under high salt concentration showed the highest mean compared to control (Fig. 3. 3. 8 b). Salt significantly affected the intercellular CO_2 on September 5 where salt treated plants showed lower C_i compared to control and on October 7 and December 4 when C_i in plants at high salt concentration was higher (Fig. 3. 3. 8 c).

Before the start of the salt treatment, Damazin plants significantly differed from Um Abdalla plants showing higher stomata conductance and C_i but two weeks after the start of salt treatment stomata conductance and C_i were higher in Um Abdalla plants with no difference during the rest of the experiment (Fig. 3. 3. 8 b & c).

Table 3. 3. 3: Significant effects (only $p < 0.05$ shown) of gas exchange measurements by date (empty cells were no significant difference) A_{sat}: photosynthesis, g_s: stomata conductance, C_i: intercellular CO_2 (-: not significant).

A_{sat}	**19 Aug**	**5 Sep**	**9 Sep**	**12 Sep**	**2 Oct**	**16 Oct**	**7 Nov**	**13 Nov.**	**4 Dec**
Provenance	-	-	0.072	-	-	-	-	-	-
Salt	-	-	-	-	-	-	-	-	0.004
Provenance*Salt	-	-	-	-	0.073	-	-	-	-
	-	-	-	-	-	-	-	-	-
g_s	-	-	-	-	-	-	-	-	-
Provenance	0.024	0.051	-	-	-	-	-	-	-
Salt	-	-	-	-	-	-	-	0.03	0.001
Provenance*Salt	-	-	-	-	0.031	0.058	-	-	-
	-	-	-	-	-	-	-	-	-
Ci	-	-	-	-	-	-	-	-	-
Provenance	< 0.0001	0.025	-	0.026	-	-	-	-	-
Salt	-	0.027	-	-	-	-	0.001	-	0.011
Provenance*Salt	-	-	-	-	-	-	-	-	-

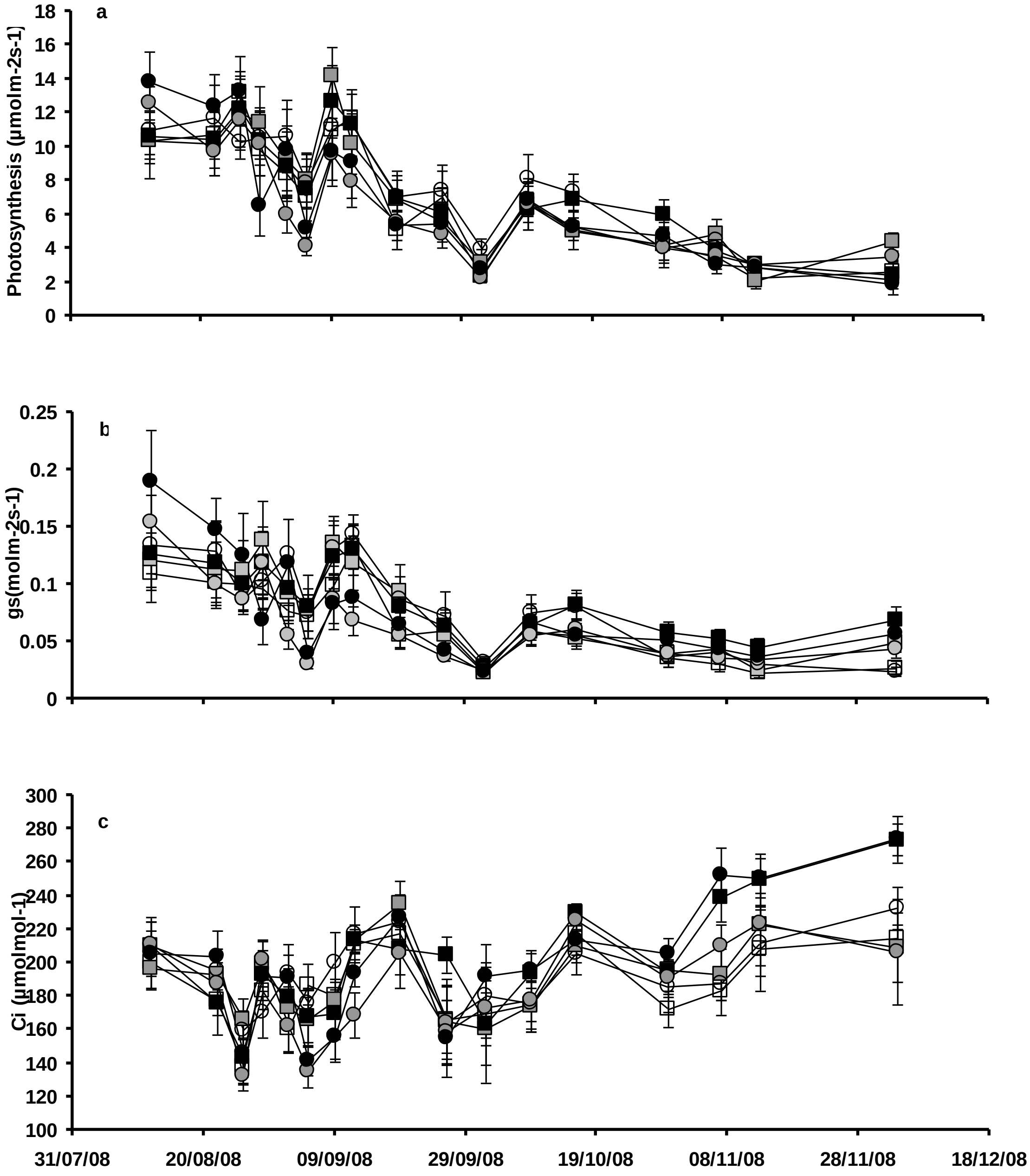

Fig. 3. 3. 8: Effect of salt treatment (control: white, low salt concentration: gray, high :black) on photosynthesis, stomata conductance (g_s) and intercellular CO_2 (C_i) on tow provenances of *Balanites aegyptiaca* Damazin (O) and Um Abdalla (□). Error bars indicate SE.

3.4 Effect of salt on *Populus nigra*

3.4.1 Biometry

Plants under low and high salt treatment showed visible symptoms of stress after two weeks (Fig. 3. 4. 3), starting with leaf necrosis (Fig. 3. 4. 4) followed by complete drying and loss of some leaves after three weeks. After five weeks all leaves of plants of the 8 mS were shed and most leaves of plants at 4 mS were lost. At the last measurement after seven weeks all old leaves of plants at 4 mS had been shed and plants had developed new leaves which were used for measurements.

There was no salt effect on stem height. There was a significant difference between clones in stem height (P = 0.0009 in August) where LO50 plants had the highest and EF50 plants had the lowest stem height (Fig. 3. 4. 1).

Clones showed no significant difference on shoot dry weight. There was significant decrease of shoot dry weight (P < 0.0001) in response to salt concentration, where shoot dry weight decrease with the increase of salt concentration (Fig. 3. 4. 2).

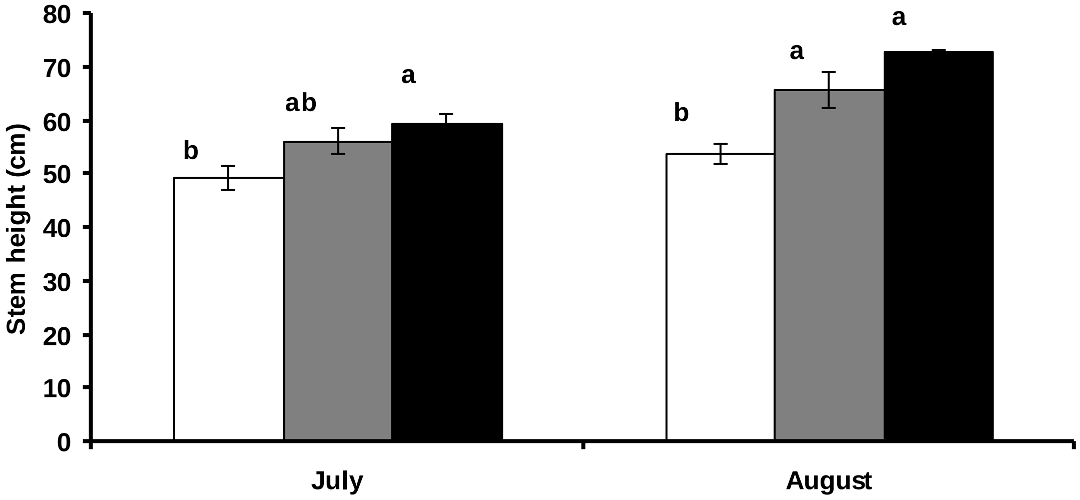

Fig. 3. 4. 1: Variation of stem height (cm) among three clones of *Populus nigra* EF50 (white), LO14 (grey) and LO50 (black). Letters indicate the Duncan's Multiple Range Test, means with the same letter are not significantly different.

Clones nearly significantly differed in root dry weight (P = 0.0835), where LO14 plants had the highest mean and LO50 plants had the lowest mean of root dry weight, which was not differ from EF50 plants. Root dry weight significantly (P < 0.0001) decreased with increasing salt concentration (Fig. 3. 4. 2).

Clones significantly differed in root: shoot ratio (P = 0.016), which were higher in EF50 and LO14 than in LO50 (Fig. 3. 4. 2). Root: shoot ratio decreased with the increase of salts concentration.

Clones showed no significant difference in total plant dry weight. Total dry weight was significantly decreased (P < 0.0001) with the increase of salt concentration (Fig. 3. 4. 2).

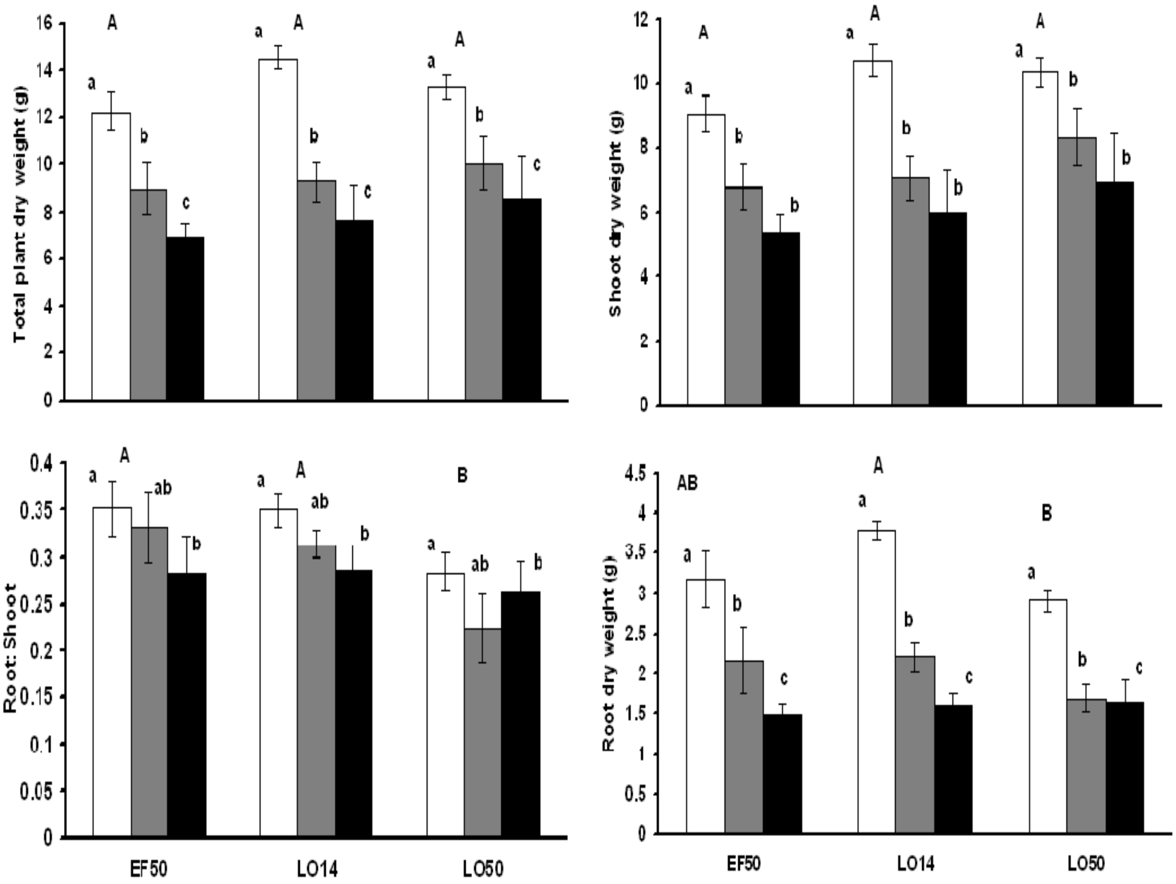

Fig. 3. 4. 2: Effect of salt treatment; 0 (white), 4 (gray) and 8 (black) mS/cm and three clones of *Populus nigra* , EF50 LO14 and LO50 on total plant weight, shoot weight, root weight and root:shoot ratio. Error bars indicate SE. Letters indicate the Duncan's Multiple Range Test, capital letters for clones and lower case letters for salt, means with the same letter are not significantly different.

3. 4. 2 Plant physiology

3. 4. 2. 1 Chlorophyll content

After three weeks leaf chlorophyll content at 8 mS had declined in all clones, while control and 4 mS were not significantly different (Fig. 3. 4. 5). At 4 mS chlorophyll content of all clones declined after 4 weeks. New leaves, produced by the end of the experiment in the 4 mS treatment by all clones, had much lower chlorophyll content compared with the control (Fig. 3. 4. 5). Clones showed significant difference before the start of salt treatment and continued with the same trend throughout the experiment, where clone LO14 had the highest mean and clone EF50 had the lowest mean of chlorophyll content (Table 3. 4. 1, Fig. 3. 4. 5).

Fig. 3. 4. 3: Effect of salt stress (control, 4 and 8 mS/cm) on three clones of *Populus nigra*, EF50, LO14 and LO50. Photos were taken after 25 days from the start of salt treatment.

Fig. 3. 4. 4: Symptoms of salt injury on leaves of *Populus nigra* seedling exposed to 8 mS/cm salt. Photo was taken after 25 days from the start of salt treatment.

Table 3. 4. 1: ANOVA significance of clone and salt affecting leaf chlorophyll content of three clones of *Populus nigra* EF50, LO15 and LO50 and three salts treatment 0, 4 and 8 mS/cm. - : before salt treatment, n.s.: not significant.

	24-Jul	01-Aug	05-Aug	18-Aug	25-Aug	31-Aug	16-Sep
Clone	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.0008
Salt	-	n.s	n.s	< 0.0001	< 0.0001	0.0014	< 0.0001
Clone*Salt	-	n.s	n.s	n.s	0.066	0.0227	0.014

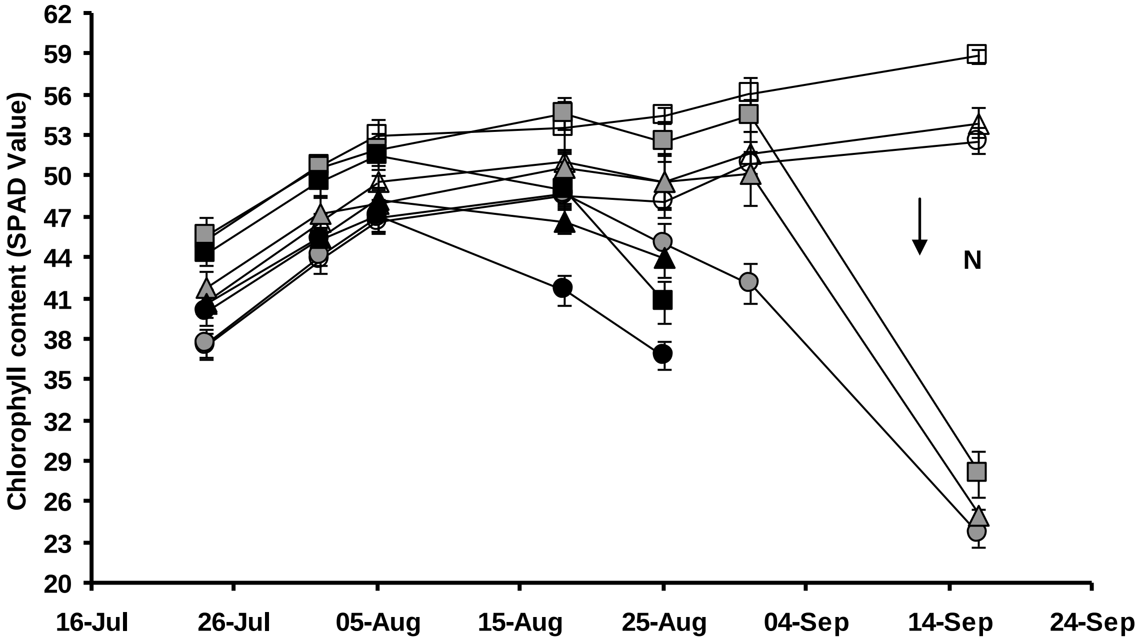

Fig. 3. 4. 5: Effect of salts treatment, 0 (white), 4 (gray) and 8 (black) mS/cm on leaf chlorophyll content of three clones of *Populus nigra*, EF50 (O), LO14 (□) and LO50 (Δ). Error bars indicate SE. N: new leaves developed and measured in 4 mS treatment.

3. 4. 2. 2 Gas exchange

From the first to the fourth week, net photosynthesis (A_{sat}), stomata conductance (g_s) and intercellular CO_2 (C_i) declined in all plants but increased in fifth week. In the seventh week A_{sat}, g_s and C_i in plants at 4mS decreased, but increased in control plants (Fig. 3. 4. 6). There was significant difference among clones in A_{sat} after one and three weeks from the start of the salt treatment (Table 3. 4. 2), where clone EF50 had the lowest mean and clone LO14 and LO50, which were not significantly different, had the highest photosynthesis. A significant effect of salt treatment was evident after three weeks when plants at 4mS had the highest A_{sat} while plants at 8mS had the lowest A_{sat} (Fig. 3. 4. 6).

There was a significant effect of salt on stomatal conductance (Table 3. 4. 2). One week after the start of the salt treatment control plants had the highest stomatal conductance and in the third and fifth week plants of 4 mS group had the highest gs. Clones significantly different after one week to the fifth week, where clone EF50 had the lowest mean of stomata conductance.

Salt treatment also had a significant effect on intercellular CO_2 (Table 3. 4. 2). In the first week the control plants had the highest intercellular CO_2 and in the third and fifth week plants at 8mS and 4mS had the highest mean. In the seventh week plants at 4mS had the highest mean of intercellular CO_2 (Fig. 3. 4. 6). There was no significant difference among clones in intercellular CO_2 in all measurements (Table 3. 4. 2).

Table 3. 4. 2: Probability from ANOVA test, showing the effect of *Populus nigra* clones (EF50, LO15 and LO50) and salt (0, 4 and 8 mS/cm) on photosynthesis (A_{sat}), stomata conductance (g_s) and intercellular CO_2 (C_i). - : before salt treatment, n.s.: not significant.

	27-Jul	**04-Aug**	**17-Aug**	**31-Aug**	**16-Sep**
Clone					
A_{sat}	n.s.	0.003	0.0012	0.013	n.s
g_s	n.s	0.013	0.0043	0.0122	n.s
Ci	n.s	n.s	n.s	n.s	n.s
Salt					
A_{sat}	_	n.s	< 0.0001	n.s	0.04
g_s	_	0.0006	< 0.0001	0.009	n.s
C_i	_	0.001	0.0001	0.0001	0.014
Clone x salt					
A_{sat}	_	n.s	0.0105	n.s	n.s
g_s	_	n.s	0.031	n.s	n.s
C_i	_	n.s	n.s	n.s	n.s

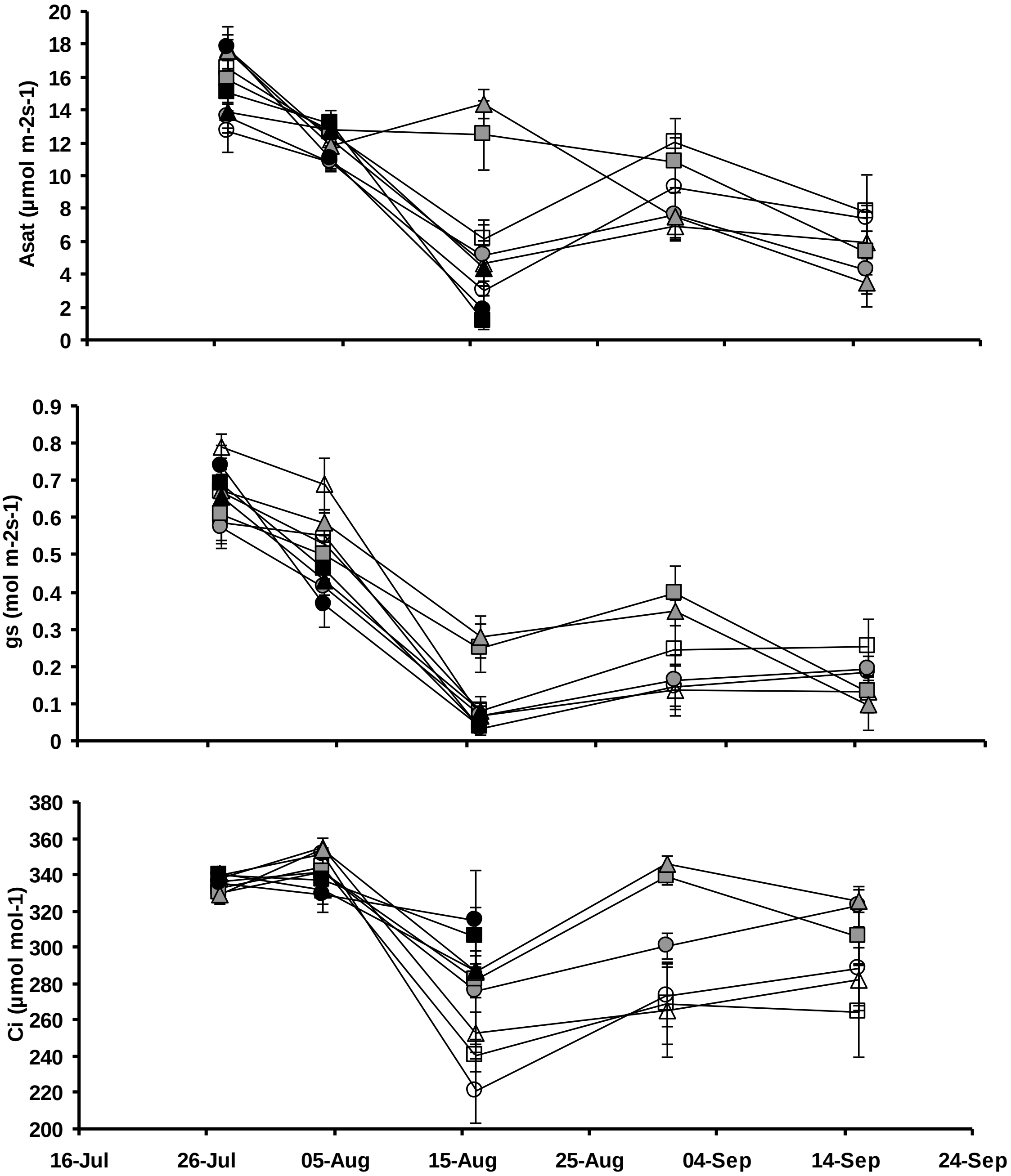

Fig. 3. 4. 6: Effect of salt treatment; 0 (white), 4 (gray) and 8 (black) mS/cm on net rate of photosynthesis (A_{sat}), stomata conductance (g_s) and intercellular CO_2 (C_i) on three clones of *Populus nigra* , EF50 (O), LO14 (□) and LO50 (Δ). Error bars indicate SE. New leaves developed and measured in 4 mS treatment in the last measurement. 8 mS plants were not shown in the last two measurements because all leaves were shed.

3. 4. 2. 3 Chlorophyll Fluorescence

Clone and salt had a significant effect on F_0 after two weeks from the start of the salt treatment to the last measurement, except on August 20 and September 9 where clones were not significantly different (Table 3. 4. 3). EF50 was the clone with the highest F_0 and plants at 8 mS had the highest f F_0 except for September 2 where plants of 4 mS group had the highest F_0 and in the last two measurements, where the control plants had the highest F_0 (Fig. 3. 4. 7).

F_v/F_m differed between clones only on August 1 (Table 3. 4. 3), but salt had a significant effect after two weeks onwards, except for the last two measurements (Table 3. 4. 3). Plants at 4 mS and control plants did not significantly differ except for the measurement on September 2 when $F_{v/}F_m$ was highest in control plants (Fig. 3. 4. 7) Plants at 8 mS had the lowest $F_{v/}F_m$.

Salt showed significant effects on Tf_m after four days from the start of the salt treatment to the last measurement, except for August 26 (Table 3. 4. 3). On August 1 and August 12 plants at 4 mS had the highest Tf_m, while plants at 8 mS and control plants did not differ. After three weeks from the start of the salt treatment to the last measurement control plants had the highest Tf, while plants at 8 mS and 4 mS, which were not significantly different, had the lowest Tf_m (Fig. 3. 4. 7). Clones were not significantly different except the last measurement (Table 3. 4. 3).

Clones differed significantly in PI, except for the last two measurements (Table 3. 4. 3). LO50 plants had the highest and EF50 plants had the lowest PI, except for September 2 when LO14 plants had the highest PI (Fig. 3. 4. 7). Salt showed significant effects on PI after three weeks to the last measurement (Table 3. 4. 3). Plants at 8 mS had a significantly lower PI than control plants (Fig. 3. 4. 7).

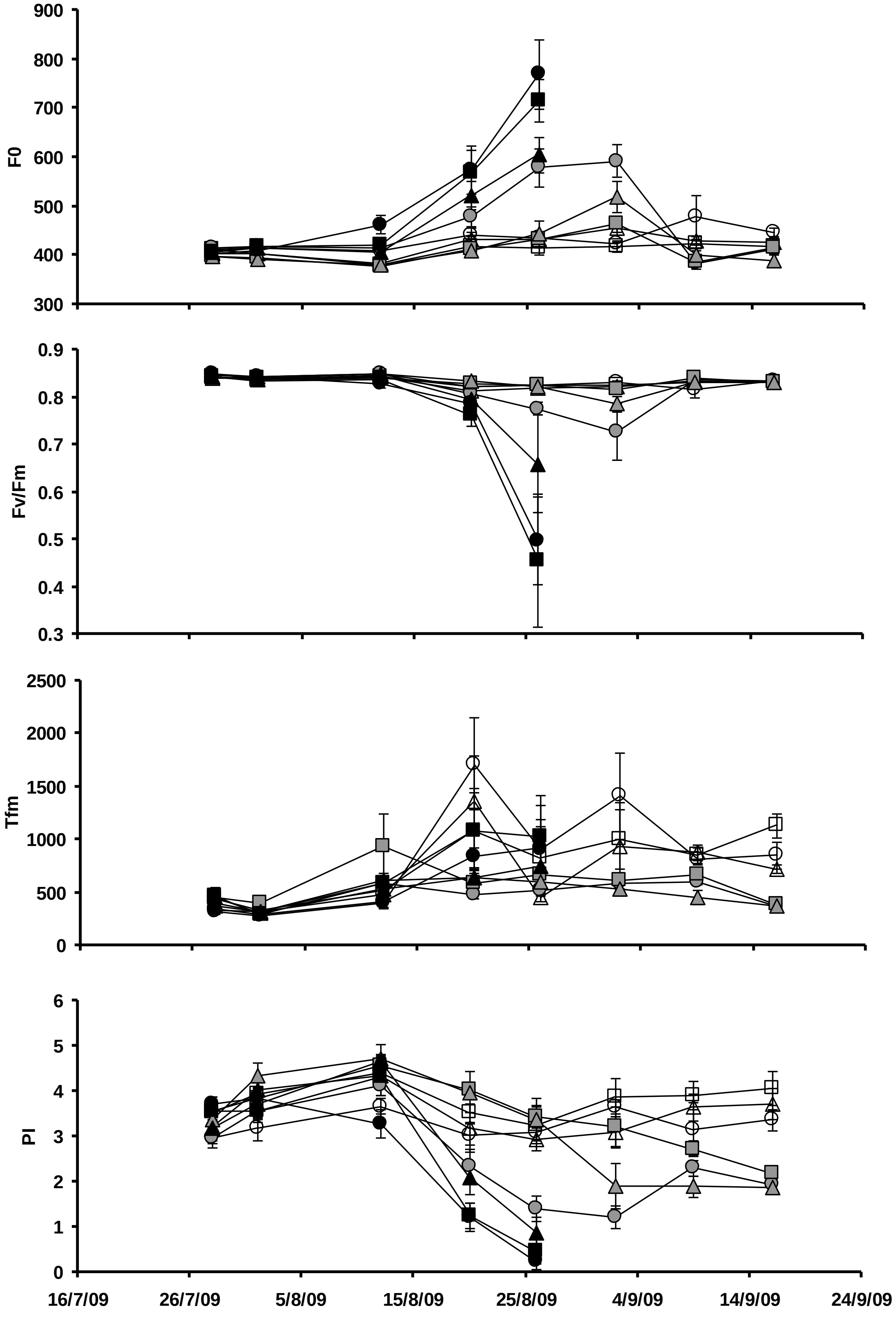

Fig. 3. 4. 7: Effect of salt treatment; 0 (white), 4 (gray) and 8 (black) mS/cm on F_0, F_v/F_m, Tf_m and PI from chlorophyll fluorescence measurements on three clones of *Populus nigra* , EF50 (O), LO14 (□) and LO50 (Δ). Error bars indicate SE. New leaves developed and measured in 4 mS treatment in the last measurement.

Table 3. 4. 3: Probability from ANOVA results showing the effect of salt 0, 4 and 8 mS/cm and three clones of *Populus nigra* EF50, LO15 and LO50 on F_0, F_v/F_m, Tf_m and PI from chlorophyll fluorescence measurements. - : before salt treatment, n.s.: not significant.

Clone	**28-Jul**	**01-Aug**	**12-Aug**	**20-Aug**	**26-Aug**	**02-Sep**	**09-Sep**	**16-Sep**
F_0	n.s	n.s	< 0.0001	n.s	0.001	0.008	n.s	0.029
F_v/F_m	0.01	0.025	n.s	n.s	n.s	n.s	n.s	n.a
Tf_m	0.013	n.s	n.s	n.s	n.s	n.s	n.s	0.046
PI	n.s	0.029	0.001	0.008	0.002	0.007	n.s	n.a
Salt								
F_0	-	n.s	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.003	0.001
F_v/F_m	-	n.s	0.01	0.0001	< 0.0001	0.03	n.s	n.s
Tf_m	-	0.05	0.03	0.004	n.s	0.017	< 0.0001	< 0.0001
PI	-	n.s	n.s	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Salt*Clone								
F_0	-	n.s	n.s	n.s	0.0251	0.0113	n.s	n.s
F_v/F_m	-	n.s	n.s	n.s	n.s	n.s	n.s	n.s
Tf_m	-	n.s	n.s	n.s	n.s	n.s	n.s	0.039
PI	-	n.s	n.s	0.02	0.004	0.048	n.s	n.s

4 Discussion

As the condition in the glasshouse was not completely controlled during all experiments, this likely resulted in substantial variation or noise in the results of experiments running over many weeks due to day-to-day variations in the climate.

4.1 Pre-experiment of *Balanites aegyptiaca*

The pre-experiment served to test germination requirements and showed that the seeds provided can be successfully germinated in sand after soaking in water. In this experiment only few growth parameters were evaluated because of the short duration. It is common that salt reduces growth of shoots and roots of salt sensitive plants (Kramer, 1983) and increases root growth in salt tolerant plants (Chartzoulakis, 2005). In this experiment salt reduced root dry weight while there was no significant reduction in shoot growth or number of leaves which could be attributed to the short duration of the experiment. Generally salt reduces the productivity and growth of plants by reducing photosynthesis which is the vital process in plant growth.

The quantum yield of chlorophyll fluorescence is the proportion of absorbed energy by chlorophyll associated with PSII being used in photochemistry and by measuring the maximum quantum yield of chlorophyll fluorescence or the maximum efficiency of photosystem II (F_v/F_m), information about changes in the efficiency of photochemistry and heat dissipation can be gained (Maxwell and Johnson, 2000). Therefore chlorophyll fluorescence turned out to be a sensitive fast indicator of salt stress and some physiological responses could be investigated after a short time as seen by the strong effect the increasing salt concentrations had on quantum yield of photosystem II and consequently ETR (Fig. 3. 1. 2 and 3.1. 3). Maximum efficiency of photosystem II (F_v/F_m) of healthy un-stressed and dark-adapted leaves often is ca. 0.8 (Maxwell and Johnson, 2000). In the pre-experiment the reduction of the F_v/F_m at close to 0 PAR was surprisingly high also the control plants and very high in salt stressed leaves. This could be because leaves were measured under low light but not in complete darkness and this low external light reduced F_v/F_m.

In salt tolerant *Grewia tenax* seedlings exposed to 0 (control), 40, 80, or 160 mM NaCl, the electron transport rate was reduced with the increase of salt concentration (Hunsche *et al.*, 2010).

4. 2 Effect of nutrient and drought interactions on *Balanites aegyptiaca*

Prolonged drought resulted in reduction of stomata conductance, reduction of water potential and reduction of yield of chlorophyll fluorescence and increased root:shoot ratio. The addition of nitrogen resulted in increased plant growth in *Balanites* (Fig. 3. 2. 5) as nitrogen is a major component of plant chemical components (proteins, nucleic acids; chlorophyll and other organic compounds). Plant dry weight increased with the increased nitrogen in rice, spring wheat and maize (Osaki, 1993). At low water stress nitrogen fertilized *Balanites* had higher stomatal conductance (Fig. 3. 2. 12), which could be due to the increased production of the photosynthetic enzyme Rubisco that has a major role in CO_2 uptake; with prolonged drought there was no effect of added nitrogen. A contradicting result was reported by Liu and Dickmann (1996) where addition of nitrogen to progressively drying soils induced more stomatal closure in two hybrid *Populus* clones. High nitrogen also increased F_v'/F_m' in both provenances before plants experienced water stress (Fig. 3. 2. 8), indicating the increased photosynthetic capacity due to increased nitrogen containing components involved in the light reaction at photosystem II or subsequent steps such as light harvesting Chl-protein complexes, the electron transport and photophosphorylation membrane complexes and the enzymes of the reductive pentose phosphate (RPP) pathway and carbohydrate synthesis.

Generally, potassium had no effect on stomata conductance, except once at low water stress (Table 3. 2. 8) when stomata conductance increased under low potassium. Low potassium increased stomata conductance in olive tree and sunflower and other plants and may be widespread in plants under drought stress, though the physiological mechanism is not understood (Benlloch-González *et al*., 2008). This is surprising because potassium is important for stomatal opening and it was therefore expected that under potassium deficiency, stomatal opening might be impaired, resulting in lower stomata conductance (Mengel and Kirkby 2001).

Potassium is a macronutrient and relative large concentrations in plant dry matter are typically required for plant growth (Epstein and Bloom 2005, Marschner, 1995). If a nutrient solution without K did not negatively affect growth of *Balanites*, this must be due to the fact that there was enough potassium in the planting substrate or stored in the large seeds. Added potassium did not affect plant growth (according to parameters measured), this could be explained according to the nutrient response curve, where high application of K increases plant growth to a point after which any increase in K will not result in increase of growth also will not retard growth.

The root dry weight of Um Abdalla plants was higher in control plants than of Damazin plants (Fig. 3. 2. 6), while the total number of leaves and branches were high in Damazin plants (Fig. 3. 2. 3 and 3. 2. 4). Reduced number of leaves of *Balanites* under drought stress is a mechanism of tolerance where plants shed their leaves to reduce transpiration area.

Declined total dry weight of *Balanites* seedlings under drought stress, could be attributed to the leaf shedding and reduction in shoot growth due to the reduction of synthesis in protein metabolism that suppresses cell division as a result of water deficit. Root:shoot ratio increased under drought in both provenances, as it is commonly observed that water deficit reduced shoot growth before root growth is reduced, resulting in increased root:shoot ratio in moderately water- stressed plants. Mild plant water stress may reduce leaf growth before photosynthesis is reduced, resulting in a surplus of carbohydrates available for root growth (Kramer and Boyer, 1995). When roots experience water stress they loosen their walls and increase cell extension through ABA- induced activity of xyloglucan endotransglycosylase (XET), the enzyme putatively involved in wall loosening, resulting in increased root growth, which is an adaptation to growth in dry soils that allows exploitation of a falling water table (Lambers *et al*., 1998). Rao *et al* (2008) reported increased root: shoot ratio in *Dalbergia sissoo* under very high water stress (irrigation interval of 21 days), which was interpreted as a measure of its tolerance to water stress.

Otieno *et al* (2005) reported increased absolute root growth, root depth and root: shoot ratio of *Acacia tortilis* plants under low soil water content during the dry season in the semi-arid area in Kenya. When seedlings of six *Acacia* species were grown in a site of 50 mm annual rain fall in the region of Riyadh, Saudi Arabia, and subjected to water stress by controlling irrigation once a week, three species (*Acacia asak*, *Acacia negrii* and *Acacia seyal*) showed reduced stem height, diameter, leaf area and dry matter production compared to the other three species (*Acacia ampliceps Acacia karroo*, and *Acacia stenophylla*) which showed greater biomass production (Aref and El Juhany 2005).

Drought reduced plant water potential of *Balanites* seedlings (Fig. 3. 2. 7). Xiloyannis *et al* (1999) reported that olive trees were able to resist water stress by lowering the water content and water potentials of its tissues, allowing the roots to utilize soil water up to -2.5 MPa. *Balanites* water potential became more negative (- 4.23 MPa) with prolonged water stress as a result of dryness of the planting medium. Rao *et al* (2008) reported that *Leucaena leucocephala* maintained higher water potential (-3.1 MPa) under irrigation interval of 21 days which designated as severe stress indicating some drought avoidance, while in *Tectona grandis* and *Albizzia lebbek* water potential declined to -5.0 MPa and -5.5 MPa, respectively under the same water stress, indicating their sensitivity to drought. When comparing the two provenances of *Balanites*, water potential in plants form Um Abdalla became more negative than in Damazin plants, which could be attributed to their stomata closing relatively less than in plants from Damazin (Fig. 3. 2. 11).

Gebrekirstos *et al* (2006) found that *Balanites* (followed by *Acacia tortilis* and *Dichrostachys cinerea*) had a wide diurnal (1.16 – 2.25 MPa) range of plant water potential in the dry season and conclude that they had high capacities to withstand changes in water availability and hence can be considered as a suitable candidates for reforestation in drought prone areas in Ethiopia comparing to *Acacia senegal* and *Acacia seyal* which had a narrow diurnal (0.32 – 1.1 MPa) that categorized them as drought sensitive species.

Stomata conductance declined with increased drought stress and duration (Fig. 3. 2. 11). Under maximum drought at the end of the experiment, stomata conductance was reduced by 62% and 48% compared to irrigated control plants for Damazin and Um Abdalla plants, respectively, (Fig. 3. 2. 11), while Um Abdalla plants had reached a more negative water potential (-4.71 MPa) compared to Damazin plants (-3.74 MPa). This reduction in stomata conductance could be attributed to reduced water at the root area where plants respond to this water deficit by producing abscisic acid, a signal for the stomata to close, therefore reduce the loss of water through transpiration, a mechanism by which plants could tolerate drought stress.

Stomata closure starts when the water potential falls between -0.8 and -1 MPa in some plants and much lower in others (Kramer 1983). Comparing to other tropical drought tolerant plants such as *Leucaena leucocephala*, *Balanites* kept the stomata open until a very negative water potential.

A decline in F_v/F_m at a water deficit of -400 g per pot, which approximately corresponds to a water potential of -2.47 MPa for drought stress plants, and a decline in F_v'/F_m' at the end of the experiment when the water potential was -4.23 MPa for drought stressed plants, indicates a decline in the quantum yield of photosystem II photochemistry and a disturbance in or damage to the photosynthetic apparatus. While F_v/F_m decreased in both provenances, Um Abdalla plants had significantly higher F_v/F_m (Fig. 3. 2. 9) at the end of July and increased g_s at the end of the experiment (Fig. 3. 2. 11), although it had more negative water potential than in plants from Damazin, which indicated higher dehydration tolerance of Um Abdalla plants and more efficient mechanisms of drought avoidance in plants from Damazin. The latter more efficiently avoid very low water potential by closing their stomata. Similar differences were found by Fotelli *et al* (2000) in *Quercus macrolepis* and *Quercus ilex*, where the former responded to drought stress by increased g_s and lower water potential and the latter responded by closing stomata and maintaining a higher water potential.

The reduced values of F_v'/F_m' measured at PAR between 70 – 760 µmol m^{-2} s^{-1} in August (Fig. 3. 2. 10) could be attributed to drought stress and other environmental factors that combined and reduced the efficiency of photosystem II such as increased temperature and radiant heat load on the leaf that lead to increased transpiration to which plants responded by stomata closure in order to avoid water deficit and consequently inhibited photosynthesis. Increased leaf temperatures and rising photosynthetic photon flux density (PPFD) caused reduction in F_v'/F_m' in three alpine trees (Braun and Neuer 2004).

In *Callistemon citrinus,* subjected to water stress by irrigating the plants with half of the water of the control plants, F_v/F_m showed the lowest values (0.71) in drought stress plants compared to (0.78) in control plants (Mugani *et al*., 2009).

Reduced yield of chlorophyll fluorescence and stomatal conductance under drought stress could be attributed to reduced CO_2 uptake, therefore reduced CO_2 assimilation and reduced efficiency of photosystem II. Percival *et al* (2006) reported reduction in F_v/F_m ratio and net CO_2 assimilation rate in *Fraxinus* genotypes under drought stress. Severe drought (where watering stopped for 42 days) significantly affected the primary photochemical processes in Norway spruce needles where F_v/F_m declined by 77% (Pukacki and Kamińska-Rożek 2005).

Although there was a reduction in various physiological processes that affects plant growth such as stomata conductance, chlorophyll fluorescence and plant water potential, more than one third of the drought stressed plants continued developing new leaves after shedding old ones but in a low rate, which indicated that *Balanites aegyptiaca* has the ability to grow under drought stress using different tolerance and avoidance mechanisms.

The two provenances showed different strategies of growth where Um Abdalla plants had increased root:shoot ratio (Fig. 3. 2. 6), increased F_v/F_m (Fig. 3. 2. 9), higher stomata conductance compared to Damazin plants especially with prolong drought stress (Fig. 3. 2. 11) and decreased plant water potential (Fig. 3. 2. 7) and had a stronger reduction in plant biomass (34% in root dry weight and 38% in total dry weight). Plants from Damazin developed more leaves and branches (Fig. 3. 2. 3 and 3. 2. 4) and reduced stomata conductance and showed less reduction in plant biomass (11% in root dry weight and 21% in total dry weight) and higher water potential compared to Um Abdalla. The variation and differences between the tow provenances in their response to drought stress could be attributed to genetic factors as each provenance belonged to a different genecological seed zone according to the classification of Aelbaek and Kenanji (1995).

4. 3 Effect of Salt on *Balanites* and *Populus*

Balanites treated with different concentrations of mixture of salt developed thick, succulent leaves with high water per leaf area, and low specific leaf area (Fig. 3. 3. 1) and also developed small white hairs on the leaf surface (visual observation). The white color could result from salt excreted through these hairs since these white hairs were not visible on leaves of control plants that may have also hairs since leaves of *Balanites aegyptiaca* tend to have trichomes on both leaf surfaces (Radwan, 2007). High water content is a mechanism developed by plants to avoid salt stress by dilution in cell water. Leaves that had reached a too high concentration of salts died and were shed. The amount of light absorbed by a leaf, and the diffusion pathway of CO_2 through its tissues depend, partially, on its thickness (Vile *et al*., 2005).

Some coastal plants, mangroves and xero-halophytes of dry regions develop succulence to dilute the high concentrations of salt; others have salt glands or special trichomes which excrete salt from the epidermal surface of leaves as in *Tamarix* species (Larcher, 1995; Lambers *et al*., 1998). Succulence of shoot was increased in two clones of salt tolerant *Eucalyptus microtheca* with increased NaCl, however at 140 mM NaCl, succulence increased by 90 and 70% in clones 43 and 42, respectively, compared to control (Morabito *et al*., 1994).

The adaptation of *Aster tripolium* to salinity may be accomplished by osmotic adjustment by accumulated Na^+ and Cl^- (Ueda *et al*., 2003). Thick leaves can be attributed to having more layers of mesophyll cells, larger cells, or both (Kozlowsky, 1997), which could have increased the photosynthetic capacity of leaves in salt-treated *Balanites* towards the end of the experiment (Fig. 3. 3. 8). Naz *et al* (2009) reported that *Aeluropus lagopoides* was adapted to salinity by excretion of Na^+ through leaf surface and accumulation of useful ions like Ca^{2+} and K^+ in the shoot, which is certainly advantage of keeping the osmotic balance. Siew and Klein (1968) reported an increase in number and size of mitochondria in succulent leaves of beans treated with 0.2 M NaCl, which could be an emergency regulatory mechanism in order to overcome the detrimental effect of salt, requiring extra energy for salt compartmentalisation and excretion. Gebauer *et al* (2004) reported that salt induced leaf succulence in *Tamaridus indica* treated with different concentration of NaCl. Sohail *et al* (2009) reported increased leaf water content of 14, 16 and 17% of *Ziziphus spina-christi* (L.) treated with 40, 80 and 160 mM NaCl respectively. Ramoliya and Pandey (2002) reported an increase in leaf thickness and decrease in specific leaf area on *Acacia nilotica* seedlings subjected to salt stress.

In spite of the obvious negative effect of salt on old leaves that had grown under salt stress, *Balanites* treated with salt often grew stronger than control plants. A mixture of salts in the proportion used in this experiment could be beneficial to plants, where these salts served as nutrients and are important for maintaining the selective permeability of membranes. Hansen and Munns (1988) reported that $CaSO_4$ counteracted the toxic effect of NaCl resulting in greater plant height, leaf number and biomass of salt treated plants of *Leucaena leucocephala*. Sun *et al* (2001) reported that NaCl treatment greatly increased the Mg uptake rates and increased rate of net photosynthesis and biomass production of stem and roots in *Pinus radiata*. Mg is the central component of the chlorophyll molecule, and is therefore essential in photosynthesis and many other metabolic processes. With increased salt concentration and time, plants under salt treatment shed their leaves and the rate of loss of leaves was higher in plants under high salt concentration.

Leaf chlorophyll content was somewhat higher in plants under salt treatment (Fig. 3. 3. 5) although the difference was not statistically significant. Similar results were observed in *Prosopis articulata* (Garcia-Carreno *et al*., 1992) and in salt tolerant plants such as *Penisetum typhoides* (Reddy and Vora, 1986) and mustard (Singh *et al.*, 1990). Increased chlorophyll content could be attributed to the activity of the oxidative enzymes involved in the biosynthesis of pigments, or simply because the thicker leaves of plants under salinity have a greater amount of photosynthetic pigments per leaf area (Terry and Waldron, 1984).

At the end of the experiment, salt treated plants showed increased net photosynthesis, stomata conductance and intercellular CO_2 (Fig. 3. 3. 8). This could be attributed to the salt avoidance mechanisms developed by plants (modification of leaf anatomy or leaf succulence and the increase in energy and photosynthetic apparatus therefore increased photosynthesis. Radwan *et al* (2000) reported that *Balanites aegyptiaca* seedlings are sensitive to salinity of 12 dS/m while 24 dS/m was harmful to seedling growth where it reduced the photosynthesis and transpiration rate.

Effect of salt on F_v/F_m was seen earlier (in the first month of salt treatment) than on gas exchange, where plants under salt treatment showed increased maximum quantum efficiency of photosystem II and higher performance index (PI) compared to control (Fig 3. 3. 6 & 3. 3. 7) which mean salt had a positive effect on plants where they increased the efficiency of photosystem II and the potential photosynthetic activity of plants. Three weeks after doubling salt concentration, plants at high salt concentration showed reduction in the maximum quantum efficiency of photosystem II, performance index and in the time at which maximum fluorescence was reached (Fig 3. 3. 6 & 3. 3. 7), that indicated the negative effect of salt on the activity of photosystem II and the activity and the number of light and dark reaction centres, also shows that plants started to suffer from salt stress. However, three weeks later plants showed no significant difference in the F_v/F_m, suggesting that *Balanites* adapted to high salt and pointing to the high ability of this species to tolerate salinity. Provenances showed significant differences in the fluorescence parameters with plants from Damazin generally appearing to perform better.

In salt tolerant *Grewia tenax* and moderately salt tolerant *Tamarindus indica* seedlings exposed to 0 (control), 40, 80, or 160 mM NaCl treatments the electron transport rate was reduced in *Grewia* while there was no significant effect on *Tamarindus*, this result showed that the salinity effect on ETR is species and salt concentration dependant (Hunsche *et al*., 2010).

Plants under salt treatment also had higher stems, number of leaves and branches (Fig. 3. 3. 3 & 3. 3. 4), thus the positive effect of salt on photosynthetic parameters was translated to a positive effect on growth.

When comparing the different ages of leaves (young and old leaves) it was clear that at the beginning of salt treatment young leaves performed better in different parameters of fluorescence than old leaves, but with time old leaves could better tolerate salt stress. The reason could be the morphological, physical and physiological modification especially of old leaves, which increased the capability of plants to reduce salt stress. This involves shedding old leaves where the salt concentration had increased too much. Small plants of *Balanites* not only resisted salt stress, but they increased their efficiency and growth at moderate to high salt treatment.

Populus under salt treatment showed loss of old leaves and formation of new leaves; the rate of loss was high on 8 mS plants and the rate of formation and survival of new leaves was high on 4 mS plants. 8 mS were too high under the conditions of the experiment; therefore plants lost leaves and died. While maintaining same salt concentration in substrate and with the continuous evapotranspiration salts will accumulate in leaves that later drop off to eliminate salts from plant.

Salt stress resulted in a moderate reduction of the chlorophyll content, faster in the 8 mS than in the 4mS treatment (Fig. 3. 4. 5), the new leaves that developed in salt stressed plants had much lower chlorophyll content. Reduced chlorophyll content with increased salt concentration was reported by many researchers (Dhanapackiam and Muhammad Ilyas, 2010; Stepien and Klobus, 2006; Al-Sobhi *et al*., 2006; Jamil *et al*., 2007; Zhao *et al*., 2007). The reduction in chlorophyll content could be attributed to the effect of salt in increasing the activity of chlorophyll degrading enzyme (Rao and Rao, 1981), destruction of the chloroplast structure and instability of the pigment protein complexes (Sing and Dubey, 1995). Clone EF50 was more sensitive to salt stress, where it had reduced chlorophyll content.

After one week plants under salt treatment responded to salt stress by reduced stomata conductance and declined C_i compared to control but the photosynthesis (A_{sat}) was not affected (Fig. 3. 4. 6, Table 3. 4. 2), then after three weeks 4mS plants performed better than control, showed higher photosynthesis, stomata conductance and intercellular CO_2. The reason for this could be that the mixture of salts used in the experiment served as nutritious compounds to plants and maintained the selective permeability of membranes. The 8 mS plants showed reduced net photosynthesis and stomata conductance and had increased C_i intercellular CO_2 compared to control. Increased C_i in most plants experienced internal water deficits that resulted in stomata closure and inhibition of photosynthesis indicated that the use of C_i by the enzymes involved in photosynthetic carbon assimilation and reduction was inhibited more than the supply of CO_2 to the leaf interior (Berkowitz, 1998), while the increased g_s and C_i in 4mS plants after one month could be due to the damage of photosynthetic system and reduced chlorophyll content because of salt stress, where plants cannot use much of the CO_2 entering through the stomata. The reduction in photosynthesis under salinity can be attributed to a decrease in chlorophyll content (Delfine *et al*., 1999) and stomata closure, depression of specific metabolic processes in the carbon uptake and inhibition in photochemical capacity (Dubey 1997).

Sixto *et al* (2006) reported a significantly declined net photosynthesis and stomatal conductance at high salinity levels on five *Populus alba* clones, similar results were reported by Fung *et al* (1998) on four poplar clones and by Zhao *et al* (2007) on Naked Oat (*Avena sativa*). In new leaves of 4mS plants lower A_{sat} and higher C_i indicate that A_{sat} is limited by biochemistry rather than reduced stomatal conductance. Clone EF50 was more sensitive to salt stress where it showed the lowest net photosynthesis and stomata conductance.

Chlorophyll fluorescence is often used as a sensitive fast indicator of stress including salt stress. There was marginal significant effect on T_{fm} (not on other parameters) after 4 days and highly significant effect on most parameters after 15 days (Fig. 3. 4. 7). In 8 mS F_v/F_m dropped substantially below 0.8 after 23 days. With 4 mS dropped only a bit, more in clone EF50 (which had lowest A_{sat}). Maximum efficiency of PSII (F_v/F_m) of healthy leaves often is 0.8 (Maxwell and Johnson, 2000).

Plants at 8 mS showed increased F_0 and decreased F_v/F_m, performance index (PI) and Tf_m, while control and 4mS plants had increased F_v/F_m, although there was significant difference between different treatments on F_v/F_m but the range was above 0.8 at the beginning of the salt treatment, with time F_v/F_m of 8 mS was decreased to 0.55 which indicated reduction of the efficiency and photo-inhibition of PSII. Plants also showed reduction of PI that indicated a reduction in the potential photosynthetic activity due to salt stress, where PI combines the density of working photosystems (reaction centres per chlorophyll) with the performance of both the light reactions and the dark reactions.

Increase of F_0 associated with a corresponding decrease of F_v/F_m and energy dissipated via photochemical pathway could be attributed to decrease of photosynthesis, dissociation of the light-harvesting chlorophyll complexes from the reaction centre of PSII (Yamane *et al*., 2000) and to photoinhibition due to limitations on photochemical quenching in the light (Bolhar-Nordenkampf and Öquist, 1993). Gao Jian and Peng Zhen-hua (2006), reported that PSII functions under salt stress were significantly inhibited with time. Jamil *et al*. (2007) and Ranjbar (2006) reported decreased F_v/F_m on high salt concentration.

The fastest chlorophyll fluorescence parameter that shows the effect of salt on plants was the Tf_m that was significantly reduced in three days of salt treatment. Also on the last measurements 4 mS plants reached the maximum fluorescence earlier than the control and the reduced Tf_m could be attributed to the age of leaves, as the measured leaves were small new leaves, as salts accumulated with time as long as the leaves transpired, the new leaves could not have high salt concentration and the effect on T_{fm} could attributed to low chlorophyll content compared to old mature leaves in control plants.

Clones were significantly different on the F_0 and PI parameters in most of the measurements, where clone EF50 had the highest F_0 and the lowest PI which indicated the sensitivity of this clone to salt stress than the other two clones.

Salt reduced shoot, root and total dry weight of plants (Fig. 3. 4. 2). At 4 mS shoot and root weight were reduced by 26% and 39%, respectively, relative to control, at 8 mS final shoot and root biomass reduction was 39% and 52% respectively, relative to control. Root:shoot ratio was decreased in plants under salt stress because root growth was reduced more than shoot growth and this could be due to the allocation of carbohydrates to production of new leaves that developed in plant under salt stress. Clones were significantly different in stem height on August where clone EF50 had the lowest stem height. However, reduced plant biomass could be due to reduced chlorophyll content, stomata conductance and photosynthesis and the reduced efficiency of photosystem II and also loss of leaves as a result of salt. Watzinger (2004) found increased shoot biomass and reduced root biomass of *Populs nigra* and three *Salix* species irrigated with landfill leachate of the municipal solid waste landfill Rautenweg in Vienna/ Austria, of 9.9 mS/cm electric conductivity and approximately the same proportions of salts as in our experiment. Watzinger (2004) reported that *Populus nigra* plants did not lose their leaves which is contradictory to our results where plants lost their leaves and had reduced shoot biomass, since Watzinger (2004) used a mixture of compost and sand, that could explain the difference due to the effect of the solid phase of the soil where cation exchange complex, also the pore size distribution can affect the availability of nutrients in the soil solution, matric potential and water uptake.

Plant total dry weight was significantly decreased in *Ceiba pentanda* seedlings at salt concentration of 12 and 15 dSm^{-1}, while seedlings under 9 dSm^{-1} were less affected, however seeds germinate and seedlings tolerate the salinity of up to 9 dSm^{-1} (Immanuel and Ganapathy, 2007). Plant dry weight was greatly reduced with increased NaCl concentration in two naked oat (*Avena sativa* L.) genotypes subjected to different salt concentrations ranging between 0, and 250 mM NaCl (Zhao *et al*., 2007).

Decreased root: shoot ratio was reported in Safflower (*Carthamus tinctoriusl*) where root growth was severely inhibited by salt (Demir and Arif 2003). The root, shoot and root: shoot ratio was reduced with increased salt concentration in seedlings of *Butea monosperma* subjected to NaCl at 0.3, 1.9, 3.9 and 6.2 dSm–1, (Hirpara *et al*., 2005).

6 Conclusion

Balanites aegyptiaca and *Populus nigra* are both multipurpose tress. Although they grow under different climates, they face similar physiological stress through drought and salts. The aim of this work was to study the physiological and morphological performance of *Balanites* seedlings and *Populus* cuttings under drought and salinity stress and to identify stress-resistant varieties that can withstand such stresses and help in conservation and cultivation of degraded land.

Since the condition in the glasshouse was not completely controlled during all experiments, this likely resulted in substantial variation or noise in the results of experiments running over many weeks due to day-to-day variations in the climate.

In the pre-experiment with *Balanites,* where only one salt (NaCl) was used, plants under high salt were strongly affected after a short time and salt strongly affected quantum yield, electron transport rate and root dry weight.

Balanites and *Populus* plants treated with mixture of salts (NaCl, $CaCl_2$ and $MgSO_4$) showed different response to salt. In *Balanites*, plants developed thick, succulent leaves with high water per leaf area and low specific leaf area and also developed small hairs on the leaf surface. Leaf chlorophyll content was somewhat high in plants under salt treatment. The effect of salt was seen earlier (in the first month of salt treatment) in F_v/F_m than in gas exchange, with plants under salt treatment showing increased maximum quantum efficiency of photosystem II and higher performance index (PI) compared to control. After doubling salt concentration, plants at high salt concentration showed a temporal reduction in the maximum quantum efficiency of photosystem II, performance index and in the time at which maximum fluorescence was reached (Tf_m), but with time the plants did not differ in F_v/F_m and also increased net photosynthesis, stomata conductance and intercellular CO_2. This shows that *Balanites* has a high ability to tolerate salinity. Old leaves appeared to tolerate salt stress better than young leaves because of their morphological, physical and physiological adaptation. Adaptations for high salt tolerance in *Balanites* include developing leaf succulence, small hairs on the surface that may aid in salt excretion, and the shedding of old leaves where salts have accumulated. Thus, according to the results of this experiment, *Balanites* could be a useful plant to be grown in saline areas where salinity is not more than 16 mS/cm.

When comparing the two provenances Damazin plants tolerated salinity better than Um Abdall plants and showed higher growth (stem height, number of leaves and branches) and increased efficiency of photosystem II as indicated by $F_{v/}F_m$, Tf_m and PI. Apparently, the mixture of salts (NaCl , $CaCl_2$, $MgSO_4$) used in the second experiment was more favourable for *Balanites* than NaCl alone.

Unquestionably, these greenhouse experiments should be followed by field trials to study the germination and seedlings development of different provenances under different soils, different salt concentrations and different nutrients, supported by physiological measurements such as chlorophyll fluorescence, gas exchange, plant water potential and leaf water content followed by nutrients chemical analysis to test the ion toxicity to plants.

Populus responded differently to salt stress according to the different physiological parameters measured. High salt concentration induced reduction in most evaluated chlorophyll fluorescence parameters, reduced chlorophyll content, reduced stomata conductance and declined intercellular CO_2 (C_i) and also reduced plant biomass compared to control. While plants tolerated low salt concentration up to three weeks, at longer treatment the damaging effect of salt on leaves was pronounced and reflected in the chlorophyll fluorescence parameters. Plants under salt treatment dropped old leaves but formed new leaves with the rate of loss higher at high salt concentrations and the rate of formation and survival of new leaves higher at low salt concentrations. Also *Populus* clones differed in their response where clone EF50 was the most sensitive to salinity, thus clone LO14 and LO50 appear better suited to be grown on landfills sites or saline areas, provided that irrigation water is < 8 mS/cm, which is lower than reported for landfill leachate.

For future research field trials on landfills site are recommended that should be accompanied by physiological parameters including the plant water status (water content, water potential and leaf osmotic potential), gas exchange and chlorophyll fluorescence measurements in addition to a chemical analysis of plants.

Prolonged drought reduced growth of *Balanites* seedlings by interfering and suppressing the physiological processes that affect the assimilation of CO_2. Plants showed reduced stomata conductance, declined quantum yield of photosystem II and a disturbance in or damage to the photosynthetic apparatus and in the end reduced total dry weight and increased root: shoot ratio.

Nitrogen resulted in increased plant biomass and increased photosynthetic capacity in both provenances indicating increased nitrogen containing components involved in the light reaction at photosystem II or subsequent steps such as light harvesting Chl-protein complexes, the electron transport and photophosphorylation membrane complexes and the enzymes of the reductive pentose phosphate (RPP) pathway and carbohydrate synthesis. Generally, potassium had no effect on stomata conductance, except once at low water stress when stomata conductance increased under low potassium.

The two provenances showed different strategies of growth where Um Abdalla plants had increased root:shoot ratio, increased F_v/F_m and higher stomata conductance and decreased plant water potential and had a stronger reduction in plant biomass (34% in root dry weight and 38% in total dry weight). By comparison, plants from Damazin developed more leaves and branches, had lower stomata conductance and showed less reduction in plant biomass (11% in root dry weight and 21% in total dry weight) and a higher water potential. This indicates a higher dehydration tolerance of Um Abdalla plants and more efficient mechanisms of drought avoidance in Damazin plants.

Although the negative effect of drought stress was seen in various physiological processes, that affect plant growth, more than one third of the drought-stressed plants continued developing new leaves after shedding old ones, albeit in a low rate. Different tolerance mechanisms such as reduced stomata conductance to reduce water loss and reduced transpiration area by developing small new leaves and shedding of the old leaves and increased root/shoot ratio enable *Balanites* to survive drought.

It would be useful for this greenhouse experiment to be followed by field trials comparing a range of provenances of *Balanites* under different water stress regimes and testing the effect of different levels of fertilizers on drought stress tolerance. It would be useful when combining water and salt stress in the field, stress effects and plant responses should be evaluated by measuring different parameters such as the gas exchange, chlorophyll fluorescence, plant water potential and plant biomass.

7 References

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8 Abbreviations

A_{sat}	Photosynthesis under saturating light
ABA	Abscisic acid
AIT	Austrian Institute of Technology
ANOVA	Analysis of variance
CAM	Crassulacean acid metabolism
C_i	Intercellular CO_2
DW	Dry weight
EC	Electrical conductivity
EDTA	Ethylene Diamine Triacetic Acid
EF	Efferding, location in Upper Austria
ETR	Electron transport rate
F_0	The initial fluorescence that occur at time 0
F_m	Maximum fluorescence
FTSCS	Forest Tree Seed Centre Soba-Sudan
F_v	Variable fluorescence
F_v/F_m	Maximum efficiency of photosystem II
FW	Fresh weight
g_s	Stomata conductance
L	Leaf age
LA	Leaf area
LO	Lobau locations in Vienna / Austria
MPa	Mega Pascal
MSW	Municipal Solid Waste
OP	Osmotic pressure
PAM	Pulse- Amplitude-Modulation
PAR	Photosynthetic Active Radiation
PEA	Plant efficiency analyser
PI	Performance index
PPFD	Photosynthetic photon flux density
ppm	Parts per million

PSII	Photosystem II
S	Salt treatment
SAS	Statistical Analysis Software
SD	Standard deviation
SE	Standard error
SLA	Specific leaf area
TDI	Total dissolved ions
TDS	Total dissolved solids
Tf_m	The time at which the maximum fluorescence value (F_m) is reached
TOC	Total organic carbon
XET	Xyloglucan endotransglycosylase enzyme
Ψ	Plant water potential

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