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COMPARATIVE ANALYSIS OF SALINITY TOLERANCE IN
TAMARIX AFRICANA AND TAMARIX GALLICA ORIGINATING
FROM TWO ITALIAN PROVENANCES

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ABSTRACT

In recent times, one of the starring abiotic stresses for plant survival in the ecosystem is salinity. This has resulted in massive loss of habitat, biodiversity, native vegetation and water resource value. Halophytes form 2% of plants and can tolerate 200-1000mM levels of salt concentration. Among them is *Tamarix* species which have been reported to be highly tolerant to salinity, occupying coastal dunes and the riverbanks of Southern Italy. They are use in the conservation and restoration practices under the perspectives of global climate changes. Different authors carried out experiments on *Tamarix* species, however their tolerance to salinity stresses are incompletely known or few studies reported. To address this gap, this thesis analyses the salinity tolerance in different provenances of *Tamarix africana* and *Tamarix gallica* available in the EcoPhysLab in-vivo collection. Here, we analyse the growth and physiological responses of *Tamarix africana* and *Tamarix gallica* of two Italian provenances (Simeto and Basento) over time across saline treatments (control, 200 mM, and 550 mM).

The finding of this study showed treatment effects on *Tamarix* species for cumulative shoot length, dominant shoot length, stem diameter and harvest biomass in both Basento and Simeto provenances. The study shows that under control treatment, *Tamarix africana* had 5.2% average leaves biomass and 2.3% wood biomass more than *Tamarix gallica*. On the other hand, *Tamarix gallica* had 13.4% average leaves biomass in moderate salinity and 27% average wood biomass in high salinity more than *Tamarix africana*. Furthermore, *Tamarix gallica* had 7.5% average root biomass in control and 13.5% average root biomass in high salinity more than *Tamarix africana*. Under moderate, salinity *Tamarix africana* had 2.5% average root biomass more than *Tamarix gallica*. The study also indicates that *Tamarix gallica* had the ability to resist high saline stress as compared to *Tamarix africana*. Furthermore, the study also revealed physiological decline of *Tamarix* species in both Basento and Simeto provenances over time across moderate and high saline treatments.

At the end of the experiment, the average quantity of salt accumulated outside the twigs of *Tamarix* species increased from 31.05 mg/g in control, to 112.21 mg/g in moderate and 169.24 mg/g in high salinity treatment. Under control, *Tamarix gallica* salt accumulated outside the twigs was 39.8% more than *Tamarix africana*. Similarly,

in moderate salinity *Tamarix gallica* salt accumulated outside the twigs was 25.0% as well as 9.7% in high salinity more than *Tamarix africana*.

The results of the study provide additional information about the intrinsically fascinating *Tamarix africana* and *Tamarix gallica* at both Basento and Simeto provenances in Italy as well as new insights for the conservation and restoration of potential fragile ecosystems, such as those occupied by *Tamarix* species. This ability has increasing potential in a world where the human population is still increasing and land use practices and changes in the climate are likely to lead to increased salinization of the land surface in many regions.

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TABLE OF CONTENTS

ABSTRACT.....	II
ACKNOWLEDGEMENTS.....	IV
LIST OF FIGURES.....	VIII
CHAPTER ONE.....	1
1. 0 INTRODUCTION.....	1
1.1 BACKGROUND.....	1
CHAPTER TWO.....	4
LITERATURE REVIEW.....	4
2.0 DEFINITION OF HALOPHYTE.....	4
2.1 HABITAT OF HALOPHYTE AND ADAPTATION.....	4
2.2 CLASSIFICATION OF HALOPHYTE.....	5
2.3 SOIL SALINITY, SALINITY THRESHOLDS AND ELECTRICAL CONDUCTIVITY.....	6
2.4 TAMARIX IN THE MEDITERANEAN.....	7
2.5 Botanical Description of the Family Tamaricaceae.....	8
2.6 The Genus Tamarix: Taxonomy, Distribution and Ecology.....	8
2.7 TAMARIX SPECIES IN ITALY.....	10
2.8 <i>Tamarix gallica</i> L.....	11
2.9 <i>Tamarix africana</i>	12
2.1.0 Tamarix Poulations in Italy.....	13
2.1.1 IMPORTANT OF HALOHYTE IN THE ECOSYSTEM.....	13
2.1.2 PHYSIOLOGICAL RESPONSES OF HIGHER PLANTS TO SALINITY.....	15
2.1.3 Physiological responses of higher plants to flooding.....	21
2.1.4 The use of tolerant plants for the ecological restoration of degraded ecosystems.....	24
2.1.5 EFFECTS OF HIGH SALT CONCENTRATIONS OF THE SOIL ON PLANTS.....	28
2.1.6 Stomatal closure.....	28
2.1.7 hyper osmotic shock.....	28
2.1.8 Inhibiting photosynthesis.....	28
2.1.9 Nutrient imbalance.....	29
2.2.0 Osmotic effect.....	29
2.2.1 Toxicity.....	29
2.2.2 Effects of salinity on plant yield.....	29
2.2.3 MECHANISM OF SALT RESISTANCE IN HALOPHYTES.....	30
2.2.4 Salt tolerance.....	30
2.2.5 Aspects of salt tolerance.....	31

2.2.6 Homeostasis.....	31
2.2.7 Salt avoidance.....	32
2.2.8 Exclusion:.....	32
2.2.9 Secretion.....	32
2.3.0 Shedding:.....	33
2.3.1 Succulence:.....	33
2.3.2 Stomatal response:.....	33
2.3.4 CELLULAR ADAPTATIONS OF THE PLANTS AGAINST SALT STRESS SYNTHESIS OF COMPATIBLE SOLUTES (OSMOLYTE PRODUCTION).....	34
2.3.5 Proline analogues.....	34
2.3.6 Aquaporin.....	34
2.3.7 Glycine betaine (GB):.....	35
2.3.8 Protection of cell wall integrity.....	35
2.3.9 ION COMPARTMENTALIZATION AND SELECTIVE TRANSPORT AND UPTAKE OF IONS AT THE PLASMA MEMBRANE.....	35
2.4.0 Molecular mechanisms involved in providing resistance against salinity in plants:.....	36
2.4.1 WHOLE PLANT LEVEL ADAPTATIONS OF HALOPHYTES AGAINST SALINITY	38
GERMINATION RESPONSES.....	38
2.4.2 GENETIC MODIFICATION OF PLANTS TO MAKE THEM SALT RESISTANT.....	39
CHAPTER THREE.....	40
3.0 Materials and Methods.....	40
3.1 Experimental Design and Procedures.....	40
3.2 Gas exchange measurements.....	42
3.3 Chlorophyll fluorescence measurements.....	42
3.4 Growth measurements and Biomass.....	43
3.5 Salt accumulation outside the twig.....	43
3.6 Statistical analysis.....	43
CHAPTER FOUR.....	44
4.0 RESULTS.....	44
4.1 Cumulative shoot length of <i>Tamarix africana</i> and <i>Tamarix gallica</i> under different saline conditions.....	44
4.2 Dominant shoot length of <i>Tamarix africana</i> and <i>Tamarix gallica</i> under different saline conditions.....	46
4.3 Stem diameter of <i>Tamarix africana</i> and <i>Tamarix gallica</i> under different saline conditions....	48
4.4 Biomass of <i>Tamarix africana</i> and <i>Tamarix gallica</i> under different saline conditions.....	50
4.5 Physiological performances of <i>Tamarix africana</i> and <i>Tamarix gallica</i> before saline treatment	52

4.6 Photosynthesis responses of <i>Tamarix africana</i> and <i>Tamarix gallica</i> to the salinity treatments	54
4.7 Stomatal conductance responses of <i>Tamarix africana</i> and <i>Tamarix gallica</i> to the salinity treatments.....	56
4.8 Maximum carboxylation rate (V_{cmax}) responses of <i>Tamarix africana</i> and <i>Tamarix gallica</i> to the salinity treatments.....	58
4.9 Photosystem II, Fv/Fm responses of <i>Tamarix africana</i> and <i>Tamarix gallica</i> to the salinity treatments.....	60
5.0 Salt extrusion from <i>Tamarix</i> species.....	62
CHAPTER FIVE.....	64
5.0 Discussion.....	64
5.1 Growth and biomass of <i>Tamarix gallica</i> and <i>Tamarix africana</i>	64
5.2 Gas exchange and chlorophyll fluorescence of <i>Tamarix gallica</i> and <i>Tamarix africana</i>	65
5.3 Salt extrusion from <i>Tamarix</i> species.....	67
CHAPTER SIX.....	68
Conclusion.....	68
References.....	70

LIST OF FIGURES

- Figure. 1** Map of Italian *Tamarix* species collection site of Basento and Simeto.....42
- Figure. 2** Cumulative shoot length (a) *Tamarix africana* of Basento and Simeto (b) *Tamarix gallica* of Simeto and *gallica* over time across saline treatments (control, 200 mM, and 550 mM). Different letters above the symbols indicate significant difference at $p<0.05$46
- Figure 3** Dominant shoot length (a) *Tamarix africana* of Basento and Simeto (b) *Tamarix gallica* of Simeto and *gallica* over time across saline treatments (control, 200 mM, and 550 mM). Different letters above the symbols indicate significant difference at $p<0.05$48
- Figure 4.** Stem diameter length (a) *Tamarix africana* of Basento and Simeto (b) *Tamarix gallica* of Simeto and *gallica* over time across saline treatments (control, 200 mM, and 550 mM). Different letters above the symbols indicate significant difference at $p<0.05$50
- Figure 5.(a)** leaf biomass (b) wood biomass (c) root biomass and (d) root/shoot biomass of *Tamarix* species under saline treatments (control, 200 mM, and 550 mM). Different letters above the symbols indicate significant difference at $p<0.05$52
- Figure 6** Physiological responses of *Tamarix africana* and *Tamarix gallica* at Basento and Simeto (a) Photosynthesis rate in morning (b) Photosynthesis rate in midday (c) Stomatal conductance (gs) in morning (d) Stomatal conductance (gs) in midday (e) Maximum carboxylation rate (V_{cmax}) in morning (f) Maximum carboxylation rate (V_{cmax}) in midday before saline treatment. Different letters above the symbols indicate significant difference at $p<0.0$54
- Figure 7.** Figure 7. Photosynthesis responses (a) *Tamarix africana* at Basento morning (b) *Tamarix africana* at Basento midday (c) *Tamarix africana* at Simeto morning (d) *Tamarix africana* at Simeto midday (e) *Tamarix gallica* at Basento morning (f) *Tamarix gallica* at Basento midday (g) *Tamarix gallica* at Simeto morning (h) *Tamarix gallica* at Simeto midday over time across saline treatments56
- Figure 8.**Stomatal conductance responses(a) *Tamarix africana* at Basento morning (b) *Tamarix africana* at Basento midday (c) *Tamarix africana* at Simeto morning (d) *Tamarix africana* at Simeto midday (e) *Tamarix gallica* at Basento morning (f) *Tamarix gallica* at Basento midday (g) *Tamarix gallica* at Simeto morning (h) *Tamarix gallica* at Simeto midday over time across saline treatments.....58
- Figure 9.**Maximum carboxylation responses(a) *Tamarix africana* at Basento morning (b) *Tamarix africana* at Basento midday (c) *Tamarix africana* at Simeto morning (d) *Tamarix africana* at Simeto midday (e) *Tamarix gallica* at Basento morning (f) *Tamarix gallica* at Basento midday (g) *Tamarix gallica* at Simeto morning (h)

Tamarix gallica at Simeto midday over time across saline treatments60

Figure 10. Photosystem II, Fv/Fm responses(a) *Tamarix africana* at Basento morning (b) *Tamarix africana* at Basento midday (c) *Tamarix africana* at Simeto morning (d) *Tamarix africana* at Simeto midday (e) *Tamarix gallica* at Basento morning (f) *Tamarix gallica* at Basento midday (g) *Tamarix gallica* at Simeto morning (h) *Tamarix gallica* at Simeto midday over time across saline treatments.62

Figure 11. Salt extrusion from *Tamarix* species of Simeto and gallica of three treatment (control, 200 mM, and 550 mM). Different letters above the symbols indicate significant difference at $p < 0.05$ 64

CHAPTER ONE

1. 0 INTRODUCTION

1.1 BACKGROUND

In recent times, one of the starring abiotic stresses to plant survival in the ecosystem is salinity. The salt-affected areas have devastated ecosystems resulting in massive loss of habitat, biodiversity, native vegetation and water resource value (Department of Natural Resources and Mines 2002) and as well as the effects on agricultural regions where it has severely degraded the landscape (Yeo 1999). Salt stress severely limits the plant growth and no toxic substance restricts the plant growth more than salt on global scale (Xiong and Zhu 2002). Salinity cause by natural phenomenon or human-induced processes that result in the accumulation of dissolved salts in the soil water to an extent inhibits plant growth (Munns 2002). Statistics shows that 6% of the world's land is affected by salinity and these cover 400 million hectares, which is over 6% of the world land area. The current 230 million hectares of irrigated land has a significant proportion of 45 million hectares (19.5%) cultivated land is salt-affected. The 1,500 million hectares under dryland agriculture, 32 million are salt-affected to varying degrees (FAO 2018). Salt affects plants in two major ways, the osmotic stress and ionic toxicity. Interestingly, all of them influence major plant processes (Yadav et al. 2011). The higher water pressure which is more than the soil under usual conditions enable plants to take up water and essential minerals. When salt stress occurs, the osmotic pressure of the soil solution is greater than that in plant cells. As a result, plant cannot get enough water (Kader 2010). Besides, its cells will have decreased turgor and its stomata will close to conserve water. The closure of the stomata leads to less carbon fixation and the production of Reactive Oxygen Species (ROS) such as superoxide and singlet oxygen. The Reactive oxygen Species (ROS) interrupts the cell processes and thus cause damages via the lipids, proteins and nucleic acids (Parida and Das 2005).

Plants are divided into two categories regarding salt stress: the glycophytes and the halophytes. Glycophytes are extremely sensitive to salt in soils whilst halophytes are salt tolerant and often grow in salty environments. Glycophytes forms the majority of

plant life on earth and it include major economic food crops, so the increasing salinity in soils is of major concern. Glycophytes cannot tolerate salt stress but however they can develop protective measures against it. At a high salt concentration ranging from 100-200 mM salt, these plants become extremely sensitive and results to their inhibition or death. Differently, halophytes that form 2% of plants, can tolerate levels of salt concentration anywhere from 300-1000 mM of salt (Zhu 2007). The significant differences in halophytes are their abilities to compartmentalize sodium and accumulate osmolytes whilst maintaining constant potassium concentration. The salt is accumulated in the leaves and roots, and can force sodium across the tonoplast by highly Na^+/K^+ selective protein transporters (Radyukina et al. 2007).

In the Mediterranean region the most widespread *Tamarix* species are *T. africana* and *T. gallica* (Aránzazu Prada & Arizpe 2008), naturally distributed in coastal areas, salt marshes and riverbanks of temporary and perennial streams. Even though *Tamarix spp (africana and gallica)* has salt-tolerating mechanisms, growth and physiological responses of *Tamarix spp (africana and gallica)* to salinity stress are incompletely known and few studies have reported how increasing salinity impacts these responses. According to (Carter J. M and Nippert J.B 2011) *Tamarix ramosissima* decreased photosynthesis, stomatal conductance, water potential, and the maximum quantum yield of photosystem II under saline concentrations ranging from 15 and 40 g/l NaCl compared to control treatments. Carter (and Nippert J.B 2011) also measured recovered photosynthetic rates, maximum quantum yield of photosystem II, and stomatal conductance after 35 days of exposure. These were suggested that physiological functioning of *Tamarix ramosissima* acclimated to extremely high NaCl concentrations over a relatively short period of time. Furthermore, initial evidence suggests proline synthesis may be the mechanism by which this species regulates osmotically to increasing salinity. Kleinkopf (and Wallace 1974) reported increased salt concentrations had a marginal effect on the net exchange rates of carbon and water in *Tamarix ramosissima*.

In addition, (Jaoudé et al. 2012) reported under saline conditions, the rate of the water consumption was lower compared to the non-saline treatment, as stomatal conductance was negatively affected by salinity and significantly declined over time. What is more is the decrease in mean xylem vessel area, assimilation rates and stomatal conductance comparison to the control, indicating both an osmotic stress and

a toxic consequence of NaCl on leaf gas exchange. Other studies say, majority of halophytes respond to salinity by exclusion (Yadav et al., 2011). Within the mangrove's environment, 99% of salts are excluded by the roots (Aslam et al. 2011) and secrete salt through shedding of their salty leaves as well as salt glands, specialized cells on the leaves and stem, which is then washed down by rain or wind (Aslam et al. 2011).

The *Tamarix* species are intrinsically fascinating for the ways in which they are adapted to grow under conditions lethal for most plant species. This ability has increasing potential in a world where the human population is still increasing and land use practices and changes in the climate are likely to lead to increased salinization of the land surface in many regions (Rozema and Flowers, 2008) resulting in destruction of the ecosystem. To this end, it is worth noting that several authors had carried out experiments on *Tamarix* spp, however growth and physiological responses specifically to *Tamarix* spp (*africana* and *gallica*) to salinity stress are incompletely known or few studies reported. To address this gap, the thesis analyses the salinity tolerance in different provenances of *Tamarix* spp., available in the EcoPhysLab in-vivo collection. The specific objectives are:

1. to analyse the physiology responses of *Tamarix gallica* and *Tamarix africana* (different provenances) to different salt concentration;
2. to analyse impact of salinity on growth of *Tamarix gallica* and *Tamarix africana* (different provenances)

CHAPTER TWO

LITERATURE REVIEW

2.0 DEFINITION OF HALOPHYTE

Halophytes have been recognized for hundreds of years; however, their definition remains equivocal or open to more interpretations. Halophytes are broadly seen as plants that naturally dwell in saline environments and benefit from having substantial amounts of salt in the growth media and tolerate salt concentration that kill 99% of other species. In addition, (Flowers et al., 1986) definition was based on ability 'to complete the life cycle in a salt concentration of at least 200 mM NaCl under conditions similar to those that might be encountered in the natural environment. (Breckle 2002) suggested that adopting a definition based on completion of the life cycle should allow separation of what might be called 'natural halophytes' from plants that tolerate salt but do not normally live in saline conditions

2.1 HABITAT OF HALOPHYTE AND ADAPTATION

The habitats of halophytes occur across a wide range of plant families, with the Chenopodiaceae being dominant (Flowers and Colmer, 2008). In a wide variety of saline habitats halophytes can grow. These are from coastal regions, salt marshes and mudflats, to inland deserts, salt flats and steppes. Halophytes have evolved to adapt to large range to tolerate seawater and higher concentrations of salts. These are achieved through the adjustment of their internal water relations through ion compartmentation in cell vacuoles, the accumulation of compatible organic solutes, succulence, and salt secreting glands and bladders (Shabala and Mackay, 2011). The optimal growth of halophyte is achieved at a concentration of around 50 mM NaCl for monocots, and between 100 and 200 mM for dicots (Glenn et al., 1999; Flowers and Colmer, 2008). In addition, some halophyte species do not show significant yield reduction even when irrigated with seawater e.g. *Suaeda maritime* (Greenway and Munns, 1980). This capability is well beyond of any known conventional crop species, making halophytes ideal for 'saline agriculture'. In contrast, halophytes tend to grow and survive in environments where salt concentrations are high (>200 mM NaCl) (Flowers and Colmer, 2008; Bui, 2013). These mechanisms regarding salt tolerance in

halophytes are generally recognized to be related to controlled uptake and compartmentalization of salts, synthesis of compatible solutes, and excretion of excess salts (Breckle, 2004; Flowers and Colmer, 2008).

2.2 CLASSIFICATION OF HALOPHYTE

Halophytes are classified in a variety of ways, such as classification based on general ecological behaviour and distribution, response of plant growth to salinity and quantity of salt intake etc (Waisel, 1972). In the classification of halophyte, they are classified on the basis on the internal salt content Steiner (1934). According to him, can be divided into two main types that is salt regulating types and salt accumulating types. Halophytes are also classified as excluders versus includers on the basis of internal salt contents of the plant (Ashraf et al., 2006). Again, halophytes are often classified as excretive and succulents on the basis of their morphology. This is significant because they are capable of excreting excess salt from the plant body known as excretive. The salt crystals may remain visible on the plant leaf surface; on such types of halophytes they have glandular cells that help to remove excess salt from the plant body (Marschner, 1995). Succulents' types of halophytes have a salt bladder on their leaf surface. To minimize salt toxicity, succulents store large amount of water within their body (Weber, 2008). Almost all the halophytes found in deserts belong to this category. According to Sabovljevic and Sabovljevic (2007), halophytes can be classified as obligate and facultative depending upon their demands and tolerance for sodium salts. Ecological physiological aspects can also be used to differentiate between obligate, facultative halophytes and habitat-indifferent halophytes (Cushman, 2001). The obligate halophytes need some salt for their growth, and they are also known as true halophytes and they thrive when the water contain over 0.5% to 1% of the sodium chloride (Ungar, 1978). Obligate halophytes show a clear optimization in their growth when the amount of salt is increased in their media. Members of the family Chenopodiaceae belongs to this category (Cushman, 2001). In contrast, facultative halophytes can be grown under saline stress but can grow well without salt or at least in an environment where the concentration of salt in the soil is quite low (Sabovljevic and Sabovljevic, 2007). A large number of dicotyledons as well

as members of Graminae, Cypraceae and Juncaceae belong to this category (Sabovljevic and Sabovljevic, 2007).

Furthermore, classification of halophytes on the basis of habitat can be divided into two main types based on their geographical distribution or habitat (Youssef, 2009). These are: Hydro-halophytes, plants which can grow in aquatic soil or in wet conditions. Most of the mangroves and salt marsh species along coastal lines are hydro-halophytes (Youssef, 2009). Xero-halophytes grow in environment, where the soil is saline, but the water content of the soil is less due to evaporation and many of them are succulents.

2.3 SOIL SALINITY, SALINITY THRESHOLDS AND ELECTRICAL CONDUCTIVITY

The definition of saline soil is the one in which the electrical conductivity (EC) of the saturation extract (EC_e) in the plant root zone exceeds 4 dS m^{-1} (approximately 40 mM NaCl) at a temperature of $25 \text{ }^\circ\text{C}$ and has an exchangeable sodium of 15%. According to (Jamil et al., 2011), the yeild of most plants is reduced at this EC_e , while many plants exhibit reduction at lower EC_e . Brackish water is between 0.7-2.0 dS/m and saline at levels above 2 dS/m. The conductivity of rain or distilled water is 0.02-0.05 dS/m whereas seawater, at the other extreme, averages between 45-60 dS/m. Salinity in water is also measured by the weight of its inorganic particulates or total dissolved solids (TDS), expressed as parts per million (ppm) or milligrams per litre (mg/l). When is less than 1,000 ppm is considered fresh or potable greater than 4,000 ppm saline, and between 35,000-45,000 ppm the standard for seawater. Consider when comparing the measurements of EC and TDS, one should note that that 1 dS/m is roughly equal to 650-700 ppm, and closer to 800 ppm at relatively higher levels of salinity.

In addition, according to Blumwald, Eduardo (2000) the thresholds of salinity are generally referred to as the maximum amount of salt that a plant can tolerate in its root zone without affecting growth. Other significant thresholds indicate the maximum level of plant salt-tolerance associated with reducing in yield or biomass (usually between 10-50%). The zero yield thresholds stipulate levels at which a plant can no longer survive. A continuum exists between degrees in salt-tolerance as

showed by the diverse spectrum of plants from those that flourish in seawater and higher salinities to those that cannot tolerate even marginal concentrations without significant decline. In a broader perspective, domesticated plants classified as salt-sensitive have salinity thresholds of 1-3 dS/m and zero yields at 8-16 dS/m (or less) while the 'moderately' salt-tolerant have thresholds of 5-10 dS/m and zero yields at 16-24 dS/m (Blumwald 2000). The determination of the level of electrical conductivity (EC) within our soils and water is the most common method of measuring salinity. The increases in in EC measurements are directly correlated with increases in the concentration of soluble salts or elemental ions especially sodium and chloride electrical conductivity is most often expressed in units such as deci-siemens per meter (dS/m) which quantify the ability of a sample to conduct electrical impulses with a resistance of 1 ohm (Blumwald 2000). Salinity thresholds do not necessarily have fixed indexes of salt-tolerance but sort of vary widely with environmental conditions and cultivation techniques that influence a plant's physiological response to increasing salinity. Some significant factors to consider when gauging the impact of salts on plant growth and yield are:

- soil structure and texture (sand/loam/clay) including fertility and permeability
- salt concentration variability within sub-soils and the root zone (vertical soil profile)
- field and habitat inconsistencies from gradual transitions to abrupt patchiness
- daily/seasonal deviations in the composition/level of soil and water salinity
- evapotranspiration rates including plant water requirements and soil infiltration
- life cycle variability of plant salt-tolerance from germination to maturity
- human interventions such as cultivation, irrigation (leaching), and drainage practices
- environmental and climatic factors (i.e. temperature, moisture, light, wind, etc.)

2.4 TAMARIX IN THE MEDITERANEAN

In *Tamarix* classification, is a facultative halophyte known to tolerate such harsh conditions through salinity and water tolerance mechanisms, which includes selective exclusion of salts from roots, compartmentalization and secretion in salt glands, and

as well as facultative phreatophytic ability (Ohrman and Lair, 2013). The root system of *Tamarix* often develops under conditions in which water tables are relatively high and where salts accumulate in the soil profile (Nippert et al., 2010; Glenn et al., 2012; Imada et al., 2013). Again, produce adventitious roots from stems under flooding (Jaoudé et al., 2012).

2.5 Botanical Description of the Family Tamaricaceae

Tamaricaceae belong to a small family of 4 genera and 110 species (Mabberley, 1987). It is distributed in temperate areas, usually in sandy tracts and maritime deserts of Europe, Asia and Africa (Qaiser, 1982) Furthermore, the family members have the capacity of accumulating salt in special glands in its leaves, and then excrete it onto the leaf surface. Foliage of salt cedar is often covered with a bloom of salt (Decker, 1961; Mozingo, 1987). These salts accumulate in the surface layer of soil when plants drop their leaves (Mozingo, 1987). Tamaricaceous plants are usually shrubs, sub-shrubs, or trees. Their leaves alternate, exstipulate, usually sessile and small. They are scale-like, herbaceous or fleshy, mostly with salt secreting glands and persistent. Their

Inflorescence is simple racemes, panicles or spikes (*Tamarisceae*). Flowers are bisexual, actinomorphic, 4-5 merous; sepals and petals free or connate at the base; anthers 2-celled, obtuse or apiculate, dehiscent by longitudinal slit; pollen grains tricolpate, with smooth wall; ovary superior, 1-locular; placentas 3-5, arising from the base; carpels 2-5, with parietal placenta; ovules usually numerous, anatropous; styles as many as the carpels, short, usually 2-5, free, sometimes united; stigmas capitate, sometimes sessile; fruit capsule, 3-5 angled, pyramidal, dehiscent by 3-5 valves from apex to the base. The seeds are many, hairy all around or with a tuft of hairs; endosperm absent (*Tamarisceae*) with straight embryo and flat cotyledons (Boulos, 1999; Qaiser, 1982; Yang and Gaskin, 2007).

2.6 The Genus *Tamarix*: Taxonomy, Distribution and Ecology

The genus *Tamarix* is one of the four genera of *Tamaricaceae* and comprises about 90 species of trees and shrubs (Zohary, 1972; Zhang et al., 2002). The taxonomy of the *Tamarix* genus is quite complex, considering that its members exhibit very few

external traits that are distinctive and easy to see. The diagnostic traits are usually related to the morphology of the small flowers, particularly the androecium and the bracts that subtend the flowers. For this reason, it is difficult to classify individuals as belonging to a certain species unless they are flowering. The *Tamarix* spp. grows in arid and semi-arid climates; however, it requires temporary edaphic humidity conditions from surface or ground water. Again, they are found in riverside areas with water regimes ranging from permanent watercourses to ephemeral streams, in humid depressions and in sandy shoreline areas, as scattered individuals or in continuous formation depending on water availability Aránzau Prada and Arizpe (2008). According to (Baum, 1978), *Tamarix* are either true trees with a well-developed trunk or shrubs. On average, they may grow up to 10 m tall (Frasier and Johnsen, 1991). Some of the species can live for hundreds of years and reach a large size, although the typical life span has been reported to be 75 to 100 years (Horton, 1977). *Tamarix* is usually deep and massively rooted, with tap roots reaching 30 m in depth. According to Merkel and Hopkins (1957), the development of root depends on soil water status. When the plants are growing in nearly saturated soils, the primary root began lateral growth, and secondary roots penetrated the soil to the capillary fringe under other conditions, the tap root grows downward with secondary branches arising in a conventional manner. Occasionally, very few lateral roots are produced, and the primary root grows downward until it reaches the water table, then secondary root branching becomes profuse (Zhang et al., 2002). The superficial side roots are also capable of producing adventitious buds. The adventitious roots derived from these buds are produced by the rooting of lenticels situated in the low branches when these are buried with moist soil (Zhang et al., 2002) or are under flooding conditions (Zhang et al., 2002). This is usually during the second week after flooding (Merkel and Hopkins, 1957). The re-sprouting of the roots is visible after the top of the plant has been removed (Frasier and Johnsen, 1991). This phenomenon is probably an adaptive advantageous in the vegetative breeding of *Tamarix* species in their natural habitats (Ginzburg, 1967). In addition, an adventitious root formation also enables these plants to be propagated through cuttings. The genus of the *Tamarix* is herbaceous, small, deciduous or persistent, scale-like leaves as its features (Baum, 1978). The showing of leaf glands specialized in salt extrusion is a characteristic feature of *Tamarix* species. In general, salt glands are specialised epidermal cells or

trichomes (not connected to the vascular bundles), which playing an active role in the secretion of solutions of mineral salts (Fahn, 1988). *Tamarix* species are highly plastic and tolerant to adversity (Zhang *et al.* 2002) in the sense that is able to occupy different habitats, as result showing a wide range of ecological variations: some of them are phreatophytes (Brock, 1994; Gries *et al.*, 2005), being dependent upon groundwater for growth and survival (Frasier and Johensen, 1991). Some halophytes species are thermophilic and vegetate in the warm region of the Dead Sea while others tolerate lower temperatures, extending their range to Europe.

2.7 TAMARIX SPECIES IN ITALY

According to Conti *et al.* (2005), *Tamarix* species in Italy are ten and among them *T. gallica* L. and *T. africana* Poiret are the most widespread. A key with species description and characteristics is given in Tab. 3.

The table 3 shows the morphological characteristics of *T. gallica* and *T. africana* (Aránzazu Prada and Arizpe, 2008).

Taxon	<i>T. gallica</i> L.	<i>T. africana</i> Poiret
Bark	<ul style="list-style-type: none"> ▪ Brownish-black or deep purple 	<ul style="list-style-type: none"> ▪ Black or dark-purple
Leaves	<ul style="list-style-type: none"> ▪ 1.3-2.5 mm long ▪ Glaucous 	<ul style="list-style-type: none"> ▪ 1.5-4 mm long ▪ Smooth or minutely papillose ▪ Margin scariosus
Inflorescences	<ul style="list-style-type: none"> ▪ 10-50 × 3-5 mm ▪ Usually on current year's branches ▪ Rachis usually glabrous loosely compound 	<ul style="list-style-type: none"> ▪ (15)30-70(80) × (5)6-9 mm ▪ Usually on previous year's branches ▪ Rachis sometimes papillose ▪ Margin scabrous

Bracts	<ul style="list-style-type: none"> ▪ Usually shorter than calyx ▪ Narrowly triangular, acuminate ▪ Margin denticulate 	<ul style="list-style-type: none"> ▪ Longer or shorter than calyx ▪ Narrowly oblong, shortly acute, to triangular, acuminate ▪ Margin usually papillose
Flowers	<ul style="list-style-type: none"> ▪ Pentamerous 	<ul style="list-style-type: none"> ▪ Pentamerous
Sepals	<ul style="list-style-type: none"> ▪ 1-1.8 mm long ▪ Trullate to ovate, acute; inner somewhat longer and more obtuse ▪ Margin not very denticulate 	<ul style="list-style-type: none"> ▪ 1-1.8 mm long ▪ Trullate, acute; outer slightly longer, narrower and more acute ▪ Margin subentire
Petals	<ul style="list-style-type: none"> ▪ (1.6)1.7-2 × 0.8-1 mm ▪ Elliptic to ovate 	<ul style="list-style-type: none"> ▪ 2-3.3 × 1-2 mm ▪ Trullate to ovate
Anthers	<ul style="list-style-type: none"> ▪ Slightly apiculate 	<ul style="list-style-type: none"> ▪ Muticous or slightly apiculate
Nectariferous disc	<ul style="list-style-type: none"> ▪ Synlophic ▪ Not very fleshy 	<ul style="list-style-type: none"> ▪ Synlophic

2.8 *Tamarix gallica* L.

Tamarix gallica is distributed in South-western Europe, South-eastern Europe as well as Macronesia. Within the Mediterranean region it is usually located in Portugal, Spain, France and Italy (Aránzazu Prada and Arizpe, 2008). *T. gallica* is a tree which can grow up to 8 m height and the stem of the diameter can reach up to 25 cm. Besides, it has brackish-brown to deep purple bark and the crown has an irregular shape (Pedrotti and Gafta, 1996). The flowers are classified into racemes which bloom in early spring and are situated on growing branches of the current year. *T. gallica* are commonly distributed in the coastal areas, salt marshes, on the riverbanks and on saline soils. It is heliophilus and very tolerant to salinity and drought. Nevertheless, it grows in exceedingly wet places (McAtee, 1914). *Tamarix gallica* is

prevalent in the Mediterranean basin. In Italy it is found in all the coastal regions (Conti et al., 2005)

2.9 *Tamarix africana*

Tamarix africana is distributed in South-western Europe, South-eastern Europe, Northern Africa as well as Macronesia. Within the Mediterranean region it is usually found in Portugal, Spain, France, Italy, Tunisia, Algeria and Morocco (Aránzazu Prada and Arizpe, 2008). *T. africana* is a small tree which can grow up to 6 m height and with dark bark. The flowers are classified into racemes which bloom in spring (April-June) and racemens are situated on growing branches of the current year. *T. gallica* are commonly distributed along the riversides and on saline soils. The distributions of the species are in the central and southern Italy (Conti et al., 2005).

In Italy, the populations of *Tamarix* belong to the vegetation order *Tamaricetalia africanae*. This order includes pioneer shrubby associations present on the riverbanks of temporary or perennial streams. The particular water conditions due to water table height variations and to water stagnation determined the riparian and marshy thermophile but do not follow specific climate zonation (Pedrotti and Gafta, 1996).The order of *Tamaricetalia africanae* also includes shrubby, pioneer associations of permanent and temporary watercourses (called *fiumare*). These vegetations have obvious thermophilic character, being limited only to the Mediterranean Region. The taxonomic order of these species shows a high tolerance to water stress during summer. The main species of these in this order of vegetations are *Tamarix gallica*, *Tamarix africana*, *Nerium oleander* and *Vitex agnus-castus*. This order is divided into the alliances *Rubo-Nerion oleandri* and *Tamaricion africanae*, the latter present only in thermo-Mediterranean plan, and includes three associations (Conti et al., 2005)

2.1.0 Tamarix Poulations in Italy

Taxonomical classification of *Tamarix gallica*(L.)

Kingdom [Plantae](#) – Plants

Subkingdom [Tracheobionta](#) – Vascular plants

Superdivision [Spermatophyta](#) – Seed plants

Division [Magnoliophyta](#) – Flowering plants

Class [Magnoliopsida](#) – Dicotyledons

Subclass [Dilleniidae](#)

Order [Violales](#)

Family [Tamaricaceae](#) – Tamarix family

Genus *Tamarix* [L.](#) – tamarisk

Species *Tamarix gallica* [L.](#) – French tamarisk

Taxonomical classification of *Tamarix africana* [Poir.](#)

Kingdom [Plantae](#) – Plants

Subkingdom [Tracheobionta](#) – Vascular plants

Superdivision [Spermatophyta](#) – Seed plants

Division [Magnoliophyta](#) – Flowering plants

Class [Magnoliopsida](#)– Dicotyledons

Subclass [Dilleniidae](#)

Order [Violales](#)

Family [Tamaricaceae](#) – Tamarix family

Genus *Tamarix* [L.](#)– tamarisk

Species *Tamarix africana* [Poir.](#)– African tamarisk

2.1.1 IMPORTANT OF HALOHYTE IN THE ECOSYSTEM

The *Tamarix species* is use in afforestation projects or programme in arid and saline areas (halo forestry) and is also flooding tolerance as documented in *Tamarix* spp. (Tallent-Halsell and Walker, 2002). These features provide a good starting point in the study of biodiversity and the performance of some ecotypes. *T. aphylla* has often been used in the past for afforestation of degraded lands because of its high growth rates (under high water availability, i.e. irrigation) and its ease of reproduction (Eshel

et al., 2010). The tender branches and leaves of the *Tamarix* species provide high value forage, especially during the dry period as fodder. However, a high salt content necessitates additional watering of livestock. Besides, in apiculture, the honey produces dark brown with minty aroma (Orwa et al. 2009). It is used as fuel (firewood and charcoal), burns reasonably well though slow to catch fire (calorific value, 4835 kcal/kg). However, the leaf litter and small branches burn poorly and perhaps because of their high salt content, giving an offensive odour if burnt green. It is used to produce fibre, the wood chips easily with little dust and are of good quality and colour, suitable for manufacture of particleboard. Twigs are used for basket making (Orwa C, Mutua A, Kindt R, Jamnadass R, Simons A. 2009). It is used in timber making, the wood is close-grained light-coloured, fibrous, fairly hard, heavy (specific gravity 0.6-0.7.5) strong, density of about 700 kg/m³, 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, turnery and fruit boxes. The galls, mainly from flowers are used for tanning leather and bark is also a rich source of tannin and mordant for dyeing. The flower galls are used as an astringent and gargle, bark for treating eczema and other skin diseases. The tribe of Tuaregs in Niger sweeten the water with branches which carry manna. *Tamarix* spp plays important role in ecological services, is highly valued for stabilizing sand dunes due to its fast growth, deep and extensive root system and ability to resist burial by shifting sand. *Tamarix* spp is also very useful for obtaining temporary shelter as quickly as possible, which can be removed once the adjacent longer-term shelterbelt has attained sufficient size. In addition, tree sheds leaves and twigs abundantly forming a compact litter that improves water holding capacity of the sand. However, it is reported to have a high-water output through transpiration. (Orwa et al. 2009). *Tamarix* spp is an attractive ornamental tree. Their thin branches and blue-green leaves give a feather-like appearance and well as a drooping clusters of finely coloured inflorescence are attractive, used to screen farm buildings and for street planting. *Tamarix* once again 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 wildfires and also hold the spread of fires along highways or railway lines caused by sparks or cigarettes. Another important thing to note here is that *Tamarix* are produced by

cuttings and plantations of this species are uniform from a genetic point of view. Consequently, there is a need in maintaining adequate variability of *Tamarix* genetic resources. Although the genus *Tamarix* has been widely used, there is no knowledge about its genetic variability. The knowledge of the genetic diversity of this genus could provide valuable information about the genotypes which can be more efficient in carbon sequestration and biomass production under stressful conditions

2.1.2 PHYSIOLOGICAL RESPONSES OF HIGHER PLANTS TO SALINITY

Many saline habitats contain sodium chloride and plants response to salinity depending on the salt concentration of the available water for root uptake, on the duration of the stress and on plant salt tolerance (Neumann, 1997; Munns, 2002). This phenomenon varies among species and, within the same species, among genotypes. According to their tolerance level, plants are divided into glycophytes and halophytes. The species that cannot tolerate high concentrations of soluble salts are called the glycophytes and are subjected to a stress condition that results in the decrease of growth and, in some cases, in plant death (Flowers and Colmer, 2008). In fact, when the stress is caused by a non-fundamental element (Schulze et al., 2005), such as sodium (Na), plants response to nutrients imbalance is particularly evident. In addition, sodium and chloride ions are considered biologically aggressive osmolytes because of their small ionic diameter and high surface charge densities, which give them a strong tendency to attract water molecules. These aggressive osmolytes cause water and ionic imbalances when they are present in high concentrations (Schulze et al., 2005).

The effects of salinity that is detrimental on plant growth concern with the followings:

1. the decrease of water potential in the root medium, due to an osmotic effect,
2. the toxicity caused by excessive Na⁺ and Cl uptake and accumulation, and the nutrient ion imbalance, owing to the trouble of essential intracellular ion concentrations especially K⁺ (Marschner, 1995; Mansour, 2000; Zhu, 2001).

The high salinity conditions cause stress. This stress is therefore due to a water deficit-like stress induced by the high concentration of solutes in the soil and to an ionic stress caused by an altered K⁺/Na⁺ ratio and too much cellular concentration of Na⁺ and Cl⁻ (Blumwald et al., 2000). Schulze et al. (2005) and Blumwald et al. (2000) described accurately the primary and secondary effects of salt stress. These authors

suggested that the toxicity of Na^+ is linked to its passive entry in the cell along a concentration gradient. Na^+ enters the cell through K^+ uptake mechanism, these two ions is characterised by a same radius size and by the same ion hydration energy. Obviously, charged ions do not move through the lipid bilayer and for that matter must cross the membrane via specialised proteins. The molecular tools revealed the identity of a large number of potassium channels and transporters that, however, are not equally permeable to Na^+ anions (Schachtman and Liu, 1999). Some transporters seem to be highly selective for K^+ over Na^+ , while other non-selective cation channels in plant roots cells might play a role in mediating Na^+ uptake. The uptake of Na^+ by these channels is highly dependent on external Ca^{2+} concentration. This is demonstrated that calcium inhibited the Na^+ permeation through non selective channels. As a result, other pathways might be involved in Na^+ uptake, because in most soils calcium levels are high enough to significantly inhibit Na^+ transport through non selective channels (Schachtman and Liu, 1999).

The high levels of Na^+ or high Na^+/K^+ lead to the alteration of many enzymatic processes in the cytoplasm (Tester and Davenport, 2003), Since the ion K^+ is responsible for the activation of about 50 enzymes. Moreover, the accumulation of positive charges in the cytoplasm results to the loss of a natural barrier to Cl^- , which is normally linked to the membrane potential, causes a substantial influx of negative ions through the anion channels. The high concentration of Na^+ ions within the cytoplasm also leads to an increased activity of the proton pumps, and especially of ATP-ase associated with the plasma membrane and antiport systems Na^+/H^+ of the tonoplast. This leads to an increased ATP consumption, an alteration of the cellular enzymatic activity and an accumulation of Na^+ in the vacuole that causes its alkalinisation. This alkalinisation which detrimentally affects the activity of various enzymes, particularly those involved in the catabolic energy metabolism. The high cellular concentration of Na^+ in due cause leads to an increased uptake of calcium, with a consequent increase in the cytosolic pools of this ion, a signalling function and triggers some regulatory processes in the cell. A decrease in cell growth by division is attributable to the secondary effects, caused by decreased energy availability in photosynthesis, due to the damage of the photosynthetic electron transport system and to the formation of ROS and leaf necrosis. Furthermore, the increased cellular salt

concentration detrimentally affects the stomatal guard cells, reducing stomatal conductance as well as mesophyll conductance (Bongi and Loreto, 1989; Delfine et al., 1998; Nandy Datta et al., 2007). The sensitivity of a leaf also depends on leaf age, for instance in old leaves high salinity increases the costs of maintaining photosynthetic rates and salt accumulation capacity (Suárez and Medina, 2005) because of ageing leaf and salt stress. Glycophytes are more affected as compare to halophytes by salt stress, although these two differently tolerant kinds of plants are characterised by common reactions of adaptation. The enzymes in halophytes are more tolerant to high concentration of NaCl than are those of glycophytes and at a concentration above 100 mM (Munns, 2002) Na⁺ starts to inhibit most of them. The adaptation of halophytes is faster and is more tolerant to severe salinity and vice versa (Schulze et al., 2005). Munns (2002) however stresses the importance of temporal variation in the response to salt stress. The author suggested that the primary responses concern with immediate variations (taking place in minutes or hours) in leaves and roots growth rates (quickly recovered). This is linked to changes in cell water relations, mediated by a root hormonal signal (Munns, 2002). Again, at a hormonal level, an increase in cellular concentration of ABA and ethylene take place, while cytokinin suffers a decline. As a result, there is the possibility that the increase in the production of ABA is induced by a water stress, rather than by the presence of Na⁺ and Cl⁻ (Schulze et al., 2005).

The expression of many specific proteins occurs as result of water stress, which may or may not be linked to the signal of ABA but, again, is not known whether this mechanism represents a response to salt or water stress (Schulze et al., 2005). (Munns, 2002) suggested the reduction in leaf growth rates, can be related to the presence of salt in the nutrient solution, which causes a decrease in the absorption of Ca²⁺. Subsequently, leaf growth is more influenced by the presence of salt than root growth (Tester and Davenport, 2003). This observation fact occurs even under water stress and could therefore be tied to this. Other than to a salt specific effect which was confirmed in some studies by the fact that the concentrations of Na⁺ and Cl⁻ never reach high levels of toxicity in growing cells (Munns, 2002). Under salt stress, after few days plants growth seems to be more influenced by their hormonal activity rather than by their water status. The leaves expansion within 24 hours under saline conditions does not undergo any change after an increase in leaf water status. In

subsequent weeks or months, damages may be visible at leaf level, which can result to death and phylloptosis of the leaf itself (Munns, 2002). As a result, salinity affects plants in two major ways, by reducing the leaf area for photosynthesising and by reducing leaf gas exchanges (Sharma, 1996).

In contrast to glycophytes, halophytes are defined as plants that undergo complete their life cycle in soils with salt concentrations above 200 mM and form about 1% of the world's flora (Flowers and Colmer, 2008). In order for halophytes to re-stand salinity, it preserve water uptake under very low soil water potentials and prevent the detrimental effect of high intracellular salt levels. Salt tolerance has two main mechanisms: those that reduce the entry of salt into the plant and those that reduce the concentration of salt in the cytoplasm. As a result, higher salt tolerance of halophytes is fully dependent on specific characteristics of the plant, which may concern (Waisel et al., 1986; Fahn, 1988; Poljakoff-Mayber and Lerner, 1994; Ramadan, 1998):

a) The control of root uptake and transport of some ions into the branches and leaves
Plant transpires about 30-70 times the amount of water that it actually uses for cell expansion, according to Munns (2002). This suggests that solutes which are not excluded at the root or at the xylem level will be 30-70 times more concentrated in the leaf than in soil. As a result, selective uptake by the root cells may prevent sodium to be accumulated into the transpiring organs, while maintaining potassium uptake. This may be achieved by increasing the highly selective K^+ transporters at the root level (Schachtman and Liu, 1999). Furthermore, Na^+ is retained in the upper part of the root system in many species and in the lower part of the shoot, showing an exchange of K^+ for Na^+ by the cells lining the transpiration stream (Munns, 2002).

b) The selective accumulation, exclusion or extrusion in order to decrease the toxic effects of Na^+

The toxicity of Na^+ ions has been widely accepted that Na^+ ions must be removed from the cytoplasm (Cheeseman, 1988). The most important mechanism of cellular adaptation to salt high concentration in halophytes is salt exclusion, even though, those species characterized by the presence of salt secretory glands (Munns, 2002). These mechanisms are used reduce the rate of salt accumulation in transpiring organs. The apoplast and vacuole can be the final compartments for salt deposition, and this is

achieved through Na^+/H^+ antiports, which are both in the plasma membrane and in the tonoplast. The proton concentration as well as the High Na^+ induce the formation of new antiports. Several H^+ pumps are then synthesised under salt stress conditions. When homeostasis is attained again; there is a low in H^+ -ATPase.

During this phase, the concentration of Na^+ in the cytoplasm is returned to its original value, while Na^+ concentration in the vacuole rises. Nevertheless, the original values of pH and membrane potential are not restored. The weakness of the cell ionic budget is the supply of K^+ .

The limited growth under salt stress of halophytes and glycophytes is probably due to a low supply of K^+ (Schulze et al., 2005). The removal of NaCl from the apoplast generally occurs through salt glands. Salt glands are specialized epidermal cells or trichomes, which play an energetic role in the secretion of mineral salt solutions, and repeatedly contain organic substances. Ions found to be secretions of these glands are Na^+ , K^+ , Mg^{2+} , Ca^{2+} , Cl^- , SO_4^{2-} , NO_3^- , PO_4^{3-} and HCO_3^- (Fahn, 1988). Although salt extrusion raises leaf tolerance under high salinity and is considered an active mechanism. It is suggested that 10-12 mol ATP are needed to extrude 1 mol NaCl in *Tamarix ramosissima* (Kleinkopf and Wallace, 1974). It was observed by Hirano et al. (1995) that, salt accumulation on the leaf surface may decrease stomatal conductance during the light by plugging the opened stomata, and increase stomatal conductance during the dark, by preventing the stomata from closing in leaves covered by dusts. Plants under saline conditions may also get rid of salt by leaf abscission. This phenomenon usually takes place in old leaves rather than in younger leaves and that is to say leaves that have been transpiring the longest (Munns, 2002). Moreover, elimination of leaf is different from leaf salt excretion through salt glands, as salt glands excrete salt, inducing a reduction in organs salt concentration, while abscission removes salt and its associated plant biomass, without decreasing salt concentration (Cram et al., 2002).

c) The accumulation of Na^+ and compatible solutes to absorb water and nutrients from the soil

Tester and Davenport (2003) suggested that plants must have a more negative water potential comparable to the medium in which they live and to achieve this condition,

they must increase their osmotic potential. For this reason, it is therefore necessary that internal changes in the concentration of solutes take place. Such changes occur as a response to water rather than to a saline stress result from two mechanisms: an increase of solutes uptake from the soil and the synthesis of solutes (Compatible solutes) (Schulze et al., 2005). Tester and Davenport (2003) suggested that, this factor poses a dilemma: Na⁺ and Cl are energetically cheap solutes (Cram et al., 2002). Though, they are toxic if they accumulate in the cytosol over a certain threshold. Compatible solutes are not toxic, but their synthesis requires a very high energy cost. In fact, although the compartmentalization of this ion in the vacuole requires energy, the number of necessary moles of ATP would be one order of magnitude smaller than that required for the synthesis of compatible solutes (Raven, 1985). These molecules do not have a very high charge, they are polar, highly soluble and as well as characterised by a high hydrating external surface (Sairam and Tyagi, 2004). On the whole, halophytes inclined to use Na⁺ and to compartmentalize it in the vacuole. Nevertheless, plants might reduce their ability to adjust osmotically under high salinities because of saturation of solute uptake systems (Munns et al, 1983), resulting in an overall decrease in plants growth.

The other adaptations to salinity by plants may be related with changes in wood anatomy. The excess salinity causes osmotic stress and as result shared some similarities with drought stress, as the increasing risk of vessels cavitation. (Pockman and Sperry, 2000) stated for photosynthesis to continue, uninterrupted transport of water through the xylem is essential for plant growth and survival, because it replaces the water lost by transpiration and allows stomata to remain open. Thus, vessel characters should be adapted to reduce the impact of cavitation on sap flow by preventing gas expansion and by reducing the loss of conductive area upon embolization (Schmitz et al., 2007). Safe hydraulic structures concern with the construction of vessels characterised by a small diameter (Junghans et al., 2006; Sobrado, 2007) as small vessels have an equally small wall area, resulting in a relatively low pit area per vessel and thus in a lower cavitation risk (Hacke et al., 2006). In effect, as the cavitation resistance is established by the maximum and not by the average pore diameter of the pit membrane, the bigger is the pit area, the larger is the chance of an exceptional large pore increasing cavitation vulnerability (Hacke et al., 2006). What is more, when vessels diameter is high, a lower xylem pressure is

enough to reduce water conductivity (Wheeler et al., 2005). Pore size can be regulated by changes in the chemical composition of the xylem sap: depending on the ionic composition of the xylem fluid, pectins swell under low ion content and shrink under high ion content, with consequent increase in the dimensions of pores in the pit membranes; thus, the increased ion content of xylem sap might lead to some compensation of cavitation-induced loss of water conductivity (Gascò et al., 2006). As a result, plant tolerance to salinity does not depend on a single physiological process, but on the interaction of multiple processes controlled by a group of genes, which explains the complexity of the phenomenon of tolerance (Mansour and Salama, 2004).

2.1.3 Physiological responses of higher plants to flooding

The flooding of plants events generates a series of biological, physical and chemical mechanisms on habitats, thus alter soil capacity to support plant growth by;

- (1) reducing O₂ diffusion and supply to roots
- (2) increasing mineral solubilisation,
- (3) promoting anaerobic metabolism of roots and microbes leading to the formation of toxic compounds
- (4) causing aggregates breakdown, clays deflocculation and destruction of cementing agents (Blom and Voeselek, 1996; Kozłowski, 1997; Schulze et al., 2005).

The gas exchange in well aerated soils according to these authors is the result of air diffusion inside the pores and this further accelerated by several soil processes that make the whole a relatively fast process. Regarding gas diffusion in pores filled with water seems to be totally different. Certainly, the diffusion coefficient (see Fick law) of oxygen in the water is 11300 times smaller than in air at the same temperature. Again, oxygen is characterized by having a low solubility in water (0.03 ml O₂/l H₂O). To this end, the gas exchanges in flooded soils are very slow, and the supply of oxygen turns into one of the main factors limiting plant growth in these environments. The oxygen that is trapped in the soil following flooding is rapidly inspired by aerobic processes and root fauna. The anaerobic or microaerophilic bacterial populations depend on organic matter as an energy source and decrease in oxygen availability promotes the growth. Though, they require electron acceptor ions that can be reduced. Under these circumstances, a lot of important oxygen-dependent processes such as

nitrification stop and are replaced by anaerobic metabolism of plants and bacteria, resulting to the build-up of toxic substances (sulphide, CO₂, oxides of Fe and Mn are accumulated in flooded soils; methane, ethane, propylene, fatty acids, aldehydes, ketones, heterocyclic compounds are produced by microbial metabolism; ethanol, acetaldehyde and cyanogenic compounds are produced by roots). Nitrate reduction is the first anaerobic process that occurs, which is used as an electron acceptor, to nitrogen (denitrification) and is followed by the reduction of Mn and Fe oxides. CO₂ can also be used as an electron acceptor, resulting to the formation of methane. The redox potential of the soil is decrease and is cause by the production of reduced ions. When it attains values in the range of -75 and -150 mV, sulphate is reduced by several strictly anaerobic bacteria, producing phytotoxic sulphide. Following flooding, the aggregates are reduced to smaller particles.

The newly formed particles are rearranged into a denser structure when the water level decreases and this is characterized by pores of smaller diameter, higher mechanical resistance to penetration of roots and low concentration of oxygen. The one that most limits plant growth among the processes generated by soil submergence is the lack of oxygen. The critical oxygen concentration for roots ranges between 5 and 10% (Schulze et al., 2005). Regarding the relationship among the concentration of oxygen and metabolism, normoxic is a situation where biochemical processes are not limited by lack of oxygen. A condition is called hypoxia if the mitochondrial synthesis of ATP appears to be influenced, but not inhibited, by low concentrations of oxygen. While in the complete absence of O₂ (anoxia), mitochondrial oxidative phosphorylation is negligible compared to the synthesis of ATP via glycolysis and fermentation (Schulze et al., 2005). Blom and Voesenek (1996), suggested the immediate outcome of a lack of oxygen in higher plants, flooding tolerant or not, is the decrease of roots aerobic respiration and consequently of ATP generation. According to (Liao and Lin, 2001) this causes a decrease in the energy available for root growth and, consequently, a reduction in the vegetative growth. Many heterotrophic organisms and plant tissues under hypoxic conditions are able to replace the Krebs cycle and switch to fermentative metabolism (Schulze et al., 2005).

A large amounts of energy in form of glucose is require for metabolism, so that the reserve material is quickly consumed and many toxic products such as lactic acid and ethanol are accumulated resulting in the promoting acidification of the cytoplasm

(Blom and Voeselek, 1996 , Liao and Lin, 2001, Schulze et al., 2005). During alcoholic fermentation, ethanol production is accompanied by the formation of alcohol dehydrogenase (ADH); this protein, synthesized in reply to flooding conditions and is responsible for the recycling of NAD⁺, which are necessary for glycolysis (Liao and Lin, 2001) allowing, although more slowly compared to aerobic conditions, ATP production (Blom and Voeselek, 1996). According to (Liao and Lin, 1995) t high levels of ADH activity and high ethanol production in anaerobic conditions are positively correlated with the degree of tolerance of a plant under these conditions. Kozlowski (1997) suggested flooding give rise to detrimental effects at leaf level by increasing stomatal closure and consequently limiting gas exchange and plant growth (Chen et al., 2005; Rengifo et al., 2005; Fernandez, 2006).The stomatal closure of plants growing in waterlogged soils is induced by a hormonal signal transmitted from the roots to the shoots (ABA and cytokinin) relatively than by a reduction in leaf water potential or a loss of leaf turgor (Kozlowski, 1997).

The photosynthetic rate reduction could be induced by a decrease in stomatal conductance however in the long-term, appears to be more influenced by inhibitory effects on the photosynthetic process itself, such as the loss of chlorophyll. According to (Schulze et al., 2005), two third of the earth's land mass is flooded at least occasionally, many species have developed various strategies to survive hypoxia (Schulze et al., 2005).The adjustments in the long term are based on rapid changes in physiological processes which result in a variation of the morphological and anatomical root features (Blom and Voeselek, 1996).

According to (Blom and Voeselek, 1996), in anoxia conditions ethylene production and accumulation in the root induces a tissue characterized by many intercellular spaces through the programmed death of certain cells (lysogeny), or the enlargement of intercellular spaces resulting from the separation of existing cells (schizogeny) and this tissue is called aerenchyma which is formed at the end of the elongation zone (Schulze et al., 2005).The aerenchyma provides an interconnected system of air channels which enable gases to diffuse or ventilate between plant organs particularly by allowing the descent of air or oxygen that derives from photosynthesis or from the atmosphere to the roots. On the contrary, there is a lift of carbon dioxide, ethylene and methane produced by roots and soil microorganisms on the surface (Blom and Voeselek, 1996; Colmer, 2003). (Armstrong, 1979) suggested that in stems,

aerenchyma can occur in the cortex and in the pith cavity. The oxygenation of the rhizosphere around the increasing tip reduces the harmful effects of anoxic soils on roots and supplies the demand of soil organisms that would compete with the root tip for oxygen (Evans, 2004). The aerenchyma formation does not necessary only ensures the tissues aeration, but also reduces the number of cells requiring energy in this tissue (Schulze et al., 2005). Many plant species that are tolerant to submergence are capable of producing, in response to an increasing concentration of ethylene and auxin, numerous adventitious roots, characterized by a highly developed aerenchyma (Blom and Voeselek, 1996). These roots frequently grow on the base of the shoot, on the upper part of the tap root, on stem nodes and above all searching the upper better aerated soil layers (Blom and Voeselek, 1996) or as well as floating on water surface (Kuzovkina et al., 2004). In the re-establishment of a contact between the root apparatus and the air enables stomatal opening and as a result leaf gas exchange and growth. The long-term responses of the aboveground portion of the plant also concern with branches increasing in length which is caused by low oxygen partial pressure of submerged internodes inducing an increasing of ethylene cellular concentration (Schulze et al., 2005). In addition to water logging, salinity can cause severe damage to plants (Barrett-Lennard, 2003) and the happenings of these stresses is increasing in many environments (Carter et al., 2006). Moreover the stress induced by salinity and flooding as single factors, has been demonstrated that salinity compromise flood tolerance mechanisms (Salter et al., 2010), avoiding adventitious root formation (Akilan et al., 1997; Salter et al., 2008) and increasing Na⁺ and Cl concentration in the foliage of plants under flooding with saline water conditions (Marcar et al., 2002). Again, (Barrett-Lennard, 2003) suggested that that halophytic species that typically inhabit waterlogged substrates can tolerate their shoot ions concentration in spite of the hypoxic or anoxic medium in which they rooted (Barrett-Lennard, 2003)

2.1.4 The use of tolerant plants for the ecological restoration of degraded ecosystems

According to (SERI, 2004), ecological restoration basically has to do with the process of assessing the recovery of ecosystems which have been degraded, damaged or destroyed. The goal of restoration practices is to assist ecosystem to have the capacity

to recover from stresses that is becoming once again resilient to perturbation without further assistance (SERI, 2002).

The Society of Ecological Restoration International (2004) suggested a restored ecosystem should have the following features:

- (1) It must be characterised by a similar diversity and community structure in comparison with reference sites;
- (2) It must be composed of local species; functional groups necessary for long-term stability must be present;
- (3) The physical environment must have the capacity to sustain reproducing populations;
- (4) It must have a normal functioning;
- (5) It must be integrated with the landscape;
- (6) Threats must be eliminated;
- (7) It must be resilient to natural disturbance and
- (8) It must be self-sustainable.

According Young et al. (2005) some ecological concepts which must have to be taken into account and applied in ecological restoration practices.

According to (Palmer and Bernhardt, 2006), the simplest restoration practice entails removing a perturbation as well as allowing ecosystem to recover via natural ecological processes. However, this practice is not all the case in the sense it depends on the degree of perturbation severity. The main objective for population-level restoration projects may involves the reintroduction of a species that has been extirpated, in that sense the restoration of important habitat components for desirable species or the demographic and genetic augmentation of an existing but reduced population (Falk et al., 2006). Moreover, in severely degraded ecosystems, the existing plant species would not be suitable for the restoration of the degraded ecosystems and as a result plant species identified to be suitable ecosystem succession phase should be introduced. To accelerate the restoration of habitats or to re-establish locally extinct populations species introduction is a widely used method. Again, one aspect to look at is a genetic differentiation and sometimes strong adaptation to local environmental conditions, the origin and quality of source populations must be cautiously measured if re-introduction is to be successful. To this end, the distribution

of genetic variability within and among populations should be taken into account. According to Rice and Emery, (2003), the challenge of restoration ecology is to make use of sufficient diversity so as to allow adaptation to new situations, while avoiding the severe effects of introducing genotypes that are poorly adapted to the environment (Rice and Emery, 2003). The highest priority for a restoration project in severely degraded ecosystems project is the establishment of a functional plant community for which tolerance of extreme conditions may be paramount (Stockwell et al., 2006). In restoration of an ecosystem, the concerns not only with the overall degree of variability, but also with its particular geographic distribution and phylogenetic lineage (Falk et al., 2006). According to (Fenster, 1991) the most appropriate approach is to specify a geographic range within which source material should be collected as population closer to another and growing under similar conditions will be more similar genetically owing to ecotypic variation and to the effect of gene flow (Govindaraju, 1990).

The collections of genotypes for use of regional mixtures moderately adapted to the general environment and within a broad geographic zone seem to be a good strategy in restoration projects (Knapp and Dyer, 1997). To this end, a wide genotypic variation in natural plant populations must be recognized and exploited. This ensures that genotypes used on a site are suitably adapted to local conditions and have a greater probability of survivorship than arbitrarily chosen material. According to (Handel et al., 2004), certain unusual genotypes can be located using the principles of evolutionary ecology and can be installed in areas with extreme conditions (SER, 2010) suggested that, the use of non-local source populations compromise the success of restoration measures as well as having negative consequences on existing populations, potentially disrupting ecosystem function. The use of tolerant material in the restoration of degraded lands was pioneered by Smith and Bradshaw (1979) and the authors collected seed from metal-tolerant species that had naturally evolved tolerance on metalliferous mining sites. These species that is *Agrostis capillaris* and *Festuca rubra* are faster colonisers, persisted longer and produced a better stabilising cover than non-tolerant commercial varieties in contaminated sites. According to (SER, 2010), many studies have been useful to riparian and salt marshes vegetation. The riparian and coastal areas and their associated characteristic vegetation play significant environmental roles, like filtering pollutants, stabilising soil against

erosion and functioning as a refuge and ecological corridor for plant communities and wildlife. These areas do not have a linear structure; however they show a specific down-stream/up-stream organization shaped by ecological processes which change gradually from spring to estuary. Most of the variability is due to salinity, flood frequency and soil type, factors that can vary along gradients and therefore affect plant growth (Howard, 2010). As a result, the collection of more tolerant genotypes/ecotypes along the gradients is at these present necessary for the future recovery of these areas which might be severely threatened by climate change. The growth rate under salt and flooding stresses varies between species and source populations and for that matter must first be evaluated before recommending species for restoration. Lessmann et al. (1997) did evaluation on the intraspecific variation in leaf elongation and biomass partitioning in response to flooding stress in populations of *Spartina alterniflora*, *S. patens*, and *Panicum hemitomon*, found that the analyses of the parameters showed a significant ecotypic differentiation in biomass partitioning. Hester et al. (1998) did similar study in order to highlight biomass and leaf morphological variations of different genotypes of *Panicum hemitomon* and *P. alternifolia* under salt stress. Currently, Aschenbach (2006) found a variation in growth rates under saline conditions of *Pascopyrum smithii* and *Distichlis spicata* of populations gathered in the areas in Kansas and Nebraska. The riparian and coastal vegetation in the Mediterranean basin has developed stressing adapted mechanisms to overcome changes in temperatures, water availability and salinity. Clearly, the rivers in the Mediterranean are characterised by a scarce and irregular water flow both in time and space. According to (Thuiller et al., 2005) the species that colonise these areas are likely to be well adapted to future conditions caused by the global warming effects. However, their capacity for natural survival as well as genetic biodiversity can be harnessed to sequester a significant part of the high atmospheric CO₂ content by increasing their plantation in presently unutilized arid/flooded areas, where there might be present of saline water or reused urban and industrial water waste. (Han et al., 2007) suggested that forestation and reforestation constitute a low-cost option of carbon dioxide sequestration with significant economic and social benefits. These are forest products, improved soil and air quality, reduced erosion and improved ecosystem health. In areas of particular natural values, as reserves and parks, this alternative should be oriented to protect and preserve the natural habitats and species

biodiversity. The reforestation of over-exploited soils in these areas should be made with local species in mixed plantations. To this end, the characterization of tolerant species and genotypes in the Mediterranean basin is becoming more important for the restoration of habitats which might be affected by drought, salinization as well as flooding with fresh and saline water as a consequence of climate change

2.1.5 EFFECTS OF HIGH SALT CONCENTRATIONS OF THE SOIL ON PLANTS

According to (Niu et al., 1995), the presence of sodium ions and chloride ions in soil in higher concentrations are extremely toxic for plants for the reason that their effect on potassium ions nutrition, cytosolic enzyme activities, photosynthesis and cellular metabolism. Among the different effects of salt stress some are as follows:

2.1.6 Stomatal closure

The closure of stomata of plants is caused by salt stress thereby leading to the reduction in the availability of carbon dioxide in the leaves as well as inhibiting the carbon fixation (Prida and Das, 2005). This brings about the exposure of chloroplast to excessive excitation energy which in turn could result the generation of reactive oxygen species (ROS) causing oxidative stresses (Parvaiz and Satyawati, 2008)

2.1.7 hyper osmotic shock

According to (Borsani et al., 2001) the presence of high salinity level of the soil may lead to oxidative stress as well as hyper osmotic shock for the plants leading to the loss of cell turgor.

(Bursens et al., 2000) suggested the inhibiting cell division. Salt stress may affect the expression of cell cycle progression genes thus affecting cell division and cell expansion leading to growth inhibition

2.1.8 Inhibiting photosynthesis

The inhibition of photosynthesis of plants is caused by salt stress for several reasons. For example, the effect of salt stress on the efficiency of translocation as well as assimilation of photosynthetic product and stomata closure (Xiong and Zhu, 2002).

2.1.9 Nutrient imbalance

According to (Serrano et al., 1997), the detrimental consequences of high salt concentrations of soil include ion toxicity and nutrient imbalance. The presence of high concentration of Na⁺ ion in the soil reduces the amount of available K⁺, Mg⁺⁺ and Ca⁺⁺ (Epstein, 1972) hence, leading to nutrient imbalance.

2.2.0 Osmotic effect

The water absorption of plants in soil with excess salts may affect the plant growth, flowering and fruiting of the plants. This happens because during salt stress plants must increase the energy that they get bigger to obtain water from the soil, under such conditions a plant eventually may die (Blaylock, 1994).

2.2.1 Toxicity

According to (Blaylock, 1994), certain elements like boron, sodium, chlorides and iron may have great toxic effect on the plant and if the plants are sensitive to these elements, they may be easily detrimental at relatively low levels of these ions in the soil. In various reactions K⁺ ion is used as a cofactor and Na⁺ ions interfere with the function of potassium hence causing a direct toxic effect on plant. In addition to this the other detrimental effects of Na⁺; however, seem to be similar to the structural and functional integrity of membranes (Kurth et al., 1986)

2.2.2 Effects of salinity on plant yield

Plants that are grown under high salt concentrations is adversely affect the elongation process of new cell because the excess salt modifies the metabolic activities of the cell wall leading to the deposition of various materials which causes a reduction in the cell wall elasticity. The secondary cell wall rapidly as a result of which cell wall becomes rigid hence causing a decrease in turgor pressure efficiency in cell wall enlargement (Ali et al., 2004). The high salt concentrations of the soil also bring about shrinkage of the cell contents as well as reduction in the development and differentiation of the tissues unbalanced nutrition, damage of membrane and disturbed avoidance

mechanism. All these factors contribute towards the reduction in plant yield (Ali et al., 2004)

2.2.3 MECHANISM OF SALT RESISTANCE IN HALOPHYTES

According to (Yeo, 1983), the salt resistance is basically a reaction between an organism and the salt stress. Ideally, plants generally respond against salinity at two levels and this is physiological or molecular level (Munns and Tester, 2008). According to (Hasegawa et al., 2000) the physiological mechanisms that are involved in providing resistance against salinity and drought stress are related, as the concentrations of salt in the soil is increased the availability of water in the soil is decreased causing reduction of the water potential leading to the shortage of water available to the plants. (Pastori and Foyer, 2002) suggested the adaptations to the stresses to these mechanisms can either be done by the pre-existing or either by induced defences. The mechanisms for the salt resistance in halophytes generally fall into two main categories and this is salt tolerance as well as salt avoidance Sabovljevic and Sabovljevic (2007). One additional feature against salinity of the soil includes the modifications at cellular level which involves certain mechanisms operating at molecular level and germination responses in case of young seedlings.

2.2.4 Salt tolerance

According to (Sabovljevic and Sabovljevic, 2007), salt tolerance strategy involves certain physiological or biochemical adaptations in the plants which basically aid the plant to maintain protoplasmic viability as the ions accumulate inside the cells. In order to achieve salt tolerance by either salt exclusion or salt inclusion, salt tolerant organisms make use of energy. (Ashraf et al., 2006) suggested that these species utilise the energy for the exclusion of excess salt from them so as to protect themselves from toxic effects of high salt content of the soil as for example protein aggregation etc

2.2.5 Aspects of salt tolerance

There are three important interconnected aspects of plant activity to achieve the salt tolerance. According to (Zhu, 2001), salt tolerance employs to both types of the plants either with or without salt glands. These aspects are: Detoxification: The reactive oxygen species (ROS) are generated as the result of salt stress which may cause detrimental damage to the cellular components of the cells. The plants exposed to salt stress get relieve of themselves from these reactive oxygen species by the production of stress proteins and compatible osmolytes. A lot of these enzymes and proteins with unknown function are believed to scavenge the ROS (Zhu et al., 1997). Various examples of osmolytes include proline, glycine, betaine etc

2.2.6 Homeostasis

Homeostasis restoration is another strategy that plants adopt against high salt concentration of the soil. The inhibition of enzymatic activity is one of the toxic effects of higher concentrations of sodium ions in the soil and for that reason it necessary that the concentrations of sodium ions within the plant cell cytoplasm and organelles should remain low (Zhu et al., 1997). In addition (Amtmann and Sanders, 1998) suggested that the several non-selective ionic channels present in the cells may be responsible to mediate sodium ion entry in the plant cell. In the cytosol Plants accumulate various compatible osmolytes, as a result lowering the osmotic potential to sustain water absorption from saline soil solutions (Zhu et al., 1997). According to (Chrispeels et al., 1999) the present of some water channel proteins across the cellular membrane may also involve in controlling water flux across membrane. In term of growth regulation, most of the plants slow down their growth rate during stress as it allows plants to relay on multiple resources to combat the stress (Zhu, 2001). During stress, there is inadequate photosynthesis which occurs due to the stomata closure leading to the limited carbon dioxide uptake causing the reduction in the growth rate in plants (Xiong and Zhu, 2002). Furthermore, CBF1, DREB1A (Liu et al., 1998) and ATHB7 (Soderman et al., 1996) are the genes which are expressed during stress only. The proteins which are produced by them affect cell division and expansion machinery, leading to growth regulation under stress (Zhu, 2001).

2.2.7 Salt avoidance

According to (Allen et al., 1994) salt avoidance is basically a phenomenon in which plant tries to keep away the salt ions from those parts of the plants where they may be toxic or harmful. Salt avoidance involves certain physiological and structural adaptations so as to reduce the salt concentrations of the cell or physiological exclusion by root membranes. (Allen et al., 1994) suggested that this may passive exclusion of ions by means of a permeable membrane, and the active expelling of ions by means of a pump or dilution of ions in the tissues of the plants.

Salt avoidance in halophytes can be seen in four main methods.

These are as follow:

2.2.8 Exclusion:

According to (Waisel et al., 1986), the easiest means of surviving of halophytes in high salt concentration is salt exclusion. With regards of mangroves, about 99% of the salts are excluded through the roots (Tomlinson, 1986). In addition, (Flowers et al., 1986) is with the view that exclusion of salt regarding a whole plant level occurs at the roots and the casparian strips may play role in salt exclusion from the inner tissues

2.2.9 Secretion

The specialized holophytes salt glands present are responsible for the secretion of excess salt from the plant (Weber, 2008). This is because the water in the plant evaporates through these salt glands and as a result the salt remains on the leaf surface forming crystals. These crystals are then blown away through wind or by rain (Lipshitz et al., 1974). Furthermore, (Waisel et al., 1986) emphasize that salt secretion is also called excretion and is one of the common ways to salt avoidance. The salt glands or through the cuticle or guttation fluid are ways to which salt can be released by plants. According to (Stenlid, 1956), It can then be re-transported to the plant through the phloem or become concentrated in the salt hairs. Salt glands are found either on the epidermis or may be found as depressed into it. They are more concentrated in leaves but are found on every aerial part of the plant. These glands are rich in mitochondria and other organelles but short of a central vacuole, so they are the transit cells not storage cells (Waisel, 1972).

2.3.0 Shedding:

The shedding of the old leaves of the plants according to Albert (1975) in some of the plants, which are grown under high salt concentrations, is a strategy to avoid the toxic effects of excess sodium salts, which are accumulated in leaves. (Chapman, 1968) also suggested that some of the halophytes release excess of salt through the discard of salt saturated organs.

2.3.1 Succulence:

The term succulence stands for a plant condition that involves increasing in cell size and decreasing in the growth extension. Again, succulence also involves a decreasing in the surface area per tissue volume as well higher water content per tissue volume (Flowers and Yeo, 1986).

According to Drennan and Pammenter (1982), the leaves of succulent plants are very thick, their mesophyll cells are increased in size and they have smaller intercellular spaces as compared to the plants devoid of succulence. Such leaves have extra mitochondria and are comparatively larger showing that some extra energy is essential in these plants for the salt compartmentalization and excretion (Siew and Klein, 1969). Succulence brings about an increase in cell size, reducing in extension of growth, decreasing surface area per tissue volume leading to higher water content per unit surface area and aids the plant to manage with salinity stress (Weber, 2008). For the duration of salinity some of halophytes (mostly halophytes of the deserts) experience succulence and this feature is of adaptive value for survival under stress (Waisel, 1972).

2.3.2 Stomatal response:

The sodium ions damaged the glycophytes stomatal function, and this disruption can be seen as a mechanism of their lack of survival in saline conditions (Robinson et al., 1997). According to (Robinson et al., 1997) stomatal response of plant to salinity are in two divisions, either the guard cells can make use of potassium to achieve their normal turgor regulation in place of sodium or the guard cells may use potassium to limit their intake of sodium. This type of mechanism is more necessary in those halophytes that lack glands.

2.3.4 CELLULAR ADAPTATIONS OF THE PLANTS AGAINST SALT STRESS SYNTHESIS OF COMPATIBLE SOLUTES (OSMOLYTE PRODUCTION)

Osmolytes are the organic compounds that have an effect on osmosis and play significant role in maintaining fluid balance as well as cell volume. The external osmotic pressure causes the burst of a cell and under this situation certain osmotic channels, may get open which allow the efflux of certain osmolytes through them as they move outside, they carry water with themselves preventing the cell from bursting out. According to (Rhodes and Hanson, 1993), Sugars, alcohols, amino acids, polyols, tertiary and quaternary ammonium and sulphonium compounds are different examples of osmolytes. As a result of the increase of salt contents of the soil, the flow of water in the direction of the roots of the plants is decreased causing a reduction of the cell membrane permeability (Waisel, 1972). Under such a circumstance osmotic adjustment of the plant cells is required. Plants carry out this adjustment by the synthesis of compatible solutes called osmolytes. Which play a function in the reduction of oxidative damage that may occur as a result to the production of ROS under salinity stress as well as they protect sub-cellular structures (Hare et al., 1998). Some osmolytes and their roles in stress are as follows:

2.3.5 Proline analogues

Naidu (2003) reported that production of proline analogue in Australia has resulted to some of the halophytes to cope with high salinity. *Melanleuca* species as for example *Melanleuca bracteata* which accumulate the proline analogue 4-hydroxy-N-methyl proline (MHP), (Bohnert and Shen, 1998) suggested such proline analogues increase the ability of plants to survive during salinity stress due to their ability to cause regulation, compartmentalization, and production outlay.

2.3.6 Aquaporin

According to (Maurel, 1997), aquaporin is another type of osmolyte, which believed to be involved in intracellular compartmentalization of the water. These opening forming proteins in halophytes carry out the water molecules. (Maurel, 1997) also indicates that the gating of water channels could have an impact on inter

compartmental movement of water. Such aquaporins are believed to play some role in salt tolerance by maintaining osmotic homeostasis and turgor of the plant cells under salt stress.

2.3.7 Glycine betaine (GB):

According to (Wyn and Storey, 1981), the GB is basically a quaternary ammonium compound which acts as an osmo-protectant and can offset the high salinity concentration in the vacuole. Besides, the stabilizing osmolyte play a role of protection of macromolecule of the plant under dehydration stresses ((Yancey, 1994). In addition, the GB is not found to accumulate in crops during stress, however, is generally found in halophytic members of Poaceae and Chenopodiaceae (Flowers et al., 1986).

2.3.8 Protection of cell wall integrity

According to (Iraki et al., 1989), in order to keep the cell growth during salt stress, cell wall properties maintenance such as permeability is required, and which is important for salt tolerance.

TPX2 is cell wall peroxides found in tomato, it's over expression increases the germination rate under salt stress. This piece of information indicates that the protection of cell wall integrity during stress assists the plant cell to retain water which protects the cell under stress (Amaya et al., 1999).

2.3.9 ION COMPARTMENTALIZATION AND SELECTIVE TRANSPORT AND UPTAKE OF IONS AT THE PLASMA MEMBRANE

According to (Borsani et al., 2003), when the concentration of salt outside a medium is much more as compared to the ones inside of the cell, more amount of salt move inside the plant body and as a result if this condition persists may lead to high amount of salt depositions inside the shoot therefore halophytes must have the capacity to keep the salt concentrations within their body low.

The increasing sodium efflux at the plasma membrane and by the accumulation of sodium in the vacuole (Zhu, 2000) is how plant cells respond to high salt concentrations of the soil. Moreover, in order to attain the compartmentalization of

sodium and chloride in the vacuole, sodium and the chloride ions are transported actively in the vacuole and if the tonoplast permeability to these ions is relatively low so that an ion concentration gradient can be sustained at an energy cost that can then be prolonged for months (Maathius et al., 1992). Halophyte tonoplast channels must for that reason be modified either to be increasingly discriminating against sodium and chloride, or the channels stay closed for the greater part of time, or to have a decreased number of channels per cell. (Flowers and Yeo, 1986; Cheeseman, 1988) came out with that halophytes usually utilize the control accumulation and sequestration of inorganic ions for the adjustment of osmotic potential of their internal tissues to the external salinity. In addition the way to which halophytes accumulate ions and the degree of salt tolerance is widely different among halophytes (Glenn and O'Leary, 1984; Glenn et al., 1996). Through the intracellular compartmentalization of ions cells are able to increase salt levels in the vacuoles thus preventing the high levels of salts in the cytoplasm (Gorham, 1995).

2.4.0 Molecular mechanisms involved in providing resistance against salinity in plants:

According to Xiong and Zhu (2002), the regulation of the expression of certain genes is one of the important strategies against high salt concentration. Salt stress regulating certain genes belongs to different groups based on their function.

These genes encode:

1. LEA protein (late embryogenesis abundant proteins)
2. Enzymes (involved in biosynthesis of osmolytes, hormones, detoxification, and general metabolism),
3. Transporters (ions transporters, ABC that is, ATPbinding cassette transporters, and aquaporins),
4. Regulatory molecules such as protein kinases and phosphatases.

The most widespread and the most significant stress regulated genes are LEA-like genes or LEAs. LEA genes encode LEA proteins or late embryogenesis abundant proteins (Baker et al., 1988). Even though, these genes have an extensive occurrence but the purpose of this group of genes are still not well defined except in some cases

where the over expression of individual LEA genes resulted in some degree of stress protection (Xuet al., 1996). According to (Liu et al., 1998) the expression of transcription factor that control the expression of LEA-like genes has been improved under stress in transgenic plants, it indicates that these proteins do have protective affect against abiotic stress (Liu et al., 1998). Nevertheless, the reality that these genes are not expressed under normal growth, but they are only expressed during stress (salt drought or low temperature stress) bring to bear that their products have some role in protecting the cellular structure during stress. One major hypothesis is that these genes product may operate as chaperon hence protecting the denaturation of some important proteins of the cell (Xiong and Zhu, 2002). In high salt concentration in the halophytes, a large number of enzymes are believed to be involved in providing tolerance against it. Such enzymes are identified to be sensitive against sodium chloride. When the concentration of sodium chloride is increased in *Suaeda maritima* observation the activity of various enzymes is inhibited (Munns et al., 1983) includes those enzymes that are involved in protein synthesis and it happens at 200 to 400 mM NaCl concentrations. There is above all very little evidence that an intrinsic difference exists between the enzymes isolated from halophytes and non-halophytes. ROS species is produced under salt stress conditions in plants. These ROSs have the capacity to interrelate with the cell membrane and other cellular components of the cell resulting to the damage to these cellular components. Plants contain diversity of antioxidants and antioxidant enzymes which are accountable for maintaining the level of ROSs relatively low (Gaoet al., 2008).The plants enzymes responsible in protecting the cell from oxidative damage (Mittler, 2002) include superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT). In different reports the role of CAT, POD, SOD have been reported in providing resistance during salt stress by preventing oxidative damages to the cell (Rahnama and Ebrahimzadeh, 2005). The presence of antioxidant enzymes can be the most important strategy of plants grown under high salinity levels on the basis of these findings.

The role of some of the antioxidant enzymes is given below:

1. SOD can simply repair the damage that is caused by ROS. SOD is one of the necessary enzymes responsible for the maintenance of normal physiological conditions of the plants and thus coping with the stress (Mittler, 2002). A large

number of studies have been carried out which bring about the positive correlation between the salt stress and level of SOD (Badawiet al., 2004; Shalata and Tal, 2002; Al Scher et al., 2002).

2. In the family of higher plants POD has wide distributions. (Passardi et al., 2005) stated that lignifications, oxidative metabolism, salt tolerance and heavy metal stress are various roles POD plays. The increased POD action is believed to be responsible in providing an antioxidant mechanism during the conditions of salt stress (Gao et al., 2008).
3. The most active enzyme in providing resistance against oxidative damage in the plant is CAT.

According to (Mittler, 2002) the enzyme necessary brings out the degradation of hydrogen peroxide into water and oxygen. The CAT activity may rely upon the species as well as the developmental and metabolic state of the plant as well as duration and stress greatness and hence varies (Chaparzadeh et al., 2004).

2.4.1 WHOLE PLANT LEVEL ADAPTATIONS OF HALOPHYTES AGAINST SALINITY

GERMINATION RESPONSES

A seed sown in the soil with high salinity level of soil according to Pollack and Waisel (1972), then the young seedling may face one of the following two dangers:

1. High osmotic potential of the surrounding medium may prevent the embryo from taking up of water
2. The toxic effects of some of the ions may cause poisoning of the embryo.

Under high salinity level in the soil both grown halophyte and glycophyte behave in a similar manner. According to (Ungar, 1996) both of them may cause delay in the germination as well as reduction in the seed number. It has been identified that NaCl inhibits the germination of many plants and even some halophytes. Laboratory investigations suggest that seeds of most halophytic species reach maximum germination in distilled water (Ungar, 1982). During the spring or in a season with high precipitation seed germination in saline environments usually occurs, when soil salinity levels are usually reduced (Ungar, 1982). Halophytes germinate better under

saline conditions but the specific mechanism at germination stage needs investigation if this exact mechanism is being defined that will be extremely beneficial in improving the crops resistance against salinity.

2.4.2 GENETIC MODIFICATION OF PLANTS TO MAKE THEM SALT RESISTANT

To make plants salt tolerant, plants can be modified genetically. According to (Winicov and Bastola, 1997), the transgene of tomato have been inserted into its genome successfully and the main target was that tomato plant should be able to survive under salt stress while the taste must not be affect, although not much success in this regard has yet been achieved. Classical breeding for salt tolerance has been tried but that was also not much successful. The alternate strategy now a days or modern times is to produce the salt tolerant plants through genetic engineering which is under consideration and genes which are necessary for salt tolerance are under investigation (Borsani et al., 2003). Grass has been made salt tolerant by transforming it with rice vacuolar membrane Na^+/H^+ anti porter gene via the *Agrobacterium*-mediated transformation. The resultant plant species has a better salt stress tolerance (Wu et al., 2005). Besides the advantage of transgenic salt tolerant plants, they also have resistance against other type of stresses for example chilling, freezing, heat and drought (Zhu, 2001)

CHAPTER THREE

3.0 Materials and Methods

3.1 Experimental Design and Procedures

The cuttings of *Tamarix africana* and *Tamarix gallica* genotypes were collected on the mouth (40°20'46'' N, 16°48'46'' E;) and on the riverside (40°27'00'' N, 16°31'44'' E;) of river Basento. Similarly, collection was made on the mouth (37°24'01 N'' N) and on the riverside (37.400°N 15.100°) of Simeto. In all 20 cuttings of genotypes was made, of which was made up of 10 each of *Tamarix africana* and *Tamarix gallica*. The two provenances of the *Tamarix africana* and *Tamarix gallica* was made up of five genotypes each.

The genotypes were replicated three times through cuttings; each replicate weight was taken with dimension of 10-15 cm in length. The average weight of the cuttings for *Tamarix africana* (Basento) was 13.9g, *Tamarix africana* (Simeto) 19.5g, *Tamarix gallica* (Basento) 13.8g and *Tamarix gallica* (Simeto) 13.9 g. The average diameter of the cuttings of *Tamarix africana* (Basento) was 6.89 mm, *Tamarix africana* (Simeto) 8.25 mm, *Tamarix gallica* (Basento) 6.97 mm and *Tamarix gallica* (Simeto) 6.95 mm. The cuttings were singularly planted in 1.6 dm³ plastic pots (11 * 11 * 22 cm) containing sand (35%) and standard organic matter (coconut fiber) (65%). The cuttings were grown in a greenhouse for 87 days in the pots and subsequently inserted into three plastic boxes (120 *100 * 0*58 cm). A modified half-strength Hoagland solution was supplied two weeks before the beginning of the experiment. The three boxes were divided into three blocks, each composed of three treatments. The treatments consist of control, 200 mM of saline (moderate) and 550 mM of saline (high).



Figure. 1 Map of Italian *Tamarix* species collection site of Basento and Simeto

3.2 Gas exchange measurements

The measurements of *Tamarix* Species using the gas exchange were performed on the twigs because of the presence of characterized scale-like leaves. Leaf gas exchange was measured on the tenth twig from the apical bud from the dominant sprout (time 0; time 1; time 2;). A portable infrared gas analyser (LI-6400, LI-COR Biosciences, Inc., Lincoln, NE, USA) equipped with a conifer chamber (LI-6400-05) was used to measure the gas exchange. The cuvette temperature at 25 °C, photo flux set at 1,500 $\mu\text{mol m}^{-2} \text{ s}^{-1}$ while the air flow was adjusted (400–600 $\mu\text{mol s}^{-1}$) to maintain a constant VPD ($1.5 \pm 0.2 \text{ kPa}$).

Photosynthesis (A) change in response to intercellular [CO_2] variation (C_i) was determined for ambient CO_2 concentration values (C_a) values (C_a) of 400, 300, 250, 200, 150, 50, 400, 500, 650, 750, 850 and 1000 $\mu\text{mol mol}^{-1}$ as suggested by (Long and Bernacchi 2003); the photosynthesis values were recorded as soon as C_a was stable (cv <0.7%) (Ainsworth et al. 2002). Net assimilation rates (A400) and stomatal conductance (gl400) measured at a C_a value of 400 $\mu\text{mol mol}^{-1}$ were considered as the assimilation and the stomatal conductance at growth-chamber CO_2 concentration. Intrinsic water use efficiency (A/gl) was calculated as the ratio between A400 and gl 400. The maximum carboxylation rate ($V_{c\text{max}}$) and the maximum rate of electron transport (J_{max}) were estimated according to (Farquhar et al. 1980), Following A– C_i curves and 5 minutes of adaptation to dark conditions, dark respiration (R_d400) was measured at a C_a value of 400 $\mu\text{mol mol}^{-1}$. After gas exchange measurements, the twigs were cut off, and their fresh weight was determined. The twigs were then scanned and the images analysed by the software Skyroot (Llandrindod Wells, Powys, UK) in order to obtain the total twig length. The twig area was estimated by multiplying the twig length by the measured mean twig diameter (0.7 mm). A small twig portion was dried at 70 °C for dry weight estimation, while the rest of the twig was dark-adapted for 15 minutes for chlorophyll fluorescence measurements.

3.3 Chlorophyll fluorescence measurements

Chlorophyll fluorescence was measured with a PAM 2000 fluorimeter (Walz) on the same samples used for gas exchange measurements. Small portions of the twig were placed close to each other to fill the entire surface of the fluorimeter clip. The

photochemical efficiency was estimated from the quantum yield of PSII in dark-adapted twigs (F_v/F_m). Additional far-red light (735 nm) was used to estimate ground state fluorescence (F_0). The fluorescence yield (FPSII; i.e. quantum yield of PSII in the light) was measured with a saturating pulse of white light.

3.4 Growth measurements and Biomass

Plant growth was measured periodically in the greenhouse. The mark diameters of the three dominant shoots of a species was determined using a digital calliper (0-150mm) (Stainless Hardened). The estimation of the cutting diameters was always carried out in the same point, highlighted previously by a sign during the first data collection campaign. Measuring tape was used to measure the height of the species.

At the end of the experiment, the species were harvested and separated into leaves, wood and root biomass. The dry weight (DW) was obtained after oven drying at 70°C until a constant weight was reached

3.5 Salt accumulation outside the twig

The twigs were then inserted into plastic tubes containing 25 ml of deionized water and shaken at 500 rpm for 20 minutes. Filter paper was used to separate the liquid phase of the twigs. The electrical conductivity of the liquid phase was measured using a conductometer (HI9811, Hanna Instruments, Inc., USA) equipped with an electrode probe (HI1285, Hanna Instruments, Inc., USA). The measured electrical conductivity values were converted in g salt g⁻¹ twig dry weight to determine the amount of salt secreted and accumulated outside the twig.

3.6 Statistical analysis

An analysis of variance (ANOVA) was performed on all the analysed growth parameters as well as physiological parameters, using statistical graphics software package (Systat 14.0) and Prism 5 software (GraphPad Software, Inc.). The post hoc analysis was performed using Fisher's LSD test, Dunn's Multiple Comparison test and Bonferroni's test to evaluate the main effects of treatment, species, time and their interaction among species at different provenances. Significance was considered for p values <0.05.

CHAPTER FOUR

4.0 RESULTS

4.1 Cumulative shoot length of *Tamarix africana* and *Tamarix gallica* under different saline conditions

There were significant differences among treatments ($p < 0.0007$) in *Tamarix africana* as well as *Tamarix gallica* ($P < 0.0391$), in both provenances, on cumulative shoot length (Figure 2(a) (b))

Under control treatment after 171 days, *Tamarix africana* of Simeto increased 22.2% more cumulative shoot length than *Tamarix africana* of Basento ($p < 0.05$) (Figure 2(a)).

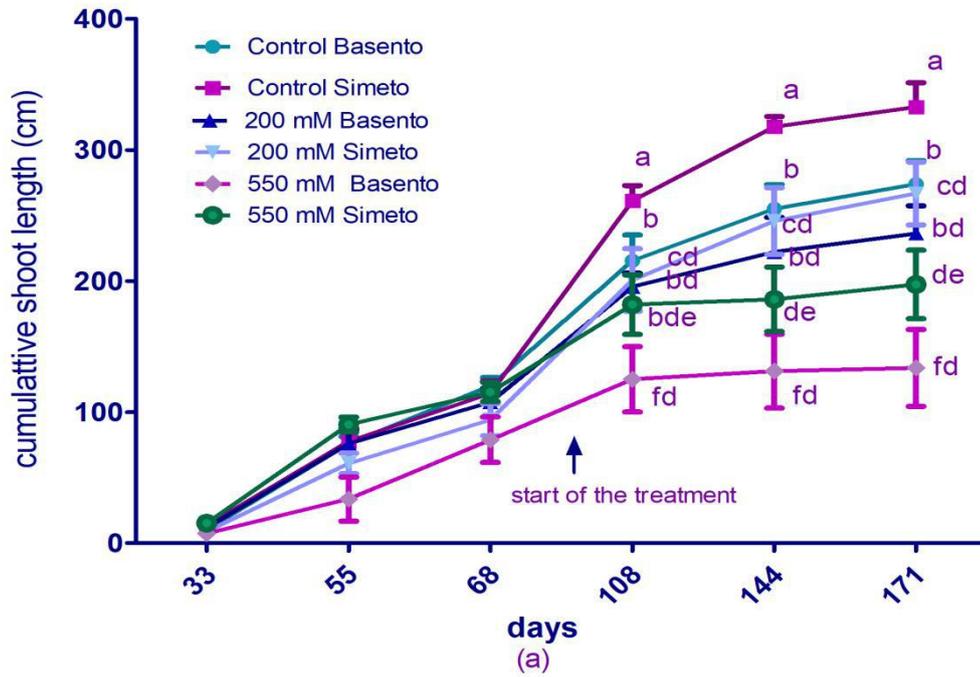
Under the moderate saline treatment of 85 days of 171 growth, no significant differences were found between the two population.

Under high salinity treatment, *Tamarix africana* of Simeto increased 10.4% more cumulative shoot length than *Tamarix africana* of Basento ($p < 0.05$)

In figure 2(b) under non-saline treatment, no significant differences were established amongst the *Tamarix gallica* population of Basento and Simeto.

This is similar to under moderate salinity treatment. However, in high saline treatment, *Tamarix gallica* of Basento increased 22.1% cumulative shoot length more than *Tamarix gallica* of Simeto ($p < 0.05$).

T. africana (Basento and Simeto)



T. gallica (Basento and Simeto)

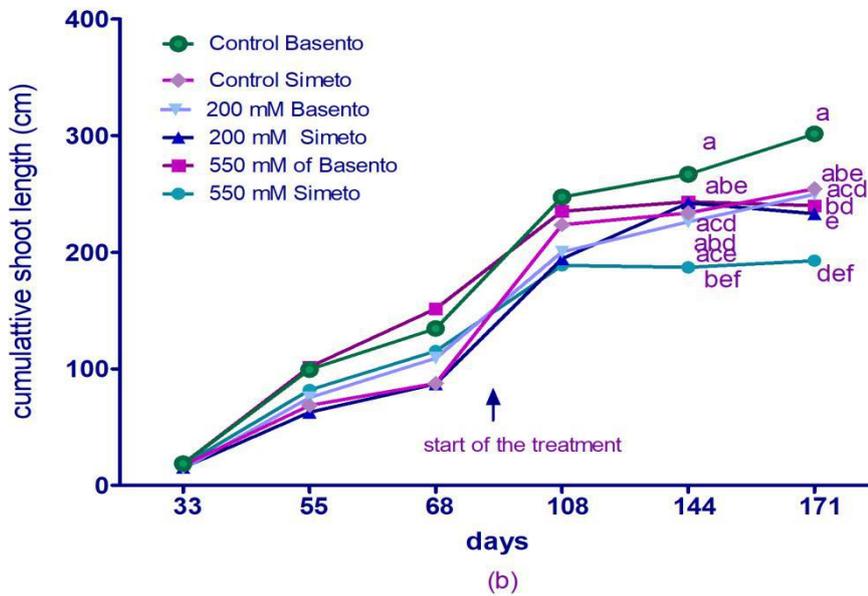


Figure 2. Cumulative shoot length (a) *Tamarix africana* of Basento and Simeto (b) *Tamarix gallica* of Basento and Simeto over time across saline treatments (control, 200 mM, and 550 mM). Different letters above the symbols indicate significant difference at $p < 0.05$

4.2 Dominant shoot length of *Tamarix africana* and *Tamarix gallica* under different saline conditions

There were significant differences among treatments ($p < 0.0001$) in *Tamarix africana* as well as *Tamarix gallica* ($p < 0.0006$), in both provenances, on dominant shoot length (Figure 3(a)(b))

After 171 days under control treatment, no significant differences were found between the two provenances.

Under the moderate saline treatment of 85 days of 171 growth, no significant differences were found between the two provenances.

Similarly, under high salinity treatment, no significant differences were established amongst the *Tamarix species* population at Basento and Simeto.

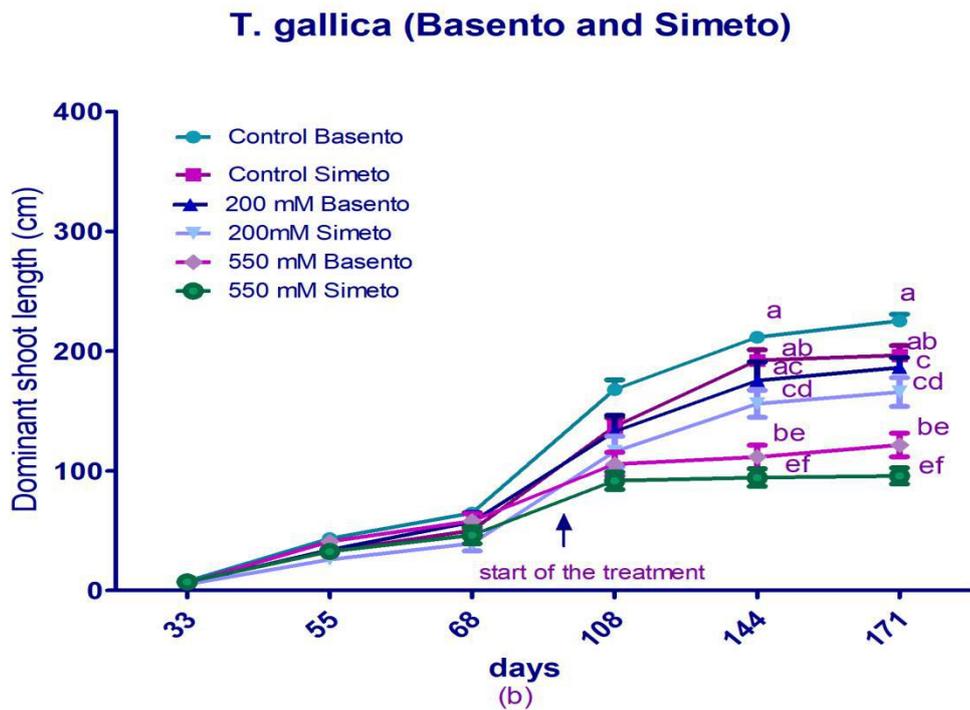
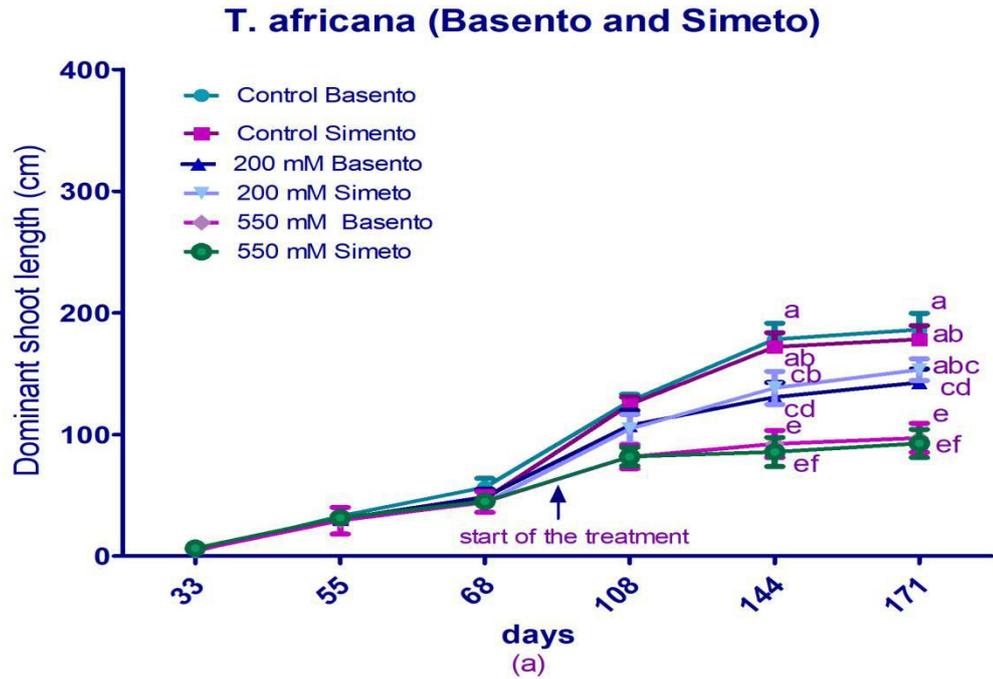


Figure 3 Dominant shoot length (a) *Tamarix africana* of Basento and Simeto (b) *Tamarix gallica* of Simeto and gallica over time across saline treatments (control, 200 mM, and 550 mM). Different letters above the symbols indicate significant difference at $p < 0.05$

4.3 Stem diameter of *Tamarix africana* and *Tamarix gallica* under different saline conditions

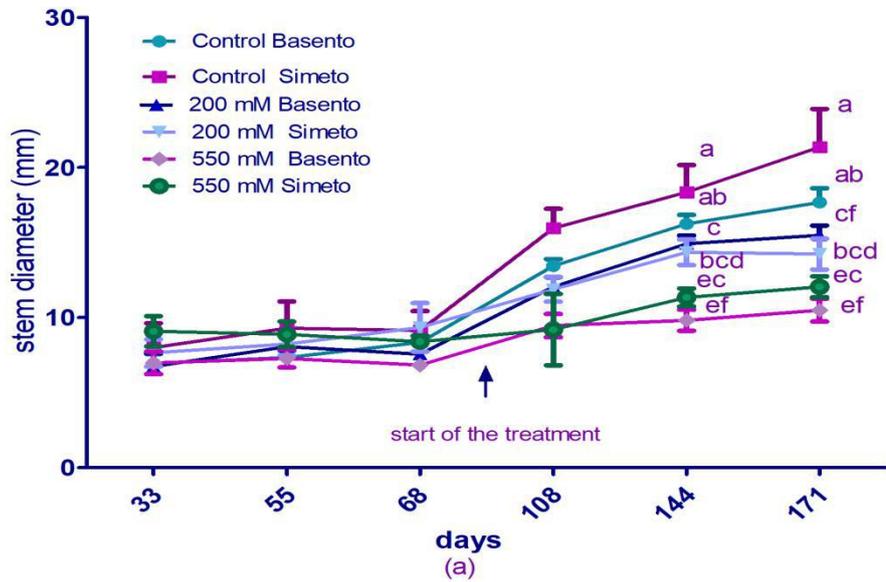
There were significant differences among treatments ($p < 0.0481$) in *Tamarix africana* as well as *Tamarix gallica* ($p < 0.0481$), in both provenances, on stem diameter Figure 4(a) and figure 4(b)

There were significant differences of variability of *Tamarix species*, treatment and time for stem diameter ($p < 0.05$) (Figure 4(a) and figure (b))

Under saline control treatment in figure 4(a) and figure (b). The growth of the stem diameter for 171 days showed no difference between the *Tamarix africana* and *Tamarix gallica* of Basento and Simeto.

Similarly, stem both moderate and high saline treatment did not affect the stem diameter of the two population.

T. africana (Basento and Simeto)



T. gallica (Basento and Simeto)

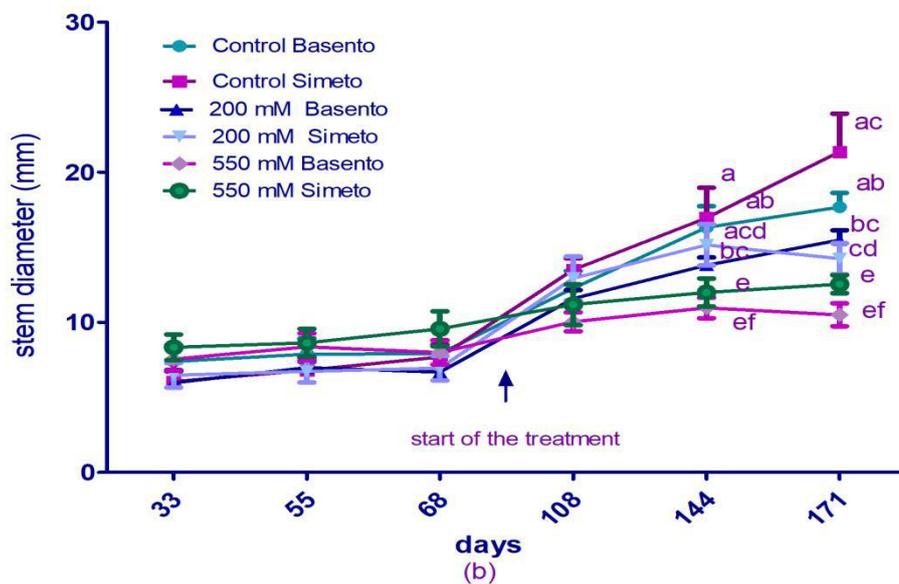


Figure 4. Stem diameter length (a) *Tamarix africana* of Basento and Simeto (b) *Tamarix gallica* of Simeto and gallica over time across saline treatments (control, 200 mM, and 550 mM). Different letters above the symbols indicate significant difference at $p < 0.05$

4.4 Biomass of *Tamarix africana* and *Tamarix gallica* under different saline conditions

There were significant differences among treatments ($p < 0.0001$) on leaf biomass, wood biomass, root biomass and root/shoot biomass of *Tamarix species* in Basento and Simeto provenances (Figure 5a-b)

The average leaves biomass of *Tamarix* species decreased from 13.6 g in control, to 11.9 g in moderate and 4.5 g in high salinity treatment (figure 5a). Under control, *Tamarix africana* increased 5.2% more in leaves biomass than *Tamarix gallica*. However, in moderate salinity *Tamarix gallica* increased 13.4% as well as 27% in high salinity in leaves biomass compared to *Tamarix africana*.

The average wood biomass of *Tamarix* species decreased from 83.5 g in control to 50.6 g in moderate and 20.2 g in high salinity treatment (figure 5b). Under control, *Tamarix africana* increased 2.3% more in wood biomass than *Tamarix gallica*. However, in moderate salinity *Tamarix gallica* increased 8% as well as 35% in high salinity in wood biomass compared to *Tamarix africana*.

The average root biomass of *Tamarix* species decreased from 51.3 g in control, to 44.3 g in moderate and 18.7 g in high salinity treatment (figure 5c). Under control, *Tamarix gallica* increased 7.5% more in root biomass and 13.5% as in high salinity treatment as compared to *Tamarix africana*. However, in moderate salinity *Tamarix africana* increased 2.5% more of root biomass compared to *Tamarix gallica*

However, average root/shoot biomass of *Tamarix* species increased from 0.52 in control, 0.79 in moderate and 1.03 in high salinity treatment (figure 5c). Under control, *Tamarix gallica* increased 28.7% more in root/shoot biomass and 10.8% in moderate salinity treatment as compared to *Tamarix africana*. However, in high salinity *Tamarix africana* increased 34% more of root/shoot biomass compared to *Tamarix gallica*

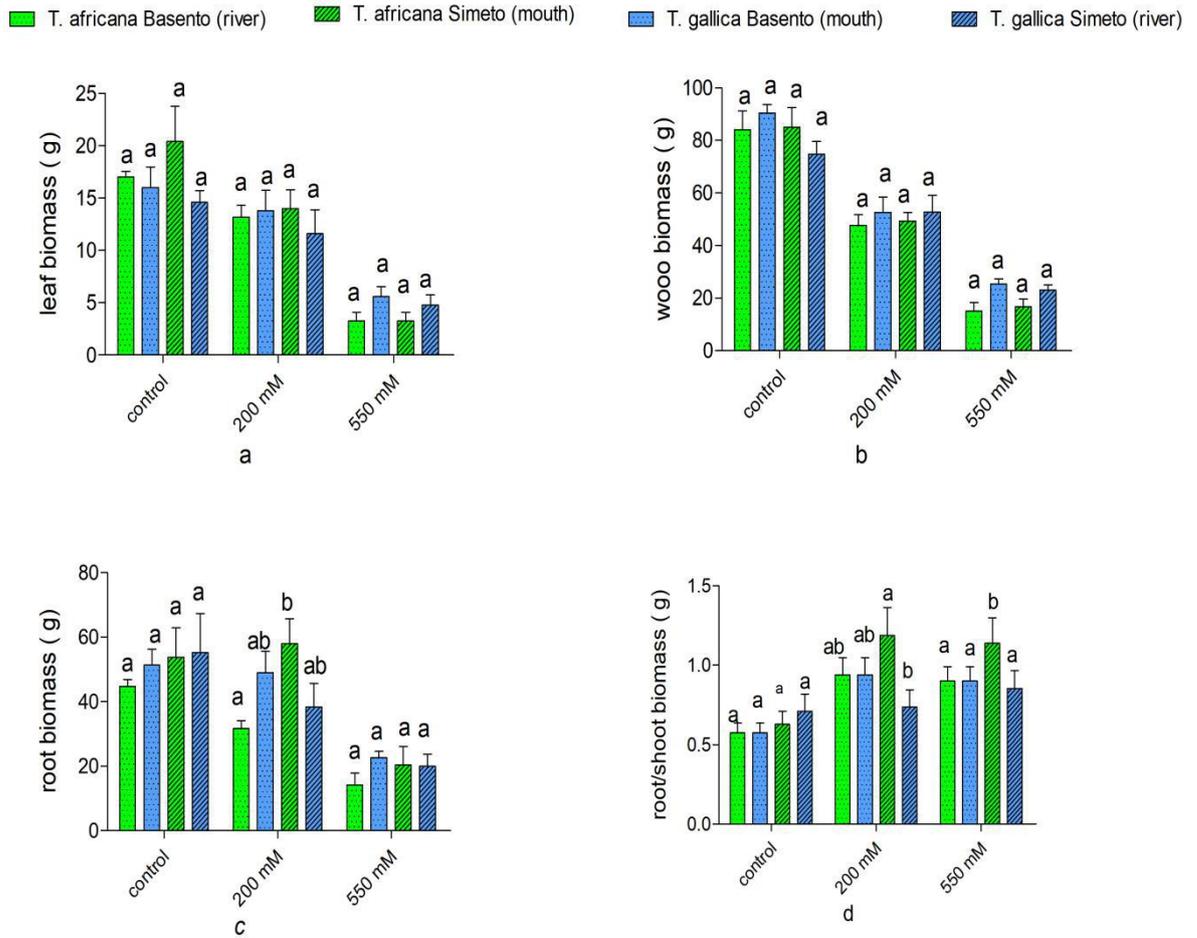


Figure 5.(a) leaf biomass (b) wood biomass (c) root biomass and (d) root/shoot biomass of *Tamarix* species under saline treatments (control, 200 mM, and 550 mM). Different letters above the symbols indicate significant difference at $p < 0.05$

4.5 Physiological performances of *Tamarix africana* and *Tamarix gallica* before saline treatment

Photosynthesis varied significantly among species and provenances in the morning, but unaffected at the midday figure 6(a) and figure 6(b). There was significant difference between *Tamarix africana* at Simeto and *Tamarix gallica* at Simeto in the morning ($p < 0.05$).

Stomatal conductance varied significantly under control in the morning, but unaffected in the midday figure 6(c) and figure 6(d) in *Tamarix* species at Basento and Simeto. There was significant difference between *Tamarix africana* at Simeto and *Tamarix gallica* at Simeto in the morning ($p < 0.05$).

Maximum carboxylation rate varied significantly under control in the morning, but unaffected in the midday figure 6(e) and figure 6(f) in *Tamarix* species at Basento and Simeto. There was significant difference between *Tamarix africana* at Basento and *Tamarix africana* at Simeto in the morning ($p < 0.05$).

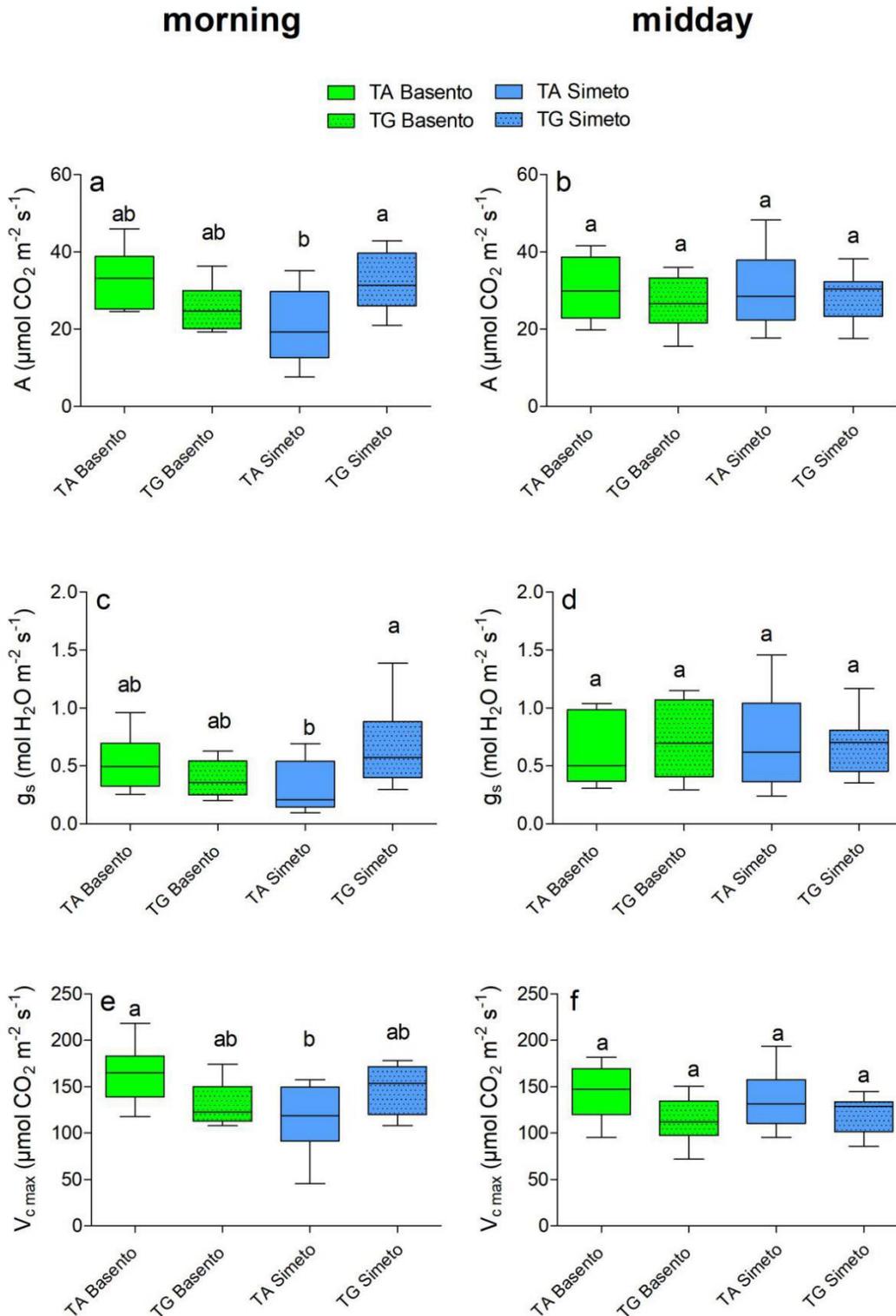


Figure 6. Physiological responses of *Tamarix africana* and *Tamarix gallica* at Basento and Simeto (a) Photosynthesis rate in morning (b) Photosynthesis rate in midday (c) Stomatal conductance (g_s) in morning (d) Stomatal conductance (g_s) in midday (e) Maximum carboxylation rate ($V_{c \text{ max}}$) in morning (f) Maximum carboxylation rate ($V_{c \text{ max}}$) in midday before saline treatment. Different letters above the symbols indicate significant difference at $p < 0.05$

4.6 Photosynthesis responses of *Tamarix africana* and *Tamarix gallica* to the salinity treatments

Photosynthesis varied significantly as salinity increased in *Tamarix africana* at Basento in both morning and midday ($p < 0.05$) figure 7(a) and figure 7(b). Photosynthesis declined from July to August and increased to September ($p < 0.05$), (figure 7(a) and figure 7(b)).

Photosynthesis varied significantly as salinity increased in *Tamarix africana* at Simeto in both morning and midday ($p < 0.05$) figure 7(c) and figure 7(d). Photosynthesis declined from July to August and increased to September ($p < 0.05$) (figure 7(c) and figure 7(d)).

Photosynthesis varied significantly as salinity increased in *Tamarix gallica* at Basento in both morning and midday ($p < 0.05$) figure 7(e) and figure 7(f). Under moderate and high salinity, photosynthesis decreased from July to August and increased to September. However, *Tamarix gallica* continued to increase from July to September under control treatment in both morning and midday.

Photosynthesis varied significantly as salinity increased in *Tamarix gallica* at Simeto in both morning and midday ($p < 0.05$) figure 7(g) and figure 7(h). Under moderate and high salinity, photosynthesis decreased from July to August and increased to September. However, *Tamarix gallica* continued to increase from July to September under control treatment in both morning and midday.

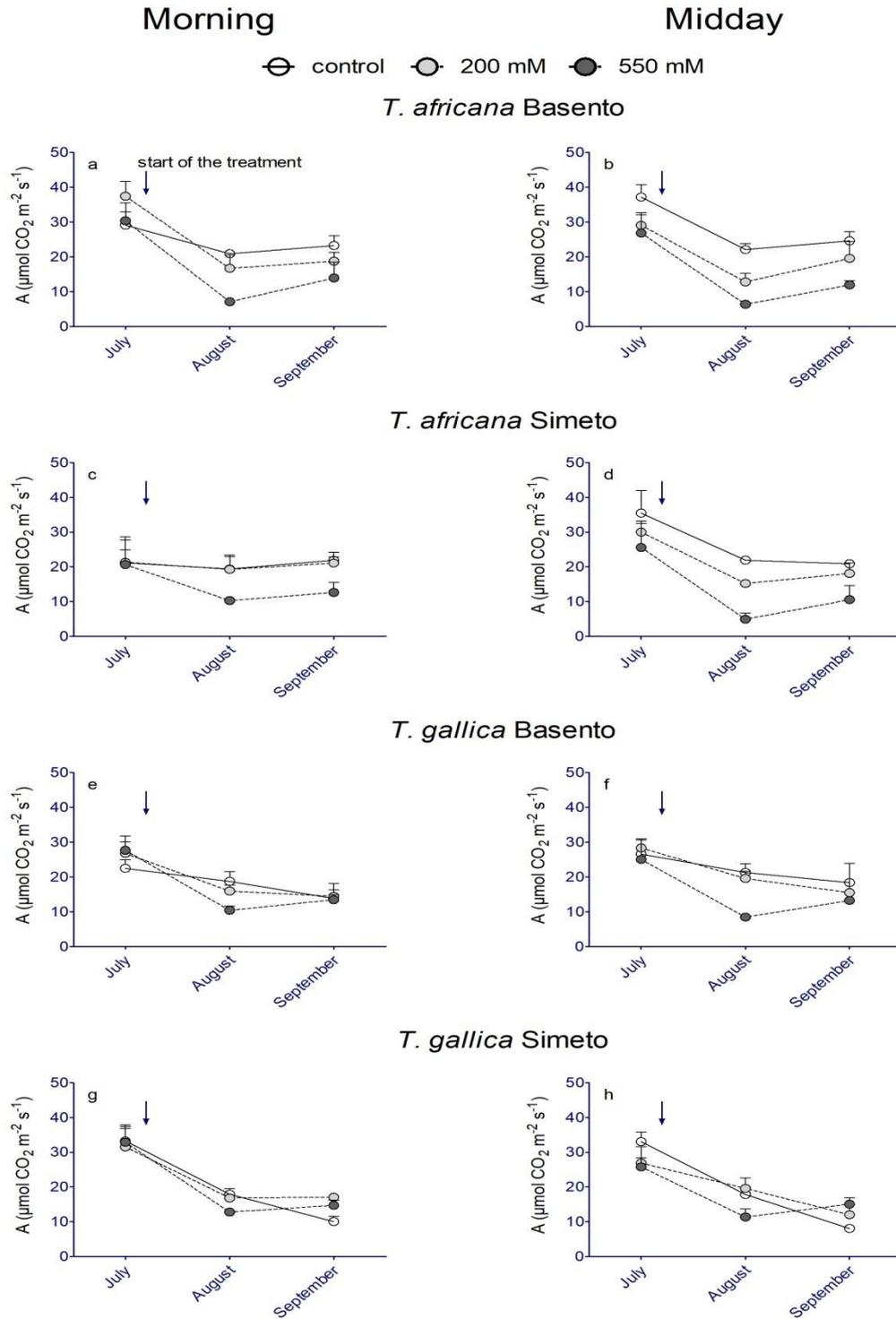


Figure 7. Photosynthesis responses (a) *Tamarix africana* at Basento morning (b) *Tamarix africana* at Basento midday (c) *Tamarix africana* at Simeto morning (d) *Tamarix africana* at Simeto midday (e) *Tamarix gallica* at Basento morning (f) *Tamarix gallica* at Basento midday (g) *Tamarix gallica* at Simeto morning (h) *Tamarix gallica* at Simeto midday over time across saline treatments

4.7 Stomatal conductance responses of *Tamarix africana* and *Tamarix gallica* to the salinity treatments

Stomatal conductance varied significantly by salinity treatment in *Tamarix africana* at Basento in both morning and midday ($p < 0.05$) figure 8(a) and figure 8(b). Stomatal conductance values declined from July to August and maintain the values to September. figure 8(a) and figure 8(b).

Stomatal conductance varied significantly by salinity treatment in *Tamarix africana* at Simeto in both morning and midday ($p < 0.05$) figure 8(c) and figure 8(d). Stomatal conductance values declined from July to August and maintain the values to September. figure 8(c) and figure 8(d).

Stomatal conductance varied significantly by salinity treatment in *Tamarix gallica* at Basento in both morning and midday ($p < 0.05$) figure 8(e) and figure 8(f). Under moderate and high salinity, Stomatal conductance values declined from July to August and maintain the values to September. However, *Tamarix gallica* continued to increase from July to September under control treatment in both morning and midday

Stomatal conductance varied significantly by salinity treatment in *Tamarix gallica* at Simeto in both morning and midday ($p < 0.05$) figure 8(g) and figure 8(h). Under moderate and high salinity, Stomatal conductance values declined from July to August and maintain the values to September. However, *Tamarix gallica* continued to increase from July to September under control treatment in both morning and midday

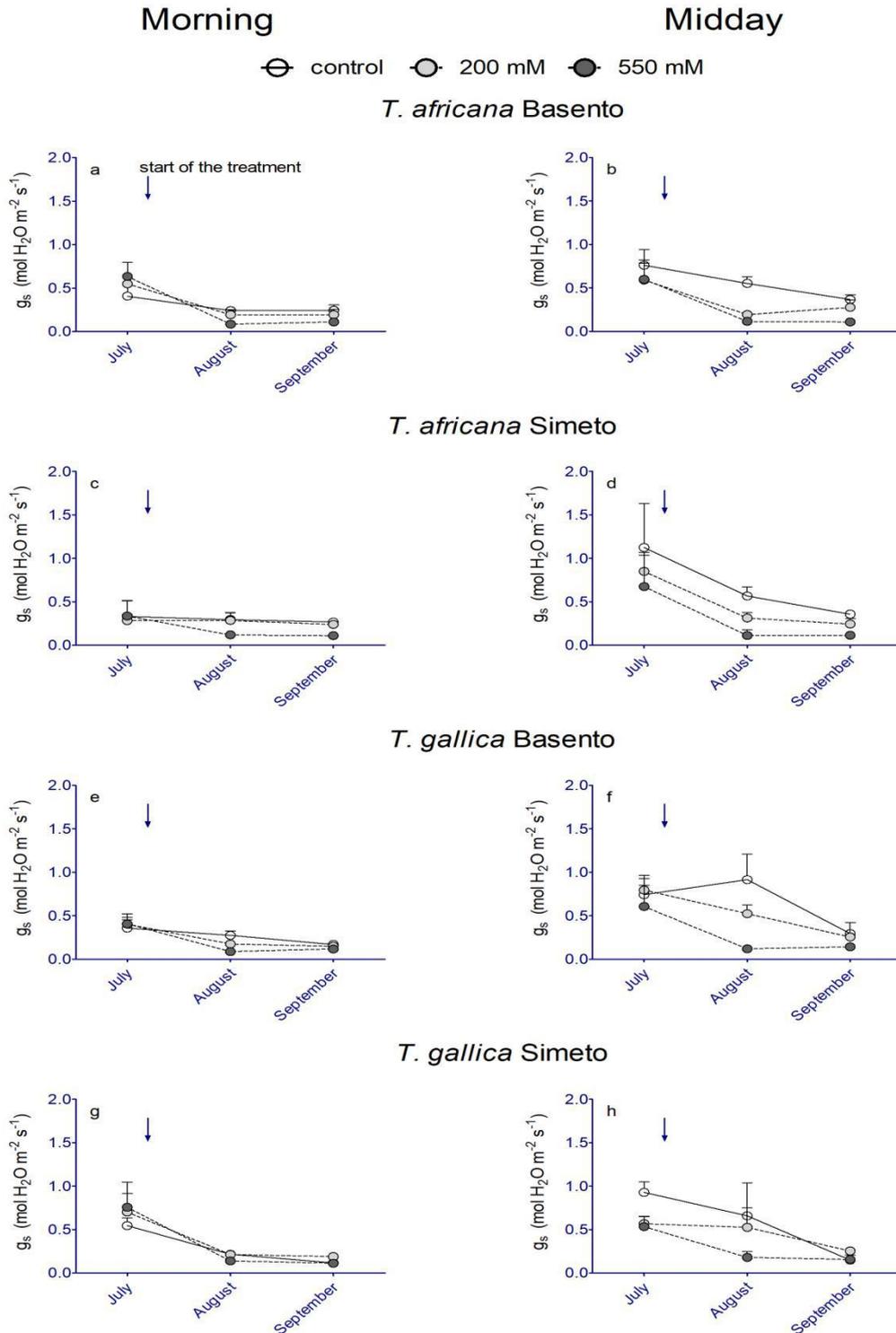


Figure 8. Stomatal conductance responses (a) *Tamarix africana* at Basento morning (b) *Tamarix africana* at Basento midday (c) *Tamarix africana* at Simeto morning (d) *Tamarix africana* at Simeto midday (e) *Tamarix gallica* at Basento morning (f) *Tamarix gallica* at Basento midday (g) *Tamarix gallica* at Simeto morning (h) *Tamarix gallica* at Simeto midday over time across saline treatments

4.8 Maximum carboxylation rate (V_{cmax}) responses of *Tamarix africana* and *Tamarix gallica* to the salinity treatments

Maximum carboxylation rate (V_{cmax}) varied significantly as salinity increased in *Tamarix africana* at Basento in both morning and midday ($p < 0.05$) figure 9(a) and figure 9(b). Photosynthesis declined from July to August and increased to September ($p < 0.05$), figure 9(a) and figure 9(b).

Maximum carboxylation rate (V_{cmax}) varied significantly as salinity increased in *Tamarix africana* at Simeto in both morning and midday ($p < 0.05$) figure 9(c) and figure 9(d). Photosynthesis declined from July to August and increased to September ($p < 0.05$) (figure 9c-d).

Maximum carboxylation rate (V_{cmax}) varied significantly by salinity treatment in *Tamarix gallica* at Basento in both morning and midday ($p < 0.05$) figure 9(e) and figure 9(f). Under moderate and high salinity, V_{cmax} declined from July to August and maintain the values to September. However, *Tamarix gallica* continued to increase from July to September under control treatment in both morning and midday

Maximum carboxylation rate (V_{cmax}) varied significantly by salinity treatment in *Tamarix gallica* at Simeto in both morning and midday ($p < 0.05$) figure 9(g) and figure 9(h). Under moderate and high salinity, V_{cmax} declined from July to August and maintain the values to September. However, *Tamarix gallica* continued to increase from July to September under control treatment in both morning and midday.

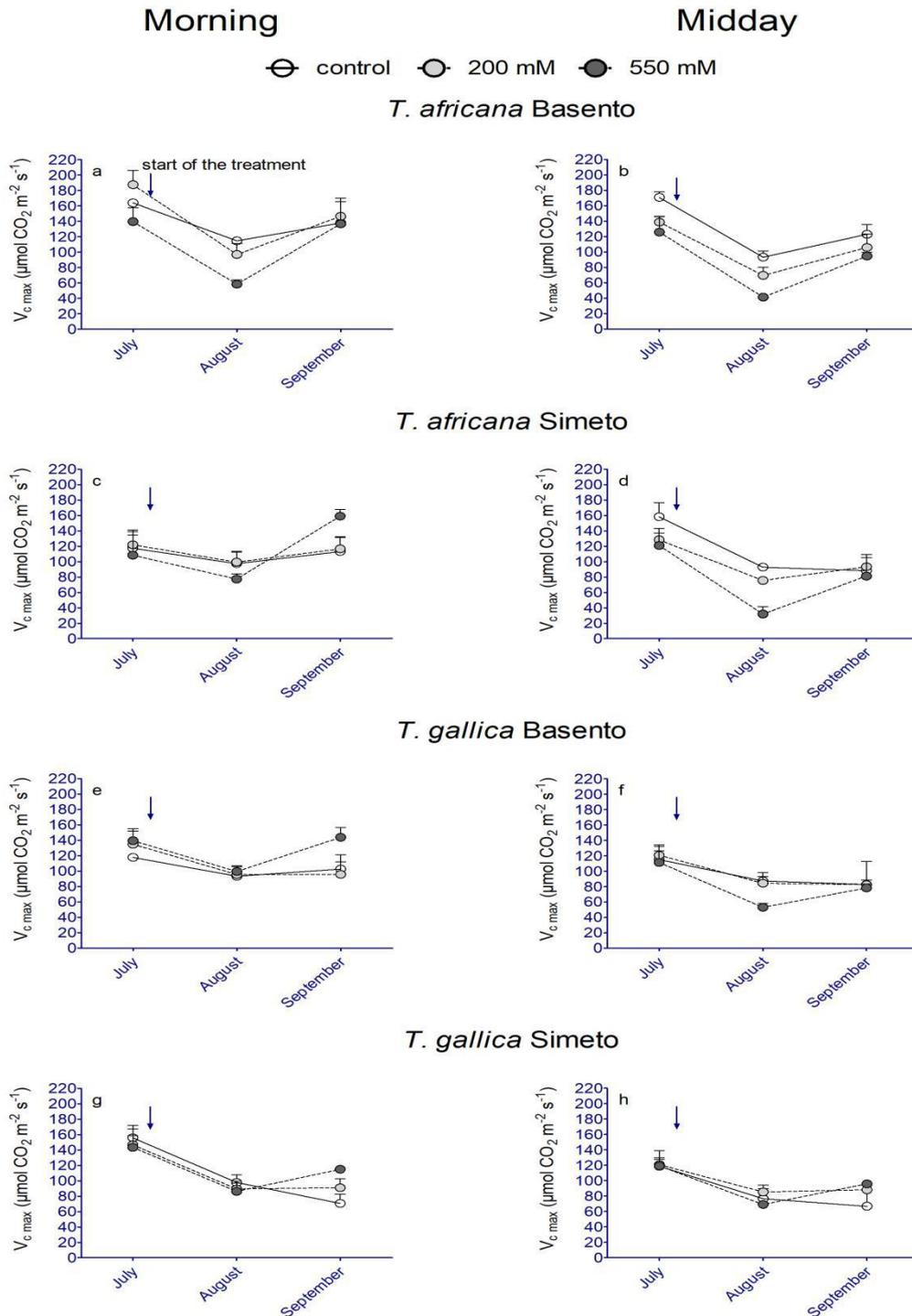


Figure 9. Maximum carboxylation responses (a) *Tamarix africana* at Basento morning (b) *Tamarix africana* at Basento midday (c) *Tamarix africana* at Simeto morning (d) *Tamarix africana* at Simeto midday (e) *Tamarix gallica* at Basento morning (f) *Tamarix gallica* at Basento midday (g) *Tamarix gallica* at Simeto morning (h) *Tamarix gallica* at Simeto midday over time across saline treatments

4.9 Photosystem II, Fv/Fm responses of *Tamarix africana* and *Tamarix gallica* to the salinity treatments

Photosystem II quantum efficiency (Fv/Fm) revealed no significant differences among treatments, both at morning and midday, in *Tamarix africana* at Basento figure 10 (a) and figure (b).

Photosystem II, Fv/Fm was not affected as among treatments in *Tamarix africana* at Simeto in both morning and midday figure 10 (c) and figure (d).

Photosystem II, Fv/Fm was not affected as among treatments in *Tamarix gallica* at Basento in both morning and midday figure 10 (e) and figure (f).

Photosystem II, Fv/Fm was not affected as among treatments in *Tamarix gallica* at Simeto in both morning and midday 10 (g) and figure (h).

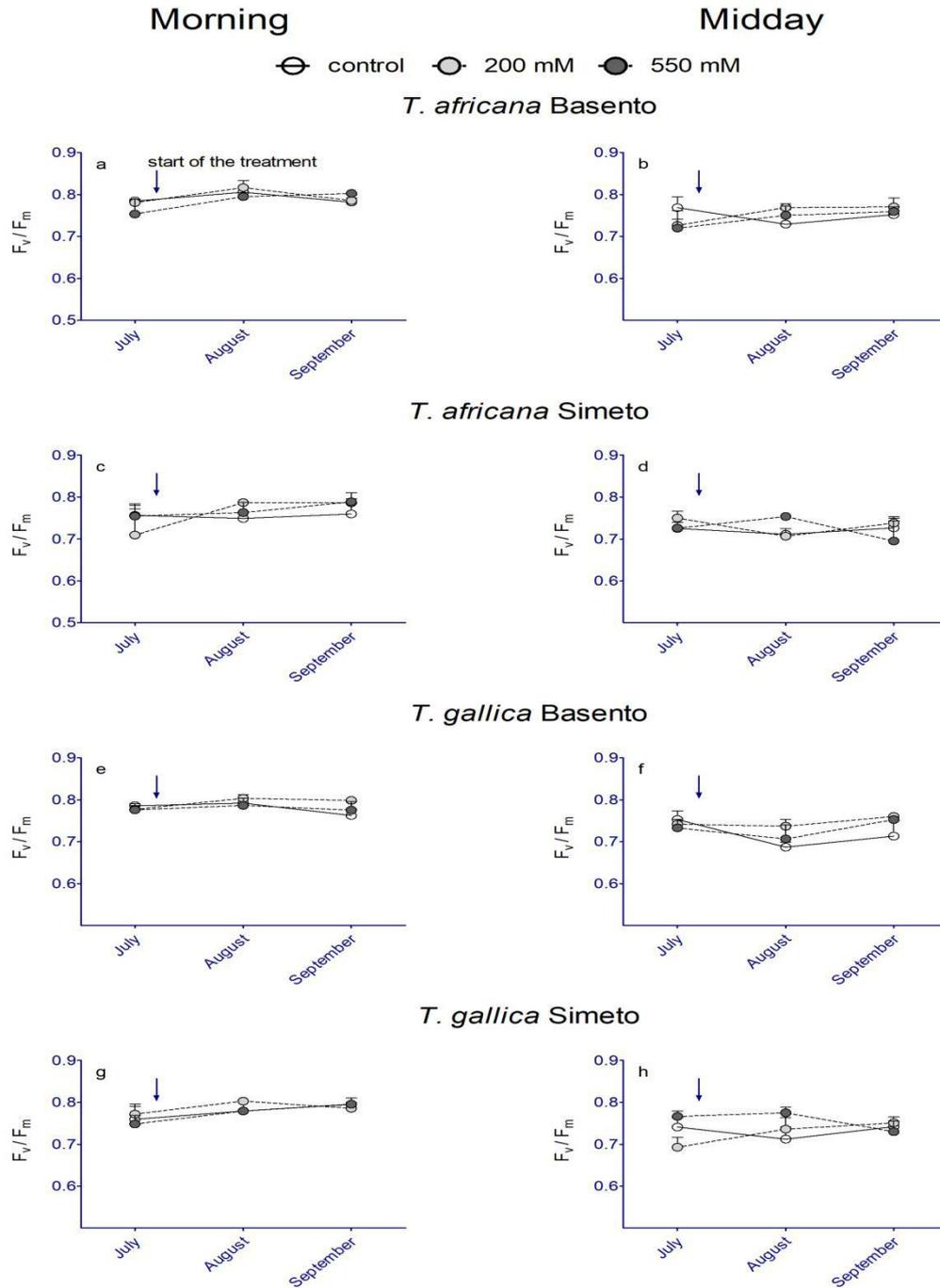


Figure 10. Photosystem II, Fv/Fm responses(a) *Tamarix africana* at Basento morning (b) *Tamarix africana* at Basento midday (c) *Tamarix africana* at Simeto morning (d) *Tamarix africana* at Simeto midday (e) *Tamarix gallica* at Basento morning (f) *Tamarix gallica* at Basento midday (g) *Tamarix gallica* at Simeto morning (h) *Tamarix gallica* at Simeto midday over time across saline treatments.

5.0 Salt extrusion from *Tamarix* species

There was significant differences of quantity of salt accumulated outside the twigs of *Tamarix* species from both provenances of saline treatment ($p < 0.05$) (Figure 11)

The average quantity of salt accumulated outside the twigs of *Tamarix* species decreased from 31.046 mg/g in control, to 112.21 mg/g in moderate and 169.24 mg/g in high salinity treatment ($p < 0.05$) (Figure 11) at Basento and Simeto

Under control, *Tamarix gallica* salt accumulated outside the twigs was 39.8% more than *Tamarix africana*. Similarly, in moderate salinity *Tamarix gallica* salt accumulated outside the twigs was 25.03% as well as 9.7% in high salinity more than *Tamarix africana*.

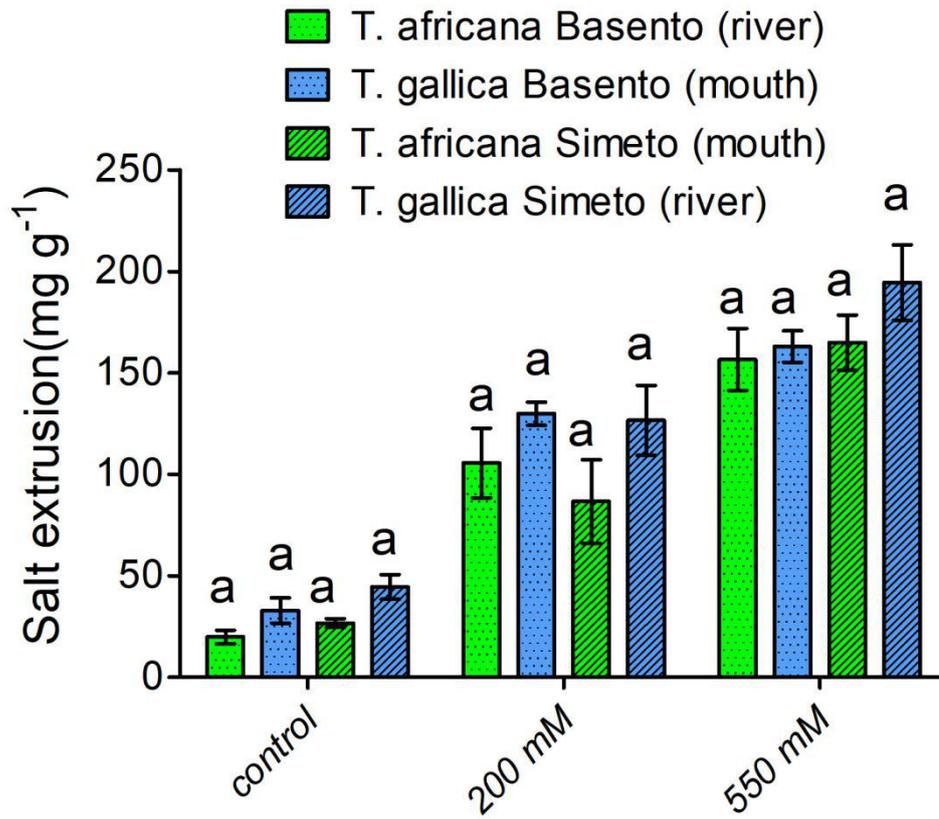


Fig. 11. Salt extrusion from *Tamarix* species of Simeto and gallica of three treatment (control, 200 mM, and 550 mM). Different letters above the symbols indicate significant difference at $p < 0.05$

CHAPTER FIVE

5.0 Discussion

The *Tamarix* species are intrinsically fascinating for the ways in which they are adapted to grow under conditions lethal for most plant species. The predominantly among the Italian *Tamarix* populations are *Tamarix africana* and *Tamarix gallica* (Kuzminsky et al. 2014). These species play an important role in the ecosystem as well as global issue of climate change, thus represents valuable material for carbon sequestration and bio-fuel production in extreme environmental conditions. To this end, they need to be preserved, enhanced and harnessed in the provenances. The present study aimed to analyse the salinity tolerance in different provenances of *Tamarix species* available in the EcoPhysLab in-vivo collection

5.1 Growth and biomass of *Tamarix gallica* and *Tamarix africana*

The finding of this study showed treatment effects on *Tamarix species* for cumulative shoot length, dominant shoot length, stem diameter and harvest biomass ($p < 0.05$) Figure 2(a) and figure 2(b), figure3(a) and figure 3(b), figure 4(a) and figure 4(b) in Basento and Simeto provenances across moderate and high salinity treatments.

The result is consistent with (Flowers and Colmer, 2008), suggesting that *Tamarix gallica* and *Tamarix africana* are highly salt-tolerant species, able to survive, grow and flower at salinities anywhere from 200-1000 mM of salt. In addition, *Tamarix* species can be considered as some of the few trees that can survive highly stressful conditions such as salinity (Kuzminsky et al 2014).

The study shows that under control treatment, *Tamarix africana* had 5.2% average leaves biomass and 2.3% wood biomass more than *Tamarix gallica*. On the other hand, *Tamarix gallica* had 13.4% average leaves biomass in moderate salinity and 27% average wood biomass in high salinity more than *Tamarix africana*. Furthermore, *Tamarix gallica* had 7.5% average root biomass in control and 13.5% average root biomass in high salinity more than *Tamarix africana*. Under moderate, salinity *Tamarix africana* had 2.5% average root biomass more than *Tamarix gallica*. Finally, *Tamarix gallica* had 28.7% average shoot/root in control and 10.8% average shoot/root biomass in high salinity more than *Tamarix africana*. Under moderate

salinity *Tamarix africana* had 34% average shoot/root biomass more than *Tamarix gallica* (figure 5)

Furthermore, study also indicates that *Tamarix gallica* had the ability to resist high saline stress as compared to *Tamarix africana*, thus maintaining higher productivity also in high saline conditions. In fact, *Tamarix gallica* had more biomass in under saline treatments compared to *Tamarix africana*. This affirms (Kuzminsky et al 2014), stating overall experimental evidence indicates that *Tamarix gallica* is characterized by a greater resistance to salt stress compared to *Tamarix africana*

The study also revealed that, differences in growth and harvest biomass under treatment amongst *Tamarix gallica* and *Tamarix africana* in both Basento and Simeto (figures 2-5) are attributed to ecological and environmental variability. (Abou Jaoudé et al. 2011) stated that plant architecture and annual growth rate indicate the growing conditions. The site of the provenance is attributed to the ecological and environmental variability. This environmental variability influences the structural characteristics of *Tamarix* species populations as well as inter-and intra-specific diversity (Kuzminsky et al 2014).

Nardini (et al. 2000) affirms that latitudinal changes of temperature, light intensity and photoperiod might have influenced the expression of plant morphological trait. Verdù (et al. 2007) suggested that the multi stemmed modification of plant structure is in response to salinity, since re-sprouting from dormant buds allows the plants to recover more quickly as compared to regeneration from seeds. Additional traits, like the formation of adventitious roots and aerenchyma, plays an important role for the adaptation to the anoxic stress induced by flooding (Colmer 2003; Evans 2004), another typical environmental constraints of estuarine environments, supporting the diffusion of *Tamarix* species (Flowers and Colmer 2008; Munns and Tester 2008)

5.2 Gas exchange and chlorophyll fluorescence of *Tamarix gallica* and *Tamarix africana*

In the study, *Tamarix gallica* and *Tamarix africana* in Basento and Simeto provenances were subjected to moderate and high salinity salt stress. This brought about induced observable changes in physiological responses as well as growth in both species compared to those growing under control treatment.

Photosynthetic capacity was significant in *Tamarix africana* at Basento and Simeto, *Tamarix gallica* at Basento and Simeto in both morning and midday ($p < 0.05$) figure 7. Photosynthetic capacity decreased in treatments from July to August and increased to September ($p < 0.05$) figure 7(a)(b) and figure 7(c) (d) in *Tamarix africana* at Basento and Simeto. *Tamarix gallica* in both moderate and high salinity decreased in treatments from July to August and increased to September. However, under control, *Tamarix gallica* at Basento and Simeto continued to increase to September in both morning and midday

The continuous decrease in photosynthetic capacity in *Tamarix gallica* is attributable to seasonal air temperature and photosynthetically active radiation changes in July, August and September in both morning and midday (figures 7-9).

Under moderate and high salinity treatment, *Tamarix* species showed yellowing and defoliation of old leaves. This is a mechanism adapted by the species to accumulate high capacity toxic ions in the oldest leaves tissues to prevent the death of young leaves. The *Tamarix* species initial responses was on the 3rd day to shock salt stress, then followed by acclimation of 25 days of treatment of 111 days plant growth in August.

The decline in photosynthesis, stomatal conductance and maximum rate of carboxylation with increasing salinity (figures 7-9) suggested that photosynthetic efficiency was reduced at high salinities (Carter J. M and Nippert J.B 2011). The evidence suggests salt-induced reduction of carbon dioxide availability inside the leaves (Prida and Das, 2005), inefficiency of translocation as well as assimilation of photosynthetic product and stomata closure (Xiong and Zhu, 2002), generation of reactive oxygen species (ROS) (Parvaiz and Satyawati, 2008), have harmful effects on cell metabolism, photosynthetic capacity and growth (Bursens et al. 2000).

The study also revealed that differences in physiological parameters amongst *Tamarix gallica* and *Tamarix africana* in both Basento and Simeto (figures 7-9) are attributed to ecological and environmental variability. Thomson (et al. 1969) suggested the mechanism by which plant effectively removes salts from internal cellular tissues to the leaf surface through the leaf glands depends on the composition of the root environment. Furthermore, *Tamarix* spp. growing at Basento, located in the most elevated position of the river-bank where flooding is less common showed the highest

value of guard cell length (Abou Jaoudé 2011). The size of stomata *Tamarix* species could be related to the flooding tolerance, since smaller stomata respond more to environmental stresses (Yordanova et al. 2005). Besides, the developing of cuticle thickenings on the outer walls of the stomatal guard of *Tamarix* species is as a result of differences in the environments. These cuticle thickenings protrude towards the stomatal pore and may increase the diffusion path through the guard cells, reducing water loss through transpiration (Struwig et al. 2011). Finally, salinity tolerance appears to be species specific, where decreasing in assimilation rates, owing to a decrease in stomatal conductance and growth is usually ascertained in *Tamarix africana* under high sodium chloride concentrations (Abou Jaoudé 2011)

5.3 Salt extrusion from *Tamarix* species

The salt secretion by specialised glands is a necessary adaptive mechanism to regulate ion concentration in leaves of halophyte (Weber, 2008). At the end of the experiment the average quantity of salt accumulated outside the twigs of *Tamarix* species increased from 31.05 mg/g in control, to 112.21 mg/g in moderate and 169.24 mg/g in high salinity treatment ($p < 0.05$) (Figure 11).

Under control, *Tamarix gallica* salt accumulated outside the twigs was 39.8% more than *Tamarix africana*. Similarly, in moderate salinity *Tamarix gallica* salt accumulated outside the twigs was 25.03% as well as 9.7% in high salinity more than *Tamarix africana*.

This phenomenon is the result of the presence of adventitious roots which may exclude Na^+ (Saqib et al. 2005), or serving as a potential reservoir for the ion, thus decreasing the quantity of salt present in photosynthetic organs. The adventitious roots of *Tamarix* species absorb translocate and secrete large amounts of salt through their leaf glands and accumulated on the leaf surface, thus completing a continuous salt movement through the plant body (Waisel, 1961; Abou Jaoudé et al. 2011).

CHAPTER SIX

Conclusion

In recent times, one of the starring abiotic stresses for plant survival in the ecosystem is salinity. This has resulted in massive loss of habitat, biodiversity, native vegetation and water resource value. The *Tamarix* species which forms part of 2% of halophyte are intrinsically fascinating for the ways in which they are adapted to grow under conditions lethal for most plant species. Here, we analysed the growth and physiological responses of *Tamarix africana* and *Tamarix gallica* of two Italian provenances (Simeto and Basento) over salinity treatments.

The finding of this study showed treatment effects on *Tamarix* species for cumulative shoot length, dominant shoot length, stem diameter and harvest biomass in both Basento and Simeto provenances. The findings also indicate that *Tamarix gallica* had large amount of biomass and the ability to resist high saline stress as compared to *Tamarix africana*.

Furthermore, finding of this study on physiology showed the impact of treatments on the photosynthetic capacity of both *Tamarix* species and provenances. Photosynthetic capacity decreased under increasing salinity in July and acclimatized in August, in both *Tamarix* species. However, under control *Tamarix gallica* continued to decrease from July to September and is attributable to seasonal air temperature and photosynthetically active radiation. At the end of the experiment the average quantity of salt accumulated outside the twigs of *Tamarix* species increased in response to the salinity.

The differences in *Tamarix* species to the increasing salinity could be related to the ecological and environmental pressures. Different environmental constraints possibly indirectly influenced the structural characteristics of *Tamarix* species provenance as well as inter-and intra-specific diversity.

The results of the study provide additional insights for the conservation and restoration of potential fragile ecosystems, such as those occupied by *Tamarix* species. Which are necessary in a world where the human population is still increasing and land use practices and changes in the climate are likely to lead to increased salinization of the land surface in many regions.

Considering the global issue of climate change, it would be interesting to conduct further studies to compare the salinity tolerance and biomass production of other *Tamarix* species at different provenances.

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