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**Prosopis juliflora as a Bio-indicator of Dryland Salinity  
in Central Myanmar**



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ကန္တာစိမ်းသစ်မျိုးအား မြန်မာနိုင်ငံအလယ်ပိုင်းဒေသ၏မြေဆီလွှာဆားငန်ဓါတ်ညွှန်းပြုနိုင်သော သက်ရှိညွှန်းကိန်းအဖြစ် လေ့လာခြင်း

ဒေါက်တာညွန့်ခိုင်  
လက်ထောက်ကထိက  
သစ်တောတက္ကသိုလ်

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စာတမ်းအကျဉ်း

မြောက်သွေ့ခြင်းကဲ့သို့ပင် မြေဆီလွှာဆားငန်ဓါတ်များခြင်းသည်လည်း ပူပြင်းမြောက်သွေ့သော ဒေသများ တွင်ကြုံတွေ့ရလေ့ရှိသည့် အဓိကပြဿနာများထဲမှတစ်ခုဖြစ်ပါသည်။ ဒေသမျိုးရင်း သစ်မျိုးများ၏ မြေဆီလွှာဆားငန်ဓါတ်လွန်ကဲခြင်းအပေါ် လိုက်လျောညီထွေရှင်သန်နိုင်စွမ်းအားသည် အကန့်အသတ်ရှိနေပါသည်။ ကန္တာစိမ်းသစ်မျိုးသည် မြေဆီလွှာဆားငန်ဓါတ်လွန်ကဲခြင်းကို ခံနိုင်ရည်ရှိသော သစ်မျိုးဖြစ်ပြီး မြန်မာနိုင်ငံအလယ်ပိုင်းဒေသ နေရာတော်တော်များများတွင် ပေါက်ရောက်လျက်ရှိပါသည်။ ၎င်းသစ်မျိုးအား လမ်းဘေးဝဲယာများ၊ ရေစီးဆင်းရာမြစ်ချောင်းကမ်းပါးတစ်လျှောက်နှင့် စွန့်ပစ်ထားသည့် မြေလွတ်မြေရိုင်းများတွင်တွေ့ရှိရပါသည်။ မြန်မာနိုင်ငံအလယ်ပိုင်းဒေသသည် သစ်တောပြုန်းတီးမှုနှင့် မြေဆီလွှာဆားငန်ဓါတ်ပြဿနာပေါ်ပေါက်ရန်အလား အလာရှိနေသောကြောင့်၊ ကန္တာစိမ်းသစ်မျိုး၏ မြောက်သွေ့ဒေသမြေဆီလွှာဆားငန်ဓါတ်အပေါ် တုန်ပြန်နိုင်စွမ်းနှင့် လိုက်လျောညီထွေရှင်သန်နိုင်စွမ်း အားနားလည်ခြင်းသည် ၎င်းသစ်မျိုးပြန့်ပွား မှုအပေါ်၌ မြေဆီလွှာဆားငန်ဓါတ်အပေါ် လိုက်လျောညီထွေ ရှင်သန်နိုင်စွမ်း၏ လွှမ်းမိုးမှုအား ခန့်မှန်းရာတွင် အလွန်အရေးပါလာပါသည်။ ယခုလေ့လာမှုသည် ကန္တာစိမ်းသစ်မျိုး၏ အမြင့်ဆုံးမြေဆီလွှာဆားငန်ဓါတ်နှင့် လိုက်လျောညီထွေရှင်သန်နိုင်စွမ်းအားကို အကဲဖြတ်နိုင်ရန်အတွက် မြေဆီလွှာဆားငန်ဓါတ်နှင့် ကန္တာစိမ်းသစ်ပင်အတွင်းဆားငန်ဓါတ်တို့အကြား ဆက်သွယ်မှုကိုတန်ဖိုးဖြတ်ရန်၊ ကန္တာစိမ်းသစ်မျိုး၏ ပြန့်ပွားမှုဖြစ်စဉ်အား မြေဆီလွှာဆားငန်ဓါတ်အပေါ် ခံနိုင်ရည်ရှိမှု အထူးပြုဖော်ပြရန်နှင့်ကန္တာစိမ်းသစ်မျိုးပြန့်ပွားမှုကိုထိ ရောက်စွာစီမံအုပ်ချုပ်ရာ၌ လေ့လာ တွေ့ရှိချက်များအား အထောက်အကူအဖြစ် အသုံးပြုနိုင်စေရန် ရည်ရွယ်လုပ်ဆောင်ခြင်းဖြစ်ပါသည်။ လေ့လာရန် သစ်မျိုးအနေဖြင့် ကန္တာစိမ်းသစ်မျိုးနှင့် ဆိုဒီယမ်ကလိုရိုက်၊ ဆိုဒီယမ်ကာဗွန်နိတ် ဆားအမျိုးအစားများအသုံးပြု၍ ကန္တာစိမ်းသစ်မျိုး၏ မြောက်သွေ့ဒေသမြေဆီလွှာဆားငန်ဓါတ်အပေါ် တုန်ပြန်မှုအားလေ့လာခဲ့ပါသည်။ ကန္တာစိမ်းပျိုးပင်ငယ်သည် မြေသားကြားခံတွင် ကောင်းစွာရှင်သန် လာသောအခါ၊ ပျိုးပင်ငယ်၏အရွက်ဟောင်းများ စတင်ညှိုးရော်လာသည်အထိ ပြင်းအားအမျိုးမျိုးရှိသော (၀.၁၂၄ မှ ၀.၅ M  $\approx$  ၀.၆ မှ ၂.၅ MPa) ဆိုဒီယမ်ကလိုရိုက်ဆားပျော်ရည်အား (၂-၃) လကြာထည့်သွင်း စမ်းသပ်ခဲ့ပါသည်။ မြေကြီးနမူနာနှင့်အတူ သစ်ရွက်နမူနာများအား စုဆောင်းခြင်း၊ စုဆောင်းရရှိသော နမူနာများအား အခြောက်ခံခြင်း၊ အမှုံကြိတ်ခြင်းနှင့် ဆားငန်ဓါတ်ပါဝင်မှုအားလေ့လာခဲ့ပါသည်။ ကန္တာစိမ်း သစ်မျိုးသည် ဆိုဒီယမ်ကလိုရိုက်၊ ဆိုဒီယမ်ကာဗွန်နိတ်ဆားအမျိုးအစားများအပေါ်တွင် တုန်ပြန်မှု မတူညီကြောင်းတွေ့ရှိရပါသည်။လေ့လာတွေ့ရှိချက်များအရ ကန္တာစိမ်းပျိုးပင်ငယ်၏ အရွက်အတွင်းရှိ ဆားငန် ဓါတ်ပါဝင်နှုန်းနှင့် မြေဆီလွှာအတွင်းရှိဆားငန်ဓါတ်ပါဝင်နှုန်းများအကြား ထင်ရှားသိသာသော ကွာခြားမှုမရှိ ကြောင်းတွေ့ရှိရပါသည်။ ဤလေ့လာမှုမှ သစ်ပင်များ၏ အပြည့်အဝပြန့်ကားသော အရွက်အတွင်းရှိ ဆားငန်ဓါတ်သည် မြေဆီလွှာဆားငန်ဓါတ်ကို ဖော်ပြနိုင်သော အညွှန်းကိန်းအဖြစ် အသုံးပြုရန်သင့်လျော်ကြောင်း အကြံပြုပါသည်။

***Prosopis juliflora* as a Bio-indicator of Dryland Salinity in Central Myanmar**

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**Abstract**

Along with drought, soil salinity is one of the major problems encountered in the dryland environment. The adaptability of native tree species to soil salinity has been limited. *Prosopis juliflora* is a salt-tolerant species that colonizes many parts of central Myanmar. It grows along roadsides, water courses, and in the wastelands. Due to the potential for deforestation and soil salinization in the area, an understanding of *Prosopis juliflora*'s responses and adaptability to dryland salinity become important in investigating the influences of salinity adaptation on invasiveness. This study aims to evaluate the relationship between soil salinity and plant osmotic potentials in order to assess the maximum adaptations of *Prosopis juliflora* to soil salinity, to delineate the invasion of the species with special reference to salinity tolerance, and contribute to the effective management of the *Prosopis juliflora* invasion in central Myanmar. *Prosopis juliflora* (Sw.) DC. (Fabaceae) was selected to analyze the correlation of soil and plant responses in relation to soil salinity. This study selected two compounds of sodium salts, sodium chloride (NaCl) and sodium carbonate (Na<sub>2</sub>CO<sub>3</sub>), to estimate *Prosopis juliflora*'s responses to dryland salinity. When the plants have adapted well to the sand medium, the application of different concentrations (0.125 to 0.5 M  $\approx$  0.6-2.5 MPa) of ionic osmotic NaCl solution was continued for 2-3 months until the oldest pair of leaves began to wilt. The leaf samples were harvested at the same time as the soil samples from the pot were collected; both were dried and pulverized, and their osmotic potentials were measured. *Prosopis juliflora* differently responded to NaCl and Na<sub>2</sub>CO<sub>3</sub>. The ANOVA revealed no significant differences between plant internal osmotic potentials (midday and saturated) and soil osmotic potential for NaCl application in the commercial potting soil medium. We suggest that the saturated osmotic potential of fully expanded leaves can serve as an indicator of rhizosphere solute concentration.

Key words: water potential, osmotic potential, salt-tolerant species, adaptation, hydroponic system

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## *Prosopis juliflora* as a Bio-indicator of Dryland Salinity in Central Myanmar

### 1. Introduction

Along with drought, soil salinity is one of the major problems encountered in the dryland environment (Ashraf and Harris 2004). Salinity by either natural causes or anthropogenic activities currently affects approximately 7 % of the total land areas of the world (Munns 2002), but this percentage is continually rising (Munns 2011). Primary salinization is driven by natural process, including the long-term accumulation of salt in the soil and groundwater (Munns 2009; Duncan et al. 2009). Secondary salinization is the results of several human-induced activities, among them the irrigation of saline water with poor drainage systems (Orcutt and Nilsen 2000) and the replacement of deep-rooted perennial native plants with shallow-rooted annual crops (Barrett-Lennard 2003; Munns 2009; Duncan et al. 2009; Vinod 2011).

Dryland salinity results from secondary salinization (Duncan et al. 2009). Plants lose 99% of water in transpiration, and use only about 1% of the water they absorbed. Deep-rooted perennial plants utilize almost all the rainwater, and some leach dissolving salts from the soil medium and accumulate them below the rhizosphere, preventing the groundwater table from recharging the saline water. Such plants keep the groundwater tables low enough away from the subsurface and maintain the hydrological balance between input and output. In direct contrast, shallow-rooted plants take up a smaller quantity of rainwater than the deep-rooted ones do. The excess rain water washes soluble salts from the root zone, causing saline water to seep into the groundwater, which in turn forces the water tables upwards. Evaporative demands and capillary actions enhance the continuous upward movement of these tables. When water transpires from the soil surface, the dissolved salt accumulates in the sub-soils. In addition, a hydrological imbalance between precipitation and evapotranspiration accelerates the secondary salinization process in the dryland environment (Tanji 2002; Munns 2009; Duncan et al. 2009). Thus far, the adaptability of native tree species to soil salinity has been limited.

The *Prosopis juliflora* invasion appears severe in both the salt-affected degraded lands and those abandoned agricultural areas which are now wastelands, where the mass germination of the species is assured by grazing cattle. *Prosopis juliflora* is a salt-tolerant species (Heuperman et al. 2002) that colonizes many parts of central Myanmar. It grows along roadsides, water courses, and in the wastelands. The results of this study reveal that no individual of this species invade the primary natural dry deciduous forests of central Myanmar: however, its expansion potential appears highest in degraded environments (Platt et al. 2003).

The results of the field measurements in Nyunt Khaing's (2013) indicated that *Prosopis juliflora* had a higher adaptability to salinity. These in situ findings were proven by conducting two independent experiments using a potometer (automatic water refilling device, hydroponic system) and commercial potting soils in the greenhouse under control conditions. In the potometer, a total of 12 individuals of two-year-old *Prosopis juliflora* seedlings ( $\approx 10$  cm height) were transplanted in summer. In a parallel experiment, 2-year-old *Prosopis juliflora* seedlings in commercial potting soils were utilized.

Due to the potential for deforestation and soil salinization in the area, an understanding of *Prosopis juliflora*'s responses and adaptability to dryland salinity become important in investigating the influences of salinity adaptation on invasiveness. Despite the fact that most scientific works have focused on areas concerning germination and productivity (Khan et al. 1987; Ahmad et al. 1994; Singh et al. 1994; Viégas et al. 2004; El-Keblawy and Al-Rawai 2005) in relation to salinity and plant

water use (Tomar et al. 2003), little is known regarding the correlation between plant internal osmotic potential and soil salinity.

## 2. Objectives

This study aims to evaluate the relationship between soil salinity and plant osmotic potentials in order to assess the maximum adaptations of *Prosopis juliflora* to soil salinity, to delineate the invasion of the species with special reference to salinity tolerance, and contribute to the effective management of the *Prosopis juliflora* invasion in central Myanmar.

## 3. Materials and methods

### 3.1 Study species selection

*Prosopis juliflora* (Sw.) DC. (Fabaceae) was selected to analyze the correlation of soil and plant responses in relation to soil salinity. The species was first introduced to central Myanmar in 1953 and has since become a noxious weed in the area. Well-adapted to higher soil salinity and drought, both of which are common to dryland environments, it is possible that *Prosopis juliflora* naturally regulates the ecophysiological processes by using superficial roots systems, the roots in these systems are well developed and occur in both the lateral and tap-root, the latter of which can grow roots up to 53 m (in the Sierrita Mountains of Arizona, USA) (Phillips 1963; Meyer et al. 1971; Canadell et al. 1996; Felker 2009; Ward 2009) in the soils. The decision to take soil samples from the active root zone was therefore a difficult one. Cells at the root tip are able to sense ion concentrations of intact soil mediums (Lynch 1995; Malamy 2005) and produce active roots at the growing medium of low concentration (Heuperman et al. 2002) to avoid higher salinity (Abrol et al. 1988; Lyuch 1995; Malamy 2005; Baluška and Mancuso 2009). In situ measurements under field conditions are time consuming, and their reliability is questionable.

In our research, the *Prosopis juliflora* (Sw.) DC. of central Myanmar revealed the highest adaptation to soil salinity (4.4 MPa  $\approx$  880 mM NaCl) with a mean minimum midday leaf osmotic potential of -5.4 MPa ( $\approx$  1080 mM NaCl). It is both highly tolerant of soil salinity in its natural habitat (Pasicznik 2001) and very adaptable to soil alkalinity (Pasicznik 2001). Singh et al. (1994) and Heuperman et al. (2002) suppose the notion that *Prosopis juliflora* (Sw.) DC. can generally endure soil salinity up to 35 dSm<sup>-1</sup>; nevertheless, salinity adaptation varies depending on the provenance. Khan et al. (1987) report that *Prosopis juliflora* (Sw.) DC. on the Makran coast of Pakistan resists soil salinity of >35 dSm<sup>-1</sup>. Independent research from Ahmad et al. (1994), Velarde et al. (2003) and Felker (2009) indicate that *Prosopis juliflora* exhibited good growth at high soil salinity levels, equivalent to 45 dSm<sup>-1</sup> ( $\approx$  450 mM NaCl).

Central Myanmar is one of the largest flat plains where the natural imbalance between precipitation and evapotranspiration is higher during the dry season. As the deforestation rate in this area is relatively high, the secondary salinization process is common. Plant's responses to soil salinity as well as the correlation between plant internal potential and soil solute concentrations were therefore evaluated with special reference to the high salinity tolerant species, *Prosopis juliflora*.

### 3.2 Salt solution application

According to Yee (2009), approximately 1.4% of the total land area in Myanmar is salt-affected, corresponding to primary and secondary salinization. Secondary salinization and/or

alkalinization are both common in central Myanmar, although, the latter is limited to some areas dominated by dark compact soils (Vertisols). By definition, salt-affected soils are those with concentrations of soluble salts high enough to sufficiently hamper plant development (DeSutter 2008). Despite the fact that soluble salts are diverse in soils with ions compositions of sodium, calcium, magnesium, potassium, sulfate, chloride, and carbonate (Orcutt and Nilsen 2000; DeSutter 2008), sodium salts are the most soluble salts (Munns and Tester 2008; Munns 2009) occurring abundantly in nature (Hogg and Bickel 1941; Rycroft and Amer 1995; Munns and Tester 2008). Sodic soils (alkaline soils) refer to soils containing highly soluble sodium (Fitzpatrick et al 1994; Tanji 2002), and black alkaline soils develop in response to the presence of sodium carbonate ( $\text{Na}_2\text{CO}_3$ ) (Essington 2003). Stamp and Lord (1923) assume the occurrence of  $\text{Na}_2\text{CO}_3$ -dominating alkaline soils in central Myanmar in areas of < 900 mm of mean annual rainfall. This study therefore selected two compounds of sodium salts, sodium chloride (NaCl) and sodium carbonate ( $\text{Na}_2\text{CO}_3$ ), to estimate *Prosopis juliflora*'s responses to dryland salinity. Unless otherwise stated, sodium chloride (NaCl) was used in hydroponic sand culture due to time constraints.

### 3.3 Greenhouse experiments and data analysis

In May of 2010, *Prosopis juliflora* seeds were sown in a media of commercial potting soil in a greenhouse in Göttingen, Germany. After germination, seedlings with one pair of leaves were transplanted into a potted soil medium for 24 months. In May of 2011, 12 individual two-year-old *Prosopis juliflora* seedlings ( $\approx 10$  cm) were transplanted into photometer (Figure 3.1). A potometer is an automatic water refilling device constructed on the basis of hydroponic system, in which a continuous supply of water is provided to minimize the effects of water stress to plant.

When the plants have adapted well to the sand medium, different concentrations (0.125 to 0.5 M  $\approx$  0.6-2.5 MPa) of ionic osmotic NaCl solution and a standard dosage of fertilizers (NPK + trace elements) were applied. The NaCl-application was carried out for 2-3 months with the concentration level being steadily increased to avoid osmotic shock. When the older leaves of the sampled plants showed the symptom of osmotic effects and began wilting, the leaf samples were harvested along with the soil water solution through the filler tube. The soil water solution collected from the tube was directly measured with the semi-micro osmometer (Knauer, Germany).

The sampled leaf materials were dried and pulverized, and the leaf osmotic potentials measured. In Göttingen, laboratory experiments were performed to measure the plants and soil osmotic potentials cryoscopically using semi-micro osmometer (Knauer, Germany). For midday osmotic potential measurement, fresh leaves (5-10 g) were weighed using a precision balance. The leaf samples were well dried in an oven (Memmert, Germany) at 105 °C for 12 hours before being pulverized in a grinder (Fritsch, Germany). For osmotic potential measurement, each 1 g ( $\approx$  1 g) of leaf powder was mixed with demineralised water (dilution  $\times 7$ ) to make the solution dilute. Then, the output solution was stored in a water bath at 55 °C for 24 hours, and centrifuged with 4000 rpm for 15-20 minutes to separate the solute and solution. The finalized solution was stored for further analysis. About 0.15 ml solution for one sample was cryoscoped twice and its raw data from semi-micro osmometer (Knauer, Germany) was recalculated for late study. A total of 2-replications per sample sap were cryoscoped. The results from the freezing point osmometry were recalculated using the following equations developed by Kreeb (1990) (Mitlöhner and Köpp 2007):

$$\Psi_{\pi} = 0.1013 \times [0.021 (\Delta_t)^2 - 12.06 \Delta_t] \dots\dots\dots (\text{Kreeb 1990});$$

$$\Psi_{\pi, t} \text{ } ^\circ\text{C} = \Psi_{\pi, 0} \text{ } ^\circ\text{C} (1 + t \text{ } ^\circ\text{C}/273) \dots\dots\dots (\text{Kreeb 1990})$$

Where:  $\Delta_t$  is the depression at freezing point;  $\Psi_{\pi,0}$  °C is the osmotic potential at 0 °C; and  $\Psi_{\pi,t}$  °C is the osmotic potential at t °C. According to Kreeb (1990), the expected error in the whole measurement processes is approximately  $\pm 2$  bar (0.2 MPa).

To measure the saturated osmotic potential, sampled plants were harvested, labelled, and allowed to rehydrate overnight. Steponkus et al. (1982) and Mitlöhner and Köpp (2007) speculate that overnight rehydration may not affect the existing osmolytes in the leaf tissues, and little variation was expected among individuals of the sampled plants. Babu et al. (1999) suggests that the rehydration process is a qualitative measurement to quantify compatible solutes in stressed plants. The cutting edges of the branches were put into a water vessel by covering them with a black plastic bag to interrupt respiration during the rehydration process. The branches absorbed water and became re-saturated overnight; the saturated leaf samples were then cut from the branches, balanced, dried, and pulverized. The procedures involved were as described in the calculation of the midday osmotic potential measurement. Here, we assume that leaf osmotic potentials resulted from the hydroponic culture as the saturated osmotic potentials.

In a parallel experiment, ionic osmotic solutions of NaCl and Na<sub>2</sub>CO<sub>3</sub> (0.125 to 0.5 M  $\approx$  0.6-2.5 MPa) were applied to 2-years-old *Prosopis juliflora* seedlings in commercial potting soils. This salt solution application was continued for 2-3 months until the oldest pair of leaves began to wilt. The leaf samples were harvested at the same time as the soil samples from the pot were collected; both were dried and pulverized, and their osmotic potentials were measured.

A linear regression analysis was performed in STATISTICA for Windows (Version 9.0, Statsoft, Inc., Tulsa, USA) to test the correlations between the leaf- and soil osmotic potentials. The one-way analysis of variance (ANOVA) and Turkey's test were conducted to analyze the significant differences between plant internal osmotic potentials and cryoscopic soil osmotic potential. The diurnal variation between midday and predawn water potential values were treated as the relaxation range of the plants from midday water stress. The midday and predawn values of the selected tree species were compared separately in graphs for a particular sampling site. A 45° bisecting line was created on the graph to mark the threshold value of tree species' water stress.

The bisecting line approach was based on Slatyer's (1957) model (Figure 3.2) which demonstrates the permanent wilting of the plant and soil. As a result of low water potential in soil, plant water potential progressively decreases while the predawn values rise to match the midday potential. At this point, the plant root ceases to extract water extraction from the soil mass (Slatyer 1957), and plant growth diminishes. In the case of dry forest plants in the dry season, available water is already restricted due to a soil water deficit; the remaining water is strongly bound to the soil particles, and the plants must expend energy in order to extract it. Midday water potential values consequently become more negative, the overnight recovery process slows, and the predawn water potential approaches midday potential. Here, both the predawn and midday values progress towards the bisecting line, potentially characterizing the ability of tree species of a particular forest stand to withstand water stress in the dry season.

The bisecting line also represents the permanent wilting point of the plant, where plant metabolism processes fail, causing the plants to die off (Mitlöhner 1997a) if there is no further rain or available water. Based on the relative position of the values on the bisecting line on the graph, the maximum negative water potential values of a tree species for a particular site can be described by extrapolation (Mitlöhner 1997a). A paired t-test (Zar 2010) was used to compare the mean values of predawn and midday leaf water potential on the bisecting line.

## 4. Results

Munns (2002) suggests that the proper approach with which to evaluate salinity tolerance in perennial plants corresponds to survival conditions rather than biomass/yield production, the latter being more appropriate for annual crops. Soil salinity imposes two-phase stresses in plants (Munns 2002; Munns and Tester 2008; Munns 2009; Peleg et al. 2011): short-term osmotic stress and irreversible long-term ionic stress. Osmotic stress inhibits plant water uptake by lowering the soil osmotic potential (Orcutt and Nilsen 2000; Läuchli and Grattan 2008), thus contributing to a temporary reduction in growth. Nevertheless, since osmotic adjustment is able to rapidly regulate plant water relations, water absorption from the root is assured to some extent (Läuchli and Grattan 2008) in the given soil water. The ionic stress, or salt-specific effects, are time dependent as well as influenced by plants' tolerance mechanisms.

All plants require certain mechanisms to adjust the intercellular ionic concentration in their tissues (Bartels and Sunkar 2005). Plant osmotic potential is always lower than that of the growing medium to ensure the uptake of water; however, this potential in higher plant is rarely lower than -0.4 MPa (Stiles 1994). Breckle (2002) found that the differences between plant osmotic potentials and soil solute potentials fall within the range of -0.5 to 1.0 MPa. Bernstein (1961a) reported that the differentiation between the plant and soil osmotic potentials of cotton (*Gossypium hirsutum* L.) ranged from -0.3 to -1.2 MPa. In addition, plants are to some extent able to control the absorption of NaCl from (Atwell et al. 1999). *Prosopis juliflora* is considered a species capable of retaining/excluding Na<sup>+</sup> ions in the root at low salinity level (Khan et al 1987; Viégas et al. 2004), although beyond 50 mM NaCl concentration in the soil solution, *Prosopis juliflora*'s ability to breakdown the mechanism and Na<sup>+</sup> concentration in shoots tends to increase with a parallel increase in soil solute concentration (Khan et al 1987; Viégas et al. 2004).

*Prosopis juliflora* developed well in commercial mixed potting soils and the hydroponic system, to such an extent that the fully expanded mature leaves were harvested in 2011 and 2012. This species differently responded to NaCl and Na<sub>2</sub>CO<sub>3</sub>. The seedling individuals developed well in soils treated with NaCl with a gradual increase in ion concentrations. Additionally, they demonstrated high tolerance to soil salinity, with a lowest midday leaf osmotic potential of -9.4 MPa in the potting soil medium and a soil osmotic potential which reached up to -7.8 MPa (Table 4.1). In the case of Na<sub>2</sub>CO<sub>3</sub>-dissolved soils, *Prosopis juliflora* exhibited the effects of osmotic stress within a shorter period after application. The lowest midday plant osmotic potential was -5.2 MPa with a corresponding soil osmotic potential of -3.9 MPa.

The hydroponic system with an application of NaCl salt solution showed intermediate responses, with a minimum osmotic potential of -5.7 MPa in relation to a soil water osmotic potential of -4.8 MPa. The gravimetric soil water content was 31% for NaCl and 37% for Na<sub>2</sub>CO<sub>3</sub> potting soils. The hydroponic culture retained saturated condition throughout the course of the treatment, and the leaves water contents were a respective 183% and 145% for the former two treatments and 133% in hydroponic system (Table 4.1). The saturated leaf osmotic potentials for *Prosopis juliflora* from greenhouse experiments were plotted on the graphs as a function of leaf water content. The leaf osmotic potentials of the species exhibited hyperbolic functions, as with *Ruprechtia triflora* in the Paraguayan Chaco (Figure 4.1). In the graph, it can be clearly seen that the curve of *Prosopis juliflora* in the potting soil with an NaCl application was steeper than that of *Prosopis juliflora* in the potometer and in the potting soil with an Na<sub>2</sub>CO<sub>3</sub> application. In addition, the leaf water contents in the former curve were higher than those of the latter two curves. This may be due to the accumulation of osmotically active substances, which influences the tissue water content in plants.

According to Sun (2002), leaf water content (% dry weight) directly correlates to the saturation and dehydration of water in a plant tissue, and thus the variation in leaf water content during drying process corresponds to tissue water losses. With a high accumulation of osmotically active substances in plant tissues, the leaf water content was lower as a result of cellular dehydration. The plant cell wall was rehydrated during the overnight re-saturation process, indicating the higher the accumulation of osmotically active substances in the plant tissue, the higher the leaf water content after rehydration.

The ANOVA revealed no significant differences between plant internal osmotic potentials (midday and saturated) and soil osmotic potential for NaCl application in the commercial potting soil medium. The difference between the mean values of midday and saturated (plant osmotic potential at full cell hydration) leaf osmotic potential was -0.6 MPa, perhaps due to the accumulation of excess solutes in the leaf tissues in relation to the high solute concentration in soil solution and/or the air saturation deficit. In Na<sub>2</sub>CO<sub>3</sub> added potting soils and the hydroponic sand growing medium with an NaCl application, the plant internal osmotic potentials and soil solute potentials were both significant at  $p < 0.05$ . The osmotic potentials in the plants and growing media of both treatments were considerably lower than those of NaCl-applied potting soil medium, mostly likely due to the solute concentration at the root-zone.

At the given soil osmotic potentials, the plant osmotic potentials were lower in the day than the osmotic potentials at full cell turgidity, i.e., the saturated osmotic potentials (Figure 4.2). As sufficient water was irrigated to the potting soil media, the lower midday plant osmotic potentials are attributed to plant mechanisms of regulating osmotic potentials between the plant and soil. In all probability, the solute concentration in the plant cell sap became higher in response to the substantial water loss suffered during the day and may be due to the accumulation of ions in the plant tissues.

In the regression analysis, the initial plant osmotic potentials for NaCl-added potting soils were -0.5 (midday) and -0.01 MPa (saturated), while those for Na<sub>2</sub>CO<sub>3</sub>-applied potting soils were -1.1 (midday) and -0.5 MPa (saturated). Initial plant osmotic potentials ranged from -0.5 to -1.1 MPa in all treatments with the exception of the saturated osmotic potentials in NaCl-adding potting soil media. In the latter case, the plant osmotic potential approached the soil osmotic potential with an initial value of -0.01 MPa; possibly, the excess ions entered into the plant tissues and progressively accumulated. The initial plant saturated osmotic potential for the hydroponic sand culture with an application NaCl was -0.8 MPa, which was consistent with the initial osmotic potentials of plant materials in the commercial potting soil media. The correlations between plant internal osmotic potentials and soil solute potentials were significant at  $p < 0.0001$ . The regression parameters and  $r$ -values were given in Table (4.2). The regression results of a combination of saturated osmotic potentials revealed that the initial plant osmotic potential was -0.6 MPa.

## 5. Discussion

Plants adapt to root zone salinity with several physiological and biochemical processes, demonstrating responses at the molecular, cellular, and whole plant level (Bartels and Sunkar 2005; Mitlöhner and Köpp 2007; Peleg et al. 2011). The stress adaptation mechanisms taking place at the molecular and biochemical levels are complex (Reddy and Reddy 2001; Ashraf and Harris 2004) and not fully understood (Munns 1993; Reddy and Reddy 2001; Martínez-Ballesta et al. 2006). Beginning with the whole plant level is a common approach to evaluate the specific environmental stress in terms of the species' responses (Jones and Jones 1989). With the intention to contribute the most relevant information about the performance of *Prosopis juliflora*, this research work focused on whole plant responses.

Plants respond to soil salinity through the three well-known mechanisms, namely osmotic adjustment, ion exclusion, and ion compartmentation (Munns and Tester 2008). For most salt-tolerant non-halophytes species, ion exclusion is the most effective mechanism (Orcutt and Nilsen 2000) but the degree of exclusion depends on both the species and the level of solute concentration in the root zone. Excess ions accumulate in the apoplastic water and are tend to compartmentalize in the central vacuole within the capacity of plants. Beyond this capacity, solutes build up in the transpiration stream, cellular dehydration occurs, and cell death results in the older leaves (Volkmar et al. 1998; Atwell 1999; Munns 2002; Munns and Tester 2008). Nevertheless, plant growth and development are still ensured if the production rate of new shoots is faster than the death of older leaves (Volkmar et al. 1998; Atwell 1999).

Scientists have previously accepted *Prosopis juliflora* as a high salinity-tolerant species, thanks to a combination of superficial roots known as the lateral and deep tap roots. Under field measurements, the reliability of root zone soil salinity is questionable not only due to the root architectures, but also as a result of the functional attributes of the plant roots (i.e. the avoidance of a saline growing medium). *Prosopis juliflora* was no exception and it is a difficult task to decide where to collect the soil samples that correspond to the actual rhizosphere solute concentration. Mitlöhner and Köpp (2007) have evaluated the interaction between plants and soils through the coupling of field measurements and greenhouse experiments and found that plant internal osmotic potentials, e.g., saturated osmotic potential, give an approximation of soluble salt accumulation at the root zone.

The findings in this study are consistent with their observations showing a strong relationship between the plant internal osmotic potential and the root medium solute concentration. Less stressed plants revealed that the osmotic potential relation between plant and soil were within -0.5 to -1.0 MPa. The stressed plants depicted the initial potential of -0.01 MPa in the regression analysis, most likely due to the progressive accumulation of osmotically active substances in the plant organs. The minimum midday osmotic potential of stressed plants was -9.4 MPa, the most critical value we have ever recorded for *Prosopis juliflora*. The lowest soil osmotic potential reached up to -7.8 MPa, the equivalent to 1600 mM NaCl. These minimum potentials are likely to be the threshold values of *Prosopis juliflora* performance to soil salinity.

The soil salinity values corresponding to *Prosopis juliflora* tolerance seemed in our results twice as high as the previous findings as reported by Khan et al. (1987), Ahmad et al. (1994), Singh et al. (1994), Heuperman et al. (2002) Velarde et al. (2003), and Felker (2009). Their findings indicate that *Prosopis juliflora* can endure soil salinity up to 45 dSm<sup>-1</sup> without affecting its normal growth conditions. All plant materials taken in their works were less than a year old; nonetheless, we found that *Prosopis juliflora* tolerates soil salinity up to -7.8 MPa, at which significant wilting of the leaves is observed. Although wilting appears permanent, the plant itself survives. The mean and standard error of soil salinity in a greenhouse experiment were  $-4.8 \pm 0.6$  MPa. *Prosopis juliflora* appears less resistant to Na<sub>2</sub>CO<sub>3</sub>; the maximum plant adaptation to it was about -5.2 MPa in plant internal osmotic potential with a mean potential of -3.5 MPa. Regarding the highest tolerance to soil salinity, it would perhaps be worthwhile to consider the influences of plant age and environmental factors.

As reported by Abrol et al. (1988), Ashraf and Harris (2004), Khan (2004), and Blumwald and Grover (2006), plant salt tolerance varies in relation to the plant's developmental phases. Many scientists (Greenway and Munns 1980; Foolad 2004; Khan 2004; Tuberosa and Salvi 2004; Blumwald and Grover 2006; Zahran 2010) have shown that the salinity tolerance of some plants such as rice, barely, corn, tomato, *Kochia indica*, *Kochia scoparia*, *Suaeda fruticosa*, *Atriplex stocksii*, and *Haloxylon stocksii* increases with the plant age. Environmental factors greatly influence the plant's ability to tolerate salinity. Plant adaptive ability in the cold and humid environments is higher than in the hot and dry environments (Abrol et al. 1988; Shaw 1999)– in our experiment, we have kept the

environmental influences as minimal as possible. Treatments were done in the central European summer, and the average temperature in the greenhouse was around 25-30 °C. The higher salinity tolerance of *Prosopis juliflora* in our findings probably relate to the age of the plant materials.

In all treatments, we maintained both the maximum soil water content in the potting soils and a continuous supply of water in the hydroponic sand growing media. Nevertheless, the relative water contents of the leaves were lower than 80% in the potting soils plants, indicating cell dehydration. This may be partly due to the excess accumulation of solutes in the plant tissues as well as the influence of atmospheric deficits during the day. The relative water content of hydroponically grown plants was slightly higher than in plants in the potting soil media.

Plants adjust the osmotic balance between their organs and the root zone solute concentration (Subbarao and Johansen 2001). Within the adaptation ability, plants maintain a lower internal osmotic potential than the osmotic potential at in the growing media; the differences between the two generally lie between 0.3-1.0 MPa (Bernstein 1961a; Breckle 2002; Mitlöhner and Köpp 2007). Soil salinity greatly influences plant distribution (Hayward 1954; Goodin and Northington 1979; Mitlöhner 1997a; Singh 2005) and habitat preference. Mitlöhner and Köpp (2007), in evaluating the bioindicator capacity of the plant to adapt to the soil solute concentration, found the reliable interaction between plant internal osmotic potential and rhizosphere soil water concentration. With a regression error of less than  $\pm 0.2$  MPa, the measure was considered a valuable and quick method to estimate plant root zone salinity. Our findings confirm those reported by Mitlöhner and Köpp (2007); the calculation error was less than  $\pm 0.2$  MPa with the exception of the relationship between the saturated plant osmotic potential and the soil osmotic potential in the  $\text{Na}_2\text{CO}_3$  treatment, which had an error of  $\pm 0.26$  MPa. Nevertheless, the r-value was high (0.94) and significant at  $p < 0.0001$  level.

We suggest that the saturated osmotic potential of fully expanded leaves can serve as an indicator of rhizosphere solute concentration. *Prosopis juliflora* is highly resistant to soil salinity, and this ability might vary with plant age. For rapid assessment of surface soils salinity under *Prosopis juliflora*, this robust technique is applicable with the use of newly grown seedlings, as the influences of root architectural traits seem to be negligible under field conditions.

## 6. Conclusion and Recommendations

Plants reflect the environmental conditions in which they grow and can thus be used as indicators to identify the conditions of a particular site as well as the ecological adaptation of the plants to the site. *Prosopis juliflora* exhibited the highest adaptability to soil water stress and salinity. In the dryland environment, drought and salinity are the obviously principle limiting factors for plant growth and development. The plant internal osmotic potential and the root medium solute concentration show a strong relationship. With this in mind, the performance of *Prosopis juliflora* to soil salinity was analyzed through osmotic potential measurements. The saturated plant-, midday plant-, and soil osmotic potentials were calculated to compare the in situ research findings with the ex situ experiment results. The outcomes are as follows:

1. In the greenhouse experiments, *Prosopis juliflora* demonstrated a higher tolerance to NaCl than to  $\text{Na}_2\text{CO}_3$ .
2. *Prosopis juliflora* in the greenhouse exhibited the highest adaptation to salinity under good water access. The minimum midday osmotic potential of stressful plants was -9.4 MPa, the most critical value we have ever recorded for the species *Prosopis juliflora*.

3. The lowest soil osmotic potential reached up to -7.8 MPa, the equivalent to 1600mM NaCl.
4. These values were relatively lower than those of the in situ measurement. The minimum midday osmotic potential of *Prosopis juliflora* in the wastelands was -4.3 MPa, while the soil osmotic potentials reached up to -3.6 MPa.
5. Regression analysis also revealed a strong correlation between plants' saturated osmotic and soil osmotic potentials. In all treatments, the correlations between plant internal osmotic potentials and soil solute potentials were significant at  $P < 0.0001$ .
6. The plant osmotic potential was suitable to determine the root zone solute concentration.
7. Due to the constraints and lesser reliability of root zone soil salinity in field measurements, plant internal osmotic potential was examined as an indicator to characterize the rhizosphere solute concentration.
8. For rapid assessment of soil salinity under field conditions, this robust technique is applicable with the use of newly grown seedlings, as the influences of root architectural traits seem to be negligible under field conditions.

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## Tables

Table 4.1. Leaf and soil osmotic potentials of *Prosopis juliflora* seedlings grown in different growing media in greenhouse experiments, Göttingen, Germany.

Category	Salt solution application			Control
	NaCl		Na <sub>2</sub> CO <sub>3</sub>	
	Potted soils	Hydroponic *	Potted soils	
<b>Plant samples</b>				
Minimum $\Psi\pi$ (MPa)	9.4	5.7	5.2	1.8
Midday $\Psi\pi$ (MPa)	5.4 ± 0.6 (18)	No data	3.5 ± 0.2 (13)	1.3 ± 0.1 (6)
Saturated $\Psi\pi$ (MPa)	4.8 ± 0.5 (18)	3.7 ± 0.3 (18)	2.7 ± 0.2 (13)	1.2 ± 0.1 (6)
LWC (%)	183 ± 14	133 ± 9.5	145 ± 15	91 ± 2.8
<b>Soil samples</b>				
Minimum $\Psi\pi$ (MPa)	7.8	4.8	3.9	0.9
$\Psi\pi$ (MPa)	4.4 ± 0.5 (18)	2.9 ± 0.3 (18)	2.2 ± 0.2 (18)	0.7 ± 0.1 (6)
WC (%)	31 ± 1.7	Saturated	37 ± 4.2	44 ± 2.1

\* In the hydroponic sand culture, a continuous supply of water is provided to minimize the effects of water stress to plant. Mean ± standard error was shown for each variable. Number of observations in parentheses.

Table 4.2. Regression analysis ( $\hat{y} = a + bx$ ) of relationship between plant internal osmotic potentials and soil solute potentials of *Prosopis juliflora* seedlings grown in different growing media in greenhouse experiments.

Category	Regression coefficients		r	$S_{\bar{y}x}$ [MPa]	n*
	a	b			
<b><u>NaCl- Treatment</u></b>					
<b>Potted soil</b>					
Midday	0.5946	1.0913	0.99***	±0.1533	18
Saturated	0.0146	1.0708	0.99***	±0.1893	18
<b>Hydroponic culture</b>					
Saturated	0.8134	1.10158	0.99***	±0.1010	18
<b><u>Na<sub>2</sub>CO<sub>3</sub>-Treatment</u></b>					
<b>Potted soil</b>					
Midday	1.1413	1.0694	0.97***	±0.1970	13
Saturated	0.4613	1.0331	0.94***	±0.2657	13

**Pooled data**

Saturated all samples	0.586	0.9973	0.98***	±0.1098	49
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\*n=

Number of observation.

**Figures**

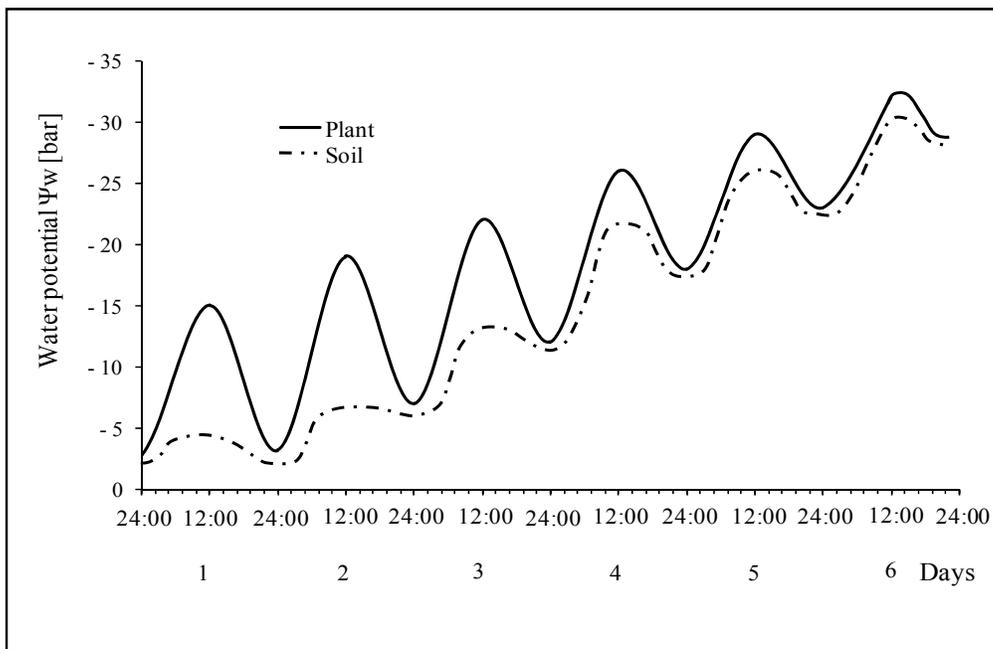


Figure 3.2. Slatyer's (1957) model, showing the diurnal relationship between plant water potential and soil water potential based on increasing drought within a week, modified [after Mitlöhner (1997a, 1999)].

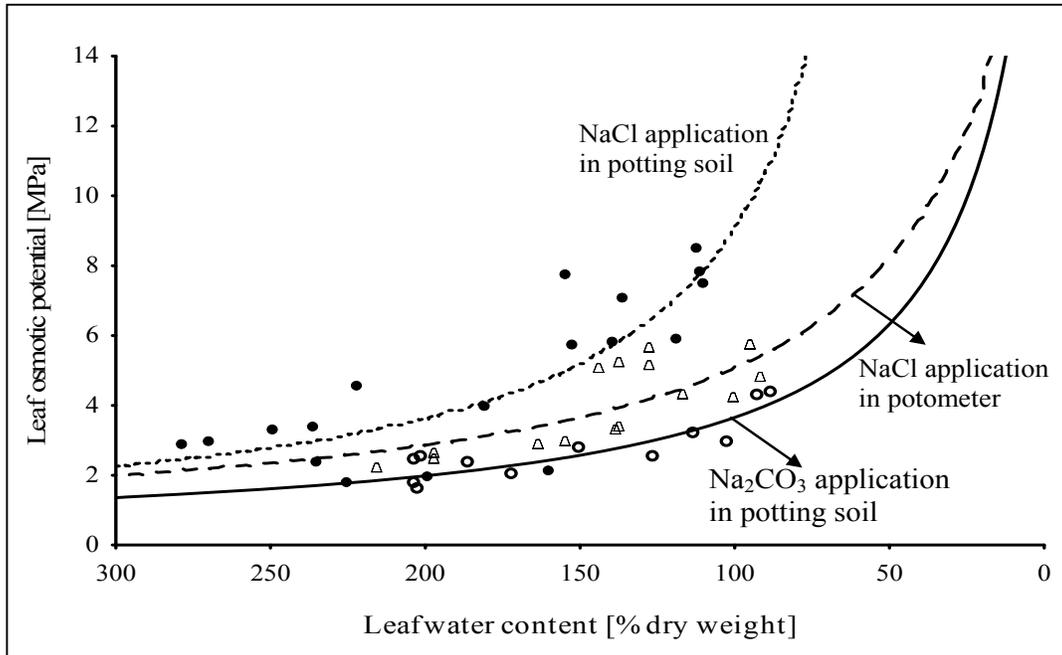


Figure 4.1. Cryoscopically measured leaf osmotic potential  $\Psi\pi$  [MPa] and related water content (% dry weight) of *Prosopis juliflora* in greenhouse experiments:  $\Psi\pi = 1/(a+b \cdot \text{leaf water content})$ .

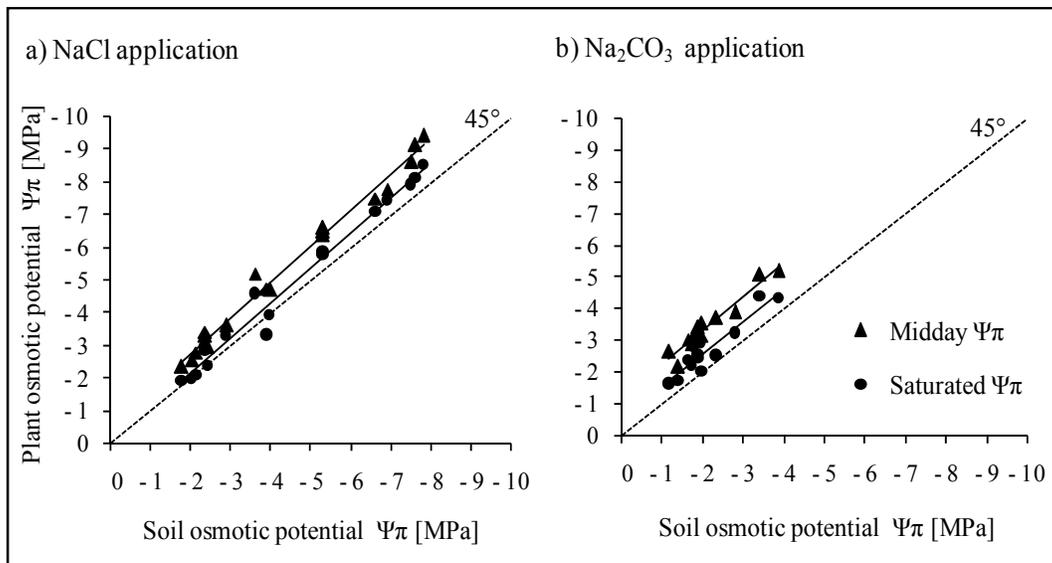


Figure 4.2. The relationship between plant osmotic potentials and soil osmotic potentials at the rhizosphere corresponding to NaCl and  $\text{Na}_2\text{CO}_3$  salt solutions in commercial potting soils. The freezing point osmometry of plant materials and soil samples were done independently at each phase of experiment. Fully expanded matured leaf samples were used.

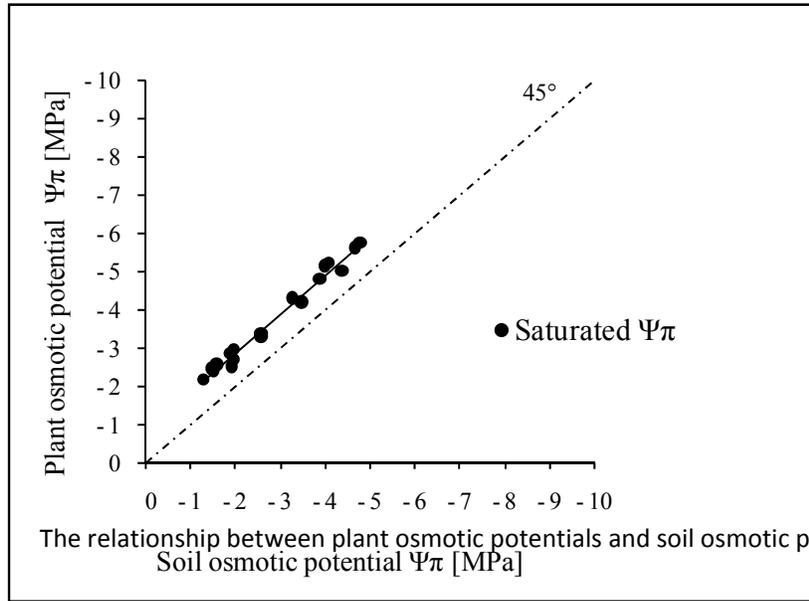


Figure 4.3.

The relationship between plant osmotic potentials and soil osmotic potentials at the rhizosphere corner was used.