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Physiological and biochemical responses and feedstock composition of *Tamarix spp.* subjected to salt and drought stress: possibilities of biomass use for energy production in arid and degraded lands.

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Dedication

This thesis is dedicated to my parents who have supported me all the way since the beginning of my studies.

Also, this thesis is dedicated to my brother Daniel who has been a great source of motivation and was always there for me.

Finally, this thesis is dedicated to all those who believe in the richness of learning and research for public's sake.

ABSTRACT

Tamarix trees are considered of particular interest for afforestation and reforestation of degraded areas, because they were noticed to establish and grow well in wide spectrum of edaphic, contrasting, and extreme environmental conditions. A better understanding of the functional mechanisms enabling Tamarix to adapt to extreme conditions is important, for a more insightful selection of the best ones, intended to be used for biomass production and conservation purposes in arid zones.

For this scope, two trials of short period were conducted in semi-controlled conditions, during which two species of Tamarix *(T. aphylla* and *T. jordanis*) collected from Negev desert were exposed to salt, drought and combined stress. The first trial was executed at mild level of stress: salt (150mM), drought (50 % F.C.); the second trial was more severe: salt (350mM), drought (30% F.C.). Physiological and biochemical responses and growth were studied regularly during the experiments.

The results of the experiments, showed the highest tolerance of *T. jordanis* to salt stress up to 350 mM. The maintenance of high amount of carbohydrates, high capacity of carbon assimilation, and active growth could be considered as potential and early markers for salt tolerance of *Tamarix spp.* On the other hand, the growth and biomass displayed highest performance of *T. aphylla* than *T. jordanis* in mild dry conditions. The highest accumulation of sugar and the highest relative water content in *T. aphylla* were suggested among their acclimatory mechanisms under mild drought (50% F.C.). Combined stress was shown to be additive in lowering performance of plants relative to salt and drought stress applied alone. Proline accumulation in both species was suggested to be a storage form of nitrogen for use under combined stress, rather than a principal osmoregulator.

Given the high productivity of some *Tamarix spp.* cultivated in arid and degraded lands (Eshel *et al*., 2010), it was also investigated the possible use of Tamarix biomass for bioethanol production, in the second step of our study. Several samples were collected from different natural and experimental plantations in Mediterranean area. Differences in ligncoelulose matrix among different locations, age, and species were evaluated. The results showed that the growing sites had significant effect on structural sugars content with no apparent significant influence on

lignin. There were no significant variations in structural sugars and lignin between different species and ages.

Despite the limited number of samples, the results, in general, have shown a good quality of lignocellulosic biomass of tamarisk for the production of bioethanol. The content of hexose and pentose sugars found in the samples analyzed was highly comparable to other species already used in the production of biofuels such as eucalyptus (43-45% cellulose).

However, some obstacles from presence of ash and extractives in high amount should be taken into account, and better understanding of cultural practices effect on these substances is essential prior to any process of sugar conversion to ethanol.

RIASSUNTO

Le tamarix sono considerate di particolare interesse per la forestazione ed afforestazione di aree aride e salinizzate. Sono specie che si distribuiscono su un ampio areale e quindi in grado di vivere e crescere in ambineti diversi, estremi e contrastanti tra loro. Una migliore conoscenza dei meccanismi funzionali che regolano la risposta adattativa a condizioni limitanti, (aridità e salinità) in *Tamarix spp*., è importante per individuare degli indici funzionali precosi, utili a selezionare specie idonee per rimboschimenti di ambienti marginali a fini conservativi e produttivi.

A tal fine, sono stati condotti due esperimenti shock di breve durata, in cui due specie di tamerici, provenienti dal deserto del Negev *(T. aphylla* and *T. jordanis*), sono state sottoposte a stress salino, idrico e alla combinazione dei due stress (salino+ idrico). In un primo esperimento le piante sono state sottoposte ad un medio livello di stress: 150mM di NaCl e 50% della capacità idrica di campo (F.C.). In un secondo esperimento i livelli di stress sono stati aumentati fino a raggiungere i 350 mM per lo stress salino e il 30% della capacità idrica di campo per lo stress idrico. I principali parametri fisiologici, biochimici e di crescita sono stati rilevati regolarmente durante i periodi di stress.

I risultati, in entrambi gli esperimenti, hanno mostrato una maggiore tolleranza alla salinità in *T. jordanis* che si evidenzia maggiormente a concentrazioni saline elevate (350 mM). Il mantenimento di elevate quantità di carboidrati, di un'elevata capacità assimilativa, e di un buon accrescimento in condizioni di salinità, potrebbe indicare un adattamento allo stress. Tali parametri possono, per questo motivo, essere considerati dei marcatori potenziali e precoci per la tolleranza allo stress salino in *Tamarix spp*. Al contrario, in condizioni di stress idrico, *T. aphylla* ha mostrato un tasso di crescita e produzione di biomassa significativamente più elevate della *T. jordanis*. Inoltre, in condizioni di stress idrico medio, è stato osservato un maggior accumulo degli zuccheri solubili e un più alto contenuto idrico fogliare (*relative water content)* a sottolineare, in correlazione con la maggiore produttività, una più elevata tolleranza all'aridità in *T. aphylla* rispetto alla *T. jordanis*.

La combinazione dei due stress ha mostrato un effetto additivo negativo su entrambe le specie. In condizioni di stress combinato il contenuto di prolina è risultato elevato. Dai calcoli eseguiti, il contributo della prolina sul potenziale osmotico fu comunque troppo basso per far considerare la prolina uno dei principali soluti, che può consentire alla pianta di tollerare lo stress salino

attraverso una regolazione osmotica. I risultati ottenuti in questa ricerca ci fanno ipotizzare una funzione della prolina di osmoprotettore e forma di accumulo di azoto e carbonio da usare in condizioni di stress successive.

Vista l'elevata e rapida produttività di alcune specie di Tamarix coltivate in ambienti aridi e salini (Eshel *et al*., 2010), in una seconda fase di questo studio è stata investigata la possibilità di usare la biomassa legnosa delle Tamarix per la produzione di biocarburanti di seconda generazione come il bioethanolo. Una serie di campioni legnosi furono collezionati nella regione arida mediterranea, in differenti siti naturali e piantagioni arboree. E' stata valutata la variabilità della matrice lignocellulosica tra specie, provenienze, e in base all'età e alle caratteristiche pedoclimatiche del sito.

Dei risultati, si è evidenziato un significativo effetto dei siti di collezionamento sul contenuto degli zuccheri strutturali senza un apparente influenza significativa sulla lignina. Non si sono evidenziate variazioni importanti tra le differenti specie e l'età. Nonostante il limitato numero di campioni analizzati, i risultati, in generale, hanno mostrato una buona qualità della biomassa lignocellulosica delle tamerici per la produzione di bioethanolo. Il contenuto di esosi e pentosi riscontrato nei campioni analizzati è risultato altamente paragonabile ad altre specie arboree già impiegate nella produzione di biocarburanti come l'eucalipto (43-45% di cellulosa).

Tuttavia le Tamarix hanno mostrato un contenuto in ceneri e estrattivi elevati, cosa che potrebbe causare alcuni problemi nei processi di trasformazione da biomassa a bioetanolo.

Si auspica un approfondimento di tali studi sia nella valutazione del contenuto in zuccheri strutturali sia dei contenuti in ceneri ed estrattivi su un numero più elevato di campioni. Inoltre implementare la conoscenza dei possibili effetti delle diverse pratiche colturali, sulla biomassa lignocellulosica delle tamarix in ambienti estremi, è fondamentale al fine di valutare qualsiasi processo di conversione degli zuccheri a bioetanolo.

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EXPERIMENTAL PART

1. Desertification

1.1 Backgound

Geographical extension

"Desertification is the degradation of land in arid, semi-arid and dry sub-humid areas resulting from various factors, including climatic variations and human activities" (UNCCD, 2007a). It is a gradual process of soil productivity loss and the thinning out of the vegetative cover. Drylands occupy approximately 40-41% of Earth's land area and are home to more than 2 billion people. Dryland subtypes are determined by the aridity index (AI=P (annual precipitation) / PET (potential evapotranspiration (Fig.1) (Millenium Ecosystems, 2005). It has been estimated that some 10–20% of drylands are already degraded, the total area affected by desertification being between 6 and 12 million square kilometres, that about 1–6% of the inhabitants of drylands live in desertified areas, and that a billion people are under threat from further desertification (world bank, 2009, UNCCD, 2007). The affected areas in Mediterranean basin extends across northern Africa into the Near East and across large parts of Europe, including Greece, southern Italy, Sicily, Corsica, and the Iberian Peninsula (UNEP, 1992; Imeson and Emmer, 1992).

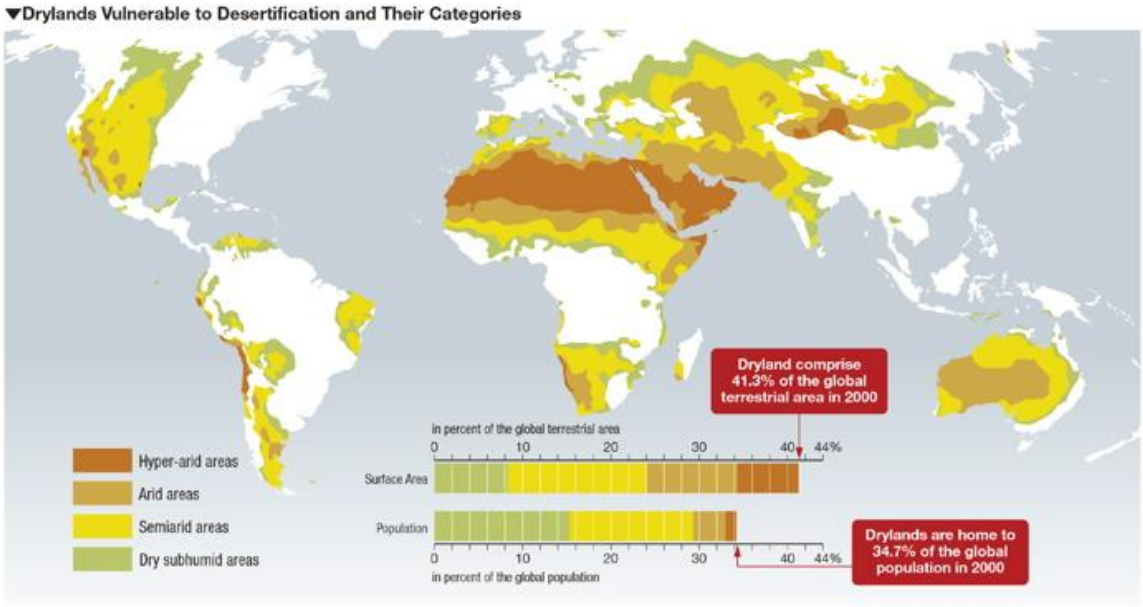


Figure 1. Global desertification map. Source: Millennium Ecosystem Assessment, 2005.

Causes

Desertification is caused by a combination of factors that change over time and vary by location. These include two direct major causes, namely natural-physical and human induced factors resulting from different activities. The former includes climatic factors of periodic droughts and climate change, natural fires and disasters and in some areas locust invasion (UNCCD, 2004). Human factors are seen as more important than the physical and many aspects considered as feedback mechanisms for physical factors (Herrmann and Hutchinson, 2005). The human induced factors are mainly related to unsustainable use of natural resources and can include: land mismanagement and fragmentation, overgrazing, over cultivation, deforestation, unsustainable irrigation practices, political acts, poverty and changeable socio-economic aspects, poor advisory services, little or no problem-solving (Millenium Ecosystem assessment, 2005).In many of the Mediterranean countries, the process is accelerated by changeable socio-economic factors including high population growth, industrialization, tourism and recreation, urbanization and intensive agricultural development. In arid and semi-arid zones of the Mediterranean, rangelands, plowing for land claim ship and barley cultivation, overstocking and uprooting of woody plants are the major causes of desertification of their fragile ecosystems (NAPCD, 2006).

Environment and Socio-economic impact

Desertification has severe environmental impacts at the global and regional scale that are summarized below in (Fig.2). Ecological unsustainable behaviors associated with desertification contribute to climate change by releasing carbon stored in the soil and vegetation, as consequence of soil and vegetation losses. It is estimated that 300 million tons of carbon are lost to the atmosphere from drylands as a result of desertification each year (Millenium Ecosystems Assessment, 2005). Desertification may also exacerbate climate change by reducing surface soil moisture and thereby increasing air temperatures in affected areas. Desertification makes land areas flood-prone, causes soil salinisation, results in the deterioration of the quality of water, silting of rivers, streams and reservoirs (Charreton, 2008). Moreover, its effects on soil erosion and fertility decline are seen due to use of unsustainable cultivation practices.

Biological diversity, which contributes to many of the services provided to humans by dryland ecosystems**,** is also diminished by desertification**.** Vegetation and its diversity are instrumental in

soil conservation and in the regulation of surface water and local climate. Different plant species produce physically and chemically different litter components and, together with a diverse community of micro- and macro-decomposers, contribute to soil formation and nutrient cycling**.** The species diversity of vegetation supports both livestock and wildlife. All plants support primary production that ultimately provides food, fiber, and fuelwood and that sequesters carbon, thus regulating global climate. Excessive exploitation of vegetation leads to losses in primary production and hence also to reduced carbon sequestration. There is a Linkage and Feedback effect among Desertification, Global Climate Change and Biodiversity Loss. For instance, for many dryland countries, climate change could mean the intensification of drought and desertification. As the global climate warms, higher rates of evaporation will cause drier conditions, increasing the frequency of droughts and vulnerability to desertification. The disruption of the interlinked services that are provided by dryland plant biodiversity is also a key trigger for desertification and its various consequences, including the loss of habitats for other species (Millenium Ecosystem assessment, 2005).

Desertification is also at the root of political and socio-economic problems and poses a threat to the environmental equilibrium in affected regions (UNCCD, 2004). Besides environmental impacts, desertification is partially responsible for population migration. Because people, who live in drylands, depend on the ecosystem services for their crops, livestock, commodities, fuel wood, etc., deterioration in ecosystem services by land degradation triggers further degradation of their livelihood and well-being.It can destroy livelihoods, render land useless, wipe out the habitat people and animals, generate conflict, and prompt migration. Although no one knows for sure how many people have had to abandon their land when it turned to dust, it appears to be in the millions. Many African countries often have serious droughts. As the land which is basis for food production is degraded, local people have no choice to exploit further natural resources such as forests and water. Over 250 million people are directly affected by desertification. In addition, some one thousand million (or one billion) people in over one hundred countries are at risk. These people include many of the world's poorest, most marginalized, and politically weak citizens. (UNCCD, 2004).

One sixth of the population of Mali and Burkina Faso has already been uprooted because of desertification. It was also a factor in the immigration of Mexicans into the United States.

Poverty forces poor people to wring as much as possible from the land in order to feed, house and warm their families. Unfortunately, over-cultivation, deforestation and other unsustainable practices degrade the land, forcing people to look elsewhere to support themselves.

From an economical point view, UNEP estimates that desertification costs the world US$ 42 billion a year. Of the total, Africa loses some US$ 9 billion a year, Asia US$ 21 billion, North America US$ 5 billion, Australia and South America US$ 3 billion each, and Europe US$ 1 billion. (UNCCD, 1997).

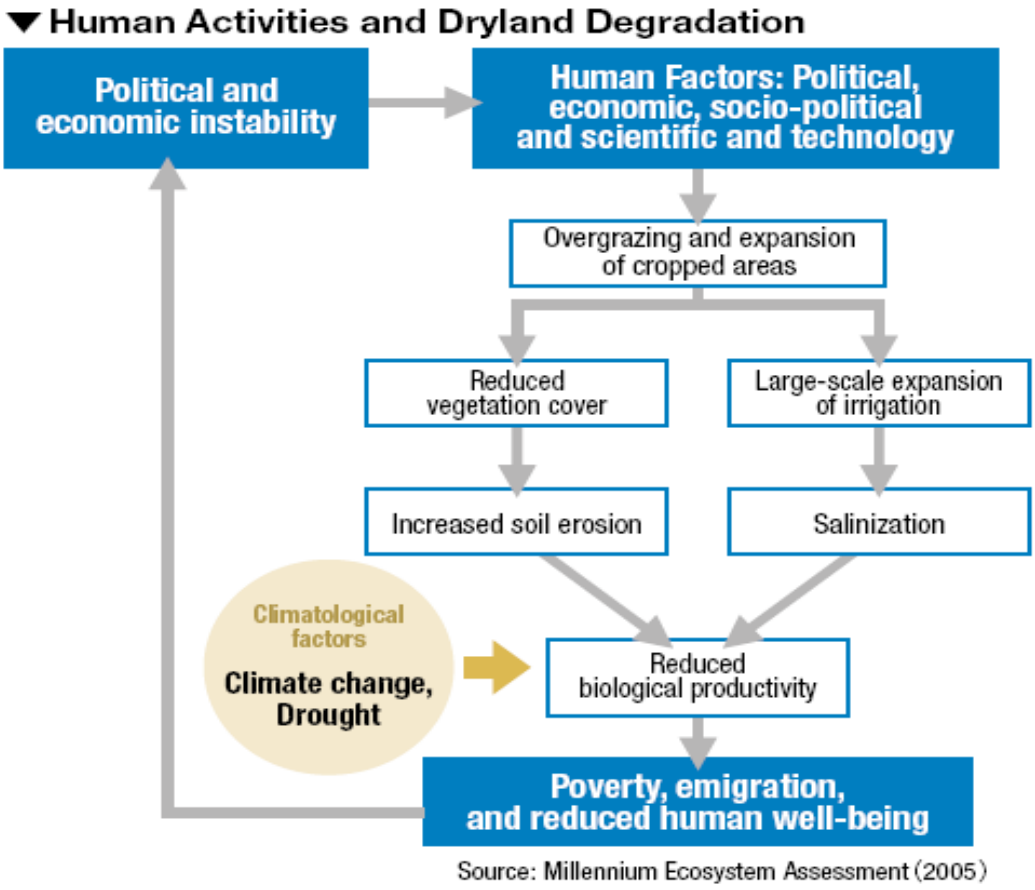


Figure 2. Cause-impact of desertification. Source: Millennium Ecosystem Assessment, 2005. **http://www.env.go.jp/en/earth/desert/leaflet_gd/index_1_4.html**.

Policies and strategies to combat desertification

The United Nations Convention to Combat Desertification (UNCCD) was adopted in June 1994. At the end of 1998, 148 countries had ratified or acceded to the Convention. Combating desertification is essential to mitigate the effects of drought in countries experiencing serious drought and/or desertification, particularly in Africa, and to ensure long-term productivity of inhabited drylands (UNCCD, 2007).The Convention aims to promote effective action through innovative local development programmes and supportive international partnerships. Governments also need to focus on awareness-raising, education, and training both in developing and developed countries (UNCCD, 1999b). To achieve the objectives of the convention, long term integrated strategies are needed, that focus in affected areas, on improved productivity of land, and the rehabilitation, conservation and sustainable management of land and water resources, leading to improved living conditions (UNCCD, 2007b).

Countries should be prompt to ratify UNCCD convention as donors or supporter; parties should work closely, in a spirit partnership and solidarity, with provincial governments, private sector, landholders, farmers and their associations, and non-governmental organizations (NGOs), to establish a better understanding of the nature and value of land in affected areas and promote conservation and the sustainable management of natural resources. Taking full consideration the special needs and circumstances of affected developing country. The provisions of this Convention shall not affect the rights and obligations of any party deriving from a bilateral, regional or international agreement. In addition to direct bilateral aid, efforts to combat desertification are also supported by local NGOs, continuous investments in science, provided through regional research facilities located in vulnerable areas, universities and colleges, companies, municipalities, and community groups as well as a wide range of multilateral and regional organizations are involved. This partnership is premised on improved communication and coordination and skills exchange. Developing countries consistently express concern about lacking the capacity to fulfill their obligations to implement the Convention to Combat Desertification.

Keeping in mind that the strategy for combating desertification must take into account certain aspects directly related to desertification, particularly the loss of biodiversity and genetic erosion, and the important role played by dryland degradation in global climate change; Should be based

on a model of sustainable development that considers the limits imposed by the prevailing environment; and should aim at slow down or stop desertification. Such strategies should, if possible, reverse the damage already done and provide decision-making tools for the diverse situations faces. For this reason is important, that the coordination of activities carried out under this Conventionare also under other relevant international agreements, particularly the United Nations Framework Convention on Climate Change and the Convention on Biological Diversity, in order to derive maximum benefit from activities under each agreement while avoiding duplication of effort (UNCCD, 2007b). All three Conventions have officially recognized that each other's objectives are interlinked and that realizing synergies is important to achieve the Conventions' objectives, and to use resources efficiently. To reach the scope, there are many key mechanisms (CDM, REDD….) to support the mitigation of climate change under the Kyoto protocol. These compliance market mechanisms allow the sale of credits to developed countries based on the establishment of a payment system for the benefit of developing States, that demonstrate the ability to reduce emissions from deforestation (COP 13 in Bali).

1.2 The Italian activities to combat the desertification processes

In the light of guiding principles of the UNCCD (United Nations Convention to Combat Desertification), the Italian Cooperation has identified several operational strategies that include: consultation and coordination with countries of high risk of desertification (such as those united in the CILSS, the Coordination against drought in the Sahel) in the context of appropriate interventions, and strengthening of partnerships with international bodies and non-governmental organizations, the orientation of action to combat desertification into development interventions, and socio-economic development.

One of the key objectives of the projects to combat desertification, concerns the protection of soil, especially agricultural areas with intensive production, areas at high risk of erosion, degraded areas by contamination, pollution, fires, and finally those uncultivated and abandoned. The areas of high risk are those that combine features of unfavorable soil conditions with negative water balance for extended periods of the year. It is essential, in this sense, to take measures and establish plans for water conservation and development of plans for prevention, mitigation and adaptation of droughts effects.

Even productive activities, if conducted in a manner inconsistent with the conservation of resources, can accelerate the processes of land degradation and desertification; which is why measures should be taken for the implementation of agricultural production systems compatible with environment of sustainable agriculture - capable of preventing physical, chemical and biological degradation of soil - and a reduction in the consumption of nonrenewable resources and, more generally, the environmental costs. Finally, since the phenomenon of desertification depends also by human activity, it is essential to carry out projects that provide a recovery of the areas currently affected by an excessive concentration of human activities (Cooperazione Italiana allo Sviluppo, 2011).

In order to implement the UNCCD the Italian Committee has signed several Memoranda of Understanding with different partners: the Food and Agricultural Organization, the Ente Teatrale Italiano, ANPA and Asinara National Park, Fondo Euro-Mediterraneo, Ministry of Foreign Affairs. Moreover, the Committee is supporting the activities of two study centers in areas that are particularly vulnerable to drought and desertification: Matera (Basilicata) and Porto Torres (Sardinia).

Within the context of bilateral cooperation between governments, the Italian ministry of environment was involved by the united convention that urges action to be taken to combat desertification as the convention underlies. Like many other signed projects, it signed in Rome on May 2003, the project "*harnessing the Biodiversity of Mediterranean Plants for mitigating the Effects of Climate Change and Desertification''* through a Memorandum of Understanding. Within the framework of the project, fast growing species (Tamarix, Populus) were chosen as potential plants to combat desertification and rehabilitate the degraded land; they are good candidates due to their capacity to withstand severe environmental conditions maintaining high yields, they are able to control soil erosion, and have economical and environmental value. The project aims to identify and select the best species for afforestation programmes in the salt and desert areas. In addition the project is looking for genetic resources for the development of fast genetic fingerprints for the selection of vegetal species. The project has started in 2008 and completed in 2011.

On the other side, the Italian ministry signed another project *"Bioforeta Project"* that scrambles to provide sustainable use of wood xerophile trees and consequently improve livelihood and

economic situation of local people in marginal lands-through launched recent studies related to possible exploitation of Tamarix biomass for bioenergy production.

2. Soil degradation induced by abiotic stress

It should be kept in mind that desertification is not a single term but a combination of one or more processes that might take place simultaneously and/or in succession. The major processes are vegetation degradation, soil erosion by water and wind, salinization and alkalinization of soil, soil compaction and crust formation.

Drought and salinity are already widespread in many regions, and are expected to cause serious salinization of more than 50% of all arable lands by the year 2050 (Ashraf, 1994).

2.1 Soil degradation by drought

The defining feature of dryland climates is the low average rainfall and the variability rainfall patterns. Both of these features substantially limit the opportunities for plant growth and the productive capacity of the lands and increase the risk of crop failure, livestock losses and resource degradation. Defined as regions where annual potential evaporation and plant transpiration exceed annual precipitation, drylands constitute about 40% of the word's land area.

In the last few years, several regions in Europe have been affected by drought. The drought of 2005 was the worst for large parts of the continent but was especially bad in the Mediterranean region (Fig. 3). In 2006, drought affects the major part of Spain and Portugal and large parts of the United Kingdom, Italy and France (WWF, 2006).

Drylands may be divided into four climatic zones: hyperarid, arid, semi arid and dry subhumid. Hyperarid regions compose the true climatic deserts, such as the Sahara, Atacama, and Namib deserts. Here irrigation is practiced in Oases, fed by rivers rising in the humid regions and by fossil groundwater. Arid regions are almost exclusively used for extensive grazing. Semiarid lands are rarely pastoral, but include extensive rainfed cropping in the wetter parts. Dry subhumid zones are woodlands and forested lands where intensive cropping is practiced, along with livestock production.

Dryland soils are frequently characterized by low fertility with low levels of organic matter and nitrogen. However, water deficiency is the principal limiting factor in plant growth with vegetative cover varying from forests in the dry subhumid zone to virtually nothing in the

hyperarid zone. In the arid and semiarid zones, vegetation tends to be patchy and to vary widely in productivity from year to year.

Dryland ecosystems apport a wide variety of plants and animals which frequently exhibit wide genetic variation within species due to the selection pressure brought about by the harsh nature and variability of the drought environment. As a result of selection pressures, dryland species exhibit a wide range of morphological, physical, and chemical adaptations to their harsh environment. This makes them a vital source of genetic material to improve crop tolerance to drought and disease.

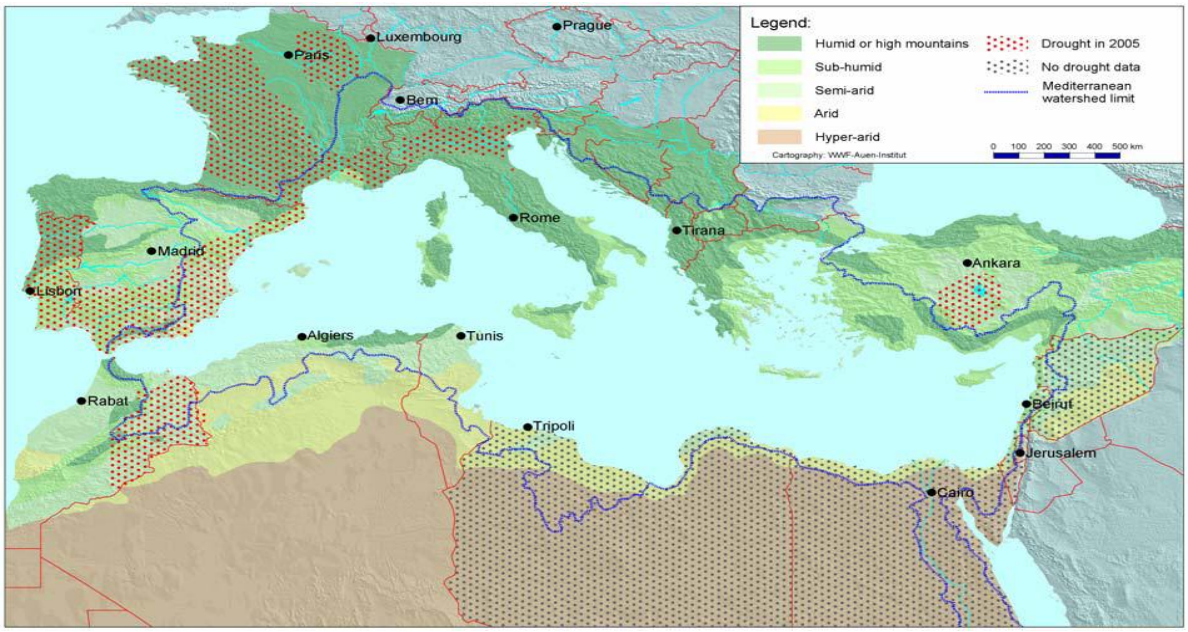


Figure 3. Drought areas of the Mediterranean region. (WWF, 2006)

2.2 Soil degradation by salinity

Soil salinization is one of the most important processes in land degradation and represents a major environmental hazard. About 41% of the world lands are subjected to desertification and land salinization because of excessive exploitation of the land (Monteverdi, 2008). Saline soils of various nature and degree occupy over 80 million hectares in the Mediterranean basin (Hamdy, 1995), and are among the major degradation processes endangering the potential use of European soils (Fig. 4). Soil degradation resulting from salinity is a major environmental constraint with severe negative impacts on agricultural productivity and

sustainability, particularly in arid and semiarid regions (Pitman and Lau¨ chli, 2002; Qadir *et al*., 2006; Suarez, 2001; Tanji, 1990).

Salinisation results from the accumulation of water-soluble salts in the soil. These salts include potassium (K^+), magnesium (Mg^{2+}), calcium (Ca^{2+}), chloride (Cl^-), sulphate ($SO4^{2-}$), carbonate ($CO3^{2-}$), bicarbonate ($HCO3^-$) and sodium (Na^+) (European Communities, 2009). Salt-affected soils may contain an excess of watersoluble salts (saline soils), excess exchangeable sodium (sodic soils) or both an excess of salts and exchangeable sodium (saline-sodic soils). The accumulation of salt in the soil is the final product of a long series of processes. It includes different processes driven by different causes.

In general the salinization occurs when, depending on the characteristics of soil and groundwater, the balance between precipitation / irrigation and evaporation goes towards the evaporation processes.

The main processes causing the salinization are:

- The increase of groundwater level
- Salt accumulation for irrigation
- Intrusion of saline water

It is possible to distinguish primary salinisation that involves salt accumulation through natural processes. Secondary salinisation caused by human interventions such as inappropriate irrigation practices, for example with salt-rich irrigation water and/or insufficient drainage (European Communities, 2009).

Over the last few decades, salt-prone soil degradation has increased steadily in several major irrigation schemes throughout the world. Examples include Indo-Gangetic Basin in India (Gupta and Abrol, 2000), Indus Basin in Pakistan (Aslam and Prathapar, 2006), Yellow River Basin in China (Chengrui and Dregne, 2001), Euphrates Basin in Syria and Iraq (Sarraf, 2004), Murray-Darling Basin in Australia (Herczeg *et al*., 2001; Rengasamy, 2006), and San Joaquin Valley in the United States (Oster and Wichelns, 2003). Salt- and irrigation induced soil degradation is prevalent in the Aral Sea Basin of Central Asia with the consequent environmental changes in that region being considered as the largest ones caused by humanity (Cai *et al*., 2003).

Therefore, it is imperative to find ways to improve sodic and saline-sodic soils to ensure that they are able to support highly productive land-use systems to meet the challenges of global food security. Over the past 100 years, several different approaches—involving chemical

amendments, tillage operations, crop-assisted interventions, water-related approaches, and electrical currents have been used to ameliorate sodic and saline-sodic soils.

Nearly a century-old record reveals amelioration of sodic soils through the provision of a readily available source of calcium (Ca^{2+}) to replace excess Na^{+} on the cation exchange complex; the displaced Na^{+} subject to leaching from the root zone through the application of excess irrigation water in the presence of a drainage system. However, due to its negligible solubility, natural dissolution of calcite does not provide sufficient quantities of Ca^{2+} to affect soil amelioration with routine management practices. Consequently, amelioration of these soils has been predominantly achieved through the application of chemical amendments such as gypsum (CaSO4 _ 2H2O), supply soluble sources of Ca^{2+} to the soil solution, which then replace excess Na^{+} on the exchange complex. However, amendment costs have increased prohibitively over the past two decades due to competing demands from industry and reductions in government subsidies for their agricultural use in several developing countries A number of tillage options, such as deep plowing and subsoiling, have also been used to break up the shallow, dense, sodic clay pans and/or nitric horizons that occur within 0.4 m of the soil's surface (Abdelgawad *et al*., 2004; Rasmussen *et al*., 1972). However, in recent decades, the crop-based approach, phytoremediation, has shown promise as an effective low-cost amelioration intervention (Ghaly, 2002; Ilyas *et al*., 1993; Robbins, 1986), as it is much cheaper than chemical amelioration. Typical plant-based strategies for contaminated soils, such as those containing elevated levels of metals and metalloids, work through the cultivation of specific plant species capable of hyperaccumulating target ionic species in their shoots, thereby removing them from the soil.

Phytoremediation of sodic soils is achieved by the ability of plant roots to increase the dissolution rate of calcite, thereby resulting in enhanced levels of Ca^{2+} in soil solution to effectively replace Na^{+} from the cation exchange complex. Phytoremediation has shown to be advantageous in several aspects:

(1) No financial outlay to purchasechemical amendments,

(2) accrued financial or other benefits from crops grown during amelioration,

(3) Promotion of soil-aggregate stability and creation of macropores that improve soil hydraulic properties and root proliferation,

(4) Greater plant-nutrient availability in soil after phytoremediation,

(5) More uniform and greater zone of amelioration in terms of soil depth,

(6)Environmental considerations in terms of carbon sequestration in the post amelioration of soil. Phytoremediation is particularly effective when used on moderately saline sodic and sodic soils. It is a viable solution for resource-poor farmers through community-based management, which would help in strengthening the linkages among researchers, farm advisors, and farmers (Qadir *et al*., 2007).

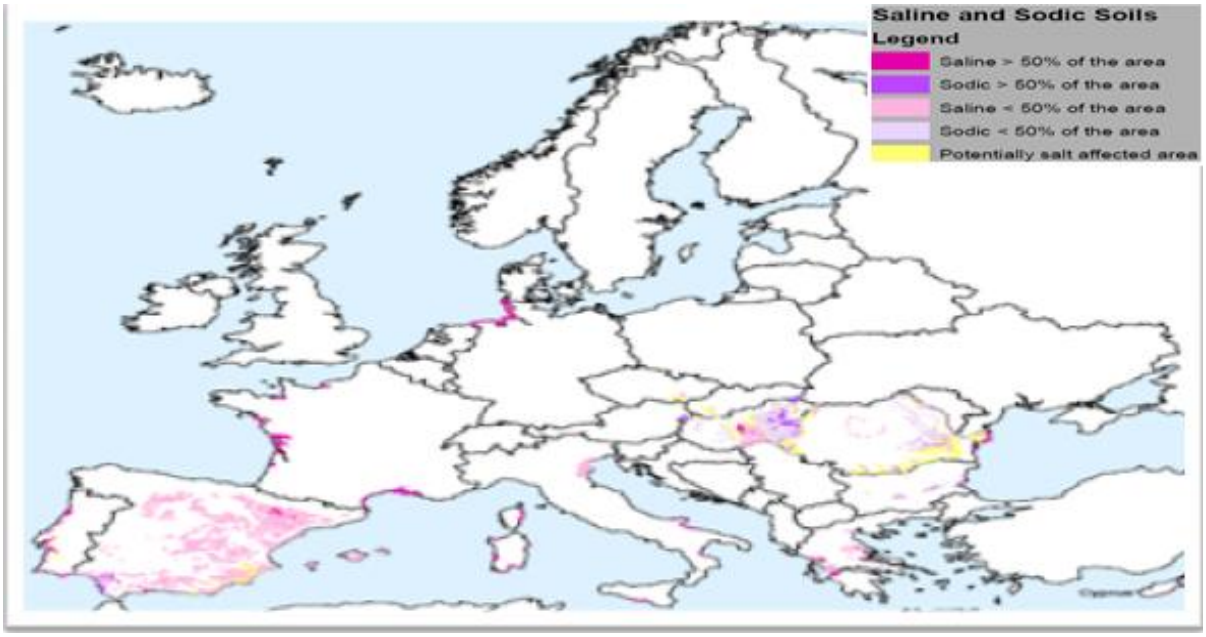


Figure 4. The area distribution of saline, sodic and potentially salt affected areas within the European Union (Tóth *et al*., 2008).

3. The adaptation of plants to drought stress and their classification

Dry habitats present a constraint for plant growth. Water is one of the basic raw materials of photosynthesis. It is the major component of plant tissues, making up 90% of the plant body. Water is the substance in which most materials enter and leave the cells of plants, and it is the solvent for the biochemical reactions which occur in living cells. Many types of habitats can be found in nature with respect to water supply.

These can be divided into xeric, mesic, and hydric habitats. The plants that are adapted for living in these habitats are called *xerophytes*, *mesophytes*, and *hydrophytes*, respectively. Xerophytes include a number of species that live in habitats where the supply of water is deficient. Mesophytes inhabit regions of average water conditions, and include the majority of wild and

cultivated plants. Hydrophytes form an extensive flora living on the surface of water or submerged at various depths.

There are numerous means by which different plants respond to drought according to comprehensive physiological indices, accordingly, they can be classified as summarized in Fig. 5).

(1) Drought avoidance, these plants restrict water consumption and/or develop a vast root system to avoid being killed by drought. They often maintain high and stable transpiration and photosynthetic rates, such as deep-rooted desert shrubs.

(2) Drought tolerance, these plants can survive without available water for a long period of time, depend on water in storage tissues, *e.g*. cactus (Deng *et al.*, 2008).

In the frame of "physiological window" mild drought induces in plants regulation of water loss and uptake allowing maintenance of their leaf relative water content (RWC) within the limits where photosynthetic capacity and quantum yield show little or no change. (Yordanov *et al*., 2003). On the other hand, the most severe form of water deficit is desiccation when most of the protoplasmic water is lost and only a very small amount of tightly bound water remains in the cell. Depending on the differences in behavior of the photosynthetic apparatus (PSA) during desiccation two groups of desiccation tolerant plants (DT) were distinguished:

- homochlorophyllous desiccation tolerant (HDT)
- poikilochlorophyllous desiccation tolerant (PDT) (Bewley, 1979; Gaff, 1989).

The most essential difference between HDT and PDT plants during desiccation seems to be that the PSA of the HDT plants is retained in a recoverable form, while in PDT plants the chlorophylls and thylakoid systems are degraded (Tuba *et al*., 1996).

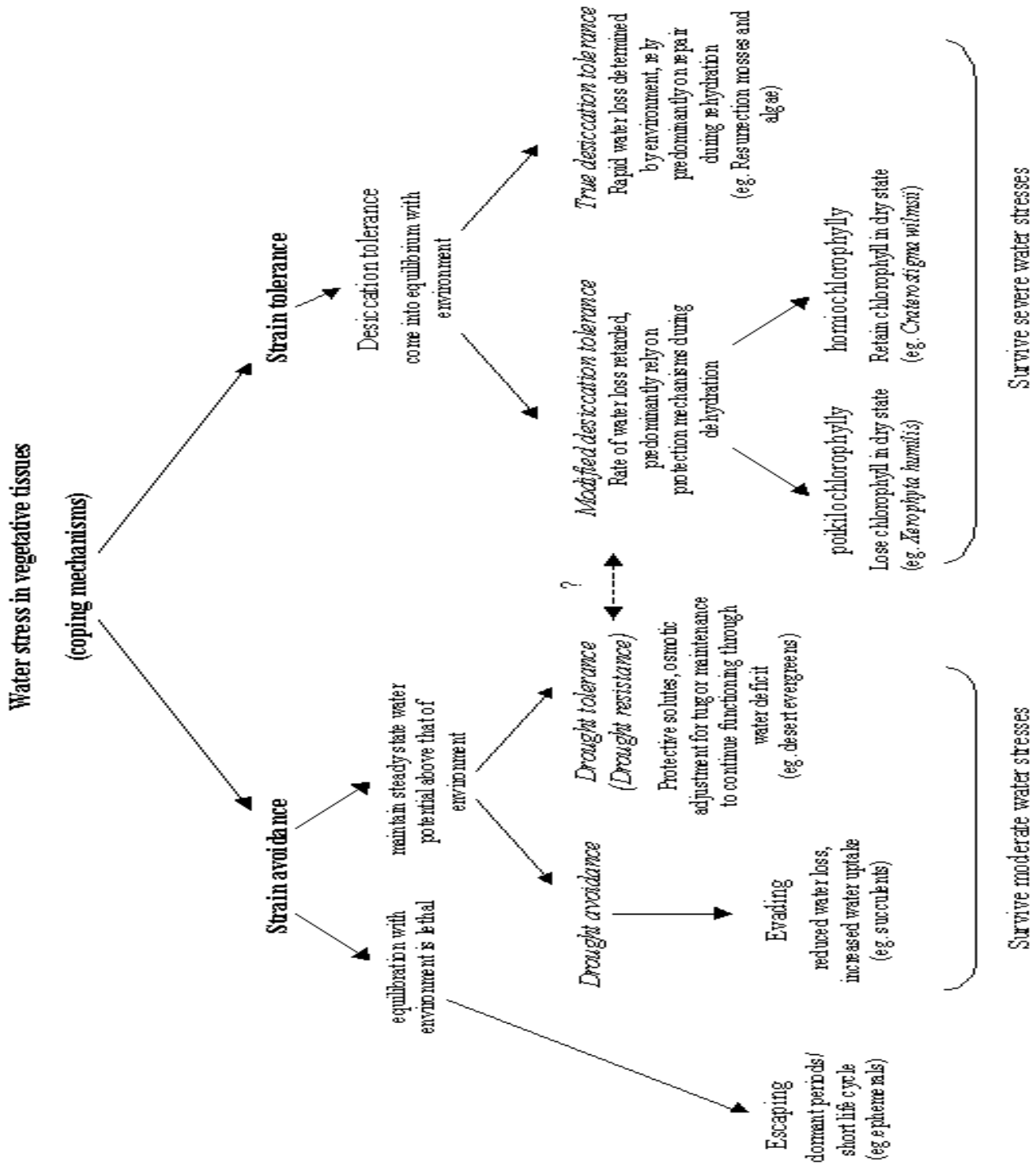


Figure 5. Classification of mechanisms utilized by plants in coping with water stress (Mundree *et al*., 2002)

4. The adaptation of plants to salt stress and their classification

Munns (2002) refers to several studies reporting as rapid responses to salt (responses that occur within a few hours of application of salt) often resemble responses to low water potential imposed using non-ionic solutes. However, longer-term responses that occur over a time frame of days to weeks are more salt specific.

Plants adapted to saline environments are known to have a plethora of mechanisms to ensure their continued growth and survival by either restricting or minimizing salt ion accumulation in leaves.

For most glycophytes, salt tolerance, although limited, is a combination of osmotic adjustment, retention of salts at the root system, compartmenting salts within the leaf vacuole, and accumulation of excess salt into older leaves which are then shed (Greenway & Munns, 1980, Munns & Termaat 1986).

For halophytes, salt tolerance is often a combination of those mentioned above for glycophytes and succulence and the use of salt glands, bladders or hairs to aid in the removal of excess Na^+ and Cl^- (Greenway & Munns 1980, Gorham, 1996, Subbarao *et al*., 2002).

Halophytes are plants which tolerate or even demand sodium chloride concentrations in the soil water they absorb. Halophytes are able to remain alive for long periods in high salt concentrations, with little or no additional growth (Hayward and Wadleigh, 1949).

Depending on the habitat conditions they have developed different strategies to survive in sometimes in very high salt conditions. Halophyte plants can be divided in obligate and facultative. The obligated halophytes need some salt to live and growth, they need NaCl concetrations $\geq$ 200 mM to survive (Flowers *et al*., 1986a), and meanwhile the facultative are able to settle on salty soils, but their optimum lies in a salt-free or at least low-salt media.

Further divisions are hydro-halophytes and xero-halophytes. Hydro-halophytes grow in aquatic conditions or on wet soil. Most mangroves and saltmarsh species along coastlines are hydro-halophytes. Xero-halophytes may grow in habitats where the soil is always saline but where the soil may dry out so much as to cause problems with water availability for the plant.

Still another division is morphologically based. One distinguishes succulent halophytes, halophytes with salt bladders on the leaf surface, and those which excrete the salt with evaporation water, where the salt crystals remain visible on the leaf surface. Many plants fall

under several of the above listed categories. They possess genes and physiological properties which enable them to cope with the salt concentration.

As a soil becomes progressively more saline it becomes more difficult for a plant to extract water from the soil. This is caused by lower osmotic potential that increases the solution entropy and forms associations between water molecules and the solute. This creates water stress in plants as solute content of the soil/groundwater increases and the ability of the roots to take up water decreases (Lambers *et al*. 1998).

Generally, Salt resistance is the reaction of an organism to salt stress. Resistance can involve either:

(1) Salt avoidance involves structural with physiological adaptations to minimize salt concentrations of the cells or physiological exclusion by root membranes.

(2) Salt tolerance involves physiological and biochemical adaptations for maintaining protoplasmic viability as cells accumulate electrolytes.

In principle, salt tolerance in halophytes can be achieved by salt exclusion (salt excluders) or salt inclusion (salt includers) (Fig. 6) (Koyro *et al*., 2011).

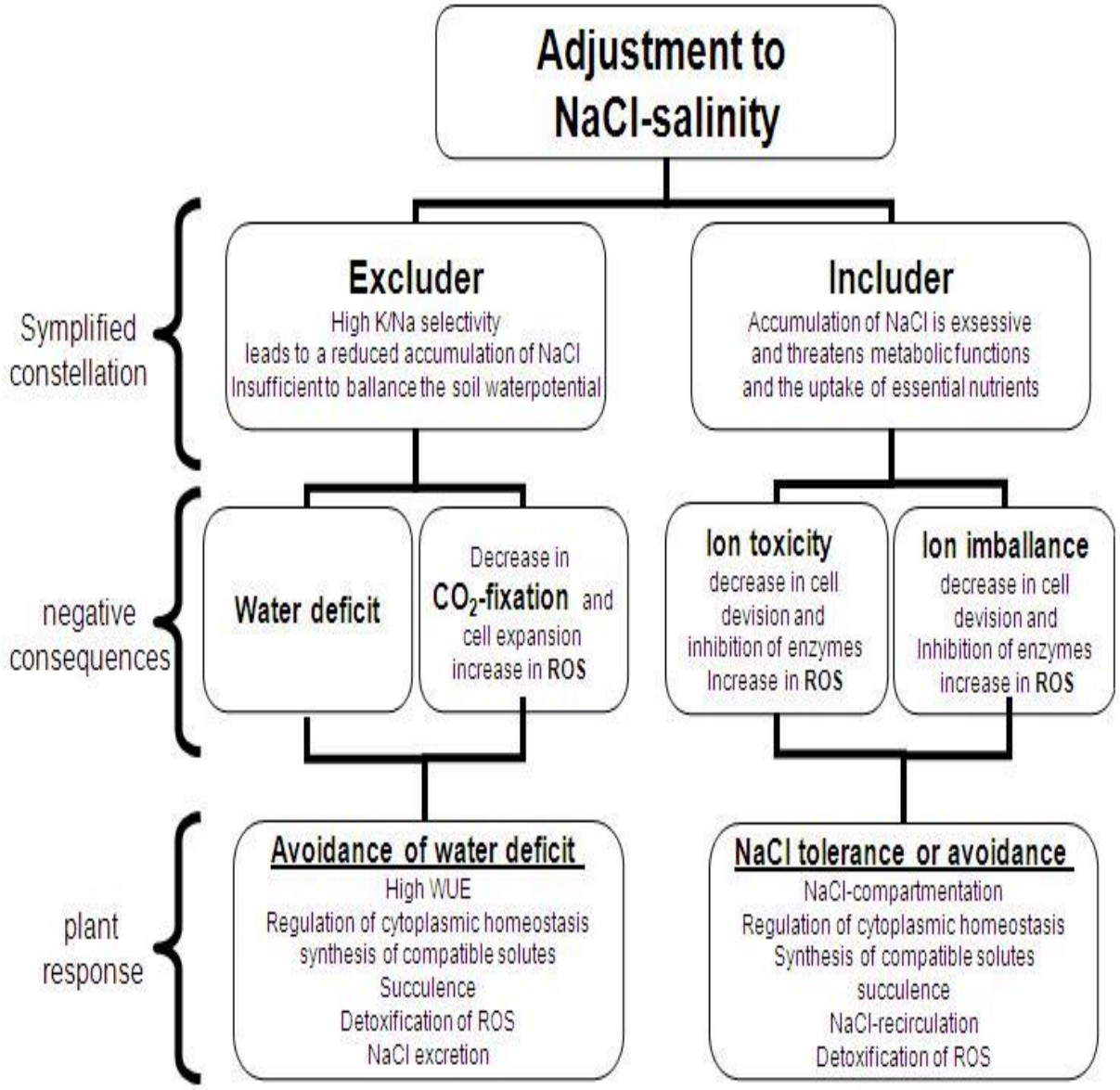


Figure 6. Flow chart showing the possible mechanisms of vascular halophytes to adjust at high external NaCl salinity (Koyro *et al*., 2011).

5. Plant responses to salt and drought stress

5.1 Photosynthesis and Stomatal Conductance responses

Photosynthesis is among the primary processes to be affected by drought (Chaves, 1991) or by salinity (Munns *et al.***,** 2006). The effects can be direct, as the decreased CO_2 availability caused by diffusion limitations through the stomata and the mesophyll (Flexas *et al.***,** 2004, 2007) or the alterations of photosynthetic metabolism (Lawlor and Cornic, 2002) , or they can arise as secondary effects, namely oxidative stress. The latter are mostly present under multiple stress conditions (Chaves and Oliveira, 2004) and can seriously affect leaf photosynthetic machinery (Ort, 2001).

Stomatal responses

Stomata close in response to leaf turgor decline, to high vapour pressure deficit in the atmosphere or to root generated chemical signals. Supply of CO_2 to carboxilation sites is therefore impaired, which implies a down-regulation of photosynthesis and increased energy dissipation. Under mild stress, a small decline in stomatal conductance (g_s) may have protective effects against stress, by allowing plant water saving and improving plant water-use efficiency by the plant.

It is possible to identify two stomatal adaptations of halophytes to salinity

(1) To use sodium instead of potassium as the principal ion determining their changes of turgor.
(2) To exclude sodium, enabling potassium to retain the principal role.

Surprisingly, studies of the ionic relations relations of halophyitic stoma, with particular respect of sodium, are rare, but the literature nevertheless provides evidence that both these methods exist, and also that the second of them could make a contribution to the control of salt uptake at the whole plant level (Robinson, *et al*., 1997).

Under dry conditions stomatal closure is like to be mediated by chemical signals (like absciccic acid) travelling from the dehydrating roots to shoots (Chaves, *et al*., 2003).

Non stomatal responses

A decrease in (g_s) is one of the earliest responses to environmental stress, but if this situation continues for long, the mechanisms involved are more complicated than simply reduction of

(g_s) and implicates nonstomatal limitations to photosynthesis, namely biochemical limitations (Graan & Boyer 1990; Giménez, *et al*., 1992; Quick *et al*. 1992; Tezara *et al*. 1999).

The second step that can limit CO_2 diffusion toward the sites of fixation (the chloroplasts) is the conductance inside the leaf mesophyll (g_m) (Flexas *et al*., 2004, 2007). Although measures of mesophyll conductance (g_m) is not as straight forward as (g_s) measurements, estimations of g_m seem to be very important to understand better the real answer to the stress (Warren, 2006). The variations in (g_m) in stress conditions may be linked to physical alterations in the structure of the intercellular spaces due to leaf shrinkage (Lawlor and Cornic, 2002) or to alterations in the biochemistry (bicarbonate to CO_2 conversion) and/or membrane permeability (aquaporins).

An increasing body of evidence has been accumulated that (g_m) is reduced under stress conditions, and particularly in response to drought (Flexas *et al*. 2002) and to salinity (Bongi & Loreto 1989; Delfine *et al*.1998; 1999).

Biochemical and photochemical limitations to photosynthesis could be get involved in long term studies. Changes in leaf biochemistry that result in down-regulation of the photosynthetic metabolism may occur in response to lowered carbon substrate under prolonged stresses (Chaves and Oliveira, 2004; Flexas *et al*., 2006). For example, a de-activation of the carboxylating enzyme Rubisco by low intercellular CO_2 concentration (C_i) has been observed (Meyer and Genty, 1998). Following stomatal closure and the fall in CO_2 concentration in the intercellular airspaces of leaves, other enzymes have been shown to decrease their activity (e.g. SPS or nitrate reductase); this change was quickly reversed when increasing CO_2 in the surrounding atmosphere (Sharkey *et al*., 1990). Under salt stress, metabolic limitations of photosynthesis resulting from increased concentrations of Na^+ and Cl^- in the leaf tissue (in general above 250 mM) do occur (Munns *et al*., 2006). Early biochemical effects of water deficits that involve alterations in photophosphorylation (a decrease in the amount of ATP leading to a decreased regeneration of RuBP) have also been described (Tezara *et al*., 1999) and seem to be dependent on species showing different thresholds for metabolic down-regulation (Lawlor and Cornic, 2002).

5.2 Water use efficiency

Following Turner (1997), we define water use efficiency as the dry matter produced per unit evapotranspiration.

At the leaf level, instantaneous water use efficiency can be estimated by gas exchange measurements and calculated as the ratio of carbon assimilation to transpiration (A/E, μmol CO_2 mol $^{-1}H_2O$). However the ratio A/E is largely dependent on the environmental conditions, for example, changes in humidity deficit of the atmosphere altering the evaporation rate even if stomatal aperture and hence assimilation rate do not change.

Water use efficiency can be calculated as the ratio of carbon assimilation to stomatal conductance (A/g_s) (referred to as intrinsic water use efficiency). (A/g_s) is often used as a normalized valued when comparing instantaneous WUE measured at different VPD. It is of particular interest to determine any underlying intrinsic differences in water use efficiency that might exist independent of the specific environmental conditions. Such a measure is particularly useful for those situations where we are interested in improving WUE, for example through plant breeding (Bacon, 2004).

The use of carbon isotope discrimination also gives a measure of intrinsic water use efficiency because it primarily measures the ration (A/g) (Where g is the total gas phase conductance to CO2) (Bacon, 2004).

Plants with higher WUE can use water economically and has a higher drought resistance (Bierhuizen and Slatyer, 1965). For example, the study of *Medicago sativa* illustrated that WUE can reflect the adaptive ability of plants to stress (Liu, *et al*., 2006).

Halophytes can also increase their water use efficiency in response to salt, thereby minimizing the amount of water that must be transpired for each unit of growth (Guy *et al*., 1980; Guy and Reid, 1986; Ayala and O'Leary, 1995; Glenn *et al*., 1997). C3 and C4 species increase water use efficiency by lowering their stomatal conductance in response to salt, which decreases rates of both photosynthesis and transpiration but not in direct proportion, resulting in an increase in water use efficiency (Osmond *et al*., 1980).

5.3 Mineral content

Both drought and salinity disturb the mineral-nutrient relations in plants through their effects on nutrient availability, transport, and partitioning in plants. Additionally, salinity stress also induces iron deficiency or imbalance due to the competition of nutrients such as K^+, Ca^{2+}, and $NO3^-$ with the toxic ions Na^+ and Cl^-. Mineral nutrients play a vital role in determining plant resistance to drought or salinity.

5.3.1 Nitrogen

Nitrogen is the mineral element that plants require in the largest amounts and a constituent of many plant cell components, including amino and nucleic acids. Therefore, nitrogen deficiency rapidly inhibits plant growth. An early study of the relationships between water availability and the N-fertilizer responses from Smika *et al*. (1965) demonstrated that fertilizer N will not increase yield without sufficient water being available to the plant, and increasing soil-water availability will not increase production without adequate N supply.

A study on wheat grown in a sandy soil under different moisture-stress levels showed similar interactive effects of N supply and drought stress. However, whereas in most cases the total shoot N uptake (mg N per plant) decreases under saline conditions, the N concentration (mg N per kg dry weight) increases or remains unchanged under optimal N conditions (Munns and Termaat, 1986; Hu and Schmidhalter, 1998). Many studies showed that, in contrast to the total N in the plant, salinity reduces the $NO3^-$ concentrationin the leaves without affecting the total N content (*e.g.*, Hu and Schmidhalter, 1998), and that the addition of

$NO3^-$ results in a reduction in Cl^- uptake and accumulation due to $NO3^-/Cl^-$ antagonism (Bernstein et al., 1974; Hu and Schmidhalter, 1997). Furthermore, the form of N supplied to salinized plants might influence not only the Cl^- concentration, but also that of other nutrients such as Ca^{2+} and K^+. The N form might also influence the sensitivity of the plant to salinity (Speer and Kaiser, 1994). For example, wheat and maize were more sensitive to salinity as the ratio of $NH4^-$: $NO3^-$ increased (Leidi *et al*., 1991; Botella *et al*., 1997).

5.3.2 Phosphorus

Phosphorus is a constituent of nucleic acids, phospholipids, phosphoproteins, dinucleotides, and adenosine triphosphate. Hence, P is required for processes including the

storage and transfer of energy, photosynthesis, the regulation of some enzymes, and the transport of carbohydrates. The data of the interactive effect between P nutrition and salinity on plant growth in the literature are frequently contradictory. However, the addition of P or the strategies employed to increase P uptake in most cases seem to be more important in drought conditions than in saline ones. Soils in arid areas are often calcareous and have high pH (*e.g.*, those in Mediterranean regions). It is generally accepted that the uptake of P by crop plants is reduced in dry-soil conditions (Pinkerton and Simpson, 1986). For example, the translocation of P to the shoots is severely restricted even under relatively mild drought stress (Rasnick, 1970). Turner (1985) pointed out that P deficiency appears to be one of the earliest effects of mild to moderate drought stress in soil-grown plants. The positive effects of P on plant growth under drought have been attributed to an increase in water-use efficiency, stomatal conductance (Brück *et al*., 2000), and photosynthesis (Ackerson, 1985), to higher cell-membrane stability, and to effects on water relations (Sawwan *et al*., 2000).

5.3.3 Potassium

Potassium is an essential factor in protein synthesis, glycolytic enzymes, and photosynthesis; an osmoticum mediating cell expansion and turgor-driven movements; and a competitor of Na^+ under saline conditions (Marschner, 1995). Because both drought and salinity affect plant growth similarly through water deficit, K^+ is equally important for maintaining the turgor pressure in plants under either stress. Additionally, higher K^+: Na^+ ratios will also improve the resistance of the plant to salinity (Asch *et al*., 2000). The availability of K^+ to the plant decreases with decreasing soil water content, due to the decreasing mobility of K^+ under these conditions. Under drought conditions, wilting in plants suggests possible K^+ deficiency (Beringer and Trolldenier, 1978). This differs from salt-stressed plants, which are characterized by the absence of wilting at the same water potentials as that for drought stress (Shalhevet and Hsiao, 1986). Potassium increases the plant's drought resistance through its functions in stomatal regulation, osmoregrulation, energy status, charge balance, protein synthesis, and homeostasis (Beringer and Trolldenier, 1978; Marschner, 1995). It also maintains turgor pressure (Mengel and Arneke, 1982) and reduces transpiration under drought conditions (Andersen *et al*., 1992). In plants coping with drought stress, the accumulation of K^+ may be more important than the production of organic solutes during the initial adjustment phase, because osmotic adjustment

through ion uptake like K^+ is more energy efficient (Hsiao, 1973). Working with wheat, Morgan (1992) showed that the lines displaying high osmotic adjustments had a high accumulation of K^+ in their tissues. In these lines, K^+ accounted for about 78% of all osmotica. By contrast, amino acids, which were the only other important contributor, constituted only about 22%. High Na+ concentrations in the external solution cause a decrease in both K^+ and Ca^{2+} concentrations in the tissues of many plant species (Hu and Schmidhalter, 1997). These decreases could be due to the antagonism of Na^+ and K^+ at uptake sites in the roots, the effect of Na^+ on K^+ transport into the xylem (Lynch and Läuchli, 1984), or the inhibition of an uptake processes (Suhayda *et al*., 1990). Reasonable amounts of both K^+ and Ca^{2+} are required to maintain cell-membrane integrity and function (Wei *et al*., 2003). In glycophytes, which comprise most crop species, exclusion is the predominant strategy for plant resistance to salt stress. Glycophytes restrict both the uptake of toxic ions like Na^+ by the roots from the soil and also the movement of the toxic ions to the shoot by controlling their influx into the root xylem from the root cells. A high K^+: Na^+ ratio in the plants is also maintained by the roots, showing a selectivity of K^+ over Na^+ and by a preferential loading of K^+ rather than of Na^+ into the xylem. Toxic ions like Na^+ can also be transported away from the cytoplasm into the vacuole of cells (intracellular compartmentalization). In fact, the underlying mechanism for maintaining adequate tissue K^+ levels under salt stress seems to be dependent upon selective K^+ uptake and selective cellular K^+ and Na^+ compartmentalization and distribution in the shoots (Carden *et al*., 2003). Plants use both low- and high-affinity transporters to take up K^+ from the growth medium (Blumwald, 2000). The three classes of low affinity channels—K^+ inward rectifying channels (KIRCs), K^+ outward rectifying channels (KORCs), and voltage-independent cation channels (VICs)—play important roles in maintaining cellular K^+ : Na^+ ratios (Amtmann and Sanders, 1998). In addition, two families of high-affinity transporters have also been reported to play a role in K^+ transport (Quintero and Blatt, 1997) and thus in also determining the K^+: Na^+ ratio in plant cells.

5.3.4 Calcium

Calcium plays a vital role in regulating many physiological processes that influence both growth and responses to environmental stresses. Included among these are water and solute movement as influenced through the effects of Ca^{2+} on membrane structure and stomatal function, cell division and cell-wall synthesis, direct or signaling roles in systems involved in

plant defense and repair of damage from biotic and abiotic stress, and rates of respiratory metabolism and translocation (McLaughlin and Wimmer, 1999). Despite its obvious importance, the low mobility of Ca^{2+} make the rates of its uptake and distribution limiting processes for many key plant functions. Furthermore, the general lack of recognition of the limiting role of Ca^{2+} is due in part to the fact that some important plant functions are controlled by changes in very small physiologically active pools of Ca^{2+} within the cytoplasm. A recent review (Cramer, 2002) summarizes the research on Na^{+}/Ca^{2+} interactions under salinity stress from a physiological perspective. Because Na^{+} readily displaces Ca^{2+} from its extracellular binding sites, Ca^{2+} availability could be seriously reduced under salinity, especially at low Ca^{2+}: Na^{+} ratios (Cramer *et al*., 1988). Furthermore, the decreased uptake of Ca^{2+} under saline conditions might be due to its precipitation and the increase in ionic strength that reduces its activity.

Calcium deficiency, in general, can impair the selectivity and the integrity of the cell membrane and permit the passive accumulation of Na^{+} in plant tissues. In addition, the low $Ca^{2+:}$ Na^{+} ratio in saline media plays a significant role in inhibiting plant growth as well as causing significant changes in morphology and anatomy (Cramer, 1992). Supplemental Ca^{2+} has also been reported to alleviate the adverse effects of salt stress on the germination and vegetative growth of bean (Awada *et al*., 1995). By contrast, various Ca^{2+}: Na^{+} ratios had no significant effect on the uptake of Na^{+} by rice (Yeo and Flowers, 1985). The relationship between salt resistance and Ca^{2+} accumulation among different plant species was investigated by Unno *et al*. (2002) using salt-tolerant maize and squash and salt sensitive reed canary grass and cucumber. The distribution of Ca^{2+} in the shoots decreased greatly in the salt-sensitive plants under salt stress, suggesting that the ability of plants to retain Ca^{2+} is associated with their salt resistance. In particular, the inherent genetic capability to maintain Ca^{2+} in the tissues and to exclude Na^{+} from the shoots were highly heritable traits, suggesting that Ca^{2+} : Na^{+} ratios might be promising indicators of salt resistance. In recent years, intracellular Ca^{2+} has been found to regulate the responses of the plant to drought and salinity and has also been implicated in the transduction of drought- and salt stress signals in plants, which play an essential role in osmoregulation under these conditions (Knight *et al*., 1997; Bartels and Sunkar, 2005).

5.3.5 Magnesium

Although many studies have analyzed plant tissues for Mg^{2+}, few salinity–nutrient studies have directed any attention to Mg^{2+} nutrition as affected by salinity (Grattan and Grieve, 1999).
Because the transport of micronutrients to the plant roots occurs via diffusion, low soil moisture content, as with P uptake, will reduce micronutrient uptake. However, because plants require much smaller quantities of micronutrients, the effects of drought stress are not as great as for P.

5.4 Plant growth

Arrest of plant growth (plant height and diameter, leaf area, leaf and shoot number) during stress conditions largely depends on the severity of the stress. Mild osmotic stress leads rapidly to growth inhibition of leaves and stems, whereas roots may continue to elongate (Nonami and Boyer, 1990; Spollenen *et al*., 1993).
The degree of growth inhibition due to osmotic stress depends on the time scale of the response, the particular tissue and species in question, and how the stress treatment was given (rapid or gradual). Growth arrest can be considered as a possibility to preserve carbohydrates for sustained metabolism, prolonged energy supply, and for better recovery after stress relief (Bartels and Sunkar 2005).
Similar changes in plant growth occur in responses to the osmotic effect of the salt, and to drought stress. (Munns and Tester, 2008).
A sudden increase in soil salinity or water moisture causes plant cells to lose water, but this loss of cell volume and turgor is transient. Both elongation of apical shoots and expansion growth are affected. Cell dimensions change, with more reduction in area so leaves are smaller and thicker. For a moderate stress, an inhibition of lateral shoot development becomes apparent over weeks, and over months there are effects on reproductive development, such as early flowering or a reduced number of florets. During this time, a number of older leaves may die. However, production of younger leaves may continue depends on stress level and time. The mechanism that down regulates leaf growth and shoot development under may be regulated by long distance signals in the form of hormones or their precursors, because the reduced leaf growth rate is independent of carbohydrate supply (Munns *et al*., 2000) and water status (Fricke *et al*., 2006; Munns *et al*., 2000).

Root growth under salt stress is usually less affected than leaf growth. With time, reduced initiation of new seminal or lateral roots probably occurs (Munns and Tester, 2008). On the other hand, continuation of root growth under drought stress may occur as an adaptive mechanism that facilitates water uptake from deeper soil layers (Munns *et al*., 2000).

5.5 Osmotic adjustment

A metabolic response to salt stress is the synthesis of compatible osmolytes. These mediate osmotic adjustment and therefore protect sub-cellular structures and reduce oxidative damage caused by free radicals, produced in response to high salinity (Hong *et al*., 1992; Hare *et al*., 1998). Osmolyte compounds include sugars, polyols, amino acids, tertiary and quarternary ammonium, and sulphonium compounds (Rhode, 1993). In salt- acclimated plants, it was shown that primary metabolites linked to amino acid and nitrogen or carbohydrate and polyol metabolism increase; these compatible solutes play a role in osmotic adjustment, membrane and protein protection or scavenging of reactive oxygen species (ROS) and of excess accumulated ammonium ions. Some of the Australian halophytic Melanleuca species are salt tolerant, which has been attributed to their ability to accumulate large quantities of osmoprotectants known as proline analogues (Naidu, 2003). These have been used in seed treatment and foliar application to increase the salt resistance of economic crops. Melanleuca bracteata, which accumulates the proline analogue 4-hydroxy-N-methyl proline (MHP), could potentially be harvested for commercial use, playing a positive role in the control of dry land salinity, with an average yield of 393kg/ha of MHP, correlating to a gross economic return of AU$14505/ha (Naidu, 2003).
Depending on the accumulated osmolyte, halophytic plants can be classified into three physiotypes (Stewart *et al*. 1979; Briens and Larher 1982): species that produce high levels of soluble carbohydrates and/or polyols, but in which nitrogenous water soluble compounds are present at very low concentrations; plants accumulating nitrogenous water soluble compounds at higher concentrations than non-structural carbohydrates; and species that accumulate both carbohydrates and nitrogenous solutes, the first remaining quantitatively predominant. It is not clear whether interspecific variability is a sufficient explanation for why some species accumulate carbohydrates and/or nitrogenous compounds to a greater extent than others. Accumulation of carbohydrates may require a great deal of carbon, which is less readily available under stress conditions when net assimilation is decreased (Jefferies and Rudmik,

1984). Moreover, in a saline environment which is generally deficient in nitrogen, Nitrogen in salt stressed plants may not be a limiting factor for accumulation of nitrogen containing compounds, despite despite $NO3^-$ reduction in many species in response to salt stress, (Mansour 2000). Popp and Albert (1995) compared a wide range of herbaceous halophytes and found that species accumulated either nitrogenous compounds or carbohydrates, but rarely both, with the exception of *Triglochin maritima* which produced high levels of both carbohydrates and proline (Briens and Larher, 1982).

In drought tolerant plants, compatible solutes such as proline may increase dozens, even hundreds of times than the original amount in drought stress (Liu *et al.,* 2002). For instance, more proline accumulates in varieties with more drought resistance, and this index has been used in study of drought resistance about 12 species of *Caragana* spp. (Zhang *et al.*, 2006) and 4 plants in Lanzhou, including *C. korshinskii*, *E. angustifolia*, *A. mongolicus* and *R. pseudoacacia* (Jiang *et al.*, 2006).

It was documented also that the accumulation of soluble sugars (sucrose, glucose and fructose) is strongly correlated to the acquisition of drought tolerance in plants (Crowe *et al.*, 1990; Vertucci and Farrant, 1995; Hoekstra and Buitink, 2001). Earlier reports mentioned that sugars protect the cells during drought by two mechanisms. First, the hydroxyl groups of sugars may substitute for water to maintain hydrophilic interactions in membranes and proteins during dehydration. Thus, sugars interact with proteins and membranes through hydrogen-bonding, thereby preventing protein denaturation (Williams and Leopold, 1989; Leopold *et al.*, 1994). Secondly, sugars are a major contributing factor to vitrification, which is the formation of a biological glass in the cytoplasm of dehydrated cells (Leopold *et al.*, 1994; Buitink *et al.*, 1998). These intracellular glasses, by virtue of their high viscosity, drastically reduce molecular movement, impede the diffusion of reactive compounds in the cell, and may maintain the structural and functional integrity of macromolecules (Sun and Leopold, 1997).

In many higher plants under dehydration stress, carbohydrate metabolism is shifted to favor the conversion of other sugars to sucrose (Norwood *et al.*, 1999; Whittaker *et al.*, 2001). Sucrose also accumulates in response to drought stress in the resurrection plants (i.e. *Xerophyta viscosa*, *Sporobolus staphianus* and C*raterostigma wilmsii* (Mundree and Farrant, 2000; Whittaker *et al.*, 2001; Cooper and Farrant, 2002), implicating a role for sucrose in the acquisition of desiccation tolerance

5.6 Water relations adjustment

The reduced water availability induced by osmotic potential of salt or by water deficit itself, can be quantified as a decrease in water potential of plants (Kramer and Boyer, 1995).
Water potential is the work that would be necessary to move a unit of mass of bound water to the tissues of a plant, to take it from the state of union to a reference status corresponding to the pure water at same temperature and the atmospheric pressure. As the value zero it is adopted for this reference potential, all the potentials that characterize the bound water to a system are negative because it is necessary to provide a work to take this water to a potential equal to zero. The units of the water potential are those of pressure being commonly used Mpa (megapascals). Alternatively bars can be used (1Mpa=10bars) (Gonzalez, 2001).

For a plant water potential may be expressed as the sum of three components:

$\Psi = \Psi_p + \Psi_s + \Psi_m$

Where:

Ψ_p: turgor potential (Difference in hydrostatic pressure with the reference)

$\Psi_{s:}$ Osmotic potential (due to the pressure of dissolved solutes)

$\Psi_{m:}$ Matric potential (due to the pressure of matrices)

Decreased Ψ (decreased free energy of the water) makes more difficult for the plant to take up water, and this in turn elicits a range of responses that allow the plant to avoid water loss, allow water uptake to continue at reduced water potential or allow the plant to tolerate a reduced tissue water content. An overall picture of these responses must include changes in water fluxes and water relations at the whole plant and the cellular levels. In most cases, the plant's first response is to avoid low Ψ. Tissue Ψ and water content are maintained close to the unstressed level by increasing water uptake or limiting water loss such that the rates of water loss and water uptake remain balanced. Such a balance is achieved in the short term mainly by stomatal closure. In the longer term, changes in root and shoot growth occur. In this manner, the Ψ of the plant will equilibrate with that of the water source.
When water uptake and loss cannot be balanced anymore, due to a severe stress in long term, when soil water content and Ψ are low, Ψ of the plant tissue must also decrease, either through water loss or by adjustments made by the plant to achieve a low Ψ while avoiding water loss.

Such adjustments are termed 'dehydration avoidance'.The main mechanisms of dehydration avoidance are accumulation of solutes and cell wall hardening. When Ψ becomes more severe, stress becomes more severe, it becomes increasingly difficult for the plant to avoid dehydration and mechanisms to tolerate reduced water content become important. Most of the dehydration tolerance mechanisms studied to date function primarily to protect cellular structure from the effects of dehydration (Verslues, 2006).

In addition to water potential adjustment, relative water content (RWC) has been used to estimate plant water status in terms of cellular hydration under the possible effect of leaf water potential and osmotic adjustments (Kramer and Boyer, 1995).A decrease in RWC may occur in presence or absence of water potential regulation as dehydration avoidance mechanism.

6. Salt specific responses

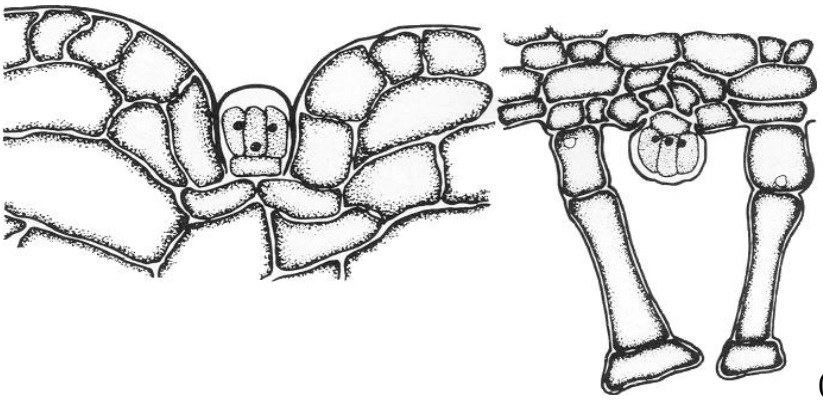
Under salinity, in addition to water deficits, plants endure salt-specific effects. Salt response follows a biphasic model, with current metabolic data indicating an early similarity with drought, whereas in the long-term plants are responding to ion toxicity. There are species-specific responses to salt. Some plants are able to prevent salt entry (salt exclusion at the wholeplant or the cellular level) or to minimize its concentration in the cytoplasm (by compartmentalizing salt in the vacuoles), thus avoiding toxic effects on photosynthesis and other key metabolic processes. When those processes do not exist or are insufficient, it was shown that Na^+ at a concentration above 100 mM severely inhibits many enzymes (including photosynthetic ones) (Munns *et al*., 2006). The enzymes that require K^+ as a cofactor are particularly sensitive to high concentrations of Na^+ or high ratios of Na^+/K^+. Salt stress differs from the low Ψ imposed by soil drying or a high molecular-weight solute in that a major factor causing long-term injury in salt stress is the ionic imbalance and toxicity caused by excess Na^+ rather than the effects of salt on Ψ (Huh *et al*., 2002; Munns, 2002). Munns, (2002) refers to several studies reporting that rapid responses to salt (responses that occur within a few hours of application of salt) often resemble responses to low Ψ imposed using non-ionic solutes. However, longer-term responses that occur over a time frame of days to weeks are more salt specific.

6.1 Na^+ and Cl^- Vacuolar Compartmentation

Internal salt concentrations can reach levels three times that of the external medium (Munns *et al*., 1983). The ability of plant cells to maintain low cytosolic sodium concentrations is an essential process for halophytes (Borsani *et al*., 2003). Plant cells respond to salt stress by increasing sodium efflux at the plasma cell membrane and sodium accumulation in the vacuole. Such compartmentalization of sodium and chloride in leaf vacuoles can only be attained if (1) sodium and chloride ions are actively transported into the vacuole and if (2) tonoplast permeability to these ions is kept low enough to sustain the ion concentration gradients at an energy cost that can be prolonged for many months (Maathius *et al*., 1992). Tonoplast conductance of higher plants can become very large, owing to the opening of unselective, slow vacuolar ion channels, as demonstrated by many patch clamp investigations (Maathius *et al*., 1990). SV channels conduct both chloride and sodium and would therefore tend to dissipate a NaCl concentration gradient across the tonoplast, causing plant death, if conductance for sodium and or chloride rises above the capability of active transport to prevent such a leakage (Maathius *et al*., 1992).

6.2 Salt excretion

Halophytes utilize salts in osmotic adjustment to mantain low water potentials of their environments. They must accumulate sufficient ions in their leaves for this purpose, while avoiding the toxic effect of those ions (Waisel, 1972). In some species, growth and ion accumulation are balanced (Flowers, 1986b) while in others excess ions are secreted via salt glands (Drenman, 1982). Mineral content of shoots is best regulated by the secretion of ions through specialized salt glands, such as in *Spartina species*. However, salts are also released through the cuticle or in guttation fluid; they are retransported back to the roots and soil via the phloem, or become concentrated in salt hairs (Stenlid, 1956). Salt glands are located on or depressed into the epidermis and are found in almost every aerial part of the plant, but tend to be concentrated on the leaves (Fig. 7).



(a) (b)

Figure 7. Segments of a leaf cross section of Avicennia marina, showing sunken glands in the upper epidermis (a) and elevated glands on the lower one (b). (Waisel, 1972)

Salt glands lack a central vacuole and have a high number of mitochondria and other organelles, suggesting that they act primarily not as storage cells, but as transit cells (Waisel, 1972). However, not all halophytes have salt glands, neither do they all discard salt saturated tissue, demonstrating that individual halophytes utilize different salt tolerance traits in different situations.

Plants can exclude salt from uptake through passive and active mechanisms. Passive exclusion occurs through having high amounts of phospholipids in their membranes, which restricts movement of sodium chloride to the shoot while allowing uptake of other ions. A benefit of active uptake of salts would be lowering the water potential of a plant. This would give the plant the ability to take up more water as the water potential of the soil/groundwater decreases. Tamarix excretes salt by use of salt hairs (trichomes) or through the use of salt glands (Lambers *et al*. 1998). Plants may employ this strategy when compartmentalization of salt no longer becomes possible. Use of salt hairs and glands are active processes and require energy, which reduces growth.

7. General features of Tamarix

7.1 Tamarix Distribution

There are thought to be approximately 54 species of *Tamarix spp.* in the world (DeLoach and Lewis 2000). The genus Tamarix consists of halophytic shrubs and trees native to a zone stretching from southern Europe and North Africa through the Middle East and south Asia to China and Japan. There are a few species in disjunct parts of Africa (Rodman 1989). Baum (1978) considers that *Tamarix* have one major centre of speciation in the Pakistan–Afghanistan–Iran–Turkmenistan–southern Kazakhstan–western China area and another in the eastern Mediterranean area (Fig.8).

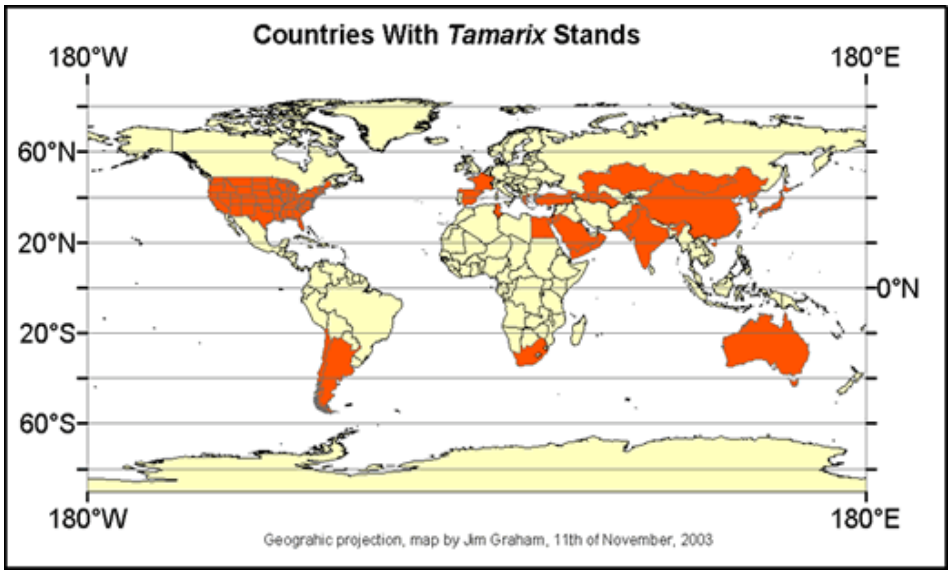


Figure 8. Tamarix distribution in the world (Graham, 2003).

7.2 General botanical and morphological characteristics

It is a shrub or shrub-like tree usually grow up to less than 6 m (20 ft) (Fig.13) commonly has numerous basal branches or trucks that are brown or blackish and younger branches that are reddish-brown or purplish. Leaves are scale-like, alternate, sessile or sheathing, with salt-secreting glands. Leaves comprise about 50% of the photosynthetic area, the rest of phothosynthesis being carried out in cladophyll stems.

Flowers are small, perfect, and subtended by a small bract (Allred, 2002). They form some racemate inflorescences. Petals are white, pinkish, or reddish - different colors often are found on

the same plant or even the same flowering branch. Flowering time varies according to species, certain species (e.g. *T. gallica* and *T. parviflora*) flower mainly in spring, rarely in summer, while other species (e.g. *T. ramosissima* and *T. chinensis*) flower in both spring and summer (Allred, 2002; DiTomaso and Healy, 2003).The species are not easily distinguishable by their vegetative features.

The root system of *Tamarix* is extensive and deep, with a strong tap root. Horton *et al.* (1960), found the root to exceed shoot length after 7 weeks of age. Gary (1963) reported that the root morphology was highly adaptable depending on water table and substrate. When growing in soils with dense clay layers, the root system tended to flatten rather than to penetrate the clay horizon vertically. Adventitious roots are produced endogensously, near the vascular cambium (Wilkinson, 1966). In order to determine variations in root depth, lateral development, and degree of branching, Tomanek and Ziegler (1960) studied 35 excavated plants that varied in age from a few days to four years. In nearly all cases, roots extended to the capillary fringe above a constant source of water. Roots were never found extending into the saturated zone except where the water table had recently risen. It was found that the degree of development of the root systems varied in several respects. Smaller roots branch and near the extremities, providing a finely subdivided and effective absorptive tap root which went down to the water table. However, on most large plants the major part of the system was made up of lateral growth of primary, secondary, and adventitious roots. The determining factor in branch root formation and development may possibly be the location of the water table.

Some species are considered phreatophytes such as *T. gallica*, and *T.canariensis* (deep-rooted to reach water table) that depend on groundwater for their water supply. However, under some conditions Tamarix spp. can grow where no groundwater is accessible. Thus, it is classified as a facultative rather than obligate phreatophyte (Kerpez and Smith 1987).

Many of the species inhabit sand dunes, and salt marshes and dry soils (Carpenter, 2000), these species are classified as xerohalophytes such as *T.tetragyna*, while other are hydrohalophytes for example *T. Africana* (Alsharhan, 2003). They produce large amounts of seed and propagate vegetatively and grow very fast.

7.3 Cultivation of Tamarix

In History, name of Tamarix was reported more than one time and used by people belonging to difficult culture. Abraham planted a tamarisk tree in Beersheba, and there he called upon the name of the LORD, the Eternal God (Genesis 21:33) In the Ancient Church holy worship was performed in places where celestial things are represented by things high and lofty, such as mountains and hills; and spiritual things, by things fruitful and leafy, such as trees (Thompson, 2002). In the Quran 35:16, the people of Saba were punished when "[Allah] converted their two gardens (rows) into gardens producing bitter fruit and tamarisks [...]" In Egyptian Mythology, the body of Osiris is hidden for a time in a tamarisk tree in Byblos, until it was retrieved by Isis. According to The New Larousse Encyclopedia of Mythology, the tamarisk plant is a favorite of the Greek god, Apollo.

The speculations on utility of Tamarix found in the history still are present nowadays; United states consider this plant as highly undesirable and aggressive phreatophyte because it invaded riparian habitats and lead to displacement of native plant species decreased wildlife habitat values, increased salinity of surface soil, and excessive groundwater consumption (Carpenter, 2003). However in Asia and Africa, they are a natural part of the ecosystem. In countries where such ornamental shrub is encouraged to be planted is used as agroforest plant (Kirk, 2008) due to its beneficial characteristics we mentioned before. In some instances, fertility and soil moisture conservation are strengthened, erosion is controlled by the introduction of versatile trees and shrubs such as Tamarix due to its fast growth (Fig.14). It could be used also as a windbreak, and shade tree. The wood may be used for carpentry or firewood. Plans are being made for the tamarisk to play a role in anti-desertification programs in China. (Tracy and Robbins, 2009). Tamarix can be planted in saline soil (Fig.15) to produce biomass for energy (eg. biofuel), (Zhiliang , 2007). Tamarix can be used as firewood (eg. wood chips) and charcoal, its calorific value is equal to 4835 kcal/kg. The biomass production can be reached maximum value of 67 t ss/ha/yr, with average values of 40 tss/ha/yr in irrigated short rotation forestry (SRF) plantation with a density of 2500 plants /ha in the Negev desert (Fig.16) (Eshel *et al*., 2010).Tamarix chips are of good quality and color, suitable for manufacture of particleboard. Twigs are used for basket making. Wood, close-grained, light-coloured, fibrous, fairly hard, heavy (specific gravity 0.6-0.7.5) strong, density of about 700 kg/m^3, high shock resistance,

splits readily when first cut and polishes well. Useful for making ploughs, wheels, carts, construction, tool handles, brush-backs, ornaments, carpentry, furniture. Tannin or dyestuff mainly from flowers are used for tanning leather. The bark is also a rich source of tannin and mordant for dyeing. In medicine field, Tamarix flowers are used as an astringent and gargle, bark for treating eczema and other skin diseases.

Tamarix is used as a plant indicator for soil type in agricultural surveys. Salt drip from the leaves kill all ground vegetation beneath the tree and litter from it is too salinized, to burn thus strips of the species can be grown to stop wild fires and also hold the spread of fires along highways or railway lines caused by sparks or cigarettes (Orwa *et al*. 2009).Tender branches and leaves provide high value forage, particularly during the dry period. The cultivation has also increased the supply of fodder as food for livestock with a consequent increase in the number of livestock per capita. In Jiashi County, for example, the average number of animals has increased from 1.7 per person in 1985 to 2.7 in 1993. Many villagers have also adopted a cut-and-carry feeding system, so that uncontrolled grazing of the forest areas on the oasis margins has been dramatically reduced.

Tamarix species has also helped rehabilitate the land for a more viable agricultural production. Agricultural productivity has increased the average annual household income in Jiashi County. Furthermore, the marketing of products which use Tamarix, such as basket making, earth carriers, and trolleys, has also improved livelihoods of the 33% of total households involved in cottage industries by generating a profit of approximately year per household (Clare parker, 2010).Many reports referred to the use of, *Tamarix*, in phytoremediation purposes (Manousaki *et al*., 2007; Al-Taisan, 2009; This plant was reported by (Manousaki *et al*., 2007) to excrete heavy metals through salt glands on the surface of their shoots., in the same time continuous harvesting of their shoots could be suitable way to recycling heavy metals. In another study the potential phytoremediation ability of Tamarix *(Tamarix tetranda* Pall.) was evaluated*;* using saline aquaculture effluent to irrigate halophytes can be a viable strategy for disposal of effluent. (Hegedüs, 2009).

Tamarix of Israeli Provenance

Sixteen species of Tamarix recorded in Israel as native , cultivated for dune fixation afforestation and ornemental purposes.*T.aphylla* and *T.jordanis* are among widely species used for cultivation (Zohari, 1956).

T. aphylla

T. aphylla is an evergreen tree (typically 5–15 m tall) (Fig.9) native to Africa and the Middle East (Waisel, 1960a) (Figs. 9, 11). It has stipule-like leaves are alternately arranged along the branches and flowers late in summer (Waisel, 1991, Zohari, 1956). Popular habitats include sand dunes, canals, riverbanks, wadi beds, salty deserts, salt marshes and coastal plains (Orwa *et al*., 2009). It is not considered a true halophyte (Waisel, 1991). It is a typical desert tree but it is also known for its very high values of transpiration, which make it a true water consumer (Waisel, 1960).

T. jordanis

T. jordanis has alternate scaly leaves and distinguishable by vernal inflorescences arising from the last year's branches (Zohari, 1956). *T. jordanis* is native to Western Asia Israel, Syria, Lebanon, Jordan, Palestinian Territory (GRIN, 2011) (Figs. 10, 12).This species is confined chiefly to the banks of the Jordan River and its affluents. *T. jordanis* is considered an extremely halophyte find mainly along the Dead Sea where the chloride content of soil is higher than 8%.

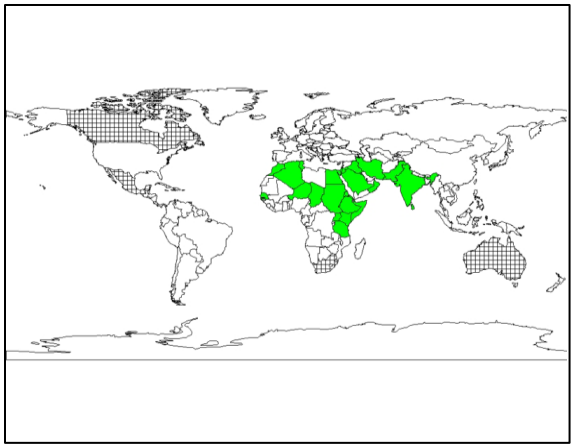


Figure 9. *T. aphylla* distribution

(Orwa *et al*. 2009) Agroforestry Database

Figure 10. *T. jordanis* distribution

http://www.gwannon.com/species/Tamarix jordanis

Figure 11. *T. aphylla*

Figure 12. *T. jordanis*

Figure 13. Mature tree of Tamarix

Figure 14. Tamarix fixing sandy soil

Figure 15. Tamarix growing in saline areas

Figure16. Tamarix Plantations in Negev desert

7.4 Adaptation mechanisms of Tamarix to salt and drought stress

Morpho-anatomical traits for adaptation to saline environments were reported in the literature. One of the characteristic features of *Tamarix* and several other halophytic plants is the presence of salt glands on the leaves (Waisel, 1972; Metcalfe and Chalk, 1950; Mcclintock, 1951). Such adaptive features play an important role in regulating ionic balance (Ramadan, 1998), maintaining or stabilizing osmotic and turgor pressure under high salinity (Ding *et al.*, 2009); another important function of these glands is the excretion of excess salt which accumulates in the tissue through transpiration (Scholander *et al.*, 1964; 1965).

Recent studies revealed also important anatomical traits (the stomatal size and density, the lignification of walls of xylem vessels and suberification of endodermis in roots) that induced

high discriminatory power of salt tolerance at high level of salinity (450 mM). The increase in stomatal density can be a way to minimize the time of opening of the stomata for gas exchange, reducing water losses; reduction of the size of the stomata ensure better stomatal control; increased thickness of the walls and lignification of xylem vessels prevent tissue collapse under stress; suberification of endodermis in roots limit apoplastic transportation of ions and water (Abbruzzese, 2011). According to Abbruzzese (2011), the greater tolerance of *T. jordanis* relative to *T. aphylla*, from morfo-anatomical side was related to increase in the thickness of the palisade and epidermis as well as increase in thickness of the endoderm at root level, which explains better carbon assimilation and lower content of sodium in leaves.

Biochemical biodiversity of *Tamarix spp.* was tackled in the past, identifying stressed induced new substances that were produced specifically by some of the ecotypes (Solomon *et al*., 1994; Jones *et al.*, 1995; Jones *et al*., 2005). *Tamarix jordanis* has been reported to contain proline analogues that can reduced the effects of NaCl increased on rubisco activity (Solomon *et al*. 1994). In another work, the pools of free amino acids and protein amino acids were investigated in *Tamarix tetragyna*; Proline accumulated in roots of *T. tetragyna* with an increase up to high salinity of 480mM, Valine and leucine accumulate in this species only at moderate salt concentration, while some other free amino acids and protein were noticed to be decreased under salt stress (Bar-Nun and Poljakoff, 1977). In a study of Arndt., *et al*., 2004 the cyclic sugar alcohol pinitol was reported as the main compatible solute in *Tamarix ramosissima*.

During periods of water stress constant physiological functioning has been reported in many studies has ability to maintain constant gas exchange and growth through periods of greatly reduced water availability (Cleverly *et al*. 1997; Pataki *et al*. 2005; Xu *et al*. 2007, Nippert, 2010).

Tamarix appear also to be a mesophyte adapted to a very narrow xeric ecosystem via a) root penetration to a deep water source, b) lowered water loss by reduced leaf area, c) increased phosynthetate production by cladophyll (Wilkinson, 1966). As a facultative phreatophyte, *Tamarix spp.* are capable of extracting soil moisture from less saturated soils in areas with deeper water tables (Busch *et al*. 1992). Besides the role of roots in water use strategy, the special behavior of stomata was seen as one of the essential mechanisms in *Tamarix spp*. (Anderson,

1982).They have been also reported to shift transpiration to earlier in the day and to decrease transpiration later in the day when exposed to lack of moisture (Devitt *et al*. in press).

8. Objectives

Our research was integrated in two scientific activities of Italian-Israeli partnership in the environmental and research development: *Harnessing the biodiversity of Mediterranean plants for mitigating the effects of climate change* and *Bioforeta* project. The first one aims to identify some species of Tamarix and Populus for the production of biomass and carbon sequestration through forestation and afforestation in the salt and desert land. In addition, the project was looking for genetic resources for the development of fast genetic fingerprints. The second one Bioforeta project, aims to assess the feasibility of using xerophile trees and shrubs susceptible of cultivation in marginal lands for the production of bioethanol.

In an effort to reassess the scarce available information regarding Tamarix responses to abiotic stress, tackling new indicators of plant tolerance, and to establish a basis for the better utilization of *Tamarix spp.* in marginal lands, the present work was divided in two parts:

1. The first part aims to investigate performance of two species of Tamarix under salt and drought conditions. It encompasses the background of the research and defines the gap in the knowledge. It covers experiments conducted during summer 2008-2009, reports ecophysiological and biochemical responses of the species under two regimes of salt and drought and combined stress.
2. The second part, reports firstly a brief description of second generation crops for bioethanol production, then encounters the methods and results of the wood's chemical composition of different population sampled from Mediterranean area.

The general objective of the current study was to identify functional index to select of suitable plants for a sustainable reforestation activities in arid and saline lands to mitigate climate changes and produce biomass for energy.

The specific objectives of our investigation were:

- Study the variability of main gas exchange parameters, and Water Use efficiency (WUE) of two Israeli *Tamarix spp.* (*T. aphylla & T. jordanis*) under different levels of salt, drought & salt + drought stress.

- Identify adaptative ecophysiological mechanisms related to carbohydrate metabolism, proline content, ion content, and their possible role in osmoregulation in *Tamarix spp.* in response to drought and saline gradients.
- Compare the performance of *T. aphylla* and *T. jordanis* in term of growth rate and biomass in the comparatives environment.
- Characterize wood composition quality to bio-ethanol production of different *Tamarix* species under natural conditions and common experimental gardens.

9. PART 1. Study of ecophysiological and biochemical responses of *Tamarix spp* to mild and severe salt and drought stress.

9.1 Introduction

Sandy soils in coastal and arid areas are of most difficult zones for plant afforestation due to temporal and spatial variation in soil salinity and superficial fresh water. In these environments, soil salinity tend to increase due to salt coming from inundation and aerosol spray, while changes in fresh water availability are primarily determined by seasonal rainfall in these areas. Salinity and water stress may occur separately or together in time such the case of halomorphic arid soils, in which salts concentrate near the surface as the soil dries between rains (Mcnaughton, 1991) and in cultivated arid soils, which can accumulate damaging levels of salts between irrigations (Mccree, 1986; Mccree and Richardson, 1987; Shalhevet, 1993).

Exposure to drought or salt stress triggers many common reactions in plants. Both stresses lead to cellular dehydration, which causes osmotic stress and removal of water from the cytoplasm into the extracellular space resulting in a reduction of the cytosolic and vacuolar volumes (Taylor and Francis, 2005). A decrease in the maximum CO_2 assimilation (A) and transpiration (E) rates is another strategy adopted by plants affected by drought and salinity (Flexas *et al*., 2004; Chaves *et al*., 2009).The decrease in (A) is primarily associated with stomatal conductance (g_s) although it has also been related to biochemical limitations (Tezara *et al*., 2003; Flexas *et al*., 2004; Chaves *et al*., 2009). Another considered adaptative mechanism which received great interest is osmotic adjustment (Serraj and Sinclar, 2002), under drought stress, the osmotic adjustment is mainly achieved by accumulation of organic solutes synthesized by the plant, while under salt stress the accumulated solutes are mainly inorganic ions (Na^+ and Cl^-) readily available in the soil solution (Flowers and Yeo, 1986; Shalhevet and Hsiao, 1986; Parida and Das, 2005).

In addition, both water deficit and salt stresses have a detrimental effect on plant growth that might be related to a reduced leaf expansion rate, lesser final leaf size, diminished leaf production rate and accelerated senescence (Greenway and Munns, 1980; Munns, 2002; Parida and Das, 2005). When both stresses are coupled, the plant is exposed to increasing levels of both water stress and osmotic stress, because the soil matrix and osmotic potentials are additive in lowering the free energy of water in the soil (Shalhevet, 1993). Hence, it is logical to think the

two stress factors could be additive in affecting plant performance (Shalhevet, 1993). However, studies in which plants are grown in drying soils at different salinities show a more complicated response, in which soil salts actually mitigate some of the negative effects of water stress. For example, plants in drying soils usually survive longer in saline than in non saline soils, because salt-stressed plants grow less and therefore deplete soil moisture more slowly than nonsalt-stressed plants (Mccree, 1986; McCree and Richardson, 1987; Richards, 1992; Shalhevet, 1993). Furthermore, salt stress can increase leaf instantaneous water use efficiency by reducing stomatal conductance to a greater extent than photosynthesis (Guy, *et al*., 1980; Guy and Reid, 1986; Ayala and O'Leary, 1995), thereby allowing plants under salt stress to produce more dry matter than plants in non saline soil on the same quantity of water (Richards, 1992). Finally, salt stress can precondition plants to low soil water potential by allowing them to osmotically adjust, enhancing their ability to survive as the soil dries (Shalhevet, 1993). Consequently, it seems a prerequisite to select plant species to have adaptations that confer drought and salt resistance to rehabilitate and reclaim degraded areas. Many of higher plants species being considered as xero-halophyte plants have adapted to a doubly harsh environment.

We mention *Tamarix spp*. which can tolerate an extreme range of environmental conditions. The ability of *Tamarix* to closely regulate photosynthesis and leaf conductance during drought increases its survivability and competitive ability in arid and semiarid rangelands (Mounsif *et al*., 2002). According to Frasier and Johnsen, (1991) *Tamarix spp.* are well adapted for survival in arid and semi-arid climates and, once established, not even dramatic changes in soil moisture will completely eliminate them, provided abundant groundwater is available. Additional advantages were reported in their fast growth and easy vegetative propagation. They have evolved various physiological processes in response and acclimation to the changes of environments. *Tamarix spp.* tolerate saline water and exclude large quantities of salt through their specialized leaves. It has a deep, extensive root system, is very proficient at accessing limited water supplies, and has higher water-use efficiency than native riparian (Zlatnik 1999).

Understanding how these plants respond to drought, salt and co-occurring stresses can play a major role in stabilizing crop performance under drought and saline conditions and in the protection of natural vegetation.

For this scope, this study was conducted to evaluate the effects of salinity and drought stress both alone and in combination, on water relations, gas exchange, osmolyte regulation and growth of

Tamarix spp. and to test if the combined stress is more or less detrimental on growth than the sum of the separate effects of salinity and water stress.

9.2 Material and methods

9.2.1 Plant material and culture conditions

The plants used in the study were obtained by multiplication of selected mother plants cultivated on Israel. They originate from extreme growing environments, cuttings of *T. aphylla* were collected from Negev Desert (31 3́0 56 .01" N 34 26' 59.62" E), and *T. jordanis* cuttings were collected from the dead sea (31 46' 03.20" N 34 30' 33.27 ″E) having less than 100 mm mean annual rainfall and a summer average temperature between 32 and 39 °C.

Individual cuttings of 20cm length were planted in pots with drain holes, of 24cm diameter filled with sandy soil. Individuals presenting homogeneous development and size were selected for this study. For each species, plants were divided into four treatments. The plants were submitted to the various stresses in the two cycles (Fig. 17).

The first experiment was executed in August 2008 and was repeated in August 2009, started 10 days after transplanting and acclimation, it includes four treatments: The first one was irrigated with water (control).The second one was irrigated with saline water 150mM. The third was irrigated to 50% of field capacity (F.C.), and the fourth one was irrigated with saline water 150mM to 50% F.C.

The second experiment was executed in September 2009, 10 days after transplanting and acclimation in greenhouse, it is also subjected to four treatments: The first one is irrigated with water (control).The second one was irrigated with saline water 350mM. The third was irrigated to 30 % of field capacity (F.C.), and the fourth one was irrigated with saline water 350mM to 30 % F.C.

Each treatment included 5-7 replicates. Pots were covered with plastic film to minimize water evaporation. Four pots without plants were used to monitor the evaporative loss from the soil surface. Plants were grown in a greenhouse for 16 days at 35 ±5°C/18 ±°C day/ night temperature. All the plants received periodically the same quantity of nutrients. The water content was monitored and adjusted daily by weighting pots. Salt concentration was analysed by collecting soil at 20cm beneath soil, then measuring its electric conductivity.

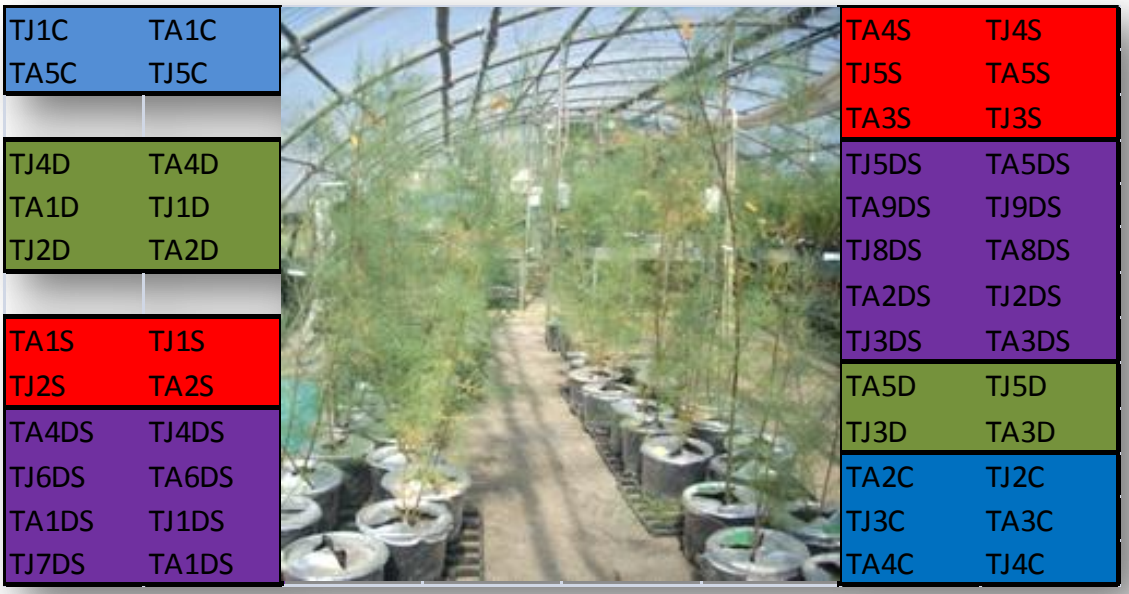


Figure 17. General layout of the experiments

TA: *T. aphylla*, TJ: *T. jordanis*, C: Control, S:Sale, D:Drought, DS: Drought salt

9.2.2 Gas exchange measurements

Net assimilation of CO_2 (A), stomatal conductance (g_s), Transpiration (E) intercellular CO_2 concentration (C_i) and photosynthetic water use efficiency
(WUE= A/gs) were measured periodically during the experiment using one leaf at middle part of the plant in a portable photosynthesis system (LI-6400) with a conifer chamber under ambient light.

All measurements were made in the morning from 9:30 to 13:00h. Measurement conditions were as follows: 1800 µmol mm^{-2} s^{-1} photosynthetically active radiation (PAR) 400µmol mol^{-1} ambient CO2 concentration and 28 ± 2 ºC leaf temperature. The vapour pressure deficit (VPD = 0.72 KPa) was calculated from saturation vapour pressure and relative humidity. Data were automatically collected after the photosynthesis rate has stabilised.

9.2.3 Carbohydrates and chlorophyll analysis

Fresh leaf tissues were finely grinded with liquid nitrogen, 40 mg were weighted and extracted with 1.5ml of 80% ethanol and 20% Buffer (Hepes 100 mM Ph 7.1 MgCl2 10mM), at

80°C for 45 minutes. After cooling and centrifugation at 14,000 g for 5 min, the supernatant is used for determination of soluble sugars and chlorophyll content. The pellet is stored at -20 °C to determine the starch content.

Chlorophyll measurements: 100µl of the supernatant is removed and diluted in 900µl ethanol. The optical density (absorbance) in a 1 cm cell against EtOH blanks at 649 and 665 nm was calibrated. Chlorophyll concentration can be calculated using the following formulas according to Wintermans and De Mots 1965.

Chl_a = ((13.95*OD665/1000)-(6.88*649/1000))* V ext / (DW/1000)* dilution factor

Chl_b = ((24.96* OD 649/1000)-(7.32*OD 665/1000))* V ext / (DW /1000)*dilution factor

Where: Chl= concentration of chlorophyll (µg / g DW^{-1}); V ext=Volume of extract (ml)

OD=Optical density; DW=Dry weight (mg).

Total soluble sugars: were measured using enzymatic activities as described by Antognozzi *et al*., 1996.

It was conducted in 96 well micro-titer plate. All sugar determination assays were modified for use with a Molecular Devices plate reader fitted with an absorbance 340nm, 10nm bandwidth interference. Assays were performed in polystyrene U or Flat well assay plates, of standard 96 well configurations. Endpoint determination for all three assays was based on the reduction of NADP to NADPH, which was detected colorimetrically at 340nm.

Glucose was first determined by converting to glucose-6-phosphate in the presence of ATP and hexokinase and then measuring its reduction of NADP in the presence of glucose-6-phosphate dehydrogenase. The NADPH formed in this reaction is stoichiometric to the amount of glucose and is measured by means of its light absorbance at 340nm.

For each sample a replicate was realized, by adding in each well:

- 200µl of a mix buffer (Hepes 100mM pH 7.4, MgCl2 5mM, DTT 0.5 mM and BSA 0.01% (W/V)), containing ATP 100mM and NAD^+ 80mM and hexokinase (0.3U/µl)
- 10 µl of the supernatant for sample analyses
- 10 µl of distilled water for blank

Absorbance zero was set with blank, and then the absorbance of the solutions in the well was read (A_1)

After that, the reaction was completed by adding in each well 0.5U of glucose-6-phosphate dehydrogenase, shaked very well and absorbance A_2 was recorded.

Absorbance of NADPH produced by transformation of Glucose was $\Delta A = A_2 - A_1$

$C = \Delta A / * \varepsilon * l$ (1)

C = concentration of glucose in mmolar (mmolle/l)

E = the absorption coefficient for NADH at 340nm is 6.22 (1 $mmol^{-1} * cm^{-1}$)

l = light path (1cm)

The concentration of glucose as mg/g DW was calculated according to the following equation:

C sugar = mmolar * V * MW * V ext / V sample * 1000 * g DW (2)

mmolar = concentration of sugar determined in the equation (1)

V = final volume in the well

MW = molecular weight of glucose (180.2)

V ext volume of extraction (ml)

V sample = volume of extract used for the assay

Fructose was phosphorylated as for glucose, the hexokinase added in the previous phase catalyses also the phosphorilation of fructose 6 phosphate in presence of ATP. The latter is converted to to glucose-6-phosphate with phosphor-gluco- isomerase (PGI). The Glucose 6 phosphate reacts with NADP with formation of gluconate-6-phosphate and NADPH. The amount of NADPH formed now is stoichiometric to the amount of fructose.

The determination of fructose directly followed the glucose by adding PGI (0.5U/µl). After 15 minutes the absorbance A_3 was recorded.

Absorbance of NADPH produced by transformation of fructose was $\Delta A = A_3 - A_2$

The concentration of fructose was calculated in mmolar or mg/g DW as in the equation (1) and (2) using

$\Delta A = A_3 - A_2$ and MW = 180.2

Sucrose was hydrolysed to glucose and fructose by adding invertase (10U/ µl) to the assay and after 15 min the absorbance A_4 was recorded.

The glucose released by the hydrolysis was assayed as above. The sucrose content was calculated from the difference of the glucose concentrations before and after enzymatic inversion.

Absorbance of NADPH produced by transformation of sucrose was $\Delta A = (A_4-A_3)/2$

The concentration of sucrose was calculated in mmolar or mg/g DW as in the equation (1) and (2) using

$\Delta A = (A_4- A_3) /2$ and MW = 343.2

The pellet is washed three times with sodium acetate 50 Mm pH 4.6 then autoclaved with 1ml sodium acetate. This step allows the gelatinization of starch, the granules swollen to favor the soulbiliztion and hydrolysation process. The pellet are hydrolysed by adding 70 u amyloglucosidase and 4u amylase. The suspension is incubated at 50°C for 1hour with regular shaking. Following centrifugation, the glucose in the supernatant is used for starch quantification.

For each sample replicate was realized, by adding in each well:

- 200µl of a citrate buffer containing ATP 100mM and NAD^+ 80mM and hexokinase (0.3U/ µl)/assay)
- 20 µl of the supernatant for sample analyses
- 20 µl of distilled water for blanks

Absorbance zero was set with blank, and then the absorbance of the solutions in the well was read (A_1).

After that, the reaction was completed by adding in each well 0.5U of glucose-6-phosphate dehydrogenase, shaked very well and absorbance A_2 was recorded.

After 15 minutes the absorbance A_5 was recorded.

Absorbance of NADPH produced by transformation of glucose was $\Delta A= A_2-A_1$

The concentration of starch was calculated in mmolar or mg/g DW as above in the equation (1) and (2) using

$\Delta A = (A_2-A_1)$ and MW = 162.1

9.2.4 Proline analysis

Free proline content was estimated following the method of Bates *et al*. (1973). Fresh leaves (0.5 g) were extracted in 3% sulphosalicylic acid and the homogenates were centrifuged at 10,000 g for 10 min. A 2 ml of the supernatant was reacted with 2 ml of acid ninhydrin reagent and 2 ml of glacial acetic acid in a test tube for 1 h at 100˚C and the reaction terminated in an ice bath. The reaction mixture was extracted with 4 ml of toluene and mixed vigorously with a vortex mixture for 15–20 s. The chromophore containing toluene was aspirated from the aqueous phase, warmed to room temperature and the absorbance measured at 510 nm using toluene as blank. Proline concentration was calculated from a standard curve using 0–100 lg L proline (Sigma).

Using the following formula: C_{final}= (C* ml toluene) /115.5) / (DW of sample /2)*Dilution, where:

C_{final} is the concentration of proline in the sample (μmoles proline /g DW)

C is the concentration of proline (μg proline /ml)

DW is the dry weight (g).

9.2.5 Mineral Analysis

At the end of the experiment, three replicates from the initial pools were selected randomly, and partitioned into leaf, stem and root for determination of ion content. The leaves, stems, and roots were washed in deionized water and dried in an oven at 80^{o}C, and then finely ground and it was taken 1.5g from each replicate of each part, to be prepared and analyzed for Na^{+}, K^{+}, $Ca2^{+}$, Mg^{2+}, P, Cl^{-}, and Fe^{2+} content, by the research institute (Gruppo CSA-Rimini).

Samples were mineralized then digested, Na^{+}, K^{+}, Ca^{2+}, Mg^{2+}, P, and Fe^{2+} content were determined by atomic absorption spectrophotometer (ICP-AES) following the standard methods (CEN/TS 15290:2006+ UNI EN ISO 11885:2009), and Cl^{-} was determined by ion chromatography following the standard methods (CEN/TS 15289:2006+ UNI EN ISO 10304-1:2009).

9.2.6 Osmotic potential

Total concentration of osmotically active dissolved particles in a solution without considering particle size, density, configuration or electrical charge is known as the expression osmolality. The addition of solute particles to a solvent changes the free energy of the solvent molecules and this allows to us indirect means (vapour pressure, freezing point or boiling point) for the measurement of osmotic potential.

The measurement of osmotic potential can only be made indirectly by comparing one of the solution colligative properties (vapor pressure and freezing point are the most common) with the corresponding cardinal property of the pure water.

Leaf osmotic potential Ψ_s meausrements were made in leaves taken from the middle part of the plant. Frozen leaves which were thawed and then washed with distilled water, were blotted dried with a paper and squeezed with a syringe of 3ml to extract cell sap. The osmotic potential Ψ_s of the cell sap was measured with a Wescor-5500 vapor pressure osmometer (Wescor, Loan, UT, USA). Readings were converted to pressure units by using the van't Hoff equation (π=-cRT), where c is the osmolality (mosmol kg^{-1}) R the gas constant and T the temperature (K) (Nobel, 1991).

9.2.7 Water potential measurements

The water potential represents a state of equilibrium between plant and soil water status, it measures status of water energy inside an organ or tissue (Tardieu *et al*., 1990).Water potential is a good overall indicator of plant health because of cell growth, photosynthesis and crop productivity are all strongly influenced by water potential and its components (Repellin *et al*., 1997; Pardossi *et al*., 1998 and Morales *et al*., 1998). Then, the use of an accurate, reliable and simple method for evaluating the water status of a plant would be an efficient tool to know the physiological condition of a plant. The pressure chamber is one of the easiest ways of following the water status in plants under natural conditions.

Predawn, usually reflect average soil moisture tension, Ψ predawn, preferred baseline measurement. Mid-day, reflects the tension by the plant to satisfy the water demand**,** Ψmid-day, indicate the maximum water potential for the day.

A relatively quick method for estimating the water potential of large pieces of tissues, such as leaves and small shoots, is by use of the pressure chamber (Scholander *et al*. 1965). In this technique, the organ to be measured is excised from the plant and is partly sealed in a pressure chamber. Before excision, the water column in the xylem is under tension. When the water column is broken by excision of the organ water is pulled rapidly from the xylem into the surrounding living cells by osmosis. The cut surface consequently appears dull and dry. To make a measurement, the investigator pressurizes the chamber with compressed gas until the distribution of water between the living cells and the xylem conduits is returned to its initial, pre-excision, state. This can be detected visually by observing when the water returns to the open ends of the xylem conduits that can be seen in the cut surface. The pressure needed to bring the water back to its initial distribution is called the balance pressure and is readily detected by the change in the appearance of the cut surface, which becomes wet and shiny when this pressure is attained. Ψ predawn was measured between 5:00 and 6:00 a.m.; Ψmid-day was measured between 12:00 and 1:00 a.m. on tissue discs punched from fully healthy and sun-exposed leaf taken from the middle part of the plant at the end, seventh and fourteenth day of the experiment.

9.2.8 Relative water content

It expresses the absolute amount of water, which the plant requires to reach artificial full saturation it is useful indicator of water balance status. (González and González-Vilar, 2001).To determine relative water content, collection of leaves was conducted between 12:00 and 1:00p.m from 4 plants per treatment. Leaves were punched from the middle part of the plant and then placed overnight in tubes full of distilled water at 20°C under dim illumination to reduce respiratory losses. Leaves were then carefully blotted and dried quickly with a paper to remove surface water then, turgid weight (TW) was recorded. Dry weight (DW) of the leaf discs was determined by drying the tissues at 75°C to constant weight. Relative water content (%) was determined as:

RWC = [(FW–DW) / (TW–DW)] × 100

Where (FW= Fresh weight), (DW= Dry weight)

and (TW= Turgid weight)

9.2.9 Growth measurements

Plant growth was monitored periodically overall the experiment, by measurement of increase plant height and counting the new emerged shoots.

At the end of experiment, stem diameter and plant height were recorded. Sprouts and shoots number were counted for four harvested plants per treatment. Then plants were divided into leaves, stems and roots and their respective fresh weight (FW) was determined. Dry weight (DW) was obtained after oven drying at 70ºC until a constant weight was reached.

9.2.10 Experimental design and statistical analysis of data

A completely randomized design, including seven replicates for each treatment was used. Data were submitted to the analysis of variance. Means were tested using a least significant difference (LSD) test ($P = 0.05$), when a significant test ($P \leq 0.05$) was obtained, the mean values were compared by using the Duncan Multiple range test.Factorial analysis was used to test any significant difference induced by treatement on species and to test effect of year on plant responses. Computations and statistical analysis were done using Statistica .

9.3 Results of ecophysiological and biochemical responses of *Tamarix spp.* to mild stress: salt (150mM), drought stress (50% F.C.), salt drought (150 mM + 50% F.C.).

9.3.1 Gas exchange measurements

Either in limiting or no limiting conditions, species differed significantly in the gas exchange parameters.

In absolute term, differences between *T. aphylla* and *T. jordanis* in the mean of carbon assimilation capacity (A) were found ($p = 0.0005$); *T. apyhlla* recorded highest value of 21% than *T. jordanis*. Moreover, observed interspecific differences in (A) were sometimes accentuated in response to stress treatments, the interaction species- treatment was significant in the 7^{th} day ($p = 0.0010$).

Salinity, water stress and the combination of water and salt stress affected assimilation rate (A), stomatal conductance (g_s) and transpiration (E) of both species.

Salt (150mM) had an effect on (A), (g_s), (E) and (C_i) in both species, these effects were highly notable at initial phase (2^{nd} day), and to less extent in the following days (Fig. 18A-D).The higher impact of salt (150mM) was higher in *T. aphylla* than in *T. jordanis.* For instance, in the 2^{nd} day, salt concentration (150mM), reduced significantly (A), (g_s) and (E) ($p \leq 0.05$) in *T. aphylla*, of 46.91%, 70.3%, and 55% respectively. Similar observations in *T. jordanis* were reported, in the second day of salt stress (150mM) (A), (g_s) and (E) decrease significantly of 51.1%, 62.6%, and 50%, respectively. Afterwards, the both species showed an improvement of these parameters in the 7^{th} day and even better restoration in the 14^{th} day compared to untreated plants. However, better improvement in these parameters was achieved by *T. jordanis* than in *T. aphylla.*; reduction in (A), (g_s), (E), in *T. jordanis* were not anymore significant relative to the control as could be seen in the 14^{th} day of salt stress (150mM) (Fig. 18A-C).

Water deficit (50% F.C.) effect on (A), (g_s), (E) and (C_i) were minimal during the experiment in both species, comparing with salt stress. Only in the 7^{th} day, water deficit (50% F.C.) showed some alterations on (g_s) and (E), without any significant effect on (A) (Fig. 18A-D).

(A), (g_s), (E), and (C_i) of plants subjected to coupled stress were the most affected among all treatments and throughout the fifteen days of the experiment (Fig. 18 A-D); On the contrary of salt stress applied alone, both species exposed to salt stress combined with drought, did not

achieve significant improvement during the experiment. At the end of the experiment, (A) was significantly lower than control in *T. aphylla* (59.8%) ($P \leq 0.05$) and *T. jordanis* (43.7%).

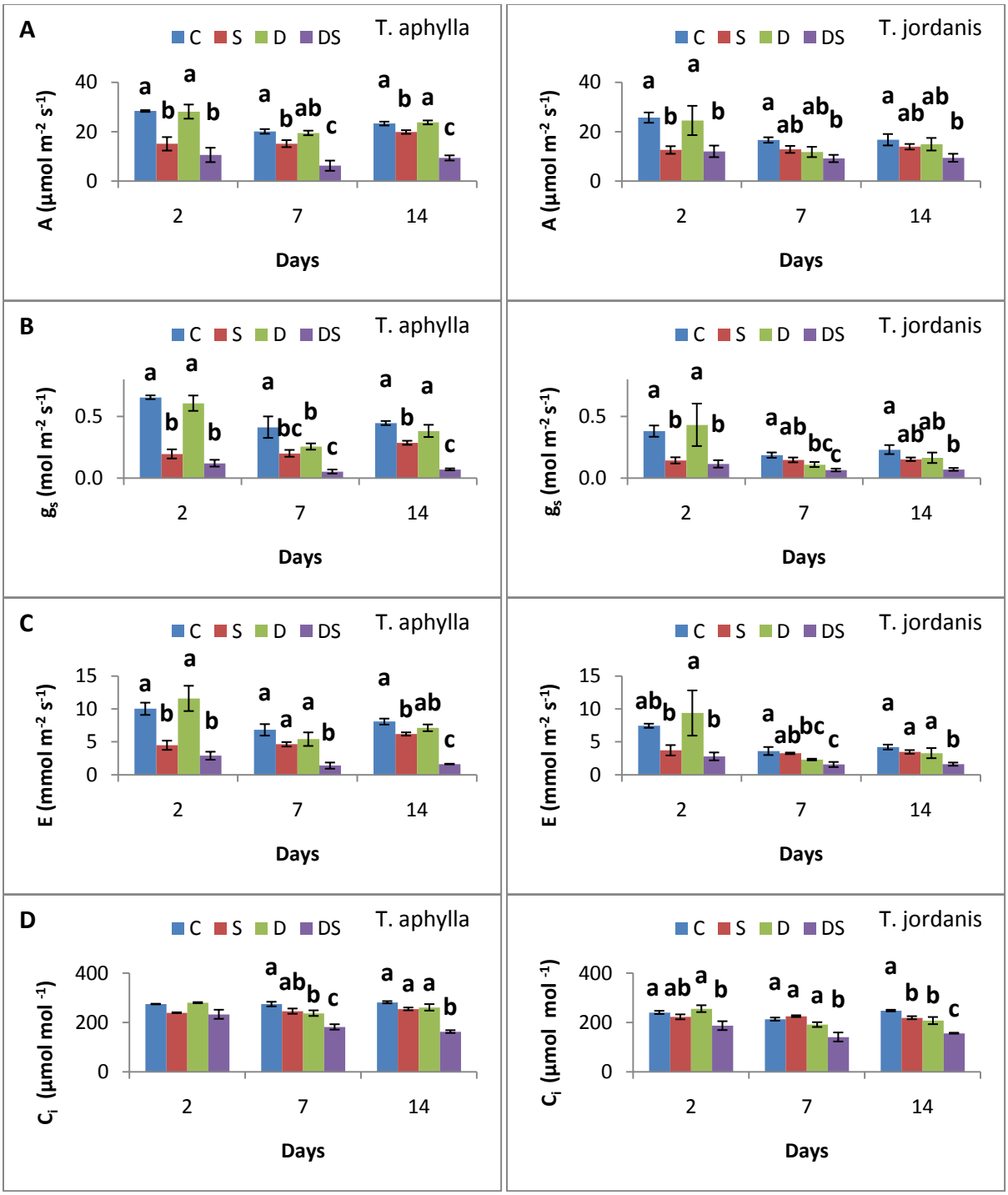


Figure 18. Gas exchange measurements (Assimilation rate (A), stomatal conductance (g_s), transpiration (E), and intercellular CO2 (C_i) in (A-D)) of *T. aphylla* and *T. jordanis* exposed to Mild stress: C: Control; S: salt (150mM), D: drought (50% F.C.) and DS: Drought salt (150mM +50% F.C). Different letters on the top of the error bars indicate statistically different means at $P \leq 0.05$

9.3.2 Water use efficiency (WUE)

Factorial analysis demonstrates that WUE (A/g_s) and (A/E) was generally major in *T. jordanis* than in *T. aphylla* independent from stress applied (p= 0.0000), due to lower (g_s) and lower (E) in *T. jordanis* as shown in (Fig. 18B-C). It was not found any interaction effect between species and treatment on WUE (either (A/g_s) or (A/E)) (p= 0.078).

Generally, better WUE was recorded by both *Tamarix spp.* in various mild stress conditions comparing with control.

The intrinsic WUE (A/g_s) was high during the experiment in salt treated plants for both species unless some fluctuations in *T. jordanis*. It increased starting from the second day in both species, this increment was significant in *T. aphylla*, (44.15 %) and non significant in *T. jordanis* (24.5%) (Fig. 19A).

(A/g_s) in drought plants was not stable it was increased at certain days of the stress; it increased in the 7^{th} day in *T. aphylla* (31.3%) and at the end of the experiment in *T. jordanis* (26.4%). Under combined treatment, both species highlighted the highest (A/g_s) among treatments that can be observed in earlier phase (2^{nd} day) till the end of the experiment (Fig. 19A).

A significant linear relationship between (A) and (g_s) ($r^2 = 0.940$, $P \leq 0.05$) was found. High (A/g_s) was attributed to decrease in (g_s) decrease in lower proportion than (A) (Fig. 20A). There was a significant relationship between (A/g_s) versus (g_s) with a regression coefficient ($r^2 = 0.91$, $P \leq 0.05$) (Fig. 20B).

In opposite to (A/g_s), the increase in WUE from the ratio (A/E) of both species under different salt and drought was not significant relative to control. (A) and (E) alterations when exist were parallel under salt and drought stress. (Fig. 19B) (In the second day of salt stress (150mM), (A) and (E) decreased at similar proportion around 50 % each in both species). (A/E) increment were more evident in both species treated with simultaneous stress due to alteration of (A) and (E) in different proportions and resulted significant only in *T. aphylla* in the 14^{th} day (Fig. 19B).

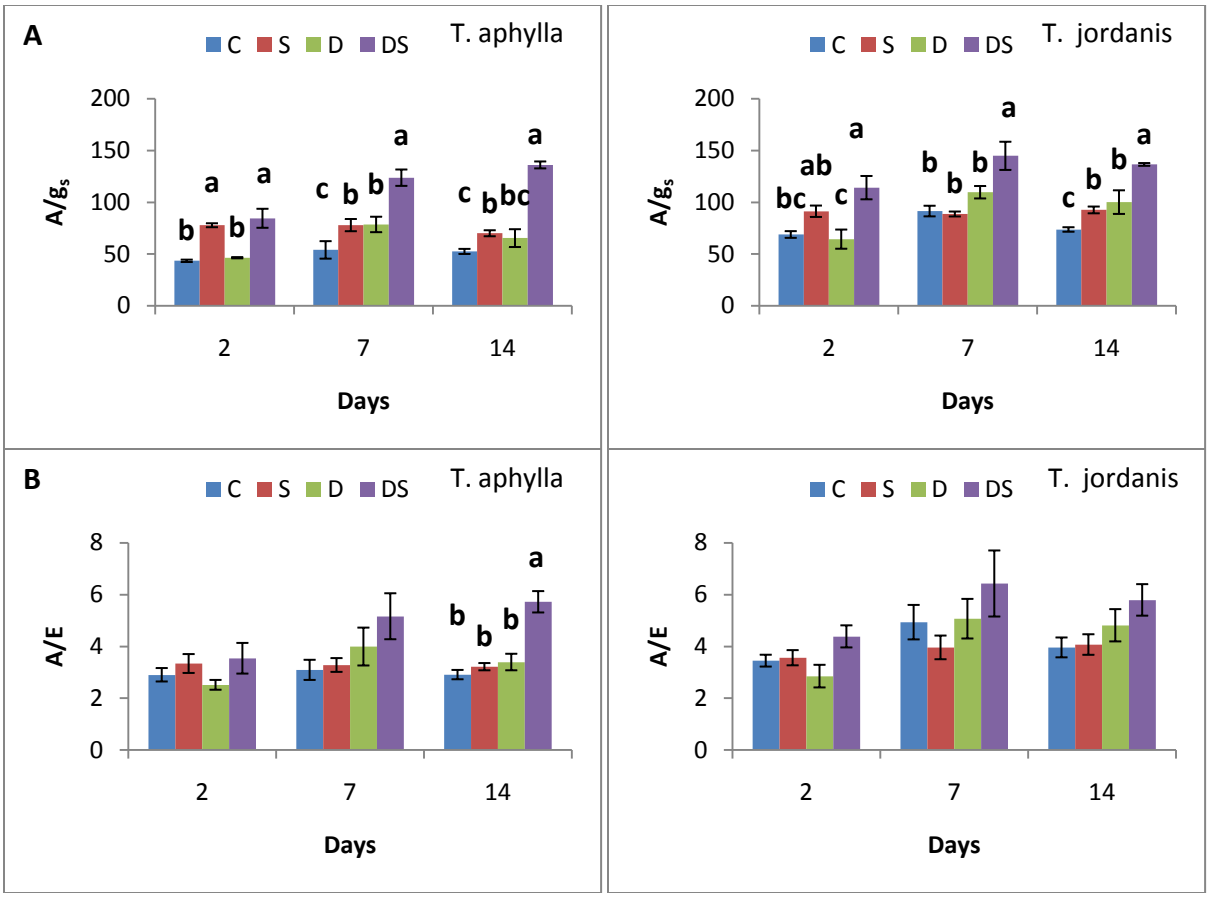


Figure 19. A/g_s ratio and A/E ratio in (A-B) of *T. aphylla* and *T. jordanis* exposed to Mild stress: C: Control; S: salt (150mM), D: drought (50% F.C.) and DS: Drought salt (150mM +50% F.C). Different letters on the top of the error bars indicate statistically different means at $P \leq 0.05$.

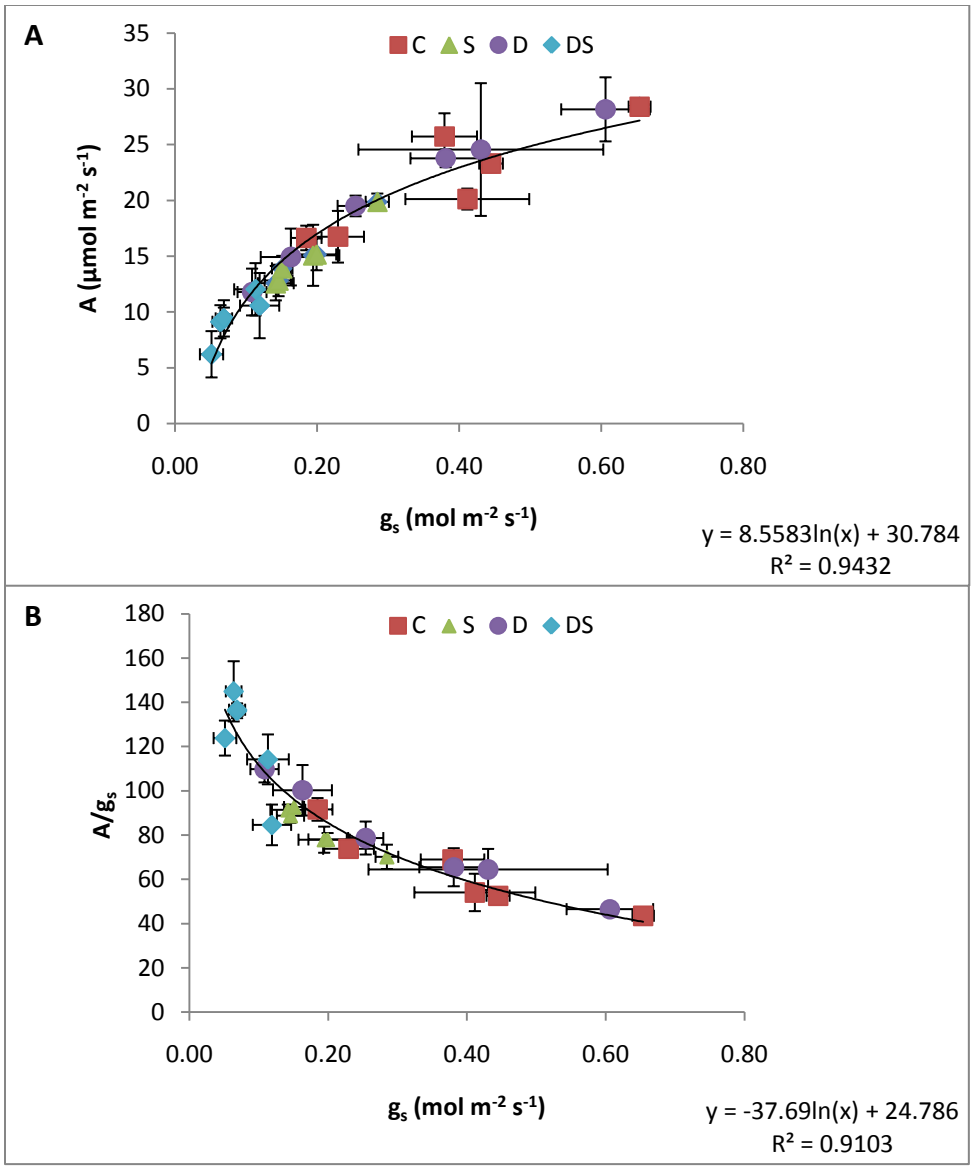


Figure 20. Maximum assimilation rate (A) and intrinsic water use efficiency (A/g_s) as a function of stomatal conductance (g_s) in plants of Tamarix exposed to C: Control; S: salt (150mM), D: drought (50% F.C.) and DS: Drought salt (150mM + 50% F.C). Data are means ± S.E. obtained at 2, 7, and 14 d after the beginning of the experiment. Lines fitted by linear regression were statistically significant at $p \leq 0.05$.

9.3.3 Chlorophyll

Overall speaking, minor or no changes occurred in Chl $_a$ and Chl $_b$ when *Tamarix spp.*were exposed to any type of mild stress applied in this experiment (Fig. 21A-C). Exceptionally, photosynthetic pigments were significantly increased, at soil water deficit exposure only in the 7th day. *T .jordanis* showed a decrease in Chl $_{a/b}$ ratio occurred in plants receiving salt and drought stress in the 14th day (Fig.21D). The decline in Chl $_{a/b}$ ratio was accompanied by insignificant increase of Chl $_b$ synthesis.

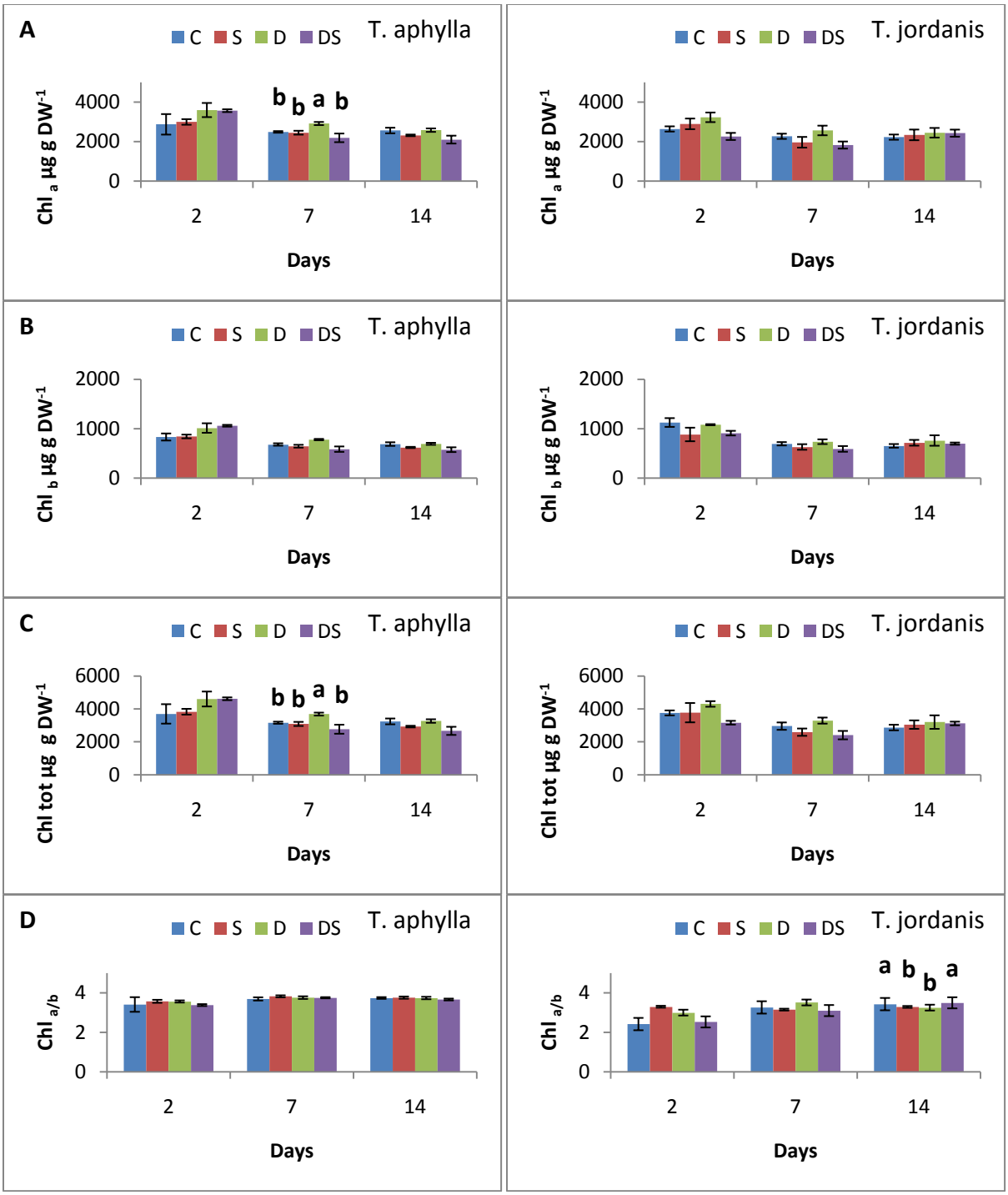


Figure 21. Chlorophyll content (chlorophyll a (Chl $_a$), chlorophyll b (Chl $_b$), chlorophyll total (Chl tot), and chlorpphyll a/b (Chl $_{a/b}$) in (A-D)) of *T. aphylla* and *T. jordanis* exposed to mild stress: C: Control; S: salt (150mM), D: drought (50% F.C.) and DS: Drought salt (150mM + 50 % F.C). Different letters on the top of the error bars indicate statistically different means at $P \leq 0.05$.

9.3.4 Carbohydrates

In leaves

Differences among species in their ability to accumulate soluble sugars and starch were evident under control conditions and stress.

The pool of total carbohydrates was dominated by sucrose and starch content in both species. In *T. jordanis*, starch level was higher than sucrose and even two times higher than sum of soluble sugars. In *T. aphylla* the starch and sucrose contents were similar, but compared to sum of soluble sugars, the starch content resulted significantly lower. Starch content was higher in *T. jordanis* than in *T. aphylla* while soluble sugars content were higher in *T. aphylla* than in *T. jordanis*. In both species glucose and fructose were 6-7 time lower of sucrose and the hexoses ratio (glucose/ fructose) is proximal to one. All non structural carbohydrates accounted for 8-12% of leaf dry matter. Generally speaking, starch content was increasing significantly during the experiment in both species under control and the different treatments. Sucrose content tended to be stable during the experiment; the only date that induced significant changes was the 7^{th} day especially in *T. aphylla*.

Sugar content in both species (*T. aphylla* and *T. jordanis*) responded to stress treatments, both species showed similar trend with slight differences, and marked response for *T. aphylla* plants.

Under salt stress (150mM), a drastic decrease in glucose and fructose content was noticed in the 2^{nd} day, in *T. aphylla*, the reduction percentage of glucose and fructose was significant, it was of 51% and 41.5%, respectively. Afterwards, at the 7^{th}day, glucose and fructose content improved in leaves of *T. aphylla*. Although the improvement of glucose and fructose synthesis achieved by *T. aphylla* in the 7^{th} day, their values turn significantly lower than control, at the end of the experiment. From 2^{nd} day, to the 14^{th} day, the reduction of glucose and fructose content was not significant in *T. jordanis* subjected to salt stress (150mM) (Fig. 22A-B).

Salt stress (150mM) never affected sucrose content of both species (Fig. 22C)

Salt stress has caused also a decline in starch content in both species. In *T.aphylla* species, starch was highly affected by salt stress, it decreased sharply at initial phase of the experiment of 49.7%, while *T. jordanis* shows a non significant reduction of starch content. A recovery in starch content in *T. aphylla* and *T. jordanis* was noticed under salt stress, in the following days as it was synthesized as control plants. However starch accumulation progress in salt treated species

was slow between the 7th and 14th day during which its augmentation was significantly higher in control plants (Fig. 23)

Under drought stress, the substantial difference in soluble sugar content was established only in the 7th day; soluble sugars (glucose, fructose and sucrose) accumulated at a significant percentage in *T. aphylla* (57.3 %) and at lower rate in *T. jordanis*, was not significant (26.4%). (Fig. 22A-C).

Water deficit initially, provoked significant decrease in starch content of *T. jordanis* and did not influence starch level of *T. aphylla*. In the 7h day, low starch was relevant for both species; it was lower of 37%, 44% in *T. aphylla* and *T. jordanis*, respectively. Between the 7th day and the 14th day, starch content increased in relevant manner, in both species under water deficit (50% F.C.) achieving the control plant values (Fig.23). Simultaneous decrease in starch content was observed with increase in sucrose content in the 2nd day in *T. jordanis* and in the in both species 7th day.

Like the effect of salt stress, combined stress induced initially a reduction of glucose and fructose in both species. Glucose was reduced of 56% in *T. aphylla* and of 46% in *T. jordanis.* Fructose was reduced of 44% in *T. aphylla* and of 31% in *T. jordanis.*

At the end of treatment, combined stress had the same effect on glucose and fructose as in salt treatment and more pronounced than of drought for both species (Fig. 22A-B).

Unlike hexose sugars, sucrose was stable whole the experiment, all treated plants were not significantly different from the control (Fig. 22C).

The couple stress reduced starch significantly for both species, reduction was estimated in the 2nd day between 49.7% and 61.3% for *T. jordanis* and *T. aphylla*, respectively. The last day of experiment, starch content under combined stress, was significant low relative to the control of 64.2%**,** 74.2% in *T. jordanis* and *T. aphylla*, respectively (Fig. 23). The starch response under combined stress was similar to salt and drought stress during. Combined stress had the most severe effect on starch content in the last day it was significantly different from salt and drought treatments in both species (Fig. 23).

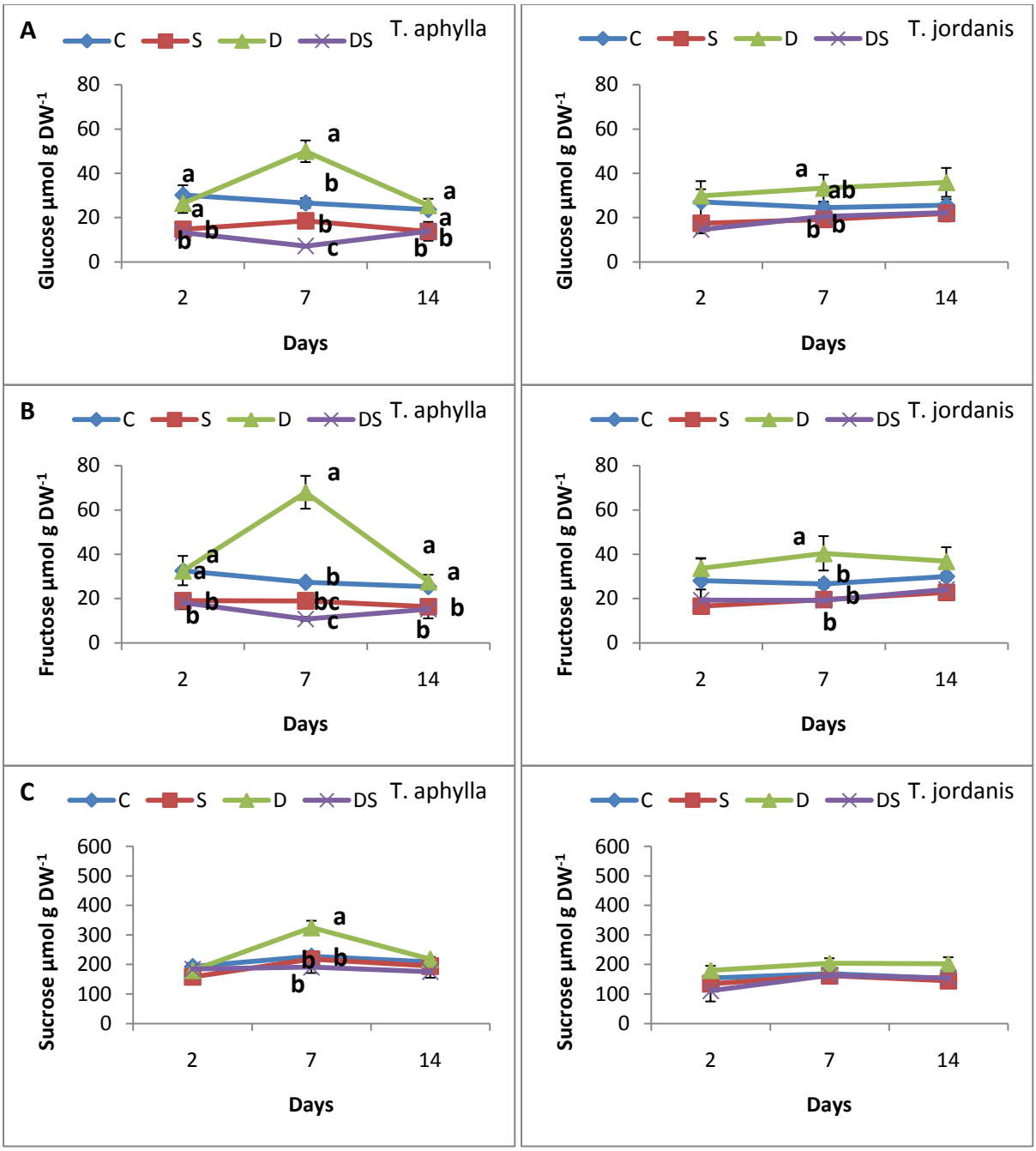


Figure 22. Soluble sugar content (glucose, fructose, and sucrose) in (A-C)) of *T.aphylla* and *T.jordanis* exposed to mild stress: C: Control; S: salt (150mM), D: drought (50% F.C.) and DS: Drought salt (150mM + 50% F.C). Different letters on the top of the error bars indicate statistically different means at $P \leq 0.05$.

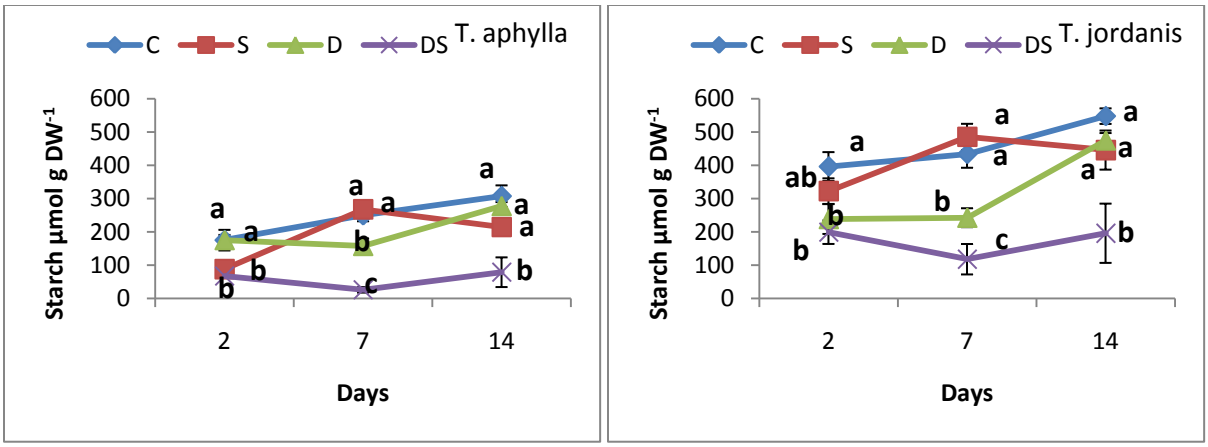


Figure 23. Starch content of *T.aphylla* and *T.jordanis* exposed to mild stress: C: Control; S: salt (150mM), D: drought (50% F.C.) and DS: Drought salt (150mM + 50% F.C). Different letters on the top of the error bars indicate statistically different means at $P \leq 0.05$.

In roots

Carbohydrates may play an important role at this level for osmotic adjustment; however we did not observe any accumulation of sugars under any stress.

The roots of both species had lower content of sucrose than leaves, at the same time ratio of glucose/fructose was equal to one. Allocation of starch in roots was lower than their allocation in leaves especially in *T. jordanis*. Roots of *T. aphylla* contained higher content of starch compared to *T. jordanis* (Table 1).

The occurred changes under stress were quiet similar in both species.

On Day 14, salt (150mM) did not induce significant differences in sugar content of roots of both species, relatively to control.

On the other hand, it was reported an non significant reduction of glucose, a significant decrement of fructose (46.3%), and a significant decrease of starch (46 %) in root of *T. aphylla* subjected to drought stress (50% F.C.), compared to the control (Table 1); *T. jordanis* showed the same result of root sugar content reduction under drought (50% F.C.), however obtained result were not statistically significant.

Sugar content of roots grown in DS treatments was not analyzed.

Table 1. Sugar contents in roots of two species (*T. aphylla* and *T. jordanis*) exposed to mild stress C: Control; S: Salt (150mM), D: Drought (50% F.C.) and DS: Drought salt (150mM + 50% F.C). Different letters indicate statistically different means at $P \leq 0.05$ at 16 days of the experiment.

	Glucose µmol g DW^{-1}	Fructose µmol g DW^{-1}	Sucrose µmol g DW^{-1}	Starch µmol g DW^{-1}
T. aphylla				
C	36.32 ± 7.13	38.12 ± 7.98 [a]	11.12 ± 2.79	156.64 ± 25.90 [a]
S	37.54 ± 4.99	38.10 ± 4.50 [a]	5.26 ± 0.45	172.47 ± 9.79 [a]
D	18.22 ± 6.65	16.66 ± 5.99 [b]	8.42 ± 4.66	84.79 ± 20.88 [b]
DS	NA	NA	NA	NA
T. jordanis				
C	16.01 ± 6.17	16.12 ± 7.21	6.65 ± 3.53	35.88 ± 13.92
S	10.13 ± 3.17	10.00 ± 3.22	2.79 ± 0.99	39.29 ± 7.33
D	6.92 ± 1.26	5.77 ± 1.30	1.33 ± 0.45	15.46 ± 4.75
DS	NA	NA	NA	NA

NA: not analyzed

9.3.5 Proline

In general *T.aphylla* accumulates larger quantity of proline than *T. jordanis.* Salt stress induced an accumulation of proline in the second day only in *T. aphylla* species. Both species did not show any significant response in proline accumulation under water deficit. For both species proline accumulation was higher under combined stress; free proline content increased approximately of 5-9 fold compared with the control (Fig. 24).

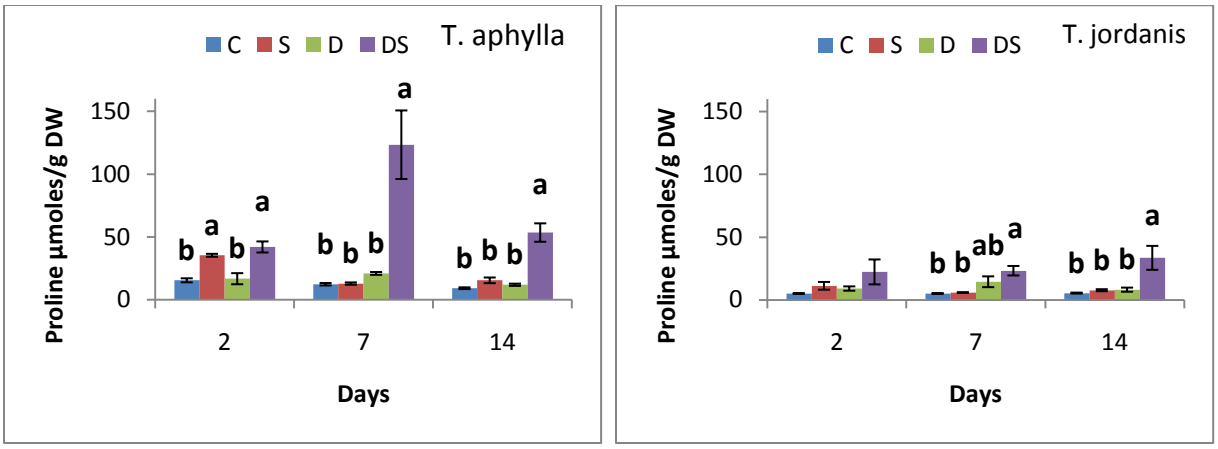


Figure 24. Proline content of *T. aphylla* and *T .jordanis* exposed to mild stress: C: Control; S: salt (150mM), D: drought (50% F.C.) and DS: Drought salt (150mM + 50% F.C). Different letters on the top of the error bars indicate statistically different means at $P \leq 0.05$.

9.3.6 Mineral content

Variations of ion accumulation in different plant organs

In both species Na^+, Cl^- showed a clear differentiation in organ accumulation where leaves build up much of these ions following by roots at lower rate, stem are the organs which contain less amounts (Fig. 25A-B). Na^+ in leaves makes up 46.9-71.84% of total plant content. Cl^- accumulation in leaves relative to other organs range between 54.54-74.5% and Mg^{2+} between 47.60-78.32%. Such wide range of percentage values, varied according to the treatment and species considered.

Mg^{2+} was also mostly found in leaves (47.60-78.32 %.) (Fig. 26A). Ca^{2+} Distribution in the different organs was the same as for Na^+ and Cl^- and Mg^{2+} in *T. aphylla* while in *T. jordanis*, roots were distinguished by their capacity to accumulate this cation over the other organs, where it was between 58.03-65.8% (Fig. 26B).

Potassium accumulated at same level in roots and stem and at higher level in leaves (Fig. 26C).

Phosphorus was the only element that its distribution among plant organs did not show large fluctuations (Fig. 26D).

Variation of minerals among treatments

In the 15-days-old plants, the accumulation of Na^+ in the root, stem and leaf of salt (150mM) treated plants was increased respectively, 5.55-5.8, 1.92-2.12 and 4.33-5.11-fold compared to control (Fig. 25A). Under combined stress, the accumulation of Na^+ in the root, stem and leaf was increased respectively by 2.96-3, 2.55-2.58 and 5.89-5.94-fold (Fig. 25A). In both species, sodium accumulation in leaves was shown significantly different between the salt and combined treatments. Although drought salt stressed plants were generally more concentrated in salt, sodium accumulation in roots was higher in salt stress rather than combined stress (Fig. 25A).

Chloride accumulates as well in higher content compared with control in root, leaves and stem for both species (Fig. 25B).

Magnesium response to treatments was highly dependent of the species involved. Magnesium reduced more in *T. aphylla* leaves than *T. jordanis* exposed to salt (150mM) and salt drought stressed plants. On the other hand, magnesium was significantly increased in roots of drought salt stressed of *T. aphylla* species. Similarly to combined stress, drought induced accumulation of magnesium in roots (Fig. 26A).

Calcium did not show any significant difference among all treatments at any different plant level, unless in *T. aphylla* where it showed an increase in calcium content when plants were subjected to drought stress. Ca^{2+}/Na^+ ratio decreased significantly in salt stress condition (Fig. 26B).

K^+ was significantly reduced in saline treatments in comparison with non-saline, and this happens more remarkably at leaf level of *T. aphylla* (Fig. 26C). In *T. aphylla*, salt treatments led to decrease of K^+ cation of 9.68% in roots, of 12.65% in stem and of 33.8% in leaves comparing with the control. Leaves of *T. jordanis* under salt show also insignificant reduction of potassium in leaves. Drought salt stress induced a non significant reduction of potassium in leaves, at lower extent than salt stress effect (Fig. 26C). ; On contrary, plants under drought salt stress increased their potassium content only at root level. The same observation at root level was noticed for drought stress where drought stress also increase their potassium content of 28.3 % at root level in *T. aphylla* species and of 37.7 % at root level in *T. jordanis*. $K^+/`Na^+$ ratio in roots resulted significantly higher in DS treatment than salt treatment for both species. The higher ratio

K^+/`Na^+ exhibited by roots of DS plants is achieved by a parallel lower content of sodium and higher potassium content than salt.

In contrast to many workers who noticed a reduction in phosphorus under saline conditions, here it was not detected at any organ plant level (Fig. 26D) whereas under water stress, phosphorus reduction was noticed at root level of *T. aphylla* (Data not shown).

Mineral variation at interspecific level

Factorial analyses did not reveal significant difference between both species in sodium accumulation in leaves ($P \geq 0.05$) (Table 2).

Interspecific differences were reported in chloride leaf content (Table 2). *T. jordanis* exhibited significantly lower Cl^- accumulation in leaves compared with *T. aphylla* ($P \leq 0.05$)

Differences in magnesium accumulation between both species were evident where leaves of *T. aphylla* accumulate in absolute term around 3 times more magnesium than leaves of *T. jordanis*. K^+ was higher also in leaves of *T. aphylla*, it was 1.5 times higher in *T. aphylla* than *T. jordanis*. Interspecific differences in Mg^{2+} and K^+ were not found great in the other organs as great as in leaves, although differences were significant.

Also calcium were higher in leaves of *T. aphylla*. Whereas Ca^{2+} was significantly higher in roots of *T. jordanis* than roots of *T. aphylla* under control, and salted soil (Table 2).

Results also showed that there were significant ($P \leq 0.05$) differences between species in K^+/Na^+ ratio of leaves independently from stress treatments. Differences in K^+/Na^+ between species and treatments were minor in stem plants (Table 2).

It was not found any particular interaction between species and treatment that may influenced Ca^{2+}/Na^+ ratio.

Roots and stem of *T. jordanis* tended to have slightly higher ions (Na^+, Mg^{2+}, K^+, $Ca^{2+}$and Cl^-) that invert and become greatly higher in leaves of *T. aphylla* (Table 2).

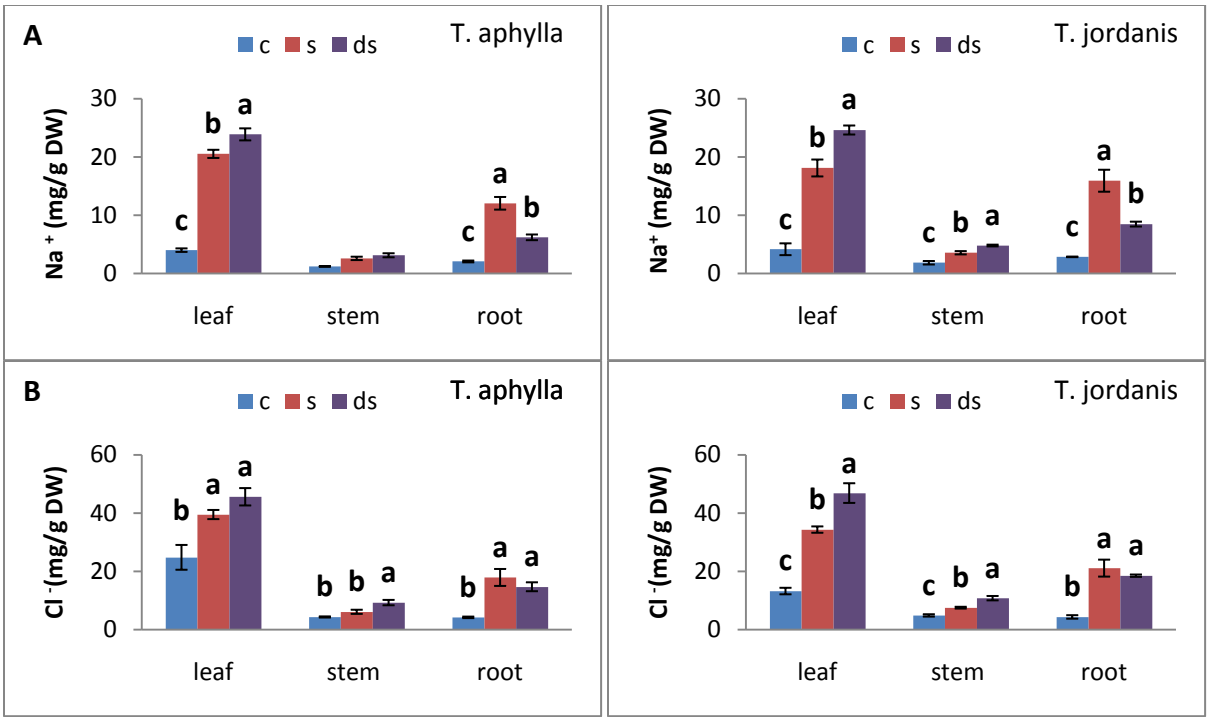


Figure 25. Mineral content (Na^+ and Cl^- in (A-B)) of plant organs (leaves, stems and roots) of *T.aphylla* and *T.jordanis* exposed to mild stress: C: Control; S: salt (150mM), D: drought (50% F.C.) and DS: Drought salt (150mM + 50% F.C). Different letters indicate statistically different means at $P \leq 0.05$ at 16 days of the experiment.

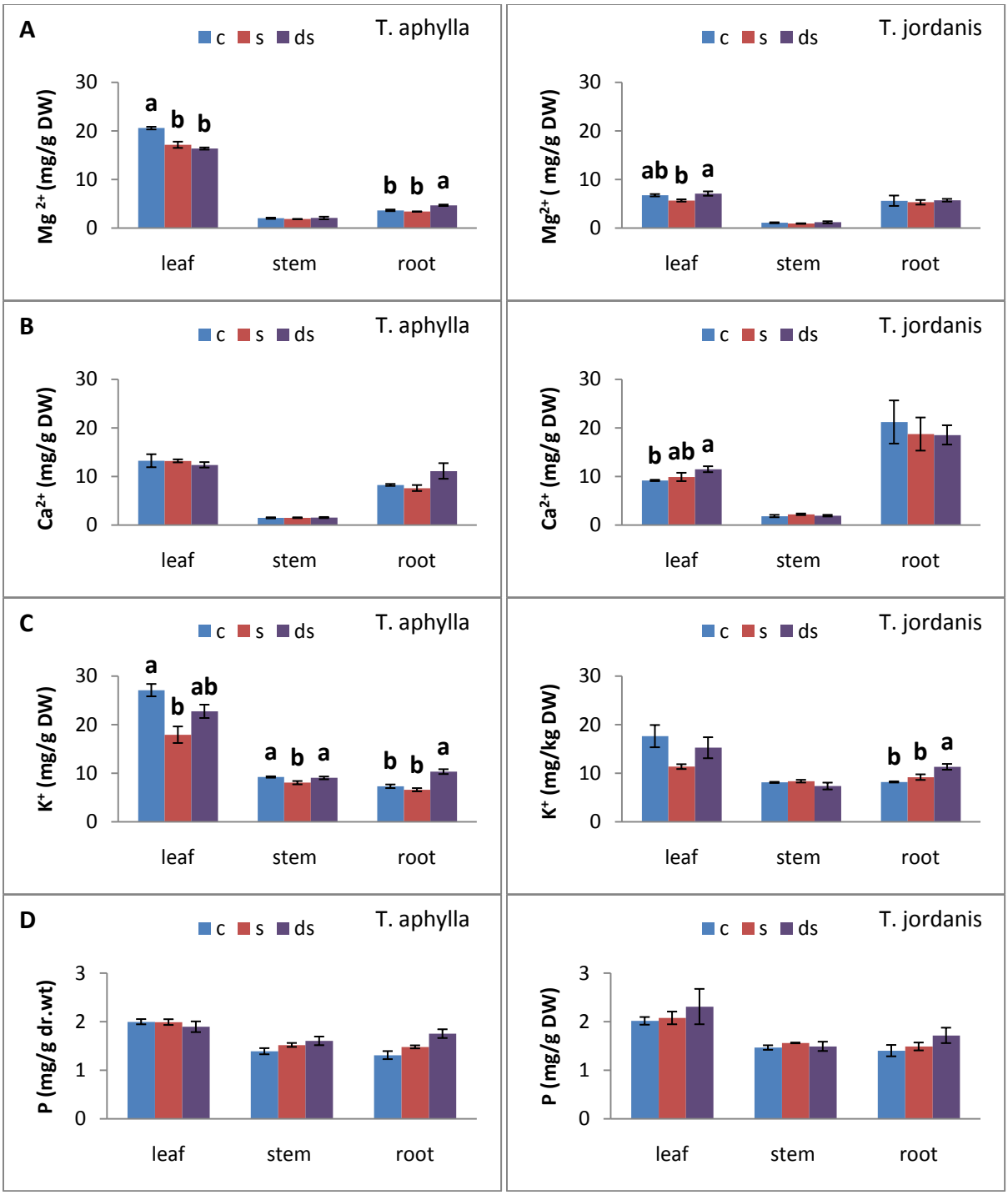


Figure 26. Mineral content (Mg^{2+},$Ca^{2+,}$ K^{+}, and P in (A-D)) of plant organs (leaves, stems and roots) of *T.aphylla* and *T.jordanis* exposed to mild stress: C: Control; S: salt (150mM), D: drought (50% F.C.) and DS: Drought salt (150mM + 50% F.C). Different letters indicate statistically different means at $P \leq 0.05$ at 16 days of the experiment.

Table 2. Analysis of variances for determined minerals in different parts (Leaves, stems and roots) of two species (*T. aphylla* and *T. jordanis*) exposed to mild stress: C: Control; S: salt (150mM), D: drought (50% F.C.) and DS: Drought salt (150mM + 50% F.C). Different letters indicate statistically different means at $P \leq 0.05$ at 16 days of the experiment.

Leaves	Na	Cl	Mg	K	Ca	P	K/Na	Ca/Na
Species	ns	**	***	***	***	ns	***	ns
Treatment	ns	***	***	***	ns	ns	***	***
Species*Treatment	ns	ns	***	ns	ns	ns	***	ns

Stems	Na	Cl	Mg	K	Ca	P	K/Na	Ca/Na
Species	***	**	***	**	***	ns	***	ns
Treatment	***	***	ns	ns	ns	ns	***	***
Species*Treatment	ns	ns	ns	**	ns	ns	**	ns

Roots	Na	Cl	Mg	K	Ca	P	K/Na	Ca/Na
Species	**	ns	***	***	***	ns	***	**
Treatment	***	***	ns	***	ns	***	***	***
Species*Treatment	ns	ns	ns	ns	ns	ns	**	ns

**P < 0.05; ***P < 0.01; ^{ns} not significant

9.3.7 Osmotic potential

A progressive increase in osmolarity was noticed in both treated with NaCl (150mM) (Fig. 27). After 14 days of the onset of salt stress (150mM), Ψ_s were -3.06 and – 3.28 MPa in *T.aphylla* and *T.jordanis* respectively.

In contrast to salt (150mM), Ψ_s of the leaves of plants subjected to water deficit plants remained close to Ψ_s of control plants, during the experiment (Fig. 27).

It was seen a parallel trend to salt (150mM) for plants under combined stress, however while it was not detected a significant difference in osmotic potential between both species under salt stress, it was shown under combined stress, where *T.aphylla* shows values of osmotic potential significantly more negative than *T. jordanis* (Fig. 27).

During the experiment, the trend of osmolarity of *T. jordanis* exposed to salt stress does not differentiate from that of under combined stress, whereas , osmolarity in *T.aphylla* plants shows a clear differentiation between these two treatments (Fig. 27).

Using the Van't Hoff equation (Nobel, 1991) to calculate the osmotic pressure for the cell sap of the leaves It was found that cations (Na^+, Cl^-, K^+, Ca^{2+}, and Mg^{2+}) made up of 1504.17 - 2730.57 µmol/g DW in *T. jordanis* and *T. aphylla* respectively, which was around 67-85% of solutes, relative to measured osmolariy, in control plants (Table 3). So it is logical to think about the role of these ions in osmotic adjustment, indeed, when salinity effect was taken into consideration, a significant increase in leaf concentration of Na^+ and Cl^- was observed in salt stressed plants, it was found that the contribution of Na^+ and Cl^- ions to the overall measured leaf osmotic potential was partial in *T. aphylla*, it increased from 28.5% in controlled plants to 41-47% in salt and combined stress, whereas the contribution of these ions in the measured osmotic potential of *T. jordanis* was higher it was increased from 23.6 % in control to 72-99% in salt and combined stress.

In contrast to inorganic ions, soluble sugars contribute little to Ψ_s, they made up 207.5- 258 µmol/g DW in *T. jordanis* and *T. aphylla* respectively which was around 7.8-9.5% of the measured osmotic potential Ψ_s in control plants (Table 4).

Also the contribution of proline to osmotic adjustment is small with respect to the total solute concentration; it accounted only 5.38-9.26µmol/g DW which is 0.24-0.28% of measured Ψ_s in control plants (Table 4).

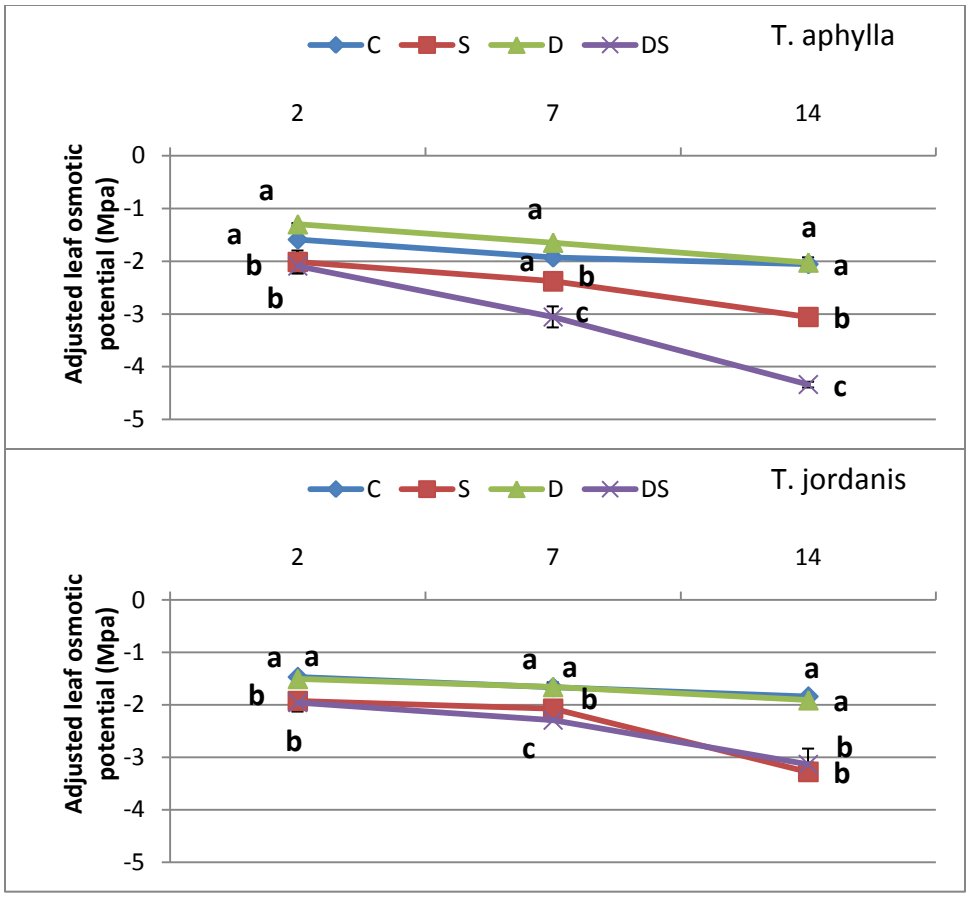


Figure 27. Adjusted leaf osmotic potential of *T. aphylla* and *T. jordanis* exposed to mild stress: C: Control; S: salt (150mM), D: drought (50% F.C.) and DS: Drought salt (150mM + 50% F.C). Different letters on the top of the error bars indicate statistically different means at $P \leq 0.05$.

Table 3. Contribution of inorganic ions concentration and contribution to the leaf osmotic potential at full turgor of two species (*T. aphylla* and *T. jordanis*) exposed to mild stress: C: Control; S: salt (150mM), D: drought (50% F.C.) and DS: Drought salt (150mM + 50% F.C), at the 16th day of the experiment.

Treatment /species	Ψ_{os} (Mpa)	Na^+		Cl^-		Mg^{2+}		K^+		Ca^{2+}	
		µmol g DW^{-1}	Ψ_{os} (Mpa)	µmol g DW^{-1}	Ψ_{os} (Mpa)	µmol g DW^{-1}	Ψ_{os} (Mpa)	µmol g DW^{-1}	Ψ_{os} (Mpa)	µmol g DW^{-1}	Ψ_{os} (Mpa)
T. apylla											
C	-1.92	174.96	-0.10	699.58	-0.45	847.43	-0.49	678.34	-0.39	330.27	-0.19
S	-3.08	893.87	-0.46	1114.25	-0.63	706.03	-0.36	448.81	-0.23	329.52	-0.17
D	-2.04										
DS	-4.18	1039.44	-0.60	1286.32	-0.85	674.21	-0.39	569.28	-0.33	309.15	-0.18
T. jordanis											
C	-1.88	181.77	-0.15	373.30	-0.29	278.60	-0.23	441.47	-0.37	229.03	-0.19
S	-3.22	788.46	-0.66	968.50	-0.75	234.27	-0.20	284.64	-0.24	246.88	-0.21
D	-2.07										
DS	-3.31	1072.06	-0.88	1322.05	-0.98	292.85	-0.24	381.94	-0.31	286.69	-0.24

Table 4. Contribution of inorganic ions concentration and contribution to the leaf osmotic potential at full turgor of two species (*T. aphylla* and *T. jordanis*) exposed to mild stress: C: Control; S: salt (150mM), D: drought (50% F.C.) and DS: Drought salt (150mM + 50% F.C), at the 16th day of the experiment.

Treatment /species	Ψ_{os} (Mpa)	Reducing sugars		sucrose		proline	
		µmol g DW^{-1}	Ψ_{os} (Mpa)	µmol g DW^{-1}	Ψ_{os} (Mpa)	µmol g DW^{-1}	Ψ_{os} (Mpa)
T. apylla							
C	-1.92	49.07	-0.03	209.07	-0.12	9.26	-0.005
S	-3.08	30.07	-0.02	194.56	-0.10	15.52	-0.008
D	-2.04	53.19	-0.03	218.32	-0.12	11.99	-0.007
DS	-4.18	29.01	-0.02	175.09	-0.10	53.55	-0.031
T. jordanis							
C	-1.88	55.55	-0.05	151.92	-0.13	5.38	-0.005
S	-3.22	44.71	-0.04	144.65	-0.12	7.66	-0.006
D	-2.07	72.74	-0.06	202.47	-0.16	8.11	-0.007
DS	-3.31	46.39	-0.04	154.15	-0.13	33.46	-0.028

9.3.8 Water potential measurements

Factorial analysis indicated significantly lower water potential in *T. aphylla* than in *T. jordanis*, independently from stress and without evidence of stress interaction water potential response of *Tamarix spp.* ($P \leq 0.05$).

The graphs showed in the figure below (Fig. 28), represented data of midday water potential of the last day of the experiment, those of the previous day of the experiment are missing due to encountered technical problems. Generally, both species showed that mean predawn water potential was higher than associated mean midday values (Fig. 28). Midday leaf water potential decreased due to salt stress (150 mM), *T. jordanis* decreased of – 1.44 Mpa while *T. aphylla* of -0.97Mpa relative to the control (Fig. 28). Under drought stress both species response to the stress quite similarly; they decrease around -1.10 Mpa. The effects of the two constraints when combined were additive, higher negative values of water potential were attained , it reached -3.81 Mpa by *T. aphylla* species and -2.32 Mpa by *T. jordanis* (Fig. 28). Larger diurnal variation was recorded for DS treatment where stress effect is more marked (Fig. 28).

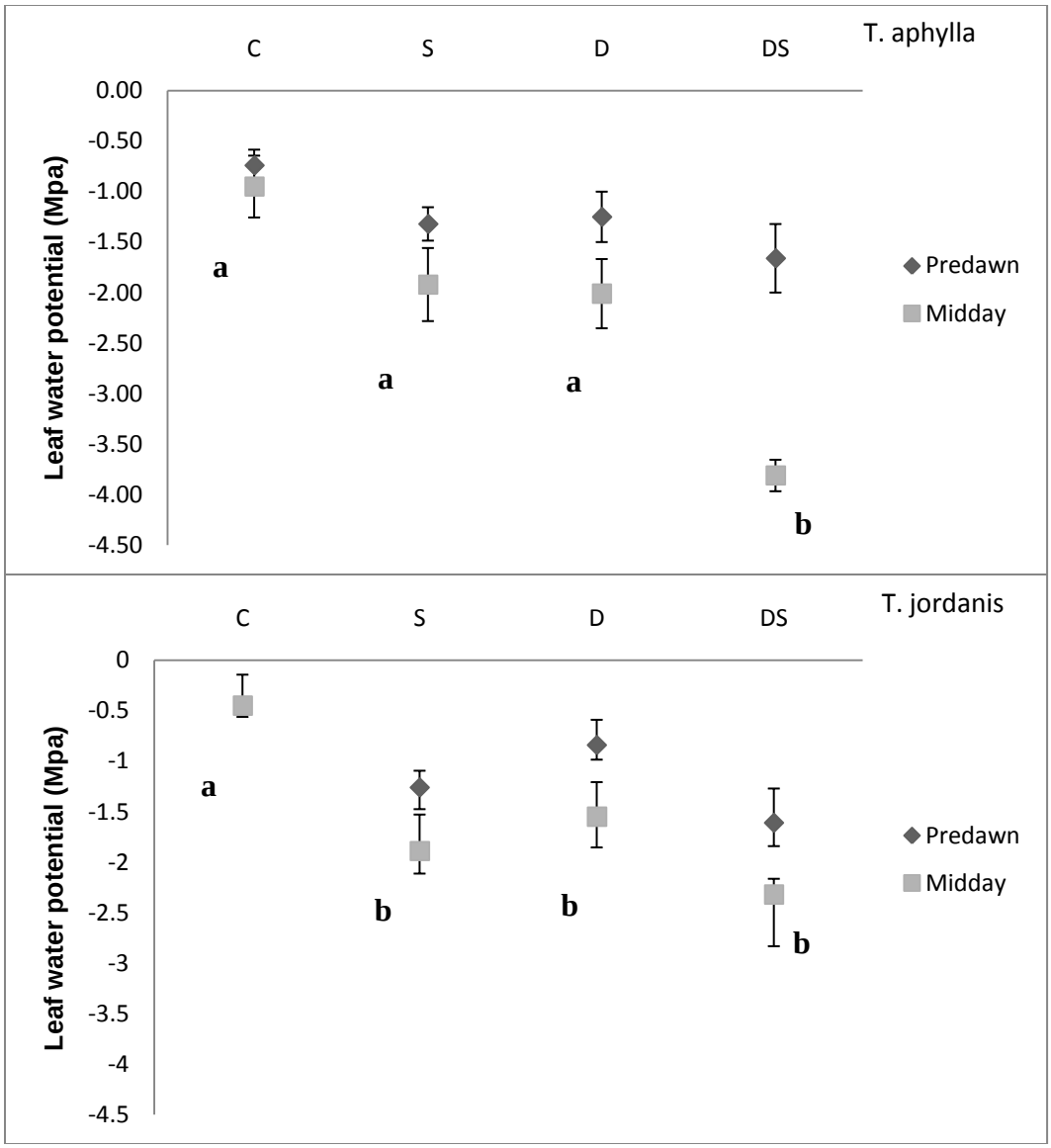


Figure 28. Water potential of leaves of *T.aphylla* and *T.jordanis* exposed to mild stress:
C: Control; S: salt (150mM), D: drought (50% F.C.) and DS: Drought salt (150mM + 50% F.C). Data are means ± standard errors. Different letters on the top of the error bars indicate statistically different means at $P \leq 0.05$.

9.3.9 Relative water content

Factorial analysis indicated a difference between both species in their water status independently from stress condition, while Relative water content (RWC) of *T. aphylla* is 98.64% and in *T. jordanis* is 85.7% ($P \leq 0.05$)

Missing data in the corresponding days (2nd day) were related to technical issues. Exposure to mild water deficit (50% F.C.), induced significant reduction in (RWC) as demonstrated by plants of *T.aphylla* in the 7th day of the experiment (Fig. 29); water content was lower than control of 8.88%, as well , it was shown for the other species although statistic analysis are not significant. The other treatments keep their water content in leaves quiet high as control plants for both species. The 14th day did not show any significant difference in (RWC) between treatments for any of the species studied (Fig. 29).

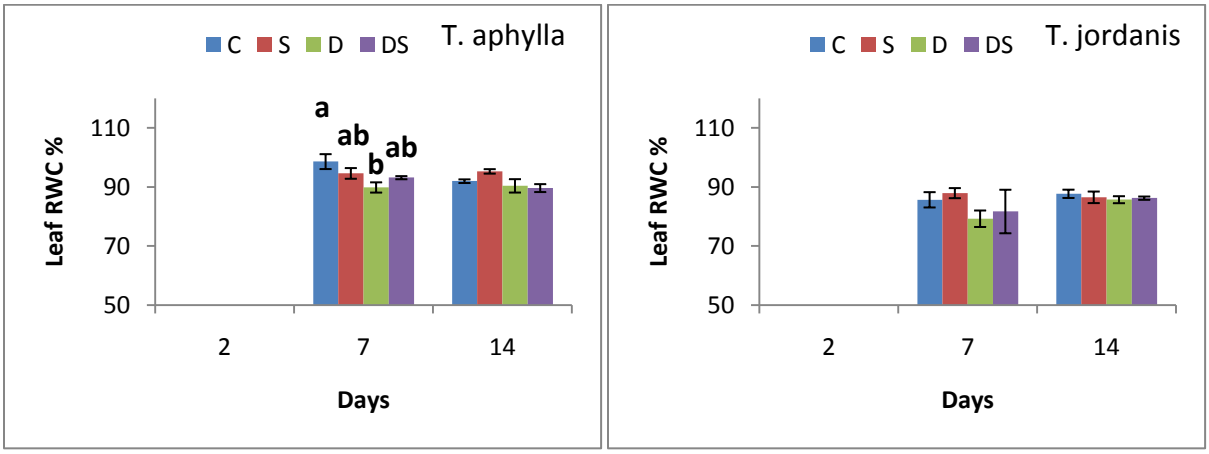


Figure 29. Relative Water Content of leaves of *T. aphylla* and *T. jordanis* exposed to mild stress: C: Control; S: salt (150mM), D: drought (50% F.C.) and DS: Drought salt (150mM +50% F.C). Data are means ± standard errors. Different letters on the top of the error bars indicate statistically different means at $P \leq 0.05$.

9.3.10 Growth measurements

The effects of drought and salt stress on growth of *T. aphylla* and *T. jordanis* were shown in Table 3. Salt impact on growth was higher on *T. aphylla* while drought impact on growth was higher on *T. jordanis.*

The negative effect of salt stress on plant height was noticed for *T. aphylla*, plant height of *T. aphylla* reduced of 28.17%, while *T. jordanis* seems to be indifferent. Throughout the experiment, new shoots were continuously emerging (Table 5).

Shoot dry weight of both species was not affected by salt stress, yet shoot dry weight of *T. jordanis* under salt stress was even higher than control. (Table 6). Roots also were not affected by salt stress, in both species.

Under drought, severe reduction of plant height (55%) was noticed in *T. jordanis* while *T. aphylla* was less affected (29.5%). Throughout the experiment, new shoots were emerged even if it was at lower rate than control (Table 5).

Drought impact on shoot dry weight was the same as for plant height highly severe in *T. jordanis,* (32.5%), and to lower extent in *T. aphylla* (19%) (Table 6).

Consequently, higher root /shoot ratio was evident in *T. jordanis,* which was not due to increase in plant roots growth to adapt stress but rather, due negative effect of water stress on above ground growth in *T. jordanis* (Table 6).

The combination of both stress had the most pronounced effect on plant growth on both species, it was detected at early stages of the experiment accompanied by clear visible symptoms of yellowness and leaf shedding at the bottom third part of the plant. At the end of experiment *T. aphylla* and *T. jordanis* decreased of 76%-84% in height, respectively. *T.aphylla* subjected to combined stress was almost prohibited to protrude new shoots (Table 5).

Under combined stress, shoot dry weight of *T. jordanis* was significantly lower than control (32.5%) whereas shoot dry weight of *T. aphylla* was not significantly different from control (35.4%). Root dry weight was not influenced by combined stress in both species (Table 6).

The root/shoot ratio resulted significantly higher in *T. aphylla* treated with combination of salt and drought stress (73.5 %) relatively to control plants; this increase was mainly due to a reduction of shoot dry weight as the root dry weight did not show any significant differences among the treatments (Table 6).

Table 5. Plant height increment and leaf number of two species (*T. aphylla* and *T. jordanis*) :exposed to mild stress C: Control; S: salt (150mM), D: drought (50% F.C.) and DS: Drought salt (150mM + 50% F.C). Different letters indicate statistically different means at $P \leq 0.05$ at 16 days of the experiment.

Experiment /species	Plant height increment (cm/15days)	Leaf number
T. aphylla		
C	0.73 ± 0.064^{a}	4.3 ± 0.78^{a}
S	0.53 ± 0.047^{b}	3.6 ± 0.37^{a}
D	0.52 ± 0.045^{b}	4.3 ± 0.29^{a}
DS	0.11 ± 0.010^{c}	2 ± 0.44^{b}
T. jordanis		
C	1.10 ± 0.160^{a}	6.1 ± 1.14^{a}
S	1.12 ± 0.251^{a}	4.4 ± 0.81^{a}
D	0.49 ± 0.082^{b}	3.8 ± 0.65^{a}
DS	0.26 ± 0.048^{b}	2.9 ± 0.59^{a}

Table 6. Shoot, root weight and root/shoot ratio of of of two species (*T. aphylla* and *T. jordanis*) :exposed to mild stress C: Control; S: salt (150mM), D: drought (50% F.C.) and DS: Drought salt (150mM + 50% F.C). Different letters indicate statistically different means at $P \leq 0.05$ at 16 days of the experiment.

Experiment /species	Shoots dry mass (g/plant)	Root dry mass (g/plant)	Root/shoot ratio
T. aphylla			
C	41.5 ± 4.4^{a}	20.4 ± 2.6^{a}	0.49 ± 0.02^{b}
S	43 ± 5.6^{a}	21.4 ± 2.3^{a}	0.5 ± 0.027^{b}
D	33.6 ± 2.9^{a}	20.9 ± 1.5^{a}	0.63 ± 0.03^{ab}
DS	26.8 ± 4.5^{a}	21.1 ± 3.7^{a}	0.85 ± 0.169^{a}
T. jordanis			
C	66 ± 7.7^{a}	30.2 ± 2.1^{a}	0.47 ± 0.04^{b}
S	77.1 ± 2.5^{a}	29.2 ± 4.5^{a}	0.45 ± 0.05^{b}
D	44.5 ± 4.6^{b}	35.8 ± 1.2^{a}	0.84 ± 0.10^{a}
DS	45 ± 4.8^{b}	27 ± 1.3^{a}	0.61 ± 0.051^{b}

9. 4 Results of ecophysiological and biochemical responses of *Tamarix spp.* to mild stress: salt (150mM), drought stress (50% F.C.), salt drought (150mM + 50% F.C.) - Replication of the experiment of mild stress.

Our experiment was repeated for the subsequent year (2009) in order to confirm our previous data, and to get any additional information, related with plant responses to salt (150mM), drought (50%F.C.) and salt drought (150mM + 50%F.C.).

However the season of the year 2009 was not favorable for plant growth as in 2008.
The interaction between year and treatment ($P \leq 0.05$, highlighted in red color) in sugar content indicated that climatic conditions in semi controlled environment had so far a great impact on plant response (Tables 7, 8).
Sugar content accumulation is the parameter which reflected to a great extent the poor conditions on which plants were grown. From the starting point of the experiment till the end, sugar content especially starch and sucrose that are reserve sugar were much lower than previous year (2008). The trend of plants response throughout the 14^{th} day was somehow the same as in the last year (2008), it could be matched but more flat with reduced statistical differences.
Any additional or peculiar responses regarded gas exchange measurements, water use efficiency, chlorophyll, water relations, and growth, were not underlined.

Table 7. Analysis of variances of soluble assessed for two subsequent years (2008-2009) during 16 days period, of two species (*T. aphylla* and *T. jordanis*): exposed to mild stress C: Control; S: salt (150mM), D: drought (50% F.C.) and DS: Drought salt (150mM + 50% F.C).

Glucose µmol g DW^{-1}/days	**2**	**7**	**14**
Effect	**p**	**p**	**p**
Species	0.104666	0.304105	0.141409
Treatment	0.00007	0.000321	0.000252
Year	0.000721	0.000451	0.00021
Species*Treatment	0.506751	0.008409	0.502165
Species*Year	0.022404	0.061448	0.027352
Treatment*Year	0.009572	0.000002	0.648665
Species*Treatment*Year	0.572126	0.001215	0.754626

Fructose µmol g DW^{-1}/days	**2**	**7**	**14**
Effect	**p**	**p**	**p**
Species	0.003626	0.03161	0.808443
Treatment	0.001881	0.000437	0.000008
Year	0.000258	0.000573	0.000035
Species*Treatment	0.520979	0.001761	0.489944
Species*Year	0.019165	0.524174	0.00042
Treatment*Year	0.026384	0.000874	0.828733
Species*Treatment*Year	0.502916	0.00275	0.601898

Sucrose µmol g DW^{-1}/days	**2**	**7**	**14**
Effect	**p**	**p**	**p**
Species	0.000004	0.0000045	0.007131
Treatment	0.261665	0.000057	0.568651
Year	0.029665	0.000763	0.00057
Species*Treatment	0.683647	0.130509	0.979523
Species*Year	0.445994	0.127522	0.257542
Treatment*Year	0.250839	0.00002	0.027555
Species*Treatment*Year	0.034685	0.026474	0.481441

Table 8. Analysis of variances of starch assessed for two subsequent years (2008-2009) during 16 days period, of two species (*T. aphylla* and *T. jordanis*) :exposed to mild stress C: Control; S: salt (150mM), D: drought (50% F.C.) and DS: Drought salt (150mM + 50% F.C).

Starch µmol g DW^{-1}/days	**2**	**7**	**14**
Effect	**p**	**p**	**p**
Species	0.0042	0.000354	0.000361
Treatment	0.000285	0.02487	0.00456
Year	0.000009	0.00127	0.00432
Species*Treatment	0.100059	0.034337	0.174044
Species*Year	0.052889	0.0264	0.105113
Treatment*Year	0.078797	0.000365	0.067167
Species*Treatment*Year	0.164162	0.570792	0.969091

9.5. Results of ecophysiological and biochemical responses of *Tamarix spp.* to severe stress: salt (350mM), drought stress (30% F.C.), salt drought (350mM + 30% F.C.).

9.5.1 Gas exchange measurements

Although the severity of different stress applied, interspecific differences in photosynthesis in response to stress (S (350mM, DS (350mM+ 30% F.C.)), (p= 0.931) were not evidenced.

However impact of different types of severe stress was evident on each species.

Significant decrease in photosynthesis under salt stress (350mM) was found in both species with some improvement remarkable only in the 7^{th} day in *T. jordanis*. However, the last day of severe salt stress (350mM), presented a significant reduction of photosynthesis of 36.8%- 41.8%, respectively in *T. jordanis* and *T. aphylla* (Fig. 30A). Similarly (g_s) and (E) showed the same behavior (Fig. 30B-C). Reduction of (g_s)was accompanied by reductions in C_i under salt stress (Fig. 30B-D).

Severe salt stress (350mM) impact on photosynthesis was high relative to mild salt stress (150mM) which showed a not significant effect on photosynthesis 14.7-16.9% of *Tamarix spp.* at the end of experiment.

Drought (30% F.C.) did not affect photosynthesis significantly in the second day in any of tested species (Fig. 30%F.C.). The following days drought (30% F.C.) provoked the wilting of plants.

Severe coupled stress DS induced high limitations of main gas exchange parameters (Fig.30A-C) (C_i) of plants treated with DS was unchanged, although the complete closure of stoma (Fig.30D).

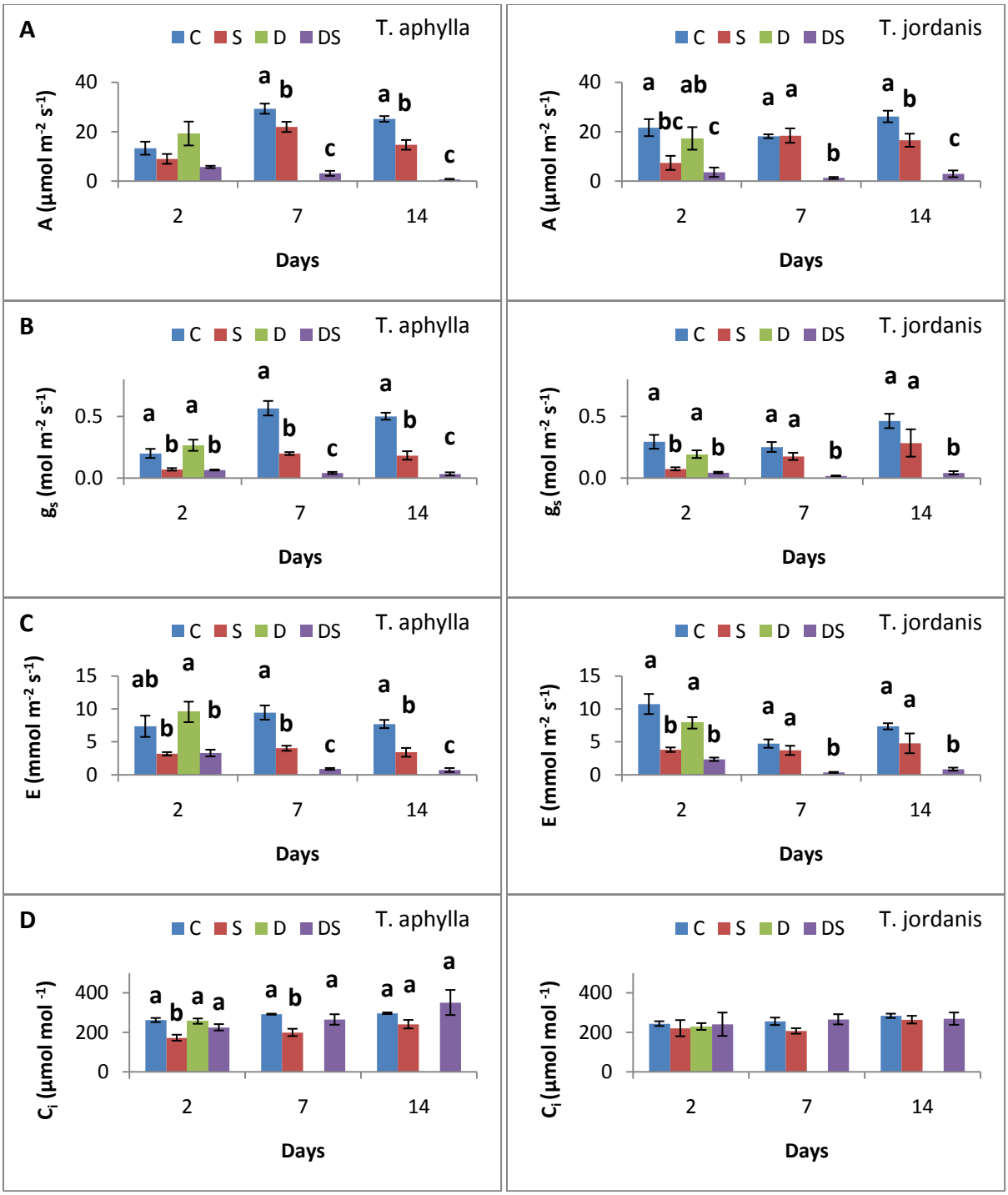


Figure 30. Gas exchange measurements (Assimilation rate (A), stomatal conductance (g_s), transpiration (E) , and intercellular CO2 (C_i), in (A-D)) of *T. aphylla* and *T. jordanis* exposed to Severe stress: C: Control; S:salt (350mM); D: drought (30% F.C.) and DS: Drought salt (350mM +30%) F. Data are means ± standard errors. Different letters on the top of the error bars indicate statistically different means at $P \leq 0.05$.

9.5.2 Water use efficiency

The intrinsic water use efficiency (A/g_s) and (A/E) were significantly higher in salt (350 mM) treated *T. aphylla*. (Fig. 31A-B). On the contrary, the intrinsic water use efficiency (A/g_s) and (A/E) in *T. jordanis* were not significantly different from control.

T. aphylla and *T. jordanis* under severe combined stress DS were not able to valorize water use; they register (A/g_s) similar to control. (Fig.31A-B)

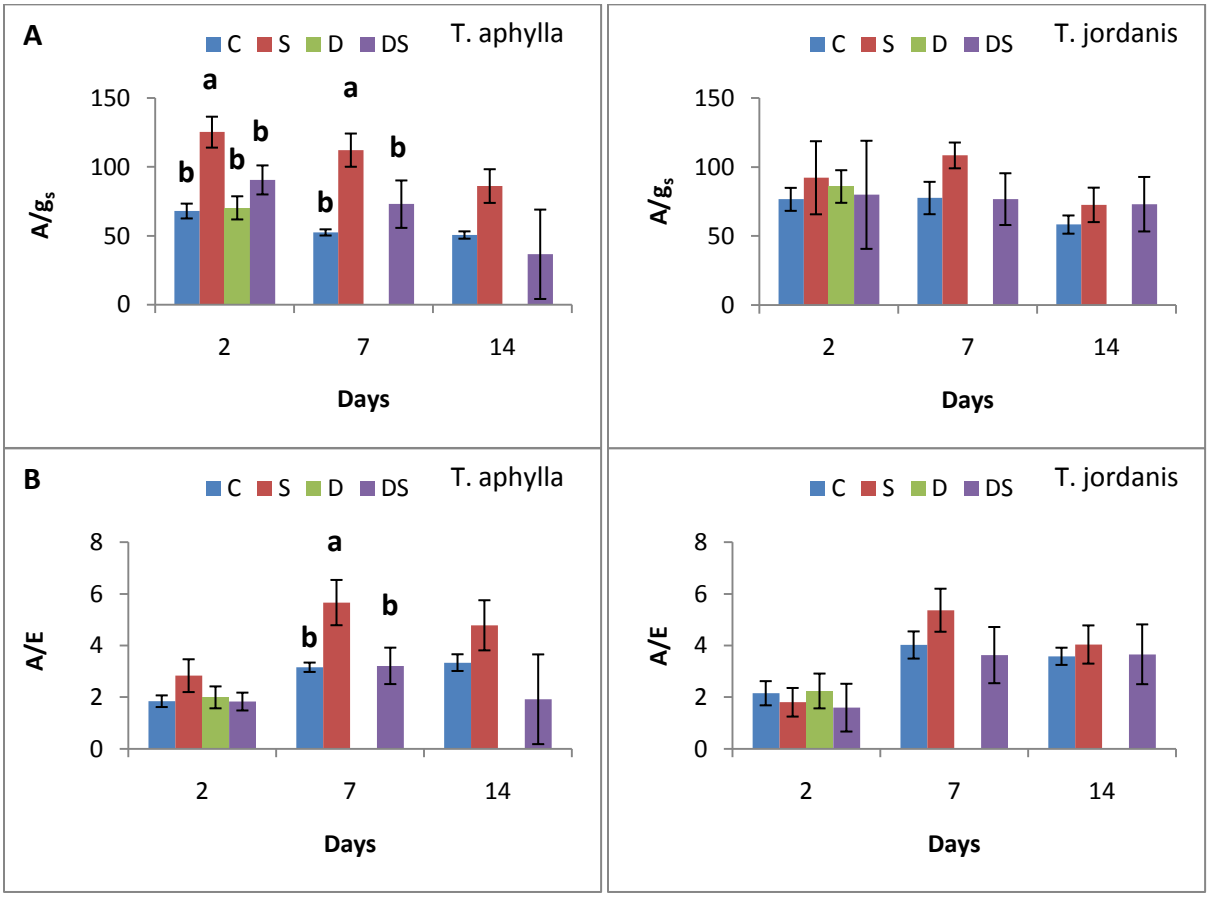


Figure 31. (A/gs) ratio, (A/E) ratio in (A-B)) of *T. aphylla* and *T. jordanis* exposed to Severe stress: C: Control; S:salt (350mM); D: drought (30% F.C.) and DS: Drought salt (350mM +30%) F. Data are means ± standard errors. Different letters on the top of the error bars indicate statistically different means at $P \leq 0.05$.

9.5.3 Chlorophyll

Pronounced effect on chl $_a$ was noticed on *T. aphylla* exposed to high salt stress (350mM) (Fig. 32A). As well, severe combined stress (DS) affected chl $_a$ of both species, (Fig.32A), however Chl $_b$ remained constant (Fig. 32B). Consequently, significant decrease in ratio of chl $_{a/b}$ was shown in DS treatments (Fig. 32D).

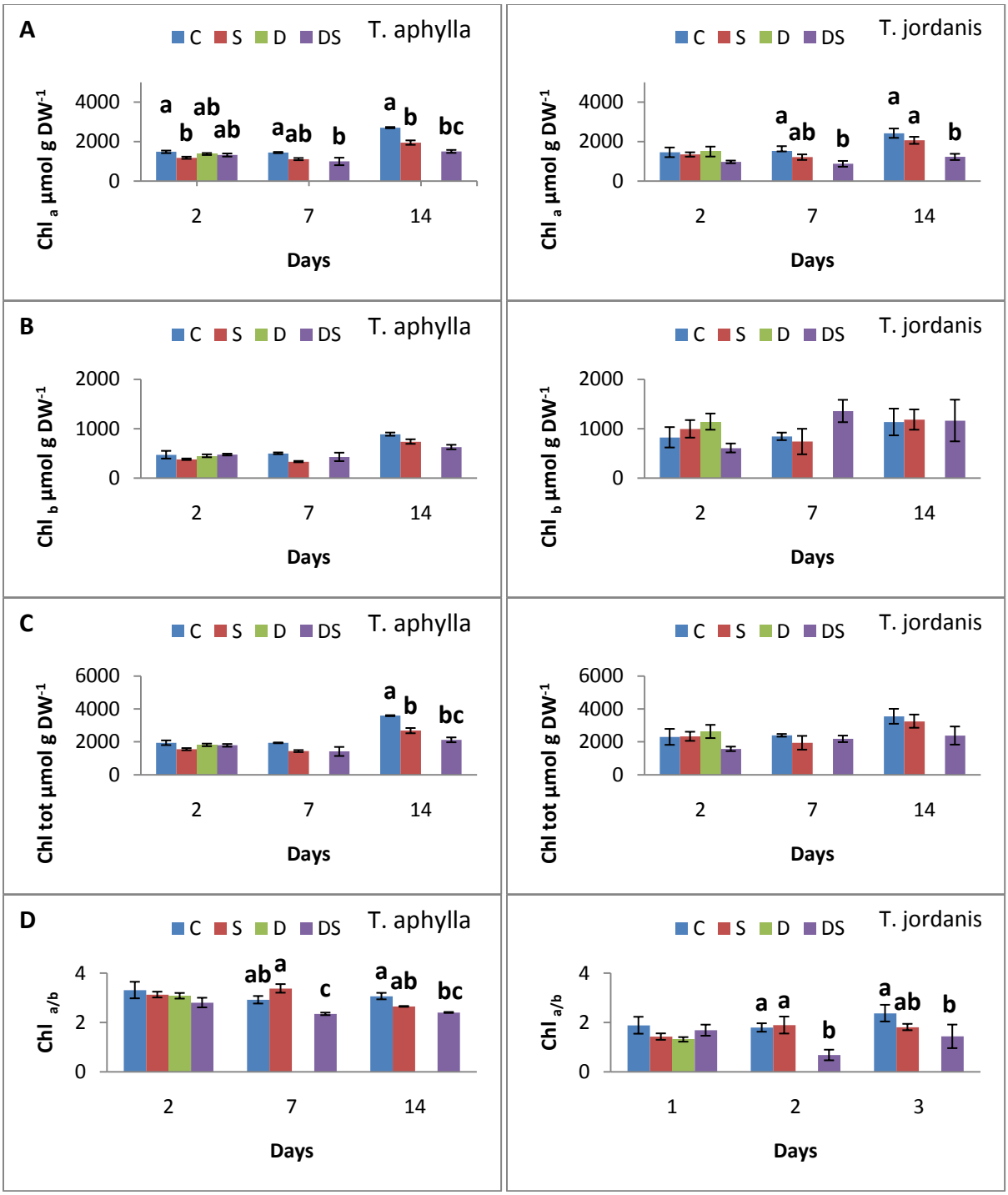


Figure 32. Chlorophyll content (chlorophyll a (Chl $_a$), chlorophyll b (Chl $_b$), chlorophyll total (Chl tot), and chlorophyll a/b ratio (Chl $_{a/b}$) in (A-D)) of *T. aphylla* and *T. jordanis* exposed to severe stress: C: Control; S: salt (350mM), D: drought (30% F.C.) and DS: Drought salt (350mM +30% F.C). Different letters on the top of the error bars indicate statistically different means at $P \leq 0.05$.

9.5.4 Carbohydrates

Soluble sugars and starch amount were always proven to be related to genotype into account, irrespective of plant treatment; the content of sucrose was higher than starch in *T. aphylla*, while starch content was higher than sucrose in *T. jordanis* (Figs. 33C, 34).

The amount of non structural carbohydrates accounted only 1.1-7.6% of leaf dry matter.

Soluble sugars and starch were reduced in 2009, relative to 2008, especially in *T.jordanis* species. Generally, the starch of the controls was about 50% lower than in 2008.

Progress of starch synthesis was induced whole the experiment in control plants, to reach its maximum in the 14th day (Fig. 34).Sucrose content lost its stability under stress conditions, differences among treatments were detected (Fig. 33C).

T. aphylla and *T. jordanis* showed similar trend in response to different stress applied, with slight differences and marked response for *T. aphylla*.

In the 2nd day, salt (350mM) induced significant reduction in glucose, fructose content in *T. aphylla* (66%, 57% respectively) and non significant reduction in *T. jordanis*. Decrement of glucose and fructose was also evident even in the 7th and 14th day, although significance was not always obvious (Fig. 33A-B). Sucrose was sensitive to salt stress (350mM), it was decreased significantly in the 7th day in *T. aphylla* and in 2nd day in *T. jordanis*, while in the 14th day sucrose values of both species were similar to control (Fig. 33C). Starch also reduced significantly in *T. aphylla and T. jordanis* treated with salt (350mM), as demonstrated in the 2nd day (Fig. 34). At the end of experiment, starch content in *T. aphylla* fails to recuperate under salt (350mM) while that of *T. jordanis* succeed again to be synthesized as control.

Drought (30% F.C) although it was severe, plants was less influenced in hexoses and sucrose than salt (Fig. 33A-C) while starch was significantly reduced in both species as shown in (Fig. 34). Data of drought in the following days were not obtained due to the death of the plants.

On the other hand, it was the first time that we can see that combined stress induced an increase in hexoses in the last day with simultaneous decrease in sucrose always yielding a ratio of glucose/fructose equal to one (Fig. 33A-C). Starch content was almost consumed in *T. aphylla* and *T. jordanis* treated with salt drought (Fig. 34).

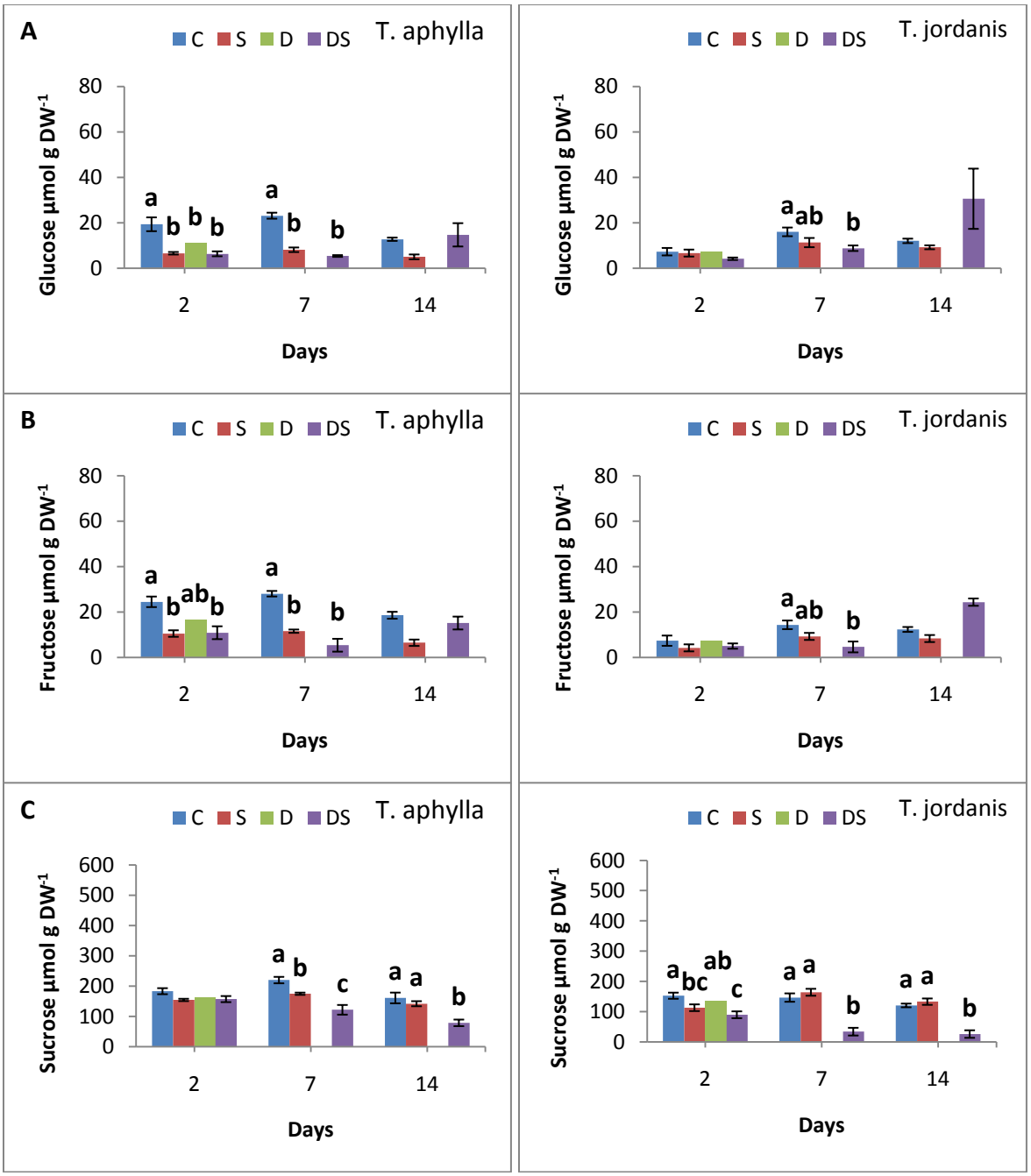


Figure 33. Sugar content (glucose, fructose, and sucrose) in (A-C)) of *T. aphylla* and *T. jordanis* exposed to severe stress: C: Control; S: salt (350mM), D: drought (30% F.C.) and DS: Drought salt (350mM +30% F.C). Different letters on the top of the error bars indicate statistically different means at $P \leq 0.05$.

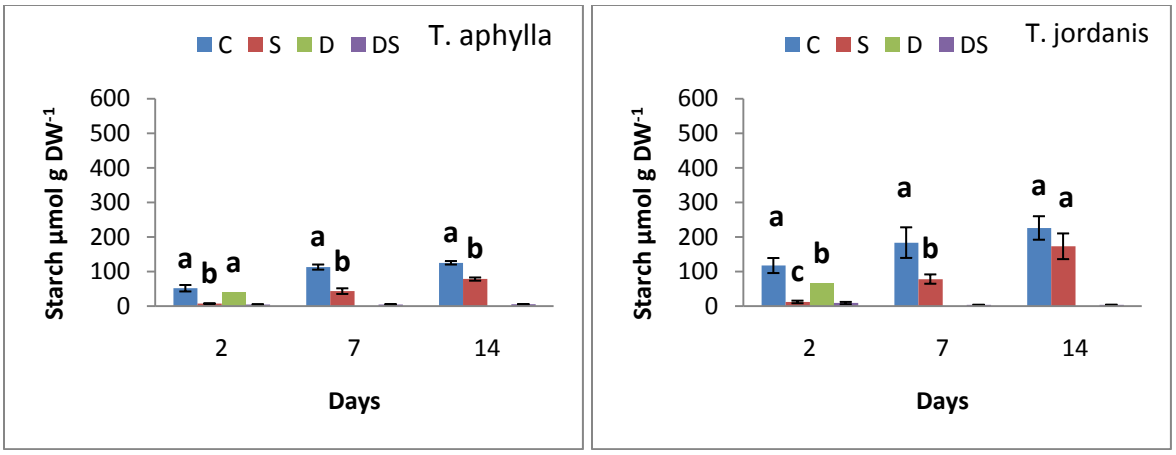


Figure 34. Starch content of *T. aphylla* and *T. jordanis* exposed to severe stress: C: Control; S: salt (350mM), D: drought (30% F.C.) and DS: Drought salt (350mM + 30% F.C). Different letters on the top of the error bars indicate statistically different means at $P \leq 0.05$.

9.5.5 Water potential

S (350mM) induced significant lowest values of midday water potential (-2.48 Mpa) in *T. jordanis* only at the end of experiment (Fig. 35).

Both species subjected to D (30% F.C.) recorded significantly low predawn (-2.00 to -2.11 Mpa) and midday water potential (-2.89 to -3.29 Mpa), this observation only done in the second day due to death of plants (Fig. 35).

DS stress showed the lowest predawn and midday water potential among treatments in both species, the last day, predawn water potential attained between -3.12 to-3.58 Mpa and midday water potential between -3.56 and -4.07 Mpa, . Plants under severe DS stress could not exhibit a high diurnal variation as they did with milder DS stress due to a lower predawn water potential attained. Plants showed found more difficulty to absorb water from soil under these levels of stress. Nevertheless, it was observed that salt presence (350mM) in severe dry soil (30% F.C.) prevented sudden leaf dehydration caused by high impact of severe drought (30% F.C.) applied alone (Fig. 35).

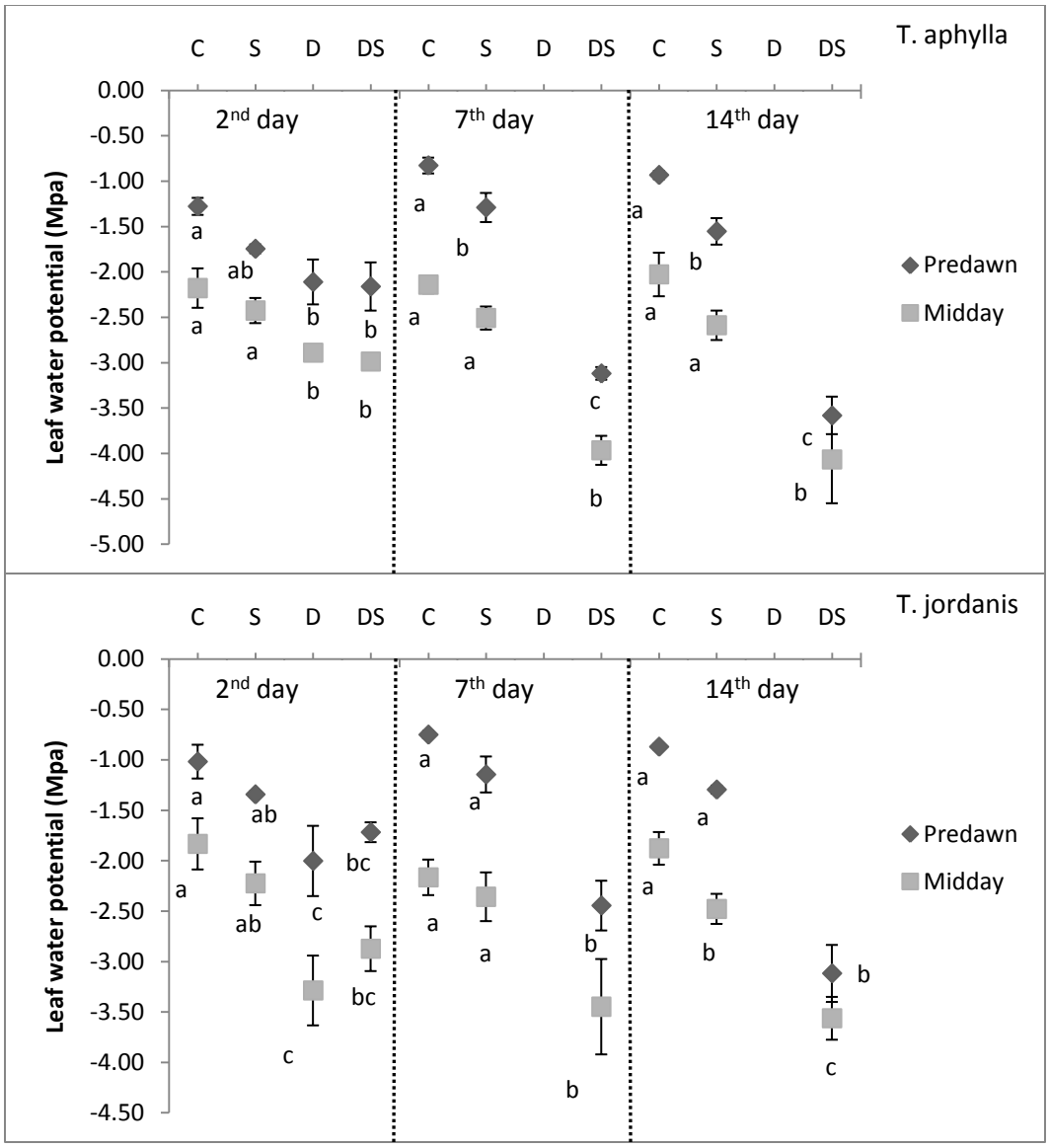


Figure 35. Water potential of leaves of *T. aphylla* and *T. jordanis* exposed to severe stress: C: Control; S: salt (350mM), D: drought (30% F.C.) and DS: Drought salt (350mM + 30% F.C). Different letters on the top of the error bars indicate statistically different means at $P \leq 0.05$.

9.5.6 Relative water content (RWC)

Salt stress (350 mM) didn't determinate a significative reduction of leaf RWC in both species (Fig. 36).

Leaf dehydration was drastic in plants under water stress of 30% of F.C., after only two days from beginning of drought treatment the leaf RWC was reduced approximately of 21-23 % in both species (Fig. 36). Few days later drought (30%F.C.) led to the death of all plants (Fig. 36).

The combined DS stress caused a high reduction in RWC of leaves in *T. aphylla* that decreased during the course of the experiment from 5.34 to 15.12 %.. Also *T. jordanis* under DS stress has highlighted a decrease of leaf RWC, although statistical significance was somehow fluctuating (Fig. 36).

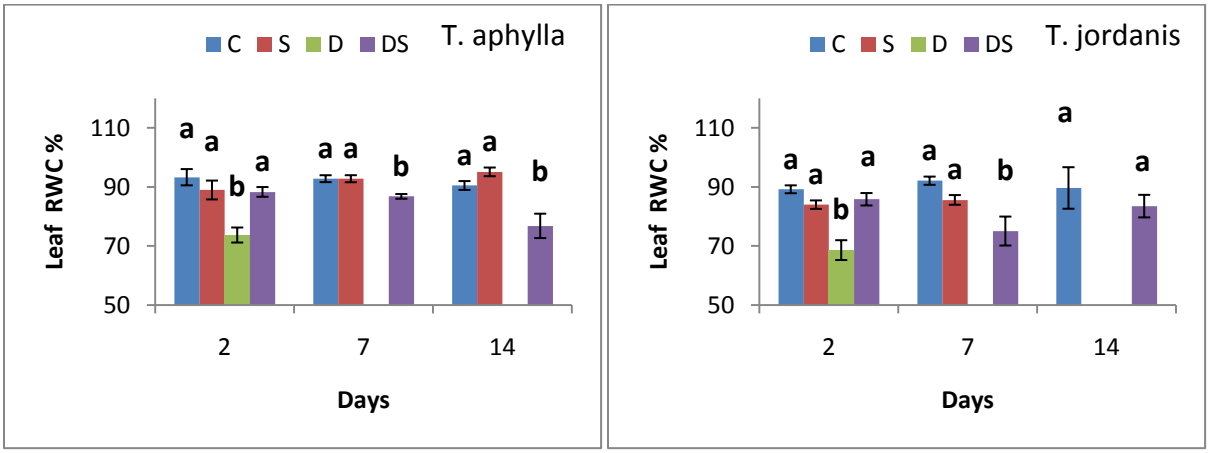


Figure 36. Relative Water Content of leaves of *T. aphylla* and *T. jordanis* exposed to severe stress: C: Control; S: salt (350mM), D: drought (30% F.C.) and DS: Drought salt (350mM + 30% F.C). Different letters on the top of the error bars indicate statistically different means at $P \leq 0.05$.

9.5.7 Growth measurements

A height growth reduction was reported in *T. aphylla* under severe salt stress (350 mM). The height growth limitation was 70.4% relative to control plants (Table 9). Shoot emergence was also highly affected in this species (Table 9). Comparing with milder salt stress (150mM), reduction percentage was higher of 42.23%. On the contrary, *T. jordanis* showed little effects on height growth and shoot emergence under severe salt stress (350mM) (Table 9).

Complete inhibition of plant growth under coupled stress DS stress was evident in both species (Table 9).

Table 9. Plant height increment and leaf number of two species (*T. aphylla* and *T. jordanis*) exposed to severe stress C: Control; S: salt (350mM), D: drought (30% F.C.) and DS: Drought salt (350mM +30% F.C). Different letters indicate statistically different means at $P \leq 0.05$ at 16 days of the experiment.

Experiment /species	Plant height reduction (%)	Leaf number reduction (%)
T. aphylla		
S	-70.37	-59.82
D	NM	NM
DS	-98.15	95.53
T. jordanis		
S	-32.94	-26.96
D	NM	NM
DS	-92.22	-88.40

NM: not measured due to due to death of plants

9.6 Discussion

Drought and salinity are two environmental constraints that often occur simultaneously in arid regions. The ability to overcome multiple and simultaneous stresses is of great importance for the plant growth and survival in stressful environments (Lichtenthaler, 1996). The present study aimed at investigating the effects of short-term salinity and/or drought imposed at two levels, on growth, water status, gas exchange, solute accumulation and mineral deficiency in *Tamarix spp.*

Salinity

It is well known, that Tamarix plants are mainly considered as halophytes they are adapted to many different saline soil types (Waisel, 1972; Hagemeyer and Waisel, 1987). One of most interesting phenomena characterizing the Tamaricacea family is the existence of an "active salt economy" (Waisel, 1961). Salt absorbed by the roots in the various soil layers are excreted on to the surface of the leaves by special salt excreting glands, thus completing a continuous salt movement through the plant body (Waisel, 1961; Kozlolwski, 1997).

In our study, submission of two different tamarix species to shock salt stress (150mM) induced notable changes in physiological responses and growth of both species compared to those growing in control conditions.

The visual symptoms were among the evident characters that reflected the highest tolerance of *T. jordanis* relative to *T. aphylla*. *T. aphylla* showed a yellowing and defoliation of old leaves much earlier and marked than *T. jordanis*. That means a high capacity to accumulate toxic ions in the oldest leaves tissues to prevent the death of young leaves.

Storage of salt in old leaves occurs probably via absorption of incoming ions from the xylem or via retranslocation through the phloem from young to older leaves. Such mechanism is a strategy reported in many glycophytes and mangroves plants, to limit the transportation of salt to active younger leaves and reduce their toxicity effect on such active organs. The shedding of old leaves may in turn eliminate the excess of salt (Mian, *et al*., Colombo, 2012).

This mechanism highlights the lower ability in partitioning toxic ions in the vacuoles in *T. aphylla* and in underlining a lower salt tolerance than *T. jordanis.*

Through the analysis, we observed an initial common responses (2^{nd} day) to shock salt stress (150mM) followed by acclimation and better performance of *T. jordanis* than *T. aphylla.*

Both species showed an immediate stomatal closure (g_s) in the 2^{nd} day (Fig. 18B) which might have resulted as a shock effect generated by a sudden osmotic stress of salt. However, some days after the beginning of the experiment, a difference in stomatal response to salt treatments (150mM) was markedly clear, between the two halophytes. A greatest reduction of (g_s) was found in *T. aphylla* while *T. jordanis* showed a non significant variation of (g_s) compared to control plants (Fig. 18B). Our results are in agreements with Abbruzzeze (2011) studies that reported also higher control of stomatal conductance in *T. aphylla* under salt stress. There are three probable explanations which could be hypothesized for such difference in stomatal response under salt (150mM):

1) The transport of ions to salt glands in *Tamarix spp.* has been shown to move via apoplastic xylem pathway (Arndt *et al*., 2004). On the other hand, *T. aphylla* extrudes more salt and could have low efficiency of compartmentation (Abbruzzeze, 2011). Thus, it could lead to a higher salt flux in the apoplast, that contributed greatly to depressing of (g_s) in *T. aphylla*. The control of stomatal opening by apoplastic Na^+ was found also in the halophyte *Aster tripolium. A. tripolium* is important in controlling transpiration, and hence the rate of delivery of salt to the leaves. There could thus be a 'top-down' regulatory mechanism which, it is hypothesized, would operate in this way: when the capacity of the shoot tissues to accumulate salt in cell vacuoles is exceeded, there is an increase in the Na^+ concentration in the apoplast, including that around the guard cells; this causes partial stomatal closure and reduces transpiration, resulting in increased water use efficiency and restricting the flow of salt into the leaves (Perera *et al.,* 1994).

2) Stomatal characters such as morphology, dimensions, and distribution play an important role in stomatal response. According to Abbruzzeze, (2011) *T. aphylla* showed a high positive correlation between (g_s) and stomatal dimensions, which were reduced under salt stress for better stomat control, while *T. tetragyna* and *T. jordanis* did not show significant correlation between these two paramaters.

3) Stomatal regulation in *T. aphylla* may also be highly dependent on potassium content in addition to sodium, which has been probably down regulated with depletion of potassium in leaves as observed in (Fig. 26C). It is well know that in halophytes Na^+ may substitute K^+ as principal ion in determining their changes of turgor or to exclude Na^+ enabling K^+ to retain a principal role (Robinson *et al*., 1997). For example, the halophyte *Cakile maritima* has the ability to use both Na+ and K^+ to support osmotic changes in driven stomatal movement (Robinson *et al*., 1997).

As result of differences in stomatal responses between the two species, the assimilation (A) and transpiration (E) under salt stress (150mM) were also affected at different rate among species.
As long with reduction in stomatal conductance, (A) and (E) were reduced in *T. aphylla* (Fig. 18A, C). The assimilation showed a high correlation with (g_s) (r^2=0.94), indicating a high intrinsic water use efficiency (A/g_s) in *T. aphylla* under salt stress from the start of experiment. On the contrary, after an initial moment of shock, *T. jordanis* have shown not significant reduction of assimilation, traspiration and stomatal conductance and maintain high biomass. This species showed higher water use efficiency in absolute term, but not significant variations between the control and salt treatment.
These results highlight the higher salt tolerance of *T. jordanis* than the *T. aphylla* which must close their stomata to limit the damage under salt stress, at the expense of assimilation rate and thus growth of the plant in the long period.

Like gas exchange parameters, sugar content under salt stress (150mM) was also affected at different rate among species. Higher impact of salt stress (150Mm) was noticed on leaves of *T. aphylla* than on *T. jordanis.*
The observed impact of shock salt (150mM) on (A), (g_s), and (E) in the 2^{nd} day, was also associated with impairment of hexoses and starch in *T. aphylla* (Figs 22A-B, 23). The important decrease in hexoses and starch in leaves of *T. aphylla* was a consequence of the decrease in CO_2 assimilation and might account for the impairment its growth found in response to salt stress (Table 5).
However, the starch content of leaves in *T. aphylla* was maintained at the same level of control after the second day, this could be related to:

1) Recovery of photosynthesis.

2) Reduction of plant sinks demands.

For instance, during the experiment a partial recovery of photosynthetic capacity has been observed in *T. aphylla*, while it was significantly reduced of 47% in the second day, it was partially recovered, so that values were significantly lower than control only of 14.7%, in the last day. On the other hand, the reduction of elongation rate of main stem and the slight increase of starch content in roots relative to control in *T. aphylla*, indicated that probably sugar export decreases according to source/sink equilibrium under salt stress (150mM).

Sugar content decrease in *T. aphylla* is in agreement with results of Parida , (2004) who reported that salinity decreases soluble sugars in the salt tolerant mangrove *Aegiceras corniculatum, and* Gadallah, (1999) who observed reduction of soluble sugars in *Vicia faba*. In our case, probably reduction of sugar under salt (150mM) in *T. aphylla* was quiet necessary to furnish a source of energy to sustain a high capacity of salt gland excretion. In view of this context, *T. aphylla* is the species which is excrete more salt than *T. jordanis* (Abbruzzeze, 2011) then it probably requires much more energy, since salt hairs and glands are active processes and require energy, which reduces growth (Kurtiss, 2003).

The content of sugars in leaves of *T. jordanis* was not significant reduced (Fig. 22A-B). Maintenance of sugars in *T. jordanis* and higher content of starch under control and salt (150mM) may have a protective role of membranes. Carbohydrates such as sugars (glucose, fructose, sucrose) and starch play a role in osmoprotection, carbon storage, and radical scavenging (Parida *et al*. 2002).

Despite the contrasting information found in the literature on the role of carbohydrates as osmolytes (Rathert 1984; Cheeseman 1988; Gucci et al. 1998; Kerepesi et al. 1998; Sultana et al. 1999; Balibrea et al. 2000; Martı´nez-Ballesta *et al*. 2004), the absence of soluble cugar accumulation under salt (150mM) in both species allows us to discard the hypothesis that assigns a role for soluble carbohydrates in maintaining high turgor potential in leaves of Tamarix under short term stress.

Data presented in this work together seem to indicate that the osmotic adjustment in Tamarix under salt stress (150 mM) mostly depended upon accumulation of inorganic ions (Table 3).

The accumulation of Na^+ and Cl^- ions may have partially accounted for the observed differences in osmotic adjustment between salinized and non-salinized plants. The high accumulation of

these ions in leaves displays the halophytic and" includer" characters of these species (Fig. 25A-B). These results are in accordance with Martinez *et al*. (2005) who bring back that in Atriplex, more than 70 % of the total Na^+ content in the plant is in the leaves.

Na^+ is not the only cation in this osmotic adjustment plants, K^+ may have also a large responsibility in this physiological reaction. Under control conditions, *T. aphylla* had higher content of K^+, which allowed us to suppose that K^+ is among essential ions in osmolyte regulation in *T. aphylla*, and probably has role in opening and closing the stoma. In response to salt (150mM), significant reduction of potassium content in leaves of *T. aphylla* was evident, while it reduction in leaves of *T. jordanis* was not significant. On the contrary, potassium was not reduced at uptake site in the roots of any species, although the well known antagonism effect between Na^+ and K^+. It is well known, that maintenance of adequate K^+ in plant tissues under salt stress is dependent upon selective K^+ uptake and selective cellular K^+ and Na^+ compartmentation and distribution in the shoots (Munns *et al*., 2000; Carden *et al*., 2003). In our case maintenance of potassium in root tissues suggest the high selectivity of K^+ at root level. The decrease of K^+ in leaves of *T. aphylla* probably resulted from interference of Na^+ to a greater extent in K^+ translocation from root to shoot, in fact potassium decrement initiated to be observed at stem levels (Fig. 26C). On the other hand, maintenance of potassium in leaves of *T. jordanis* was reported in many halophytes as common mechanism of protection against salt in halophytes plants (Munns et al., 2000; Carden et al., 2003). This cation plays vital roles in plant cells including osmoregulation, photosynthesis, enzyme activation, and formation of carbohydrates, nucleic acids, and proteins (Fageria *et al*., 1997). Accordingly, salt-tolerant species maintain K^+ uptake more effectively than salt-sensitive species (Schachtman *et al.* 1989; Colmer *et al.* 1995).
In both species Ca^{2+} acquisition was maintained high at root and leaf levels. It was reported that maintenance of calcium acquisition and transport under salt stress is another important determinant of salinity tolerance (Soussi *et al*., 2001; Unno *et al*., 2002). Calcium is important during salt stress, in preserving membrane integrity, signaling in osmoregulation and influencing K^+/Na^+ selectivity (Tozlu *et al*., 2000).

In accordance with many other halophytes species, (Flowers *et al.,* 1986), the growth of *Tamarix jordanis* was stimulated by salinity. In contrast, reduction in growth of *T. aphylla* was evident under salt conditions (Table 5). The decreased in growth was probably due to energy cost associated with salt pumping, increased respiration (Kleinkopf and Wallace 1974) and

decreased photosynthetic rates (Jackson *et al*. 1990). Our results agree with Waisel, (1961) who observed also a depression of growth of saplings of *T. aphylla* under irrigation with 0.1M and 0.2Mthat could be due to osmotic and toxic effect of NaCl solution.

Under higher salt treatments (350mM), salt impact on both species becomes much severe. Nevertheless, *T. jordanis* tolerates this level of salt as well, while *T. aphylla* sensitivity was much more evident in most of the assessed parameters

The stomatal regulation during the experiment becomes more important not only in *T. aphylla* but also in *T. jordanis*. *T. jordanis* which is as true halophyte only at salt stress (350mM) start to use this avoidance mechanism of stomatal closure while it has been used in *T. aphylla* starting from lower salinity (150mM). It was reported that a reduction of (g_s) is likely a more typical response in halophytes following exposure to salinities well above those necessary for optimal growth and development (Webb, 1966; Gorham, 1996; Koyro, 2006). The exposure of halophytes to increasing salinity may result in partial stomatal closure as a strategy to limit both transpiration and transport of salt to leaves. (Véry, *et al*., 1998). Evidently, high salt (350 mM) presented a threshold for optimal growth of *T. jordanis* where we can start to observe a certain limitation of plant growth (Table 9).

Differentiation between both species in sugar and starch response and adaptation to salinity were more evident under salt (350mM). Israeli studies reported earlier that *T. aphylla* is not a true halophyte (Waisel, 1961); in fact our findings regarding starch content demonstrate a failure of this species to adapt as it did at lower salinity, while *T. jordanis* was able to adapt even a high salinity of 350mM, they were to synthesize starch similarly to control after acclimatization. Most probably starch depletion in *T. aphylla* was quite essential for certain growth maintenance and as a source of energy to extrude the toxic levels of salt from the vacuoles which is normally slowed, as mentioned in earlier studies of salt extrusion by Tamarix, as reported by Waisel, (1961).

At such level of salt (350mM), the lower performance of *T. aphylla* was not restricted only to gas exchange parameters and sugar content, salt stress (350mM) induced as well reduction of chlorophyll content in *T. aphylla* (Fig. 32A). The finded reduction of chlorophyll content in *T. aphylla* agree with several reports of decrease content of chlorophyll by salinity as reported in a number of glycophytes (Gadallah., 1999; Agastian *et al*., 2000). The decrease in

Chl $_a$ content at (350 mM) NaCl might possibly due to changes in the lipid protein ratio of pigment–protein complexes or increased chlorophyllase activity (Iyengar and Reddy, 1996). However, Chl $_{a/b}$ ratio remained unaffected by NaCl treatment in *T. aphylla* it appears that the light harvesting complex (LHCs) of thylakoid membranes are little altered by salt exposure. On the contrary of *T. aphylla*, photosynthetic pigments in *T. jordanis* were intact (Fig. 32A). High chlorophyll content is a desirable characteristic because it indicates a low degree of photoinhibition of photosynthetic apparatus (Farquhar *et al*., 1989).

In contradiction to might have been expected, about dehydration effect arising from salt (350mM) on leaves both species maintained a good water status for all the stress period. The absence of significant changes in leaf relative water content (RWC) during the period of osmotic stress in agree with many studies on other halophytic species, where, the (RWC) showed no significant overall response after days or months of exposure to saline stress (Ramani *et al*., 2006 and Redondo-Gómez *et al*., 2007**).** On the other hand, *Tamarix spp.* are salt excretor, the excreted salt crystals which cover branches and leaves are highly hygroscopic (Waisel, 1960), which allows the absorption of water from atmosphere and maintenance of high relative water content in spite of the salt stress at which are subjected.

In the light of our findings and the available information in the literature, *T. aphylla* and *T. jordanis* prove their ability to cope with salt stress (150mM-350mM) by using different strategies. However discrimination between both species in salt tolerance level was identified. The physiological responses and growth parameters assessed in our study reflect the higher tolerance of *T. jordanis* relative to *T. aphylla* under salt (150mM-350mM).

Drought

Tamarix is not only resistant to salt but to drought as well. Many studies mentioned the important morphological features of Tamarix, characterized by small leaves and deep root system which confer their drought tolerance. Other numerous studies reported also that the great tolerance of Tamarix to drought by limiting water loss through stomatal control and by low susceptibility to cavitation of xylem (Smith *et al.* 1998, Anderson 1982), in addition to the morphological feautures.

In contrast to salt (150mM), mild water stress (50% F.C.) never induced appreciable effects on carbon assimilation, on any of studied species (Fig.18). The reported observations in maintaining optimum photosynthesis, under water stress (50% F.C.) may be related to high capacity of *Tamarix spp*. to excrete calcium carbonate in non saline soil, which cover the twig, hence trap CO_2 from the atmosphere, and moisten the twig. This phenomenon was clearly identified and explained by Waisel, (1991) who stated that the glands of *T. aphylla* grown in non saline conditions are carbon concentrating system. For instance, in non saline conditions Tamarix glands excrete large amount of calcium forming a crust of carbonate solution is formed that trap CO_2. During the night, when the pH of the solution drops to 8.5-9, a considerable quantity of the trapped carbon is released, enriching the ambient atmosphere (1000ppm) that remains high in the early hours of the day. Thus, the released CO_2 is subsequently absorbed and higher assimilation rate were recorded. Therefore salt, chalk crust may have a conspicuous adaptative value due to optimization of photosynthesis by the carbon concentrating system (Waisel, 1991).

In spite of smaller surface area of *T. aphylla*, our data of photosynthesis showed its higher capacity of assimilation under control and drought stress (Fig. 18A). *T. aphylla* excrete more calcium than *T. jordanis* (Abruzzeze, 2011), thereby it may benefits from extra source of carbon trapped by more calcium, and exploit it for maximal assimilation during the early morning hours (Waisel, 1991).

Apart the 7^{th} day of the experiment, stomatal conductance under water stress (50% F.C.) was maintained in both species as illustrated in graphs (Fig. 18B) although the lower water potential reached (Fig. 28). Maintenance of stomatal conductance under drought (50% F.C.) possibly reflected two hypotheses:

- One of them deals with the mechanism of twig moistening. In fact, analysis of the RWC did not indicate a severe dehydration of leaves except in the in middle of experiment (7^{th} day), followed by restoration of water status. The reason of maintenance of good water status is related to provision of cover of hygroscopic solutes arising from carbonates that moistens the twig as stated before. Therefore, the surrounding humid atmosphere provides an additional source of water (Waisel, 1960), allowing the restoration of leaf water status, of plants under water deficit (50%). *T. aphylla* the typical desert tree, with leathery, needle-like, and thicker leaves compared with *T. jordanis*, showed higher valued of RWC in absolute term (Fig. 29).

- The second one deals with root system of Tamarix that is extensive and deep with a strong tap. It is known that Tamarix are at the same time avoiders and spenders. According to Chaves, (1991) drought avoiding of the spending type can keep their stoma open, but they are able to extract water from the soil rapidly enough to compensate for water loss. This type of adaptation to water stress can be achieved with plants of deep and large root system.

The only date which presented some variations under drought (50%) in the parameters was at midway during which relative humidity reached its lowest values (37.9%).

In this date, a decline in stomatal conductance occurred in *T. aphylla* without influencing photosynthesis; thus (A/g_s) increased, thereby biomass of *T. aphylla* was not significantly affected comparing with biomass of *T. jordanis* (Fig. 19). It has been suggested that under mild drought stress, a small decline in stomatal conductance may have protective effects against stress, by allowing plant water saving and improving plant water-use efficiency by the plant (Chaves *et al*., 2009).

Osmotic adjustment associated with drought tolerance was not consistent (Fig. 27), probably the osmotic adjustment was unnecessary because water stressed and control plants maintained similar RWC (Fig. 29). However, the only increase in soluble sugars with concomitant decrease in RWC reduction was observed in the 7^{th} day (Fig. A-C). Accumulation of soluble carbohydrates in leaves is a common phenomenon under water stress (Chaves, 1991). Accumulation of soluble sugars under drought stress could be attributed to low translocation rates due to low carbohydrate utilization by sink as a result of decline in growth and overall energy demand by stressed plants. However, the results in our study, regarded roots as sink organs were highly active in starch remobilization thus, they did not indicate a low sink demand, despite growth was somehow inhibited. The duration of the experiment was short; nevertheless the high content of soluble sugars and their increase mainly in *T. aphylla* may reflect that soluble sugars accumulation may be one of possible developed adaptative mechanism that could be adopted by this species coming from dried areas of Negev Desert. It has also been observed that plants growing in dry habitats contain as rule more soluble sugars than those living in moist habitats. Iljin (1957) reported values of sugar content (on a dry weight basis) ranging from 1.3% in herbaceous mesophytes to 6.9% in xerophytic trees and shrubs.

In the 7^{th} day, sucrose was increased with simultaneous decrease in starch content (Figs. 22C, 23). It is also a common observation that water stress conditions induce a severe decrease in starch and, simultaneously, an accumulation of soluble sugars (Iljin, 1957; Turner, Begg, and Tonnet, 1978). Such a shift in carbon partitioning may be adaptive since it could contribute to osmotic adjustment and protection (Daie, 1996; Lawlor and Cornic, 2002). Our results are supported by Abdel-Nasser and Abdel-Aal (2002), who also reported an increase in sucrose and decrease in starch contents, in safflower (*C. mareoticus L*.).

Chlorophyll content of leaf is indicator of photosynthetic capability of plant tissues **(**Nageswara *et al*., 2001**;** Wright *et al*., 1994**).** *Tamarix spp*. resistance to drought (50% F.C.) may be associated also with resistance of pigment apparatus, minor changes in chlorophyll content and stability in chlorophyll $_{a/b}$ ratio were seen in our study (Fig. 21)

Potassium level in roots showed positive increment in mild stress, its accumulation may somehow contributed to drought resistance. Potassium increases the plant's drought resistance through its functions in stomatal regulation, osmoregrulation, energy status, charge balance, protein synthesis, and homeostasis (Beringer and Trolldenier, 1978; Marschner, 1995). It also maintains turgor pressure (Mengel and Arneke, 1982) and reduces transpiration under drought conditions (Andersen *et al*., 1992).

For instance, accumulation of K^+ in plant roots produces a gradient of osmotic pressure that draws water into the roots. K^+ is involved in maintaining the water status of plants and turgor of root cells (Anonymous, 1998).

Surprisingly, despite the limited effect of drought on photosynthesis and mineral content (phosphorus, potassium) (Figs. 18A, 26C-D), it was observed that growth of *T. jordanis* was severely limited, plant height was reduced of 55% over control and development of new leaves were slowed (Table 5). Thus, other factors not analyzed in our study imposed growth limitation; one of these could be related to hormonal factors. The mechanism that down regulates leaf growth and shoot development under drought stress may be regulated by long distance signals in the form of hormones or their precursors, because the reduced leaf growth rate is independent of carbohydrate supply (Munns *et al*., 2000) and water status (Fricke *et al*., 2006; Munns *et al*., 2000).

A scarcity in water (50% F.C.) had a great impact in above ground biomass of *T. jordanis* species. Since above ground biomass is a collective term of plant height, leaf area, number of

leaves, stem growth and water content in leaves and photosynthetic rate, therefore, decrease in all these parameters influence biomass allocation. The results reported show that reduction of above ground biomass of *T. jordanis* is attributed to reduction of plant height. Although a slight effect on plant growth was also detected in *T. aphylla*, this is do not turn any effect on biomass due to high water use efficiency of *T. aphylla* under drought content, thereby allowing plants under stress conditions stress to produce a quantity of dry matter more or similar of control plants with less water consumption.

It is well established that an increase in root/shoot ratio is a morphological adaptation to drought (Cuartero and Fernandez-Munoz, 1999); the increase in root/shoot was observed for *T. jordanis* under drought conditions. It was not caused by increase of root biomass rather it was due to significant decrease in shoot biomass (Table 6). Joly *et al*. (1989) considered this as an adaptation that restricts transpiration surface area and increases water absorption from the soil. This finding concurs with the results of with the results of Barros and Barbose (1995) for *Acacia farnesiana*, and with others for different woody species (Khalil and Grace, 1992; Ibrahim, 1995).

Although the high capacity of Tamarix to trap water from atmosphere, and reestablish water status in case of water deficit (50%). The results indicated that severe water stress (30% F.C.) affected plants so intensively in pots, resulting in drop of water potential and quick dehydration and death of plants, within few days.

One explanation for the quick dehydration of plants was the interaction between low relative humidity and drought factors. In mild drought stress (50% F.C.), the humidity was high (70%), while during the severe one (30% F.C.), lower humidity was recorded in the first days (48.16%). Probably, the amounts of water precipitated by the trees from atmospheric humidity were not quite large to compensate water deficit in soil.

Both *Tamarix spp*. were only able to endure dry conditions (50% F.C.) through many different mechanisms. This is comprises the high capacity of water absorption and conservation, increment of potassium in roots, accumulation of soluble sugars in leaves, presenting minimum alterations in photosynthetic capacity. Some identified clues like higher capacity of photosynthesis, highest capacity of sugar accumulation, and increase in water ruse efficiency attributed the higher performance and better growth of *T. aphylla* than *T. jordanis.*

Drought salinity

Controversial results were reported in literature about the benefit of salt in combination with drought stress.

In this study the presence of both constraints (salt 150 mM + drought 50% F.C.) in the soil showed to have the most severe impact in both species, relative to drought and salt stress. Visual observations and measured parameters reflected so far the additive negative effect resulting from combining both stress (salt 150mM + drought 50% F.C.).

Visual symptoms were evident at leaf level after 3 days of the onset of experiment. Both species showed yellow symptoms followed by defoliation at lower part of the plants; this would suggest that in *Tamarix spp.* salt accumulated in older tissues to prevent the death of young leaves (Mian, *et al*., 2011; Colombo, 2012).

Adverse effects on all measured parameters were reported. Photosynthesis, transpiration, stomatal conductance, sugars, starch were significantly reduced in response to combined stress (salt 150mM + drought 50% F.C.).

The combination of D (50% F.C.) and S (150 mM) induced similar effect to salt stress on reduction of hexoses sugars. In addition, both species highlighted a marked decline of the starch content which resulted significantly lower than that of salt and drought stress applied alone. This confirms the positive interaction between S and D on starch reduction in each species.

Nevertheless both species survive whole the experiment. The ability of both species to survive may be associated with high production of proline content, increase in water use efficiency, maintenance of relative water content and high selectivity of potassium.

Proline may act as osmoregulator or osmoprotector (Delauney and Verma, 1993). Other authors propose that constitute a stock of utilizable nitrogen by the plant after the period of suffering water (Dib *et al*. 1992).

In our study, high proline content was observed in *Tamarix spp.* grown under combined stress (salt 150mM + drought 50% F.C.) (Fig. 24). Most probably, proline accumulation was induced by ion stress of NaCl rather than water stress, because salt stress (150mM) alone showed as well initial increase in proline in *Tamarix spp.* (Fig. 24). However, Proline usually accumulated in cytosol, which is only 5 to 10% of the cell volume (Lee et al., 1990; Winter et al., 1993), and then this might be enough for osmotic adjustment in the cytosol (from -0.2 to -0.4 MPa in

osmotic potential). From this analysis and calculations, the osmotic potential contribution of proline calculated in our experiment was too low (-0.028-0.031 MPa in the 7^{th} day) thus was not high enough to contribute to osmotic adjustment. On the other hand, the absence of significant correlation between osmolarity and proline (r^2= 0.24) suggests also that proline was not high enough to be considered the principal solute that may allow plants to overcome effect through osmotic adjustment. Thus in this study, the proline served principally as a osmoprotector and storage forms of nitrogen and carbon for future use under stressful conditions, the peak of proline accumulation in the 7^{th} was decreased, which confirms its use in the following days. Our results are supported with Wikqiang, (2010) and Koyro (2006) who find that proline has contributed to the protection of *T. chinensis* against high salinity in the soil by scavenging the ractive oxygen species (ROS) generated by salt stress, wchich may cause oxidative damage to different cellular components including membrane lipids, proteins and nucleic acids. Similar results obtained also in studies of Sanchez *et al*., (1998) where proline does not prove to play an appreciable role in osmotic adjustment in peas cultivars, however, can act as osmoprotector of cytosolic enzymes and cellular structures.Watnanabe *et al*. (2000), find as well similar results in *Populus euphratica* under salt stress.

Although the adverse effects of water and NaCl treatments on gas exchange parameters of Tamarix, they did not affect chlorophyll content suggesting a high resistance of the photochemistry apparatus to combined stress.

One of mechanism that allows plant to tolerate and survive moderated double stress may be the increase of potassium accumulation at root level. Highest ratio of K^+/Na^+ was determined in roots relatively to salt stress in both species; the ability of plants to retain Ca^{2+} although the high concentration of sodium in the external solution may also be is associated somehow with the surviving of both species.

Under these severe stress originated from high salinity and drought plant growth was almost inhibited in both species. Such deceleration of growth could be attributed to more than one factor: hindrance in cell division and cell elongation, reduction in assimilation rate and ion toxicity due to reduction in water use and thereby reduction rate of salt flux.

Under combined stress reduction in dry matter accumulation was severe for both species because both of the above mentioned stresses are there, and plant has to face many difficulties for its survival. The increase in ion toxicity, reduction in water avalibility, and reduction in metabolites

necessary for photosynthesis are common observations under such types which not only retard plant elongation but also hindered dry matter accumulation that is why biomass production is decreased.

Overall speaking, our results agree with findings of Waisel, (1961) who found that *T. aphylla* was sensitive to change in the moisture content of salt soil. When the soil salinity reaches concentration of 0.1M-0.2M of NaCl a short drought period might causes a severe damage to these trees.

On the contrary of mildest combined DS (150mM + drought 30% F.C.), the severe combined stress (350mM + drought 50% F.C.), induced a decrease in RWC and water potential (Fig. 36). This is result of osmotic stress built up by the high salt concentration of the external solution, and dehydration at the cellular level (Greenway and Munns 1980). As well due to decrease in water flux and extrusion capacity that is a great trap of water from atmosphere in *Tamarix spp.*

By the end of the experimental period, the reduction in assimilation rate was more severe when the stress was intensified. The smaller (g_s) of these treatment plants was accompanied by slower rates of photosynthesis; but smaller (g_s) was not the sole cause of slower photosynthesis because intercellular CO_2 (C_i) was unaffected at high salinity coupled with severe drought (Fig. 30D). Must probably assimilation was reduced due also to non stomatal limitations factors. Intercellular CO_2 concentration rate increases in dehydrated leaves (Lauer and Boyer, 1992, Epron and Cornic, 1993) because the photosynthetic apparatus is unable to fix CO_2 fast enough to deplete intercellular CO_2 concentration. A higher intercellular CO_2 concentration in dehydrated leaves indicates that the demand for CO_2 diminishes since metabolism is inhibited.

It can be observed that the high levels of salinization and drought induced a significant decrease in the contents of Chl $_a$ pigment (Fig. 32). The decreased in chlorophyll content under stress is a commonly reported phenomenon and in various studies, this may be due to different reasons, one of them is related to membrane deterioration (Ashraf and Bhatti, 2000).

In the first time we observed increase in hexose sugars, glucose and fructose increase at equal ratio (Fig. 33A-C). Invertase cleaves sucrose reduction is due to the increase of invertase acitivity as is reported by Muscolo *et al*. (2003) in leaves of plants treated with highest and severe combination stress (350 mM +30% F.C.) which indicates a major request of hexoses, substrated necessary for respiratory processes.

Despite of additive impact of stress, on photosynthesis, growth, Tamarix were able to survive for whole the experiment under salt. Richards (1992) reported a beneficial effect of salinity on many halophytes and non halophytes grown to the wilting point in drying soils.One way in which soil salinity can enhance plant performance in dried soil, is by lowering growth rate of plants, thereby decreasing the rate at which soil water is depleted and thus enhancing the longevity of plants (Richards, 1992; Shalhevet, 1993). Eshel and Waisel (1984) found that similar-sized *Salsola Kali* plants took twice as long to reach the wilting point on saline comparing to non saline treatments. *Atiplex canescens* seedlings grew much more slowly at 520 mol/cm^3 than at lower salinities but lasted longer before wilting (Glen and Brown, 1998). Hence, the increased longevity could aid the survival of plants in saline soils between scattered rain.

10. Part II. Chemical characteristic of Tamarix feedstock in Mediterranean area

10.1 Introduction

In recent years, there have been active movements of bioethanol production from lignocellulose crops (2nd generation biofuels). The need has been emerged firstly, responding to the growing global energy demand and concerns about the negative effects of growing greenhouse gas (GHG) emissions from fossil fuels. Secondly, the need is responding to the generated debate on competition between food and biofuel production, which resulted due increasing use of sugarcane of sunflower seeds and of maize grains for production of ethanol, or the use of soybeans and various other oil-crops for production of biofuel (Albiac, *et al.,* 2007; Brahic, 2007).

Cellulosic ethanol can be produced from a wide variety of cellulosic biomass feedstocks including agricultural plant wastes (corn stover, cereal straws, sugarcane bagasse), plant wastes from industrial processes (sawdust, paper pulp) and energy crops grown specifically for fuel production. Roughly, two-thirds of the dry mass of cellulosic materials is present as cellulose and hemicellulose. Cellulose is the most prominent component. It is a homopolysaccharide with building blocks of glucose molecules (Fig. 37). Hemicellulose is a short, highly branched chain of heteropolysaccharides (DP 100–200) built from hexoses D-glucose, D-mannose, and D-galactose), pentoses (D-xylose, Larabionose, and D-arabionse), and deoxyhexoses (L-rhamnose or 6-deoxy-L-mannose and rare L-fucose or 6-deoxy-L-galactose). Small amounts of uronic acids (4-O-methyl-Dglucoronicacid, D-galacturonic acid, and D-glucuronic acid) are also present (Holtzapple, 1993a) (Fig. 38). The monosaccharides released upon hemicellulose hydrolysis include a large fraction of pentoses. Lignin is a phenylpropane-based polymer and is the largest non-carbohydrate fraction of lignocelluloses (Fig. 39). Unlike cellulose, lignin cannot be depolymerized to its original monomers. Lignin and hemicellulose form a sheath that surrounds the cellulosic portion of the biomass (Holtzapple, 1993b) (Fig. 40). In addition, to above components of lignocelluloses, low molecular organic (extractives) and inorganic substances (ash) represent a minor proportion.

In arid lands, it may possible to find suitable xerophytes and halophytes for biofuel industry having at the same time the capacity to influence positively soil environment and social conditions. For instance, *Prosopis laevigata* naturally distributed in semi-arid to arid lands of Mexico contains between 61.7 - 64.5% of holocellulose and Klason lignin content between 29.8

- 31.4% (Carillo et al., 2008). *Sisal* which is a semi-xerophyte belonging to Agave genus is grown mainly in Brazil, Tanzania, and Kenya, its leaf fibre contains high amounts of 43-78% cellulose, 10-13% Hemicellulose, 4-12% lignin (Mwaikambo, 2006). Another important species of arid lands, is *Euphorbia tirucalli* which grows growing in hot deserts and comprised of sugars and cellulose.

Wood crops grown such as Tamarix, under a short rotation intensive culture system may be an attractive alternate fuel source if their chemical composition proves to be convenient, for their fast growth rate, high biomass production (26 to 52 tons/ha/y of organic biomass) when irrigated with fresh, saline or brackish water fresh (Eshel, *et al*., 20110), and tolerance of both salt and arid conditions.

The variations of chemical composition of raw material may be associated to genetic variability in plant at intra-inter specific level, growing conditions, sampling method and age. These variations must be taken into account to select the optimum feedstock for ethanol production.

The main chemical characteristic of wood to produce a high quality bio-ethanol is a high concentration of sugars mainly hexoses because of their easy fermentation, while pentoses require genetically modified organisms for their fermentation with all subsequent complications that could be arised. Low lignin concentration is considered a good trait because high amount of lignin impedes enzyme accessibility and subsequently reduces the efficiency of enzymatic hydrolysis of cellulose and hemicellulose polymers.

In addition such material must come from relatively younger age classes which produces large amount of biomasss because the success in producing ethanol from lignocellulosic material depends on the feasibility of establishing low rotation harvest times (2-5 years).

The overall objective of this work was to study the environmental effect on chemical wood quality and select the best performing provenance/species on the basis of growth, establishment, and biomass quality for an intensive bio-ethanol production in arid and degraded lands. For this purpose quantitative assessment of lignocellulose components will be assessed on wood of Tamarix plants coming from diffent contrasting natural mediterranean sites.

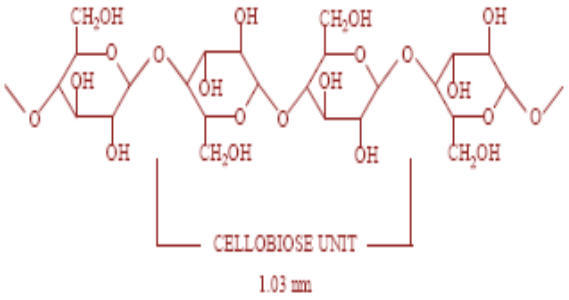


Figure 37. The structure of cellulose

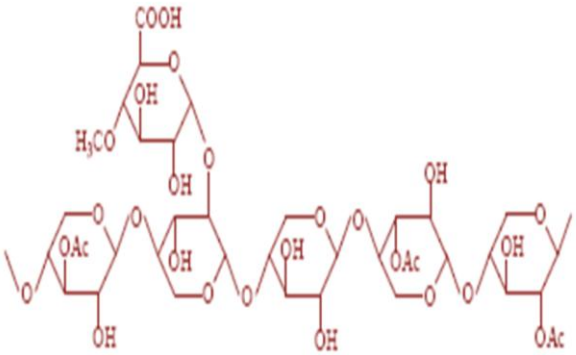


Figure 38. The structure of hemicellulose

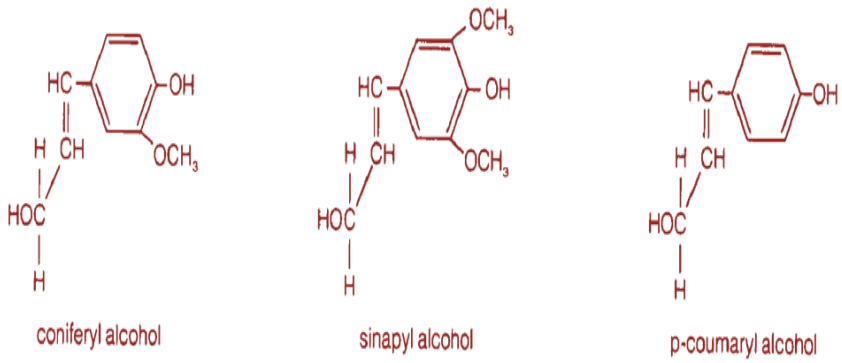


Figure 39. The structure of lignin

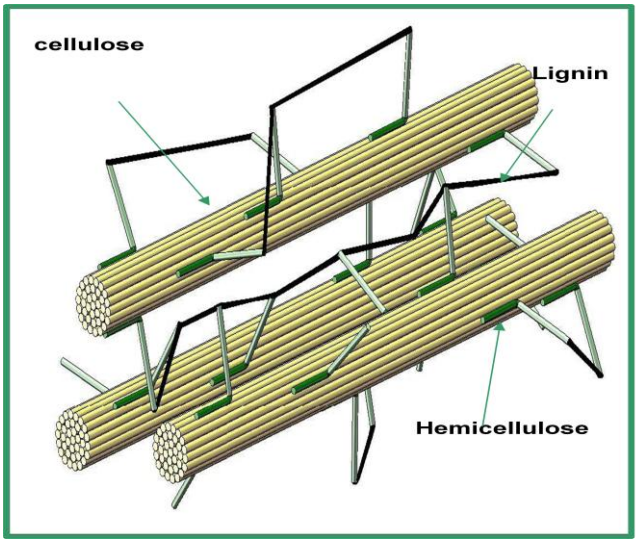


Figure 40. Components of lignocellulose

10.2. Material and methods

10.2.1 Sampling

Study material of *Tamarix spp*. originates from districts of ten geographic locations/provenances. These provenances were:

Cabo de gata (CG), Sanlúcar de Barrameda (SB) Sevillia (SE), and Tabernas (TA), (species identified *T. gallica*) in Spain.

Bou Hanifia (BOU), Kheiter (KH) and Ghassoul (GH) in Algeria, (species identified *T. gallica*).

Alcantara (ALC) and Crati (CRA) (species identified *T. africana*) in south of Italy

Yotvata (YOT) in Israel (species identified *T. aphylla* (TE) and *T. jordanis*) (TJ)), (Table 10).

Provenances were different in plant growth conditions. Samples were collected from coastal areas (CRA), (CG), (KH), (SB), from sites across the river where water table is fresh (ALC), (SE), (BOU) , from desert zones (TA), (GH) and from short rotation forestry plantation in Negev desert (YOT) irrigated with reclaimed sewage water from the city. Twenty-fourty trees were selected for straightness and absence of decay symptoms. The samples were taken at breast height (1.3 m) from each mother plant. The chosen trees had close diameters.

Table 10. Location and description of field site

Site	Latitude	Longitude	Altitude (m)	Mean annual rainfall (mm)	Mean annual temperature (^{0}C)
Spain					
Cabo de gata (CG)	36°46'N	2°14'W	21	241	18.19
Sanlúcar de Barrameda (SB)	36°78'N	6°35'W	30	451	18.16
Sevillia (SE)	37°22'N	5°59'W	31	533	20.75
Tabernas (TA)	37° 2'N	2°23'W	405	140	20
Algeria					
Bou Hanifia (BOU)	35°46'N	5° 7'W	354	471.6	17
Ghassoul (GH)	33°22'N	1°11'W	1347	243	14.8
Kheiter (KH)	35°30'N	1°25'W	1001	205.2	15
Italia					
Alcantara (ALC)	37°54’N	15°13'E	54	709	24.15
Crati (CRA)	39°43'N	16°31‘E	4	565.5	16.9
Israel					
Yotvata (YOT)	29° 53'N	35° 3' E	91	25	30

10.2.2 Milling

As a requirement for the following protocols, the biomass was air dried to a moisture content less than or equal to 10%. For this purpose, the biomass samples were left in a convection oven at 45ºC for about a week. Dried logs were sliced into thin sections. Then, slices were ground in a cutting mill, equipped with a sieve with an equivalent pore size of 1mm.The milled samples were sieved using 20/80 mesh sieves. The fraction retained on the top of pan (-20/+80 mesh fraction) was kept for compositional analysis. The material in the bottom pan is the fines (-80 mesh) fraction was retained for ash analysis. Waiting to perform the chemical analysis, sieved samples were stored inside sealed plastic bags and placed inside a cold room, at 4ºC.

10.2.3 Determination of Extractives

The samples were analyzed for ethanol extractives, according to the NREL protocols "Determination of extractives in Biomass".

The process of removing extractives present in the biomass is particularly important when performing analysis of structural components, because these chemicals can interfere and lower the precision of that analysis. For this scope a Soxhlet extraction procedure was applied to extract the interfering components (Chlorophyll, waxes, non structural sugars or othe minor components).

For the extraction procedure, five grams of milled dried sample prepared in previous steps was put into thimble and dried for 48 hours at 40ºC. After that the single thimble was inserted into a glass Soxhlet extractor for an extraction procedure (Fig.41).

The extraction was carried out, by adding 190 ml of HPLC-grade ethanol into the receiving flask. The extraction was run for about 24 hours, adjusting the heating mantles to assure that the liquid inside the Soxhlet tube refluxed 4 times per hour. Before stopping the extraction, it was verified the liquid inside the Soxhlet tube looked completely clear.

The ethanol extraction residues had their respective solvents evaporated by placing them in rotary vacuum evaporators equipped with water baths at 40ºC.The solvent-free solids were then weighted and the extractives content (in a dry weight basis) was calculated according to equation (2.1) (NREL, 2005).

$$\% \text{ Extractives} = \left(\frac{\text{Weight flask} + \text{residue after extraction} - \text{Weight receiving flask}}{\text{Weight initial dry sample}}\right) \times 100 \qquad (2.1)$$

Where: residue after extraction = ethanol extractives.

Figure 41. Soxhlet extraction setup used for water and for ethanol extractives determination

10.2.4 Sample preparation and hydrolysis

Before the extractives-free samples were analyzed for structural carbohydrates and lignin, they were prepared and acid-hydrolyzed. This process and the remaining fiber analysis steps were all made based on NREL Standard method." Determination of Structural Carbohydrates and Lignin in Biomass "

Approximately 300 mg of sample were added into tarred pressure tubes and their weight was recorded. It was then added 3 ml of 72% sulfuric acid to each pressure tube. After a brief mixing, the tubes were put into water baths at 30ºC, and the mixture was incubated for an hour, and stirred every 5 minutes. After this reaction, the acid concentration inside was diluted to 4% by

adding 84 ml of deionized water. The tubes were then autoclaved for one hour at 121ºC, using the liquids setting (Fig. 42).

To measure the background sugar degradation during this step, a set of sugar recovery standards (SRS) was prepared. The chosen concentration of sugar recovery standards resembles the concentrations of sugars in the test sample, and 4% sulfuric acid was added. The SRS was also used in duplicate and was autoclaved along with the samples.

Figure 42. Sample aspect after two-step hydrolysis

10.2.5 Determination of acid insoluble and acid soluble lignin

The portion of acid insoluble lignin was determined using the sample resultant from the last step, which had undergone concentrated and diluted acid hydrolysis. The autoclaved hydrolysates were vacuum filtered through filtering crucibles. The filtrates were captured and saved for acid soluble lignin and carbohydrate determination. The acid insoluble residues (AIR) remaining in the crucibles were rinsed with deionized water and then dried at 105ºC for 19 hours, until constant dry weight was achieved.

Their dry weight was recorded and then the crucibles were put inside a muffle furnace at 575ºC for 24 hours. After cooling inside a desiccator, the crucibles containing the ash were weighted.

Aliquots of the filtrates from the hydrolysate liquors were analyzed for acid soluble lignin by UV-Vis Spectrophotometry, within six hours of the hydrolysis, as indicated in the protocol (NREL, 2011). A blank of deionized water was run on an UV-Vis Spectrophotometer at 205nm and using a 1cm light path quartz cuvette.

The percentages of acid insoluble (AIL) and acid soluble lignin (ASL) were determined using equations (2.2) and (2.3), respectively (NREL, 2011). The total lignin was the sum of these two parcels. These results came referred in an extractives-free basis.

$$\%\ \text{AIL extr. free} = \frac{\text{Weight dried 105 °C Crucible + AIR} - \text{Weight incinerated crucible + ash}}{\text{Weight extr .free .sample}} \times 100 \qquad (2.2)$$

$$\%\ \text{ASL extr. free} = \frac{\text{Absorbance} \times \text{Volume filtrate} \times \text{Dilution factor}}{\varepsilon \times \text{Weight extr. free sample}} \times 100 \qquad (2.3)$$

Where: Volume $_{\text{filtrate}}$ =0,087 l

ε = Absortivity of soluble lignin from bagasse biomass at 205 nm

(Weights are in g)

10.2.6 Structural Carbohydrate Determination

The carbohydrate contents of the samples was determined based on the HPLC analysis of the monomeric sugars glucose (for cellulose), xylose, arabinose and mannose (for hemicellulose).

It was used a Knauer HPLC system a Shimadzu CTO-10AS equipped with an oven set and a refractive index (RI) detector (Fig. 43). It was used an Aminex HPX-87P column and a 125-0119 precolumn (both from Biorad).

Twenty mililiters of the filtrates saved in the last step were first neutralized to pH 6 by adding calcium carbonate while stirring. After reaching the desired pH, the supernatants were decanted and then filtered through 0.2 μm polyether sulfone filters into the autosampler vials. It was also prepared a calibration verification standard (CVS), containing 1g/l of each of the sugars to be analyzed. This standard was used to check that the calibration curves used were valid.

The samples and the CVS were analyzed on the HPLC system using 0.2 μm filtered and degassed HPLC grade water as the mobile phase, a flow rate of 0.2 ml/min, column temperature of 80ºC, RI detector, and a sample running time of 1hour.

The sugar concentrations were calculated based on the peak areas measured and calibration curves already available for the column and settings used.

The range of the concentration of the calibration standards, for Tamarix wood was 0.25- 0.5-1,2- mg/ml.

The sugar recovery standards (SRS) come into play to calculate the percentage of sugar recovered after the 4% acid hydrolysis. For Tamarix wood, SRS should include
D-(+) glucose, D-(+) xylose, D-(+) galactose, and L (+) arabinose. The amount of each sugar recovered was calculated with equation (2.4) (NREL, 2011).

$$\%\ \text{R sugars, x} = \left(\frac{\text{Concentration detected by HPLC}}{\text{Known concentration of sugar before Hydrolysis}}\right) \times 100 \quad (2.4)$$

Where % $R_{sugar,\ x}$ =Recovery percentage of a sugar after 4% acid hydrolysis
The sugar concentrations were then corrected with this factor, according to equation (2.5)

$$\text{Cx} = \frac{\text{Concentration detected by HPLC} \times \text{Dilution}}{\%\text{R sugar, x}/100} \qquad (2.5)$$

Where C_x = concentration of a sugar in the hydrolyzed sample after correction for loss on 4% acid hydrolysis.
Dilution Factor = 1
To calculate the correspondent concentration of polymeric sugars, it was necessary to multiply the calculated concentrations with an anhydro factor, to compensate for the water molecules incorporated during polymeric sugar hydrolysis. Therefore, for hexoses (glucose, galactose and mannose) that value was 162/180, and for pentoses (xylose and arabinose) it was 132/150. The percentage of each sugar on an extractives free basis was then calculated with equation (2.6)

$$\%\ \text{polymeric sugar extr. free} = \frac{\text{Cx} \times \text{Anydro factor} \times \text{Volume filtrate}}{\text{Weight extr.free sample}} \times 100 \quad (2.6)$$

Where Volume filtrate =0,087 L
Concentrations are in g/L
Weights are in g
The percentage of hemicellulose was considered to be the sum the percentages of xylan, mannan, arabinan, galactan while cellulose equal to glucan alone.

To correct all the above mentioned percentages (lignin, sugars) from an extractives free basis to an as-received basis it was necessary to use the percentage of total extractives (from water plus ethanol):

$$\%\ \text{Component as received} = \%\ \text{component extr. free} \times \left(\frac{100 - \%\ \text{extractives total}}{100}\right) \quad (2.7)$$

Figure 43. Knauer HPLC system used for fiber analysis

10.2.7 Statistical analysis of data and calculations

Data of chemical composition of wood were submitted to the analysis of variance. Means were tested using a least significant difference (LSD) test ($P = 0.05$), when a significant test ($P \leq 0.05$) was obtained, the mean values were compared by using the Duncan Multiple range test. Computations and statistical analysis were done using Statistica .

Ethanol yield from cellulose and hemicelluloses = cellulose and hemicellulose content (%) in dry matter × conversion factor derivation:

Conversion factor derivation= (1.11 pounds of C6 sugar/pound of polymeric sugar or 1.136 pounds of C5 sugar/pound of C5 polymeric sugar) x (.51 pounds of ethanol/pound of sugar) x (2000 pounds of ethanol/ton of C6 (or C5) polymeric sugar) x (**1 gallon of ethanol/6.55 pounds of ethanol) x (1/100%)

specific gravity of ethanol at 20ºC (Biomass Feedstock Composition and Property Database,** 2009**).**

10.3 Results

Locations effect

The analysis of variance of contents of total sugars resulted significantly different for the locations assessed. Generally, the trend of total sugars amounts tended to be higher in Tamarix trees that live in two locations of Algeria, compared with those in Spain, Italy and Israel.

(BOU) and (GH), were the sites in Algeria which recorded statistically significant highest values of total sugars (67-68%) comparing with many other collected wood from Spain (CG), (SB), (TA) (53.6- 56.33%) and Italy (CRA) (55.47%) (Fig. 44).

The observed differences in total sugar content among locations was mainly attributed to difference in glucose fraction and less to other analyzed sugar components (Table 11). The data of glucose % for two sites in Algeria (BOU, GH) were 51.57 % and 49.31%, respectively, while the glucose content of other sites was lower of 4.18-10.79%.

Glucose % of *Tamarix spp*. grown in Algeria locations agree reasonably with previous data reported in the literature, as per Zheng *et al*. (2007) who found that glucose makes 49.34% of dry basis of *T. aphylla* biomass.

Variations among locations in xylose, galactose and arabinose were not statistically significant however the average difference counted respectively of 5.02%, 1.3%, and 0.9%. (Table 11).

Some locations like those in Algeria, indicated also high content of xylose, than other sites although not significant. Most of xylose values fall into expected values of Zhen *et al*. (2007). The galactose and arabinose % found in our study were higher than galactose and arabinose found in studies of Zhen *et al*. (2007), which were 0.46 %, and 0.68% respectively,

Lignin shows a media difference of 4% between plants of different ecotypes, is a smaller range than was reported for glucose (Fig. 45). These results were quiet distant from lignin data reported by Zheng *et al.*, (2007), lignin in our study result lower of at least 10%.

On the other hand, the components of lower importance such as the extractives and ash, showed some notable differences among the sites.

Tamarix grown in Italian sites were the trees that produced the highest amount of extractives (9-10 %) while those in Israel produced the lowest quantity (3.54 %) the difference was significant of (6.62 %) (Fig. 46). As for extractives, significant difference in ash content between locations was evident. Tamarix in Italian sites contained the lowest amount of ash content (3.52-3.61%) (Fig. 47).

Differences in sugars between ecotypes growing in saline zone and non saline and desert areas were not significantly different. Such as, in Algeria the sugar content in saline site (KH) were not significantly different from the non saline one (BOU), also in Spain sugar content in the saline site (SB) were not significantly different from non saline (SE), also sugar content were not significantly different between the site which was on the vicinity of the sea (CRA) and the inland area (ALC). Nevertheless, in all the three countries, the average value of total sugars of trees growing in saline locations, were likely to be lower of 3-6% than non saline ones.

Lignin was not statically different between saline and non saline areas.

Extractives of samples coming from irrigated areas (YOT) were significantly lower than desert areas such as (GH) and non significantly different than other desert areas (TA).

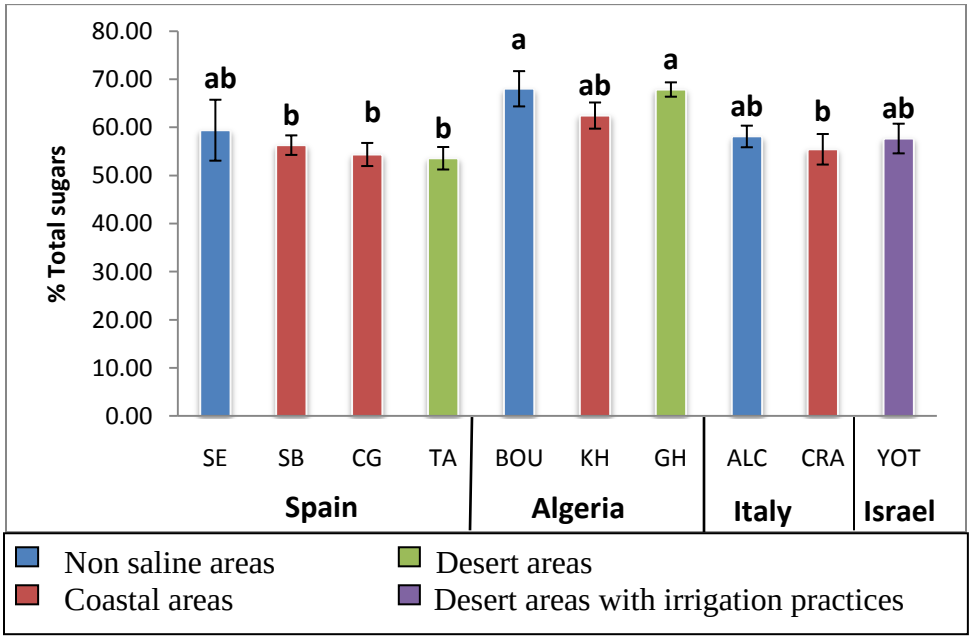


Figure 44. Total Sugars percentage of *Tamarix spp*.of different provenances. Data are means of 4 trees ± standard errors. Different letters on the top of the error bars indicate statistically different means at $P \leq 0.05$.

Table 11. The average values of sugar fractions percentage of Tamarix wood for different locations. Data reported are means ± standard errors. Different letters indicate statistically different means at $P \leq 0.05$.

Country	Location	% Glucose	% Xylose	% Galactose	% Arabinose
Spain	CG	38.55 ± 4.98^{c}	12.65 ± 1.87^{a}	1.57 ± 0.71^{a}	1.61 ± 0.34^{a}
	SB	40.89 ± 2.29^{bc}	13.05 ± 0.23^{a}	0.88 ± 0.07^{a}	1.51 ± 0.34^{a}
	SE	43.35 ± 2.45^{abc}	14.17 ± 0.65^{a}	0.68 ± 0.1^{a}	1.23 ± 0.23^{a}
	TA	38.88 ± 2.16^{c}	12.80 ± 0.68^{a}	0.52 ± 0.08^{a}	1.41 ± 0.37^{a}
Algeria	BOU	51.57 ± 3.36^{a}	14.59 ± 0.62^{a}	0.71 ± 0.06^{a}	1.18 ± 0.23^{a}
	KH	45.66 ± 2.51^{abc}	14.88 ± 0.75^{a}	0.45 ± 0.13^{a}	1.49 ± 0.25^{a}
	GH	49.31 ± 1.78^{ab}	15.92 ± 0.63^{a}	1.09 ± 0.49^{a}	1.57 ± 0.23^{a}
Italia	ALC	42.55 ± 1.04^{bc}	12.70 ± 1.38^{a}	1.56 ± 0.52^{a}	1.32 ± 0.24^{a}
	CRA	39.24 ± 1.99^{c}	13.27 ± 1.47^{a}	1.75 ± 0.6^{a}	1.22 ± 0.28^{a}
Israel	YOT	44.12 ± 2.35^{abc}	11.40 ± 1.11^{a}	1.46 ± 0.4^{a}	0.71 ± 0.09^{a}

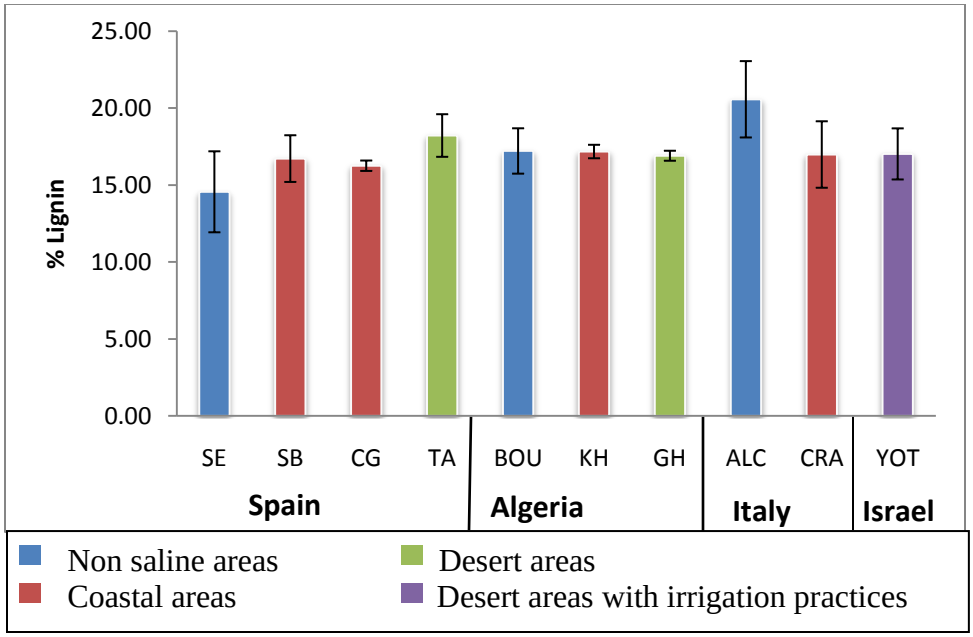


Figure 45. Lignin percentage of *Tamarix spp*.of different provenances. Data are means of 4 trees ± standard errors. Different letters on the top of the error bars indicate statistically different means at $P \leq 0.05$.

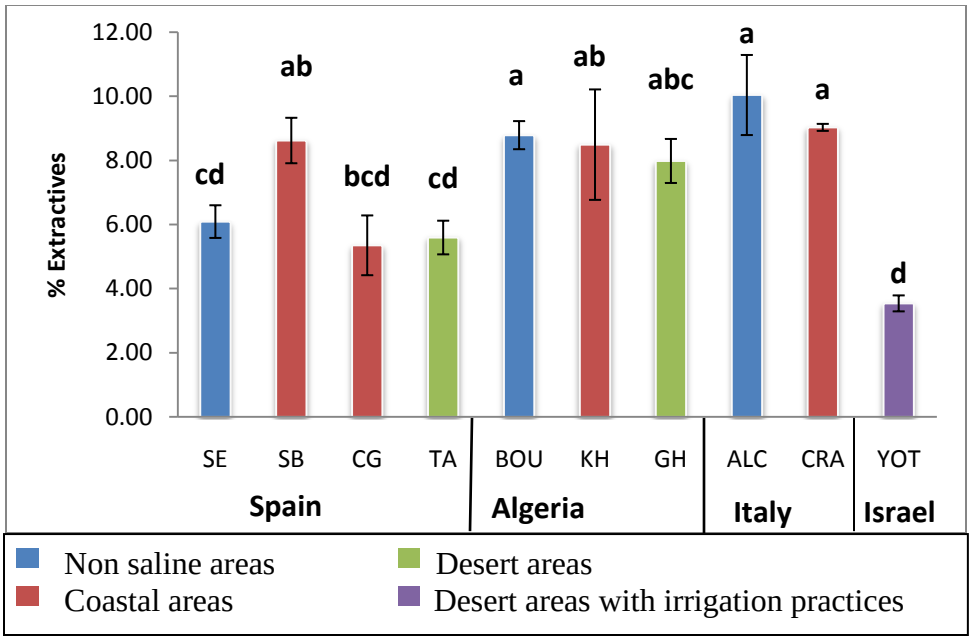


Figure 46. Extractives percentage of *Tamarix spp*.of different provenances. Data are means of 4 trees ± standard errors. Different letters on the top of the error bars indicate statistically different means at $P \leq 0.05$.

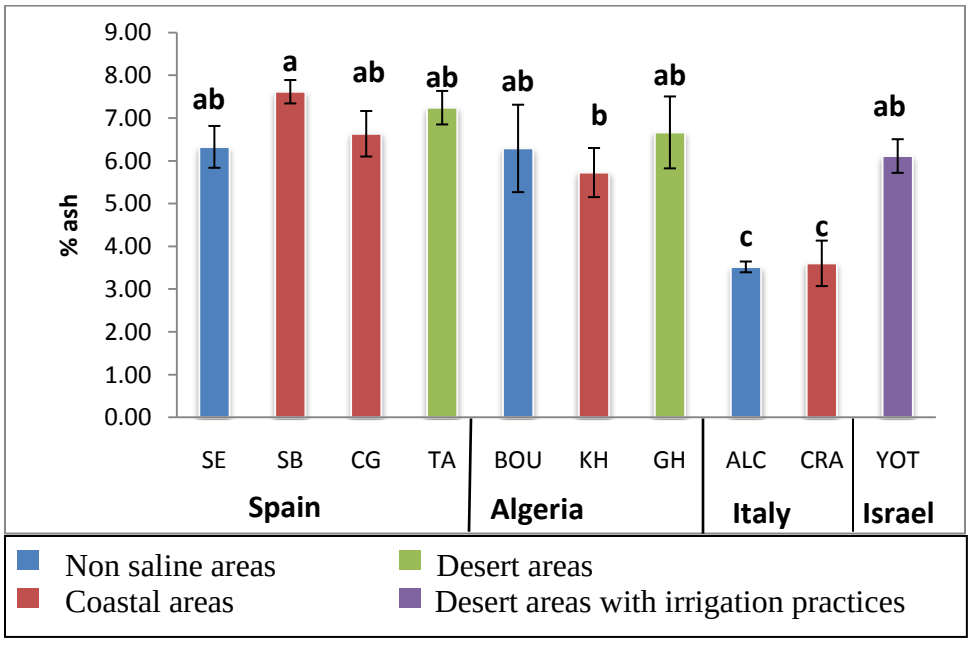


Figure 47. Ash percentage of *Tamarix spp*. of different provenances. Data are means of 4 trees ± standard errors. Different letters on the top of the error bars indicate statistically different means at $P \leq 0.05$.

Species effect

The collected samples belong to different species. Those in Italy (CRA) and (ALC) were classified as *T. africana* (Abbruzeze, 2011; Terzoli, 2011, personal communication) and those in Israel were *T. aphylla* and *T. jordanis*. Those in Algeria or Spain were classified as *T. gallica* (Abbruzeze, 2011, personal communication).

The sum of sugar was not statistically significant among the analyzed and identified species, *T. africana, T. aphylla* and *T. jordanis*. However *T. jordanis* tended to have higher content of total sugars (4.08%) than *T. aphylla*.

Lignin was not significantly different between the three species.

Extractives content were significantly different among species; *T. aphylla* and *T. jordanis* had the lowest content (Table 12).

T. aphylla, T. jordanis and *T. gallica* had highest content of ash than *T. africana.*

Table 12. The average values of sugar fractions percentage of Tamarix wood for different species. Data reported are means ± standard errors. Different letters indicate statistically different means.at $P \leq 0.05$.

Species	% Sugars	% Lignin	% Extractives	% Ash
T. gallica	60.30 ± 1.55^{a}	16.72 ± 0.51^{a}	6.65 ± 0.24^{b}	6.65 ± 0.24^{a}
T. africana	56.80 ± 1.89^{a}	18.78 ± 1.66^{a}	9.53 ± 0.61^{a}	3.56 ± 0.26^{b}
T. aphylla	55.37 ± 4.74^{a}	16.57 ± 3.39^{a}	3.42 ± 0.23^{b}	6.54 ± 0.69^{a}
T. jordanis	59.45 ± 4.16^{a}	17.38 ± 1.10^{a}	3.61 ± 0.46^{b}	5.69 ± 0.36^{a}

Age effect

Although we tried to select trees from fields that had the same diameter, however, when they were analyzed they showed to not have the same age.

Some growing trees with older age (above 6 year) like (TA), (ST) in Spain, (CRA) in Italy, and (GH) presented comparable diameters with trees of younger age (2-6 years) which indicated, the hard conditions in which they were growing.

Tamarix planted in the experimental gardens in Israel which were irrigated daily, grown fast and reach the same diameter of other older trees grown in nature, within short period of 4 years.

Nevertheless, the chemical data summarized in Table (13) did not result statistically significant according to age.

Table 13. The average values of sugar fractions percentage of Tamarix wood for different age
Data reported are means ± standard errors. Different letters indicate statistically different means.at $P \leq 0.05$.

Age class (years)	% Sugars	% Lignin	% Extractives	% Ash
2-6	60.83 ± 1.68^{a}	18.13 ± 0.94^{a}	7.20 ± 0.76^{a}	5.34 ± 0.34^{a}
6-13	57.76 ± 1.56^{a}	16.62 ± 0.66^{a}	7.19 ± 0.38^{a}	6.24 ± 0.34^{a}

Technical problems

Our analyses were not easy to perform due to lack of expertise, and machine problems. Thus several issues were encountered.

A component closure near 100% suggests that most components were accounted for and little double counting of components was occurring. The average of total mass closures was low for many of our samples. This is could be arise for the following issues.

In our case we only performed ethanol extraction however water extraction was also a prerequisite although low nitrogenous compounded were expected. (D'Annibale, A., 2011, personal communication).

We used a 0.2 ml /min flow rate of the mobile solution, the elution of glucose and xylose was better however , the sugars eluted later and the peak width were broader than using 0.6ml/min, which could lower the precision of peak integration and the calculation of sugar quantity.

Due to the low concentration of mannose in the samples, it was never detected, even using higher rate (0.6ml/min), thus hemicelluloses calculation was not possible on basis of sugar elution.

10.4 Discussion

The proportion of sugars and lignin in a biomass feedstock is a very important criterion in determining its suitability as an economically viable feedstock and in deciding on the optimum pathway for its conversion.

Wood chemical composition may be determined by genetic characters and evolutionary response to abiotic or biotic stresses acting singly or simultaneously (weed and invasive species competition, nutrition, water relation, disease and insect infestation, (Roelofs *et al*., 2008). For instance, cellulose and hemicellulose formations are linked to key genes involved in their biosynthesis, as well to biotic and abiotic stress (gravitropic stimuli, season….). Variation in lignin content is likely to be of adaptive importance **(**Gonzalez-Martinez *et al.* 2006**),** as it has primary roles in stem strength **(**Coleman *et al.* 2008**),** maintenance of water conduction **(**Gindl 2001**;** Plomion *et al.* 2001**;** Voelker 2009**;** Walker 2006**),** and possibly defense **(**Blanchette 1991; Coleman *et al.* 2008**;** Schwarze *et al.* 2000**;** Syafii *et al.* 1988**),** each of which are probably affected by spatially varying selection pressures. As with lignin, wood extractives are also thought to play a role in the tree defense against pathogens (Boddy 2001**;** Gierlinger *et al.* 2004) However extractives may interfere with the efficiency of delignifying chemicals (Wallis *et al*., 1996).

The detected variations in wood components of Tamarix plants were large in our study due to expected variations among individuals belonging to the same site that would be related to micro-climate conditions in which they were grown and genetic variability.

However, the reported variations among individuals belonging to the same site in our study, agreed with previous literature data that present variations in wood components from other commonly used biomass resources. For instance sixteen clones of poplar hybrids were analysed exhibited 7 to 15% points in cellulose content and a smaller range for lignin of 9.3% point (Dinus, 2001). Significant genetic variation in cellulose contents of 75 3 year-old *Populus deltoids* grown at a single location was also reported (Oslon *et al*., 1985). In *Eucalyptus spp.* genetic variation within species is small, only a few percentage points but sometimes as large as nine (Berolucci *et al*., 1995).

The geographic positions (Latitude and altitude) may play a role in determining sugar content in this case; those trees located in Algeria (lower latitudes) contained significantly higher total sugars than many sites in Spain, and Italy, probably due to higher sun exposure. It was found

that lowland cultivars of switchgrass provide the highest yields at lower latitudes (Casler *et al*., 2004).

Unless Italian populations, all the other populations contain a high amount of ash. High content may result from soil properties, it was stated that ash contents tend to be higher in regions having calcareous soils (Dinus, 2000). It may also result higher from thicker bark which substantially in greater amount than wood, and which thickness and proportion vary greatly with position within tree as well as tree size and age, growth rate, and site (Dinus, 2000). Ash has several undesirable properties for energy conversion processes, and some consider it to be the most limiting factor in those processes (Jenkins *et al*., 1998), it is one of major contributor to boiler scaling and slagging (Dinus, 2000).

The low content of extractives in Tamarix grown in the common garden in Israel relative to those grown in natural conditions could be related to irrigation management practices or to species into account. It was found that Polyflavonoid tannins content of many plants increased in response to drought stress (Pizzi and Cameron, 1986; Moore *et al*., 2004). Small quantities of tannins or phenolics from extractives can inhibit enzymatic breakdown of cellulose during the conversion to ethanol (Dinus, 2000).

Tamarix biomass was not significantly influenced by harsh conditions, although Tamarix grown in coastal zones tend to have lower glucose than those grown in inland areas and across rivers.

Due to the high variability found among samples, data of sugar content and lignin content of Tamarix of different sites did not allow us to determine definitely the best candidate; sample size should be increased for a more reliable estimation of wood quality. However some conclusions can be drawn;

The analyzed species (*T. aphylla*, *T. jordanis* and *T. africana*) were higher in quality than for example *T. indic*a a species reported in literature which has very low content of cellulose and hemicelluloses (12.17 and 24.67% respectively) (Abideen, *et al*., 2011).

Also, Tamarix recorded higher values of sugars compared to studied wood composition of many other halophytes (*Aleorus lagapoides*, *Aerva javanica*, *Calotropis procera*, *Suaeda fruticosa*…) (Abideen, *et al*., 2011).

On the other hand, biomass composition data of Tamarix were comparable to other hardwood feedstock such as eucalyptus which the wood cellulose content ranged from 43% to 45% (Cotterill and Macrae, 1997).

Tamarix in Algeria may yield 118.3 gallons of ethanol per dry ton of feedstock and the lowest yield may result from desertic area like Tabernas (TA) in Spain may produce 93.2 gallons per dry ton of feedstock. Of course this yield is theoretical and the efficient yield relies on many other features related to biomass recalcitrance and process of conversion. However in this first evaluation, this yield is comparable to many other considered biomass in the market. Besides their ability of use under short rotation type of agro-forestry to yield 26 to 52 tons/ha/y of organic biomass, which is not less than that obtained for common cash crops on arable land (Eshel *et al*., 2010) making it a desirable feedstock for the production of ethanol.

Lignin content was low which in turn should be easier and cost effective in pre-treatment of material and subsequently conversion process. Poplar species and hybrids have higher total lignin contents from 21 to 29% (Sannigrahi and Ragauskas, 2009).

However many technical challenges could present by the highest amount of extractives, ash and salt. The ethanol extractives content of *Tamarix spp*. were higher than poplar species (1.4-3.6%) (Sannigrahi and Ragauskas, 2009). The ash content of *Tamarix spp.* was also much higher than ash content of clone poplar wood (*P. deltoides* and *P. euramericana*) that contains between 0.47% and 1.16% (Klasnja *et al*. 2002).

If the extractives content are proved to be produced by periodic drought then, irrigation practices will be not only positive in increasing high biomass quantity but also by improving their quality for feasible production of bioethanol.

If high ash content was only attributed to soil properties, probably is better to avoid high calcareous soil, while if it is genetic therefore is necessary to breed the most suitable one.

Although this study showed that the location , species may diverge the quality of biomass several questions remain regarding best management practices for biomass production of high quality, thus further research is required to separate the effects of location species, management practices.

11. General conclusions

An understanding of physiological and biochemical responses of different *Tamarix spp.* to changes in environmental variables is important for a more insightful selection of the best ones for planting in extreme and degraded areas currently in decline, in summary this study showed that:

- *T. jordanis* tolerate salt soil up to 350mM without effective interference of its functions. However *T. aphylla* was affected even at salt stress of 150mM. The carbon assimilation the hexoses sugars content, and the growth in *T. aphylla* were impaired, in addition starch and pigments were also affected at higher concentration (350mM).
- In contrary of salt stress, water deficit of 50% showed higher impact on *T. jordanis,* while *T. aphylla* seems to acclimate and perform better. The combination of severe drought (30% F.C.) and low relative humidity was detrimental for both species.
- Combination of both stresses (salt and drought) was critical for plant growth and performance. However, after stress release, gas exchange parameters showed a substantial recovery in both species (data not shown). It could be inferred that soil drying combined with salinity did not cause permanent alterations in Tamarix plants, which can be useful species in revegetation programs in saline areas subjected to periodic drought.
- The present species employed common acclimating mechanism of salt stress avoidance, presenting initial restriction in the stomatal conductance, maintenance of relative water content, capacity to use inorganic ions for osmoregulation, maintenance of sucrose and increasing water use efficiency.
- The presented species employed also common acclimating mechanism to cope with drought stress (50%) presenting maintenance of relative water content and accumulation of soluble sugars at leaf level and increase of potassium content at root level.
- The strategy of using of compatible solutes proline or sugars in leaves of *Tamarix spp.* was more likely to be as osmoprotective role rather than osmoregualtor, the osmotic adjustment via accumulation of inorganic ions remains the principal osmotic adjustment of both *Tamarix spp.*

- From an agronomic perspective we may say that in harsh environments at moderate water salinity (150 mM), the evaluated *Tamarix spp.* were able to acclimate without impairing the biomass, whereas at moderate drought (50% F.C.) we could expect 19-33% reduction in biomass according to species, and in the absence of irrigation and higher salinity the benefits of cultivating Tamarix would be seriously jeopardized.

It could be also concluded from the study of Tamarix biomass quality for its prospective use for biomass production in arid lands that:

- The content of hexoses and pentoses of studied samples was highly comparable to other hardwood feedstock such as eucalyptus where the wood cellulose content ranged from 43% to 45%.
- Tamarix biomass may be feasible for the production of bioethanol as it showed high sugars and low lignin content. Nevertheless there are serious challenges by using Tamarix feedstock, due to high content of ash and extractives.
- The growing site may diverge significantly biomass quality of Tamarix.
- There was lack of consistency of data related to severe environmental and edaphic impact on sugar and lignin content; this could be mainly due to the limited number of samples in the experiment and possible random genetic drift in the sampling.

Given the high and rapid productivity of some species of Tamarix (Eshel *et al*., 2010), it would be interesting to conduct further studies to compare the tolerance and the biomass production of other species (*T. gallica* and *T. africana*) in harsh conditions.

To recertify the compositions of the samples, analyses should be repeated with greater sampling size of samples and to check if cultural practices such as irrigation and fertilization may influence positively the biomass quality, specific target studies should be conducted in common garden sites.

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