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Harnessing the biodiversity of Italian *Tamarix* species: populations, plants and leaf responses to extreme environmental constraints

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Part I **Italian *Tamarix* spp. populations: distribution, structure and ecology**

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Thesis outline

Studying the ecophysiology of plants growing in the Mediterranean area is a challenging subject of research. This is particularly true for the vegetation occupying coastal areas and ephemeral riverbanks, as the species present in these habitats are frequently subjected to a multitude of stresses (salinity, flooding, drought, high temperatures...) that are sometimes additive and interconnected. This background becomes more complicated as a consequence of global climate changes, which are not only increasing the strength of these stresses, but may also lead to the total disappearance of entire ecosystems, causing an unquantifiable loss of species and biodiversity.

The knowledge of the specific and intra-specific diversity of any endangered habitat is the first step for conservation practices. Moreover, some species may be selected for their natural tolerance to some particular stress, and may be conserved and used in the future for the restoration of damaged ecosystems in which these species were already present.

The *Tamarix* genus is composed of about ninety species. Though their centre of origin seems to be Central Asia, *Tamarix* species are also naturally distributed in the Mediterranean region and in the African deserts, and are characterised by high endurance of adversities. Some species are known for being invasive (especially in the United States) and, among them, the most studied are *Tamarix ramosissima* and *T. chinensis*. In Italy, *Tamarix* species are eleven, and are naturally distributed on coastal dunes and on riverbanks of Southern regions. The two most widespread species are *Tamarix gallica* and *Tamarix africana*. Although they play a fundamental ecological role in dunes fixation and in occupying salinized areas which would otherwise be subjected to desertification, their distribution, ecology and physiology are not well known, probably as a consequence of the marginal use of their products, and the difficulty in species identification.

In this work, the **Introduction** focuses on highlighting the major role of plants in the global carbon cycle and in contrasting and monitoring climate change effects, on understanding how coastal and riverside plants respond to the main stresses to which they are subjected, and on introducing the current knowledge on the use of plants in the ecological restoration of degraded areas. The final part of the introduction concerns with the genus *Tamarix*, with particular regard to its taxonomy, ecology and physiology.

The different studies undertook in this thesis have been divided into two parts: the **Part I** is focused on the *in situ* characterisation of populations distribution, structure and ecology, while the **Part II** concerns with the physiological photosynthetic and growth responses of *Tamarix* species and provenances to salinity and flooding. Every chapter, in which these two parts are divided, is structured as an article, including its introduction, conclusions and references. Although some repetitions are present, especially concerning the cited references, each chapter can be considered as a single independent work; this structure, in my opinion, can be helpful for the understanding of the whole thesis. Before the **General conclusions**, which highlight the main results obtained in this work, a brief, but not less important, **Appendix** is dedicated to the *ex situ* conservation of the collected materials, which can be considered the permanent basis for a further future knowledge of the genotypes and the genotype × environment characterization of the Italian species and provenances of *Tamarix*.

General Introduction

Background: global carbon cycle and human induced changes

The global carbon (C) cycle refers to the exchange of this element across its 4 major sinks: the atmosphere, the oceans, the continents and the fossil deposits (Schimel, 1995; Houghton, 2003). This element is present in both inorganic (carbonates in the lithosphere, bicarbonates in the hydrosphere and carbon dioxide in the atmosphere) and organic components (living matter). Together with the water cycle, the carbon cycle represents the most important biogeochemical cycle for humanity, given its vulnerability to human disturbances.

The carbon cycle begins with plant assimilation of the atmospheric carbon dioxide (CO_2) (Fig. 1).

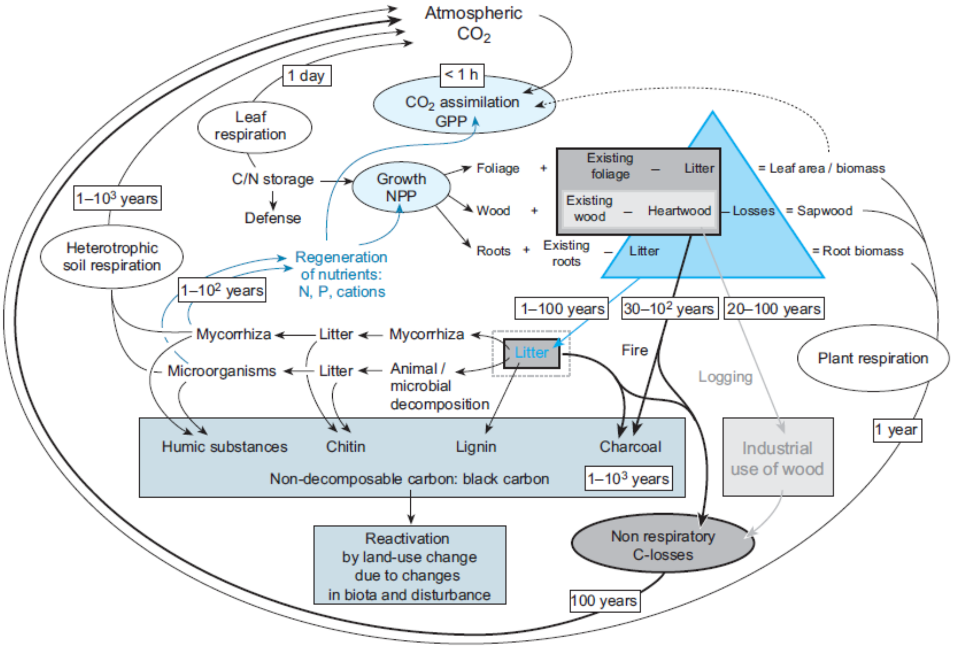


Fig. 1 Turnover of carbon ecosystems. Turnover begins with photosynthetic assimilation of CO_2 by the plant. Products are distributed to all photosynthetic and non photosynthetic plant organs. Maintenance and growth of biomass require energy that might be measured as respiration. Leaf litter fall, mychorrizae decomposition, fires and harvest release CO_2 as respiration. In ecosystems, only black carbon remains for a very long period of time (from Schulze et al., 2005).

The plant cover assimilates CO_2 from the atmosphere by photosynthesis, leading to the formation of sugars which are either used immediately for growth, to form or maintain existing structures (about 50% of the assimilated C), or are stored as starch. The CO_2 assimilation is a diffusion process: CO_2 from the atmosphere diffuses into the leaf as the CO_2 concentration in the intercellular spaces of the mesophyll where photosynthesis occurs is smaller than in the external air (Schulze et al., 2005). The leaf cuticle is almost impermeable to CO_2, thus gas exchange occurs through small adjustable pores, the stomata. As photosynthesis occurs simultaneously with transpiration, plants need to regulate stomatal opening in order to assure sufficient CO_2 to enter the leaf whilst conserving water, in order to avoid dehydration and metabolic disruption (Lawson, 2009). Carbon fixation is an energy consuming process. The energy required for the biochemical CO_2 fixation is produced during the light reactions of photosynthesis.

Photosynthesis depends on the interaction of two separated photosystems: photosystem I and photosystem II (respectively PSI or P700 and PSII or P680, according to the absorption maxima of their chlorophyll *a* molecules), consisting in a light-gathering antenna complex – containing chlorophyll *a*, chlorophyll *b* and carotenoid molecules – and a reaction centre. PSII is mainly located on the stacked membranes of the grana while PSI is located mainly on the non-stacked lamellae. PSI and PSII operate in series. According to Mc Donald (2003), the initial photochemical reaction is the excitation of P680 by the absorption of wavelengths of light <680 nm. The excited form of P680 (P680*) is photo-oxidised, and its lost electron is transferred to a primary electron-acceptor (pheophytin). P680* is then transformed into a cation radical, P680$^+$. P680$^+$ is the strongest biological oxidant known, and it is able to oxidise (to gain electrons from) H_2O. However, a single photon does not possess enough energy to bring about the photolysis of water: four photons are needed, so that PSII must be excited four times. This brings to the oxidation of two molecules of water to produce one molecule of oxygen, four protons and four electrons:

$$2H_2O \longrightarrow O_2 + 4H^+ + 4e^-$$

The four electrons released from water do not pass directly to P680$^+$, which can only accept one electron at a time. An oxygen evolving complex (OEC) passes four electrons, one at a time, to P680$^+$. Pheophytin extra electron is passed through a non-cyclic chain of electron carriers (plastoquinone Q_A, plastoquinone Q_B – plastoquinol QH_2, cytochrome b_6/f complex). P680$^+$ is also rapidly reduced, thus returning in its ground state, being again ready for excitation. The electron needed for the reduction of the P680$^+$ comes from the water molecules in the luminal side of the thylakoid membrane. The electron transport is simultaneous to the movement of protons from the stroma across the membrane into the thylakoid lumen, which establishes a proton-motive force across the membrane. Protons move down their own concentration gradient from the thylakoid lumen back into the stroma via a group of transport proteins, resulting in the formation of ATP.

Similarly to PSII, the primary event in the PSI is the excitation of the reaction centre P700 by the absorption of a photon of wavelength >680 nm. The newly formed P700* loses an electron to a primary electron-acceptor A_0, producing the strong oxidising agent P700$^+$, which is reduced to P700 by an electron passed from PSII. A_0^- passes its electron trough a chain of carriers (phylloquinone, iron-sulphur protein Fe-S, ferredoxin Fd, flavoprotein ferredoxin-NADP-oxidoreductase) and ultimately to NADP to form NADPH. Two molecules of NADPH are formed for each O_2 molecule released from H_2O. Moreover, electrons excited within PSI may be transported independently of PSII in a series of reactions, called cyclic electron transport, which yields ATP as its only product.

Light reaction processes may be summarized in the following equation:

$$H_2O + ADP + P_i + NADP \longrightarrow ATP + NADPH + H^+ + O_2$$

During the evolution of the plant kingdom, certain species have developed strategies in their way of assimilating CO_2 in order to overcome water loss; while few differences are reported in photosystems functioning from algae to land plants, there are considerable differences in the way the CO_2 is fixed during the dark reactions (Schulze et al., 2005). The possible ways of fixing CO_2 are: the C_3 photosynthetic carbon reduction cycle (PCR or Calvin cycle), the C_4 photosynthetic carbon assimilation cycle (PCA cycle) and the crassulacean acid metabolism (CAM). In C_3 plants, which constitute about 95% of the plant species, the enzyme ribulose-1,5-carboxylase-oxygenase (RuBisCO) catalyses the carboxylation of ribulose-1,5-bisphosphate (RuBP), a 5-carbon compound, by carbon dioxide in a two-step reaction. The initial product of the reaction (6 CO_2 fixed) is an unstable six-carbon intermediate that immediately splits in half, forming two molecules of 3-phosphoglyceric acid (3-PGA), a 3C compound. In C_4 plants, which constitute about 2% of the overall plant species, organic acids with four-carbon skeleton are formed from the reaction of CO_2 with phosphoenolpiruvate (PEP) catalysed by the PEP-carboxyalse. The C_4 acids are decarboxylated in the bundle sheath. The CO_2 released is then reduced by the C_3 pathway. The advantage of the C_4 pathway is in the very large concentration of CO_2 in the bundle sheath cells and also in the re-fixation of the respired CO_2. Moreover, PEP-carboxylase has a higher affinity for CO_2 compared to the RuBisCO and has no oxygenase function. Thus, C_4 plants can maintain smaller stomatal aperture at the same C_3 plants photosynthetic rate, and are therefore more adapted to the arid sub-tropical regions with temperature above 30 °C (Schulze et al., 2005). CAM plants resemble C_4 plants biochemically; however, CO_2 uptake and assimilation are not separated spatially but temporally: CO_2 uptake occurs at night, when acids are formed and stored in the vacuole, while CO_2 release and assimilation take place during the day, in the mesophyll cells. Thus, nocturnal CO_2 uptake results in a very high water use efficiency (Schulze et al., 2005). As C_3 plants are the most widely numerous and distributed plants, the Calvin cycle will be explained more in details. According to Mc Donald (2003), the CO_2 fixation by means of photosynthetic carbon reduction cycle occurs in three stages. In the first stage, the carboxylation stage, the atmospheric CO_2 enters the PCR cycle by reacting with a five-carbon RuBP to yield two molecules of 3-PGA. The carboxylation reaction is catalysed by RuBisCO, which accounts for 50% of the soluble proteins in plant leaves. Its high concentration in the chloroplast stroma coupled to its high affinity for CO_2 ensure a rapid fixation of the normally low (0.038%) concentration of this gas in the atmosphere. The second stage, the reduction stage, is a two-step reaction: first, 3-PGA is phosphorylated to 1, 3-PGA which is then reduced to 3-phosphoglyceraldehyde (3-PGAL). NADP-glyceraldehyde-3-phosphate dehydrogenase catalyses this reaction. The ATP and NADPH used in this phase are generated in the light reactions. The third stage, the regeneration, is essential for the continued fixation of CO_2. In this stage, the CO_2 acceptor (RuBP) is regenerated by the interconversion of the carbon skeleton of 3-PGAL. Five × 3C molecules (triose phosphates) are interconverted to yield three × 5C molecules (pentose phosphates). Thus, for every three turns of the PCR cycle, three molecules of RuBP (15C) condense with three molecules of CO_2 (3C) to form six molecules of 3-PGA (18C). The six molecules of 3-PGA are reduced to six molecules of 3-PGAL using six ATP (for the synthesis of 1, 3-PGA) and six NADPH (in the reduction of 1,3-PGA to 3-PGAL). One of these molecules of 3-PGAL (3C) is the net yield of PCR cycle. The other five 3-PGAL molecules (15C) are rearranged to form three molecules of RuBP (15C). This last step requires one ATP for each RuBP formed, thus three ATP.

$$\text{3 RuBP} + 3\ CO_2 \longrightarrow \text{6 3-PGA} \xrightarrow{\text{6 ATP + 6 NADPH}} \text{5 3-PGAL} \xrightarrow{\text{3 ATP}} \text{3 RuBP}$$

Therefore, the reduction of one molecule of CO_2 requires 2 NADPH (2×217 kJ) and 3 ATP (2×29 kJ) that is a total of 521 kJ, corresponding to 4 mole electron. The oxidation of one mole of hexose yields about 469 kJ mol^{-1} CO_2; this represents about 90% efficiency in the energy storage for the PCR cycle.

In C_3 plants, photosynthesis is always accompanied by photorespiration which, in the light, consumes O_2 and converts RuBP to glycolate and CO_2 during a process called the photorespiratory carbon oxidation (PCO). This reaction is catalyses by RuBisCO and yields one molecule of 3-PGA and one molecule of phosphoglycolate. Phosphoglycolate is converted into glycolate in the chloroplast, which is transformed in glycine in the peroxisome, which is decarboxylated in the mitochondrion to form serine, CO_2 and NH_3. Then, serine is converted to PGA in the peroxisome which re-enters into the PCR cycle in the chloroplast. For every two molecules of phosphoglycolate (4C) formed by photorespiration, one molecule of 3-PGA (3C) is recycled and one molecule of CO_2 is lost (-25% C of the PCR cycle due to photorespiration). The amount of NADPH and ATP consumed in the glycolate pathway is approximately equal to that used for the reduction of CO_2 in the PCR cycle. Carboxylase and oxygenase activity of RuBisCO are competitive that is, CO_2 and O_2 compete for the same active site on the RuBisCO enzyme and the outcome of the competition depends on the concentration of both gases. Under normal atmospheric pressure and when temperature is 25 °C, the rate of carboxylation is four times that of oxygenation. Although the concentration of O_2 is much higher than that of CO_2 in the atmosphere, the affinity of RuBisCO is higher for CO_2 (K_c = 12 μM) than for O_2 (K_o = 200 μM) (K_c and K_o are the Michaelis-Menten constants for carboxylation and oxygenation).

The carbon assimilated through photosynthesis over a certain period of time is called gross primary productivity (GPP) (Fig. 2a), neglecting light induced respiration (photorespiration) (Schulze et al., 2005). The balance between plant assimilation and respiration (autotrophic respiration) is called net primary productivity (NPP). About 50 % of the NPP reaches the soil each year, contributing to the formation of litter (Fig. 2b). Of the leaf litter, half remains into the soil as organic matter (SOM) with a mean residence time of more than one hundred years, while the remaining part is mineralised and returns to the atmosphere as CO_2 or methane (CH_4) through the heterotrophic respiration (R_h) of soil microorganisms (Fig. 2a, 2b).

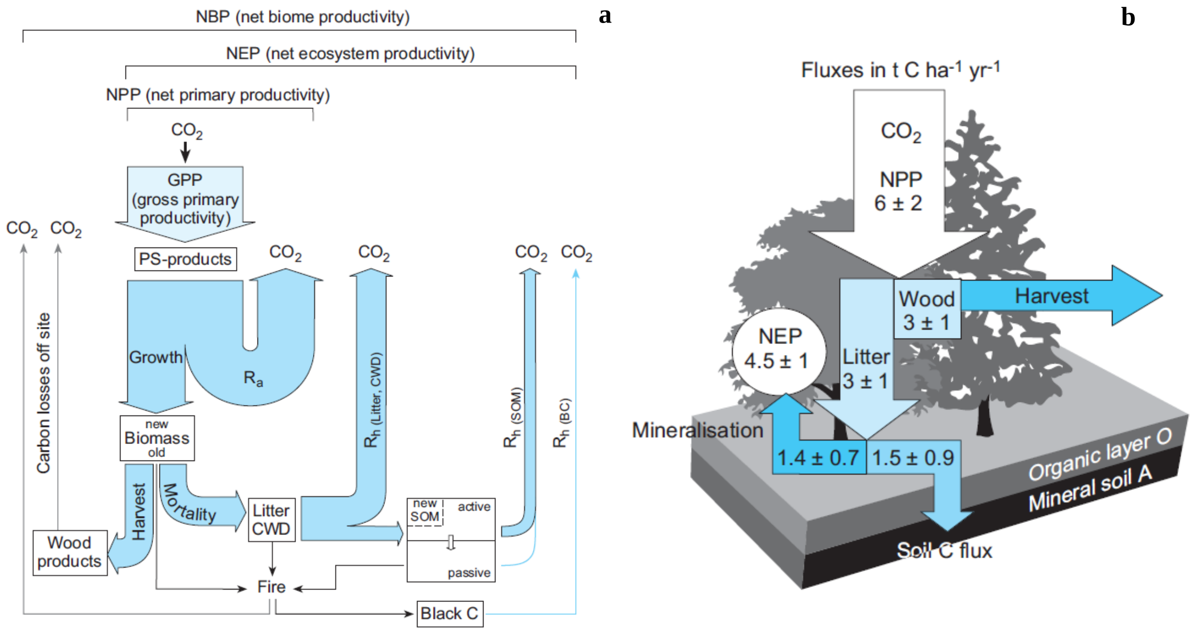


Fig. 2 (a) Productivity in the global carbon cycle (GPP: gross primary production; NPP: net primary production; NEP: net ecosystem productivity; NBP: net biome productivity; CWD: coarse woody debris; SOM: soil organic matter; BC: black carbon; R_h: heterotrophic respiration; R_a: autotrophic respiration) and (b) the flow of carbon in a forest ecosystem. Almost half of the primary production is in litter and in the other half in accumulated wood. Of the leaf litter, half remains in the soil as organic substances with a mean residence time of more than hundred years (from Schulze et al., 2005).

The net ecosystem productivity (NEP) is the balance between assimilation and total autotrophic and heterotrophic respiration (Fig. 2a, 2b), which is the process by which living organisms use energy from oxidation of organic matter for growth and maintenance (Houghton, 2003). Independent of soil respiration are processes that remove carbon from the system without appearing in the respiration term; examples of such carbon fluxes are harvesting by man, grazing and fires (Schulze et al., 2005). The difference between NEP and these carbon fluxes is called net biome productivity (NBP) (Fig. 2b).

The increase in the atmospheric CO_2 concentration since the second half of the XIX century have led to the alteration of the global carbon cycle as a consequence of vegetation photosynthetic responses to increased CO_2 availability, but also of its climatic effect on some processes involved in the carbon balance (respiration, fires…). The climate is generally defined as the average weather that occurs in a given region over time, and is expressed by the average values and variability of some physical factors, the most important of which are temperature and precipitation (Wuebbles and Jain, 2001). While climate consists of average meteorological conditions, the weather is defined as the meteorological conditions at a particular time (Dessler and Parson, 2010). Thus, the main difference between the two definitions is related to the considered time-scale. The Earth’s climate depends on the energy emitted from the sun. The distribution of the emitted energy is a function of the emitter temperature: the hotter is the emitter, the shorter is the wavelength of peak emission. Thus the Sun, which has a surface temperature of 6000 K, emits most radiation in the range of 0.2-4 µm (including ultraviolet, visible, and near infrared wavelengths), whereas the Earth at 255 K emits mainly in the range 4-100 µm (long-wave radiation) (Mitchell, 1989). On Earth, 96 to 99% of the atmosphere consists of molecular nitrogen and oxygen and the inert gas argon; these simple molecules are relatively inefficient absorbers of short waves solar radiation. Of the incoming radiation, only 45% is absorbed by the earth; 25% is reflected by the atmosphere, 5% by the reflective surfaces of the Earth (glaciers, oceans), while 25% is absorbed by the atmosphere and re-emitted in form of long wave infrared radiation (heat). Also the Earth emits energy as long wave infrared radiation. Some other gases present in the atmosphere, such as water vapour, carbon dioxide, methane, nitrous oxide, chlorofluorocarbons and tropospheric ozone, are characterized by more complex molecular shapes that allow them to absorb this long-wave radiation, and re-radiate it both back to the surface, producing an additional warming, and to space, maintaining the balance with incoming solar radiation (Mitchell, 1989; Dessler and Parson, 2010). The phenomenon is called the greenhouse effect and the responsible gases are called greenhouse gases. Although climate should be more stable than weather, any natural or human-induced alteration in the concentration of the greenhouse gases in the atmosphere leads to a modification in the radiation balance, increasing the heating caused by the natural greenhouse effect. Moreover, climate may also change over time by responding to the action of other radiative forcings, which refers to the measure of the influence of a factor in altering the balance of energy in and out the Earth system and the atmosphere, thus representing an index of the importance of the factor as a potential mechanism of climate change (IPCC, 2007), such as volcanic or solar ones. Climate change has usually occurred during the last 400 000 years, alternating ice ages to interglacial periods characterised by higher temperatures. During these natural variations, climate underwent slow changes which cannot be perceived on a human life time scale (Weart, 2006). However, there is a clear perception of the recent rapid warming at a global level (Grace, 2004). From the late nineteenth century, the average global temperature of the Earth's surface has risen by 0.74 °C (IPCC, 2007). According to the Fourth Assessment Report of IPCC (2007), the rapid observed global warming is due, with a very high confidence (90%) to external (not natural) radiative forcings and, particularly, to the observed increase in greenhouse gases concentrations in the atmosphere. Particularly, this increase seems to be induced by human activities, resulting in the enlarged greenhouse gas emissions compared to the pre-industrial period. In fact, during the past 50 years, the sum of solar and volcanic forcings would likely have produced cooling. Observed patterns of warming and their changes over time are simulated only by models that include anthropogenic forcings (Fig. 3).

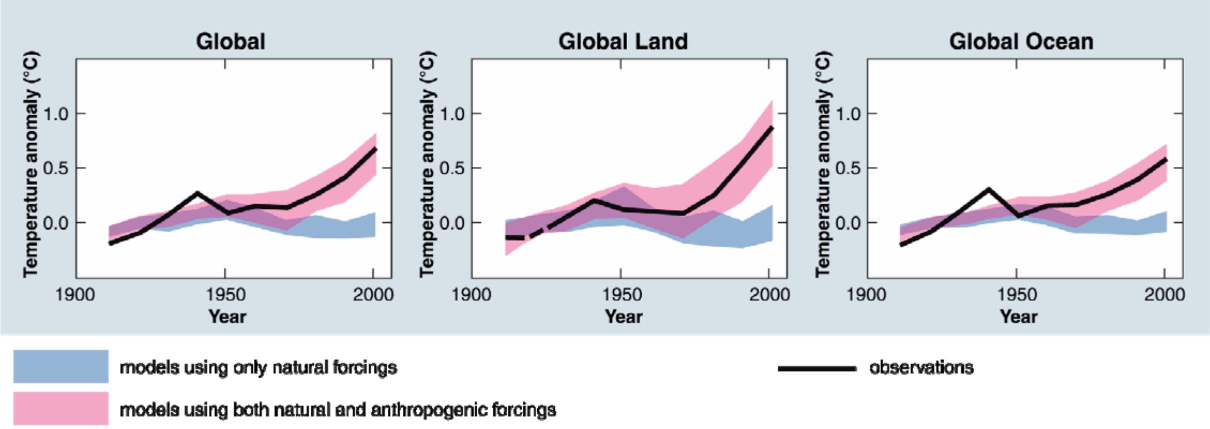


Fig. 3 Comparison between the observed global-scale changes in surface temperature and those simulated by climate models using either natural or both natural and anthropogenic forcings. Decadal averages of observations are shown for the period 1906-2005 (black line). Blue shaded bands show the 5-95% range for simulations from climate models using only the natural forcings due to solar activity and volcanoes, while red shaded bands show simulations using both natural and anthropogenic forcings (from IPCC, 2007).

After water vapour, carbon dioxide is the main contributor to the greenhouse effect (Dessler and Parson, 2010). Due to its seasonal and geographical variation, the first reliable measurement of atmospheric CO_2 concentration was performed only in 1958, when the first continuous monitoring of the concentration of this gas was made on the island Mauna Loa (Hawaii) and at the South Pole (Houghton, 2003). In that year, the measured concentration of CO_2 in the air was 315 ppm. The concentration reached 368 ppm in 2000, with an average growth rate of 1 ppm per year since 1958 (IPCC, 2001). In 2005, the atmospheric concentration of carbon dioxide reached 379 ppm (IPCC, 2007). The study of the gas bubbles trapped in ice cores extracted in Greenland indicates that the pre-industrial CO_2 concentrations ranged between 275 and 285 ppm. Between 1751 and 2000, the total emissions of carbon dioxide were 275 Pg C, most of which released after 1860. These emissions increased by 70% between 1970 and 2004 (IPCC, 2007). According to Dessler and Parson (2010), methane, the next largest contributor to greenhouse effect, absorbs infrared radiation about 20 times more strongly than CO_2 on a per molecule basis, but it is present in the atmosphere at about 1.75 ppm. Smaller contributions come from nitrous oxide, chlorofluorocarbons and ozone. Even if emissions are derived mostly from combustion and consumption of fossil fuels (industry and transport), agriculture, fires and changing land use accounts for 20% of the anthropogenic emissions of GHGs.

Forest fires and the reduction of global forest areas affect the capacity of soil and vegetation to sequester C, and are therefore in part responsible for the variation of the CO_2 fluxes between the land surface and the atmosphere (Houghton, 2003). Moreover, agriculture contributes 50% of CH_4 emissions (livestock and rice cultivation) and 70% of N_2O emissions (fertilizers, animal metabolic wastes, and biological nitrogen fixation) (IPCC, 2001).

On the whole, human activities would have caused an increase in radiative forcing of +1.6 W m^{-2} since 1750. This led to a broader definition of the phenomenon of global climate change, defined as any change in climate over time due to natural variability or as a result of human activities.

Secondary effects of climate change and plants responses

The increase in global average temperature is not the only effect of climate change. Changes in precipitation patterns (distribution, intensity and duration) are leading to the increase of heavy rain events and/or severe droughts which might damage vegetation and soil structure by increasing desertification and inundation (Kassas, 1995; Le Houérou, 1996; Knox, 2000; Nicholls, 2002; Christensen and Christensen, 2003). Moreover, the increase in global average air and ocean temperatures is causing a widespread melting of snow and ice resulting in the rise of global average sea level, which could cause the salinization of irrigation water, estuaries and freshwater systems (IPCC, 2007). Desertification, salinization, flooding and the rise of sea level represent global issues.

The causes of desertification are complex, often local, and differ depending on the region concerned (Pickup, 1998). Desertification is characterised by different features, depending on the context in which it is associated, which may be economic, social or environmental (Greco et al., 2005). For these reasons, this phenomenon has been defined by the International Convention to Combat Desertification (International Convention to Combat Desertification - CCD United Nations, 1994) as land degradation occurring in arid, semi-arid and dry sub-humid earths, as a result of various factors including climate change and human activities. The Convention defines these regions as the areas (except polar and sub-polar ones) in which the relationship between precipitation and potential evapotranspiration falls within the range of 0.05 and 0.65, i.e. those areas where rainfall appears to be less than the amount of water potentially lost (evapotranspiration and water use by plant organisms) during part or all year round (Kassas, 1995). Desertification is the result of two components: vegetation and soil degradation. The main causes of these two processes are due to soil depletion as a consequence of agriculture and overgrazing, the reduction of tree cover, and poor water management. These factors trigger the desertification process only where an inherent ecological fragility of the system is already present (Grainger et al. 2000; Kassas, 1995). The predisposing factors that make arid ecosystems vulnerable are: limited water availability (low rainfall) or a variable or seasonal water availability (recurring drought events, resulting in reduced plant growth; part of the year with the total absence of rain), poor vegetation cover, or covers varying with the seasons (which do not allow a continuous protection against soil erosion), reduced bioproductivity, a poorly developed soil with low organic matter content (Kassas, 1995). Climate change may then lead to an increased susceptibility of ecosystems to the desertification process. The result of these factors is the reduction (or loss) of productivity and biodiversity of natural affected ecosystems (Kassas, 1995; Ceccarelli et al., 2006). Mediterranean European countries are directly touched by the phenomenon of desertification (Mendizábal and Puigdefabregas, 2003). In Europe, the most arid countries are Spain and Greece, followed by Portugal and Italy. In these areas, desertification is also linked to another problem, which is in part caused by the increased temperatures and the decreased precipitation: the salinization of the soils as a result of an accumulation of salts in the irrigation water, and seawater intrusion.

Based on the FAO (1997) Soil Map of the World, the total area of saline soils is 397 million ha, while sodic soils cover 434 million ha. Saline environments are defined as those lands whose soil contains high percentages of soluble salts, and where one (usually NaCl) or more of these is normally in excess compared to the others (Poljakoff-Mayber and Lerner, 1994). Soil salinization is the result of two distinct processes: a natural geological process and a human-induced process. Geo-historically formed saline soils can be found along the seacoast, at lakesides, or in dry lands that were previously under marine or lacustrine conditions, while man-made saline soils are mostly found in (semi) arid lands, and are the result of salt accumulation after irrigation for agriculture (Oosterbaan, 2003). According to Poljakoff-Mayber and Lerner (1994), two types of saline environments can be found: the first is characterized by the presence of water, the second by its absence. Wet saline environments are usually represented by marsh sites near the sea, characterized by periodic flooding and therefore fluctuations in salt concentration. Dry saline environments are widespread in inland areas, especially in desert ones. There are also two additional types of saline environment: the coastal dunes and the dry salt lakes. The common characteristics in these areas are soil and/or water salinity, and the type of vegetation.

Flooding is a common environmental variable in many saline habitats (Colmer and Flowers, 2008). Temporary or continuous flooding conditions are the result of several phenomena including the rivers flood, melting of glaciers, precipitation characterised by high intensity and duration, poor soil drainage, water accumulating following the closure of a dam, water rising from underground and storm surges. It has been hypothesized that, in response to global change, phenomena of flooding can occur with greater frequency. In addition, Leifert (2007) suggests that reduced leaf stomatal opening, caused by the rising of CO_2 concentrations in atmospheric, can lead to a lower release of water vapour in the atmosphere, causing an increase in the amount of water remaining on continents surface, consequently increasing the duration of flooding.

According to the last IPCC report (2007), global average sea level has risen at an average rate of 1.8 mm/yr since 1961 and at 3.1 mm/yr since 1993, due to the contributions of the thermal expansion, the melting of glaciers, of the ice caps, and of the polar ice sheets. Moreover, satellite data show that the average annual Arctic sea ice extent has shrunk by 2.7% per decade since 1978, with larger decreases (7.4%) in summer. Also mountain glaciers and snow cover have declined in both hemispheres. From 1900 to 2005, precipitation increased significantly in the eastern parts of North and South America, northern Europe and northern and central Asia, but declined in the Sahel, in the Mediterranean, in southern Africa and in parts of southern Asia, causing an increase in the areas affected by drought. Furthermore, the frequency of heavy precipitation events has increased over most areas, and since 1975, the incidence of extreme high sea level has increased worldwide. Projections for the end of the XXI century indicate that the negative impacts of climate change will become stronger, according to the temperature increase that will be observed (Fig. 4). Concerning Europe, negative impacts will include increased risk of inland flash floods, and more frequent coastal flooding and erosion (due to storminess and sea-level rise). In Southern Europe, climate change is projected to worsen conditions (high temperatures and drought), and to reduce water availability. Climate change is also projected to increase the frequency of wildfires. These effects will be particularly severe in the Mediterranean countries, as they are already characterised by a high precipitation and temperature inter-annual variability (Giorgi, 2006). While a pronounced warming is projected in the Mediterranean region, with a maximum increase in temperature in the summer season (Giorgi and Lionello, 2008), the changes in precipitation patterns may lead to contrasting trends: on one hand, an increase in the occurrence of heavy precipitation events is expected all over Europe (Semenov and Bengtsson, 2002) that, coupled with an increase in land ice melting due to climate warming, may induce an increase in the occurrence of pick river flows and an increasing risk of inundation (Parry et al., 2007). On the other hand, precipitation and water availability are projected to decrease resulting in an increase of drought stress (IPCC, 2007). Furthermore, the rise of the sea level due to oceans expansion, melting of permafrost, snow and land ice may have detrimental effects on coastal areas, which will be subjected to flooding with salt seawater and to more severe saline water intrusion.

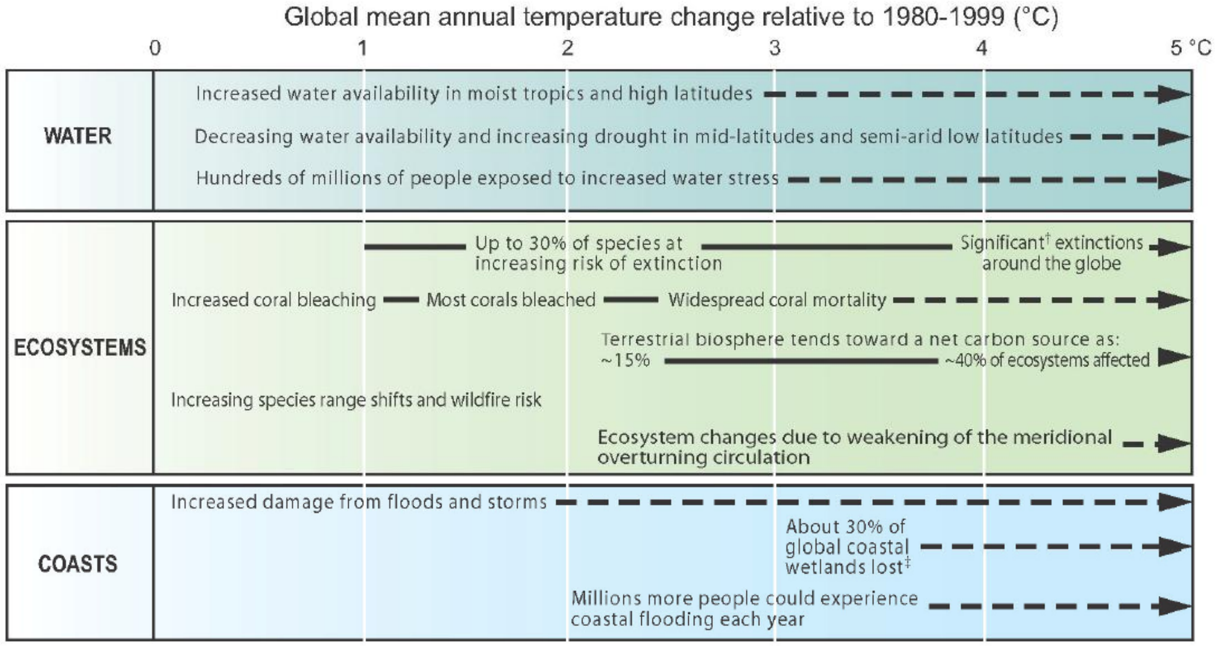


Fig. 4 Illustrative examples of global impacts projected for climate changes associated with different amounts of increase in global average surface temperature in the 21st century. The black lines link impacts; broken line arrows indicate impacts continuing with increasing temperature. Adaptation to climate change is not included in these estimations. Confidence levels for all statements are high (from IPCC, 2007).

Terrestrial ecosystems respond to constantly changing biotic and abiotic environmental factors; daily and seasonal fluctuations of light radiation, temperature and humidity are inherent characteristics of any natural ecosystem, and define the distribution of species and biomes (Norby and Luo, 2004). Plants growth, development and reproductive potential are closely related to the environmental conditions in which they live (Mills, 2002). In general, stress occurs when one or more factors (biotic or abiotic) that affect plant growth are in deficit or exceed the range of the plant physiological tolerance to the same factor. Environmental factors which do not allow an optimal level of growth are called stressors. Global change has led to the alteration of CO_2, temperature, water availability and soil characteristics in a relatively short time. The awareness of the physiological stresses induced by these phenomena and plants relative responses and adaptations may be helpful in managing, protecting and restoring the affected areas. In this session, plant responses to salinity and flooding will be discussed.

Physiological responses of higher plants to salinity

Sodium chloride is the most common salt in many saline habitats. Plants responses to salinity depend 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) which vary among species and, within the same species, among genotypes. According to their tolerance, plants are divided into glycophytes and halophytes.

Glycophytes are those species that cannot tolerate high concentrations of soluble salts 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, plants response to nutrients imbalance is particularly evident, especially when the stress is caused by a non-fundamental element (Schulze et al., 2005), such as sodium (Na). Moreover, 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, causing water and ionic imbalances when they are present in high concentrations (Schulze et al., 2005).

Salinity detrimental effects on plant growth concern with (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 (3) the nutrient ion imbalance, due to the disturbance of essential intracellular ion concentrations (especially K$^+$) (Marschner, 1995; Mansour, 2000; Zhu, 2001). The stress caused by high salinity conditions 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 an excessive cellular concentration of Na$^+$ and Cl$^-$ (Blumwald et al., 2000).

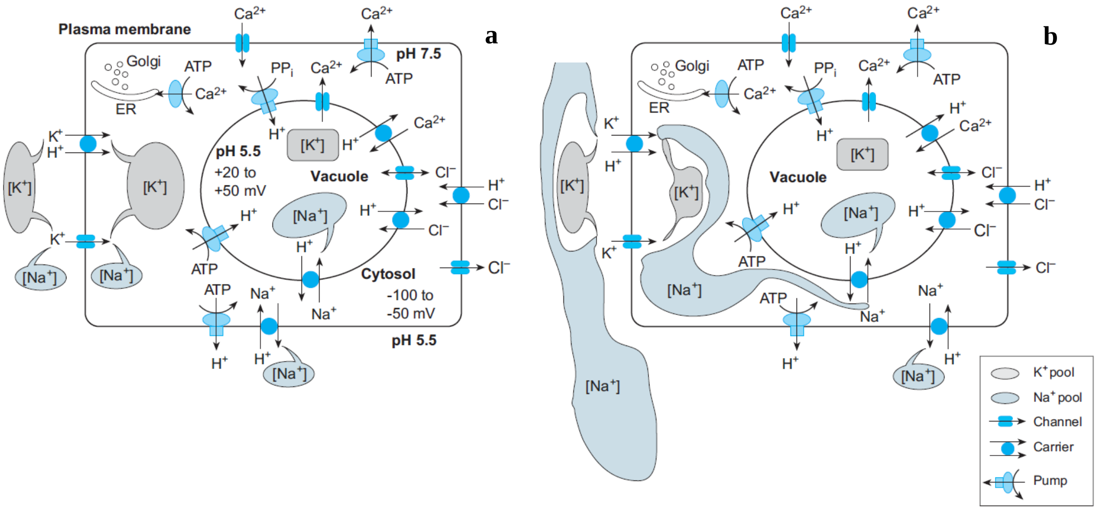


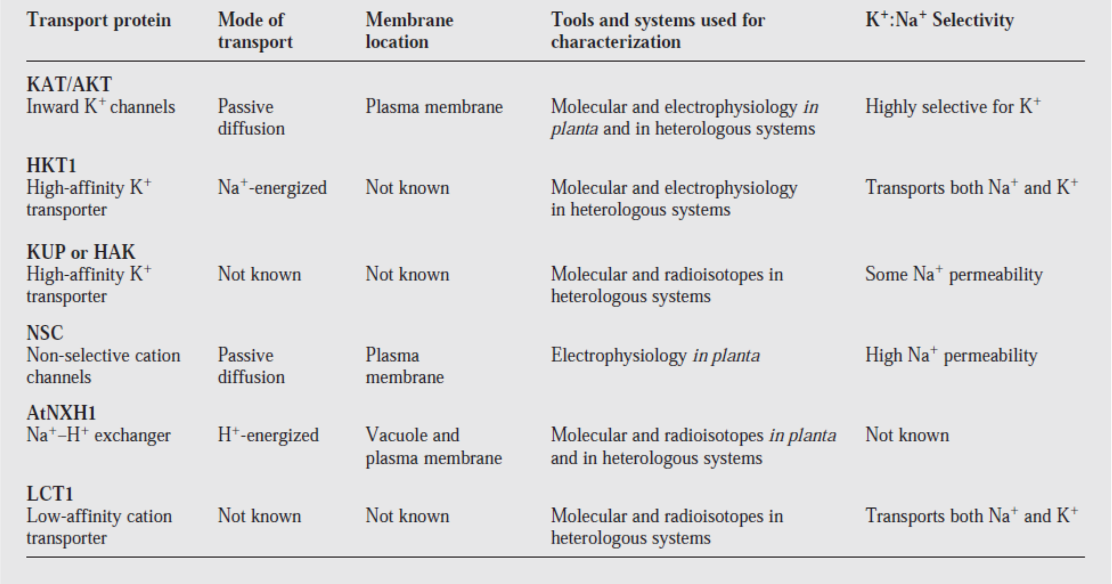
Fig. 5 Ion relations in plant cells in (a) non-stressed and (b) strongly stressed states (from Schulze et al., 2005).

The primary and secondary effects of salt stress are accurately described by Schulze et al. (2005) and Blumwald et al. (2000). According to these authors, 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 (Fig. 5), these two ions being characterised by a same radius size and by the same ion hydration energy. In fact, charged ions do not more through the lipid bilayer, and so must cross the membrane via specialised proteins; molecular tools revealed the identity of a large number of potassium channels and transporters (Tab. 1) that, however, are not equally permeable to Na$^+$ ions (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; it has been demonstrated that calcium inhibited the Na$^+$ permeation through non selective channels. Thus, other pathways might be involved in

Na^+ uptake, because in most soils calcium levels are high enough to substantially inhibit Na^+ transport through non selective channels (Schachtman and Liu, 1999).

Tab. 1 Plant K^+ and Na^+ transport (from Schachtman and Liu, 1999)



Transport protein	Mode of transport	Membrane location	Tools and systems used for characterization	K^+:Na^+ Selectivity
KAT/AKT Inward K^+ channels	Passive diffusion	Plasma membrane	Molecular and electrophysiology *in planta* and in heterologous systems	Highly selective for K^+
HKT1 High-affinity K^+ transporter	Na^+-energized	Not known	Molecular and electrophysiology in heterologous systems	Transports both Na^+ and K^+
KUP or HAK High-affinity K^+ transporter	Not known	Not known	Molecular and radioisotopes in heterologous systems	Some Na^+ permeability
NSC Non-selective cation channels	Passive diffusion	Plasma membrane	Electrophysiology *in planta*	High Na^+ permeability
AtNXH1 Na^+–H^+ exchanger	H^+-energized	Vacuole and plasma membrane	Molecular and radioisotopes *in planta* and in heterologous systems	Not known
LCT1 Low-affinity cation transporter	Not known	Not known	Molecular and radioisotopes in heterologous systems	Transports both Na^+ and K^+

Since the ion K^+ is responsible for the activation of about 50 enzymes, high levels of Na^+ or high Na^+/K^+ lead to the alteration of many enzymatic processes in the cytoplasm (Tester and Davenport, 2003). Furthermore, the accumulation of positive charges in the cytoplasm leads to the loss of a natural barrier to Cl^-, which is normally linked to the membrane potential, causing a massive 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 in particular of ATP-ase associated with the plasma membrane and antiport systems Na^+/H^+ of the tonoplast, leading to an increased ATP consumption, an alteration of the cellular enzymatic activity and an accumulation of Na^+ in the vacuole that causes its alkalinisation, which detrimentally affects the activity of various enzymes, particularly those involved in the catabolic energy metabolism. The high cellular concentration of Na^+ ultimately leads to an increased uptake of calcium, with a consequent increase in the cytosolic pools of this ion, which has a signalling function and triggers some regulatory processes in the cell. Secondary effects are attributable to a decrease in cell growth by division, caused by a decreased energy availability, and in photosynthesis, due to the damage of the photosynthetic electron transport system and to the formation of ROS and leaf necrosis. Moreover, high cellular salt concentration might have a detrimental effect on stomatal guard cells, reducing stomatal conductance as well as mesophyll conductance (Bongi and Loreto, 1989; Delfine et al., 1998; Nandy (Datta) et al., 2007). Under high salinity, leaf photosynthetic capacity could be limited by the electron transport (Bongi and Loreto, 1989; Delfine et al., 1998) and by the activity of RuBisCO (Bongi and Loreto, 1989). Leaf sensitivity also depends on leaf age: in old leaves, high salinity increases the costs of maintaining because leaf aging and salt stress both reduce photosynthetic rates and salt accumulation capacity (Suárez and Medina, 2005).

Although glycophytes are more affected than halophytes by salt stress, these two differently tolerant kinds of plants are characterised by common reactions of adaptation. In fact, enzymes in halophytes are no more tolerant to high concentration of NaCl than are those of glycophytes, and Na^+ starts to inhibit most of them at a concentration above 100 mM (Munns, 2002). Halophytes adapt faster and are more tolerant to extreme salinity and vice versa (Schulze et al., 2005). Moreover, Munns (2002) stresses the importance of temporal variation in the response to salt stress. The first responses concern with immediate variations (tacking place in minutes or hours) in leaves and roots growth rates (quickly recovered), linked to changes in cell water relations, mediated by a root hormonal signal (Munns, 2002). In fact, at a hormonal level, an increase in cellular concentration of ABA and ethylene take place, while cytokinin suffers a decline. It is therefore possible 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). As in water stress, the expression of many specific proteins occurs, 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). The reduction in leaf growth rates, however, can also be related to the presence of salt in the nutrient solution, which causes a decrease in the absorption of Ca^{2+} (Munns, 2002). Subsequently, leaf growth is more influenced by the presence of salt than root growth (Tester and Davenport, 2003). This phenomenon occurs even under water stress and could therefore be tied to this, rather than to a salt-specific effect; this 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). After few days under salt stress, plants growth seems to be more influenced by their hormonal activity rather than by their water status. In fact, leaves expansion within 24 hours under saline conditions does not undergo any change after an increase in leaf water status. In the following weeks and months, damages may be visible at leaf level, which can lead to death and phylloptosis of the leaf itself (Munns, 2002). Thus, salinity affects plants growth in two ways: by reducing leaf photosynthesising area and by reducing leaf gas exchanges (Sharma, 1996).

Contrarily to glycophytes, halophytes are defined as plants that can complete their life cycle in soils with salt concentrations above 200 mM and constitute about 1% of the world's flora (Flowers and Colmer, 2008). To cope with salinity, halophytes should preserve water uptake under very low soil water potentials and avoid the detrimental effect of high intracellular salt levels. Mechanisms for salt tolerance are therefore of two main types: those that minimize the entry of salt into the plant and those that minimize the concentration of salt in the cytoplasm. Thus, the 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

According to Munns (2002), a plant transpires about 30-70 times the amount of water that it actually uses for cell expansion, which means that the 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. Thus, the selectivity of uptake by the root cells may prevent sodium to be accumulated into the transpiring organs, while maintaining potassium uptake. This may be achieved through an increase of highly selective K^+ transporters at the root level (Schachtman and Liu, 1999). Moreover, in many species, Na^+ is retained in the upper part of the root system and in the lower part of the shoot, indicating 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^+

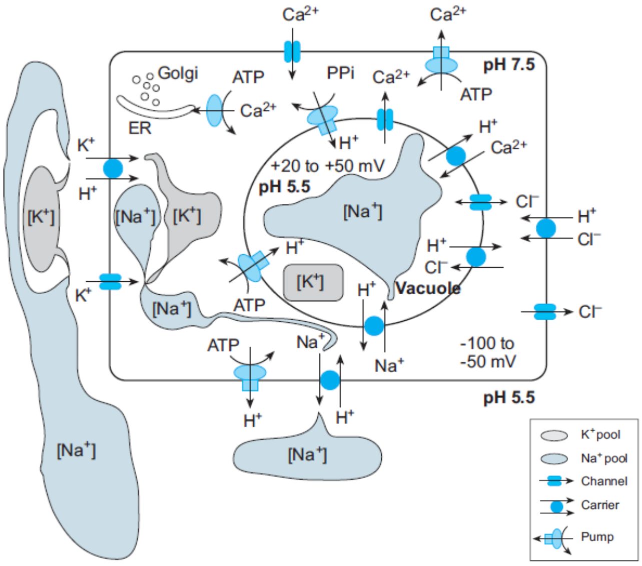
It has been widely accepted that Na^+ ions, due to their toxicity, must be removed from the cytoplasm (Cheeseman, 1988). Salt exclusion is the most important mechanism of cellular adaptation to salt high concentration in halophytes, even in those species characterized by the presence of salt secretory glands (Munns, 2002). Cell exclusion is a mechanism used to reduce the rate of salt accumulation in transpiring organs. Both the apoplast and the vacuole can be the final compartments for salt deposition; this is achieved through Na^+/H^+ antiports, which are both in the plasma membrane and in the tonoplast. High Na^+ and proton concentration induce the formation of new antiports. Several H^+ pumps are then synthesised under salt stress conditions. When homeostasis is reached again, there is a decrease in H^+-ATPase. During this phase, the concentration of Na^+ in the cytoplasm is brought back to its original value, while Na^+ concentration in the vacuole is higher. However, 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 of halophytes and glycophytes under salt stress is probably due to a low supply of K^+ (Schulze et al., 2005).

The elimination of NaCl from the apoplast generally occurs through salt glands. Salt glands are specialized epidermal cells or trichomes, which play an active role in the secretion of mineral salt solutions, and often contain organic substances. Ions found in the secretions of these glands are Na^+, K^+, Mg^{2+}, Ca^{2+}, Cl^-, SO_4^{2-}, NO_3^-, PO_4^{3-} e HCO_3^- (Fahn, 1988). Although salt extrusion increases leaf tolerance under high salinity, it is considered an active mechanisms. It has been estimated that 10-12 mol ATP are needed to extrude 1 mol NaCl in *Tamarix ramosissima* (Kleinkopf and Wallace, 1974). Moreover, 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, as observed by Hirano et al. (1995) in leaves covered by dusts.

Plants under saline conditions may also eliminate salt by leaf abscission. This phenomenon usually takes place in old leaves rather than in younger leaves, i.e. the leaves that have been transpiring the longest (Munns, 2002). However, leaf elimination 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, plants must have a more negative water potential compared to the medium in which they live (Tester and Davenport, 2003); to achieve this condition, they must increase their osmotic potential. It is therefore essential that internal changes in the concentration of solutes take place. Such changes, which occur as a response to a water rather than to a saline stress, may be the result of two mechanisms: an increase of solutes uptake from the soil and the synthesis of solutes (compatible solutes) (Schulze et al., 2005). According to Tester and Davenport (2003), this factor poses a dilemma: Na^+ and Cl^- are energetically cheap solutes (Cram et al., 2002). However, 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, are polar, highly soluble and are characterised by a high hydrating external surface (Sairam and Tyagi, 2004). Most halophytes tend to use Na^+ and to compartmentalize it in the vacuole (Fig. 6).



However, under high salinities, plants might reduce their ability to adjust osmotically as a result of saturation of solute uptake systems (Munns et al., 1983), resulting in an overall decrease in plants growth.

Fig. 6 Adaptations to high salinity concentrations (from Schulze et al., 2005).

Other adaptations to salinity may be correlated with changes in wood anatomy. Excess salinity causes osmotic stress and thus shared some similarities with drought stress, as the increasing risk of vessels cavitation. 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 for photosynthesis (Pockman and Sperry, 2000). As a result, vessel characters should be adapted to reduce the impact of cavitation on sap flow by preventing gas expansion and by minimizing 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 fact, as the cavitation resistance is determined by the maximum and not by the average pore diameter of the pit membrane, the larger is the pit area, the bigger is the chance of an exceptional large pore increasing cavitation vulnerability (Hacke et al., 2006). Moreover, when vessels diameter is high, a lower xylem pressure is sufficient 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).

Thus, the salinity tolerance of a plant 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).

Physiological responses of higher plants to flooding

Flooding events trigger a series of biological, chemical and physical mechanisms on habitats that, altogether, alter soil capacity to support plant growth by: (1) reducing O_2 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 Voesenek, 1996; Kozlowski, 1997; Schulze et al., 2005).

According to these authors, gas exchange in well aerated soils is the result of air diffusion inside the pores, but this is further accelerated by several soil processes that make the whole a relatively fast process. Gas diffusion in pores filled with water appears to be totally different. Indeed, at the same temperature, the diffusion coefficient (see Fick law) of oxygen in the water is 11 300 times smaller than in air. Furthermore, oxygen is characterized by having a low solubility in water (0.03 ml O_2 l^{-1} H_2O). For this reason, the gas exchanges in flooded soils are very slow, and the supply of oxygen becomes one of the main factors limiting plant growth in these environments. The oxygen that is trapped in the soil following flooding is quickly consumed by aerobic processes and root fauna. The decrease in oxygen availability promotes the growth of anaerobic or microaerophilic bacterial populations that use mainly organic matter as an energy source. However, they require electron acceptor ions that can be reduced. Under these conditions, many important oxygen-dependent processes such as nitrification, stop and are replaced by anaerobic metabolism of plants and bacteria, leading to the accumulation of toxic substances (sulphide, CO_2, 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). The first anaerobic process that occurs is the reduction of nitrate, which is used as an electron acceptor, to nitrogen (denitrification), followed by the reduction of Mn and Fe oxides. CO_2 can also be used as an electron acceptor, leading to the formation of methane. The production of reduced ions causes a decrease in the redox potential of the soil. When it reaches values in the range of -75 and -150 mV, sulphate is reduced by several strictly anaerobic bacteria, producing phytotoxic sulphide. After flooding, the aggregates are reduced to smaller particles. When the water level decreases, the newly formed particles are rearranged into a more dense structure, characterized by pores of smaller diameter, higher mechanical resistance to penetration of roots and low concentration of O_2.

Among the processes generated by soil submergence, the one that most limits plant growth is the lack of oxygen. The critical oxygen concentration for roots ranges between 5 and 10% (Schulze et al., 2005). Concerning the relationship between the concentration of oxygen and metabolism, a situation in which biochemical processes are not limited by a lack of O_2 is called *normoxic*. If the mitochondrial synthesis of ATP appears to be influenced, but not inhibited, by low concentrations of O_2, the condition is called *hypoxia*, while in the complete absence of O_2 (*anoxia*), mitochondrial oxidative phosphorylation is negligible compared to the synthesis of ATP via glycolysis and fermentation (Schulze et al., 2005). According to Blom and Voesenek (1996), the immediate consequence of a lack of oxygen in higher plants, flooding tolerant or not, is the decrease of roots aerobic respiration and, consequently, of ATP generation. This causes a decrease in the energy available for root growth and, consequently, a reduction in the vegetative growth (Liao and Lin, 2001). Under hypoxic conditions, many heterotrophic organisms and plant tissues are able to replace the Krebs cycle and switch to fermentative metabolism (Schulze et al., 2005). This type of metabolism, however, requires large amounts of energy in form of glucose, so that the reserve material is quickly consumed and many toxic products, such as lactic acid and ethanol, are accumulated, promoting acidification of the cytoplasm (Blom and Voesenek, 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 response to flooding conditions, is responsible for the recycling of NAD^+, which are necessary for glycolysis (Liao and Lin, 2001) allowing, albeit more slowly compared to aerobic conditions, ATP production (Blom and Voesenek, 1996). It has been demonstrated that 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 (Liao and Lin, 1995). According to Kozlowsi (1997), flooding can give rise to detrimental effects also 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). In plants growing in waterlogged soils, stomatal closure under flooding conditions is induced by a hormonal signal transmitted from the roots to the shoots (ABA and cytokinin) rather than by a reduction in leaf water potential or a loss of leaf turgor (Kozlowski, 1997). The reduction in the photosynthetic rate could be induced by a decrease in stomatal conductance, although in the long-term, this appears to be more influenced by inhibitory effects on the photosynthetic process itself, such as the loss of chlorophyll.

As two third of the earth’s land mass is flooded at least occasionally, many species have developed different strategies to survive hypoxia (Schulze et al., 2005). In fact, in the long-term the adjustments are based on rapid changes in physiological processes, resulting in a variation of the morphological and anatomical root features (Blom and Voesenek, 1996). Under 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) (Blom and Voesenek, 1996): this tissue, called aerenchyma, is formed at the end of the elongation zone (Schulze et al., 2005). The aerenchyma provides an interconnected system of air channels, enabling gases to diffuse or ventilate between plant organs (Fig. 7a); particularly, it allows the descent of air or oxygen that derives from photosynthesis or from the atmosphere to the roots and, conversely, the lift of CO_2, ethylene and methane produced by roots and soil microorganisms on the surface (Blom and Voesenek, 1996; Colmer, 2003). In stems, aerenchyma can occur in the cortex and in the pith cavity (Armstrong, 1979). The oxygenation of the rhizosphere around the growing 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 not only ensures the tissues aeration, but also reduces the number of cells requiring energy in this tissue (Schulze et al., 2005).

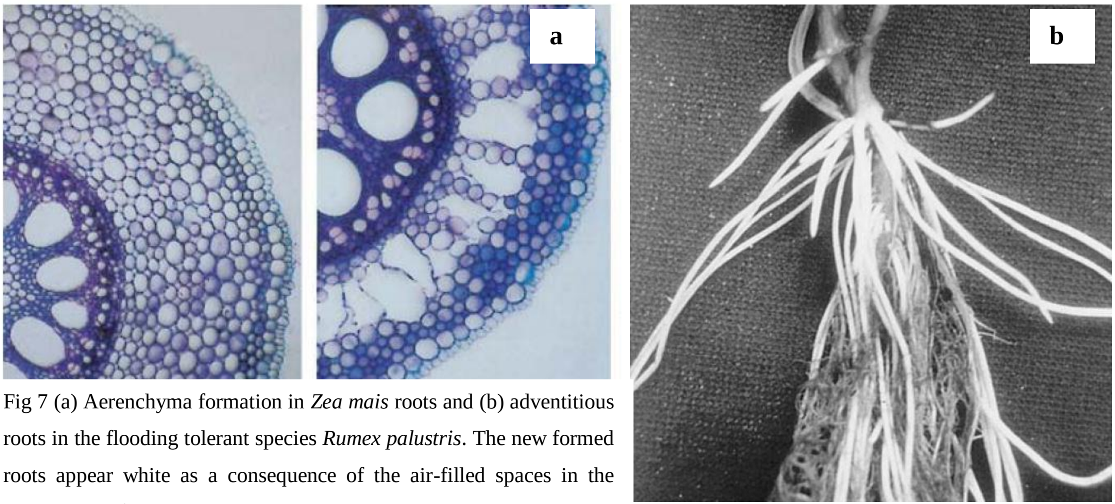


Fig 7 (a) Aerenchyma formation in *Zea mais* roots and (b) adventitious roots in the flooding tolerant species *Rumex palustris*. The new formed roots appear white as a consequence of the air-filled spaces in the aerenchyma (from Schulze et al., 2005)

Many species tolerant to submergence are capable of producing, in response to an increase in the concentration of ethylene and auxin, numerous adventitious roots, characterized by a highly developed aerenchyma (Blom and Voesenek, 1996) (Fig. 7b). These roots usually grow on the base of the shoot, on the upper part of the tap root and on stem nodes, mostly exploring the upper better aerated soil layers (Blom and Voesenek, 1996) or floating on water surface (Kuzovkina et al., 2004). The re-establishment of a contact between the root apparatus and the air enables stomatal opening and, thus, leaf gas exchange and growth. The long-term responses of the aboveground portion of the plant also concern with branches increase in length, caused by low oxygen partial pressure of submerged internodes which induces an increase of the ethylene cellular concentration (Schulze et al., 2005).

Together with waterlogging, salinity can cause severe damage to plants (Barrett-Lennard, 2003) and the occurrence of these stresses is increasing in many environments (Carter et al., 2006). Besides the stress induced by salinity and flooding as single factors, it has been demonstrated that salinity compromise flood tolerance mechanisms (Salter et al., 2010), preventing 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). However, it has been demonstrated 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).

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

Ecological restoration is the process of assessing the recovery of ecosystems which have been degraded, damaged or destroyed (SERI, 2004). The aim of restoration practices is to help ecosystem in having the capacity to recover from stresses, i.e. becoming once again resilient to perturbation without further assistance (SERI, 2002). According to the Society of Ecological Restoration International (2004), a restored ecosystem should have the following attributes; (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; (2) functional groups necessary for long-term stability must be present; (4) the physical environment must have the capacity to sustain reproducing populations; (5) it must have a normal functioning; (6) it must be integrated with the landscape; (7) threats must be eliminated; (8) it must be resilient to natural disturbance and (9) it must be self-sustainable. Young et al. (2005) propose some ecological concepts which must have to be taken into account and applied in ecological restoration practices (Tab. 2).

Tab. 2 Ecological concepts that are generally understood by restoration practitioners (from Young et al., 2005).

Ecological concepts	
Competition	Plant species compete for resources, and competition increases with decreasing distance between individuals and with decreasing resource abundance
Niches	Species have physiological and biotic limits that restrict where they are thrive
Succession	In many ecosystems, communities tend to recover naturally from natural disturbances following the removal of these disturbances. Restoration often consists of assisting or accelerating these processes
Recruitment limitation	The limiting stage for the establishment of individuals of many species in often early in life, and assistance at this stage (irrigation, protection from competitors) can greatly increase the success of planted individuals
Facilitation	The presence of some plants (such as N-fixers) enhances natural regeneration
Mutualism	Mychorrizae, seed dispersal and pollinators are understood to have useful roles in plant regeneration
Herbivory/predation	Seed predators and herbivores often limit regeneration
Disturbance	Disturbance is a natural and sometime essential component of many communities
Island biogeography	Larger and more connected reserves maintain more species and facilitate colonisations
Ecosystem functions	Nutrient and energy fluxes are essential components of ecosystem function and stability
Ecotypes	Populations are adapted to local conditions; matching ecotypes to local conditions increases restoration success
Genetic diversity	Populations with more genetic diversity have grater evolutionary potential and long-term perspectives than genetically depauperate populations

The simplest restoration practice involves removing a perturbation and allowing ecosystem to recover via natural ecological processes (Palmer and Bernhardt, 2006). However, this practice is not always possible and depends on the degree of perturbation severity. The primary goal for population-level restoration projects may be the reintroduction of a species that has been extirpated, the restoration of critical habitat components for a species of interest or the demographic and genetic augmentation of an existing but reduced population (Falk et al., 2006). Nevertheless, in severely degraded ecosystems, already existing plant species may not be suitable for the restoration of the degraded ecosystems; thus, plant species adapted to the corresponding ecosystem succession phase should be introduced. Species introduction is a widely used method to accelerate the restoration of habitats or to re-establish locally extinct populations. However, as there is a genetic differentiation and sometimes strong adaptation to local environmental conditions, the origin and quality of source populations must be carefully considered if re-introduction is to be successful. From this point of view, the distribution of genetic variability within and among populations should be taken into account. The challenge of restoration ecology is to utilise sufficient diversity to allow adaptation to new circumstances, while avoiding the adverse effects of introducing genotypes that are poorly adapted to the environment (Rice and Emery, 2003). Moreover, in severely degraded ecosystems, the highest priority for a restoration project is the establishment of a functional plant community for which tolerance of extreme conditions may be paramount (Stockwell et al., 2006). Restoration may be concerned not only with the overall degree of variability, but also with its particular geographic distribution and phylogenetic lineage (Falk et al., 2006). The most common approach is to specify a geographic range within which source material should be collected (Fenster, 1991) as population near one another and growing under similar conditions will be more similar genetically due to ecotypic variation and to the effect of gene flow (Govindaraju, 1990). The use of regional mixtures deriving from collections of genotypes, moderately adapted to the general environment and within a broad geographic zone seems to be a good strategy in restoration projects (Knapp and Dyer, 1997). Thus, the wide genotypic variation in natural plant populations must be recognized and exploited. This will ensure that genotypes used on a site are best adapted to local conditions and have a greater probability of survivorship than arbitrarily chosen material. Also, certain unusual genotypes can be located using the principles of evolutionary ecology and can be installed in areas with extreme conditions (Handel et al., 2004). Moreover, the use of non-local source populations may not only compromise the success of restoration measures, but may also have negative consequences on existing populations, potentially disrupting ecosystem function (SER, 2010).

The use of tolerant material in the restoration of degraded lands was pioneered by Smith and Bradshaw (1979); the authors collected seed from metal-tolerant species that had naturally evolved tolerance on metalliferous mining sites. These species (*Agrostis capillaris* and *Festuca rubra*) colonised faster, persisted longer and produced a better stabilising cover than non-tolerant commercial varieties in contaminated sites. More recently, many studies have been applied to riparian and salt marshes vegetation. In fact, riparian and coastal areas and their associated characteristic vegetation play an untold number of environmental roles, like filtering pollutants, stabilising soil against erosion and functioning as a refuge and ecological corridor for plant communities and wildlife (SER, 2010). These areas do not have a linear structure, but 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). Thus, the collection of more tolerant genotypes/ecotypes along these gradients is currently necessary for the future recovery of these areas which might be severely threatened by climate change. However, differences in how the growth rate under salt and flooding stresses varies between species and source populations must first be evaluated before recommending species for restoration. Lessmann et al. (1997) evaluated the intraspecific variation in leaf elongation and biomass partitioning in response to flooding stress in populations of *Spartina alterniflora, S. patens*, and *Panicum hemitomon* finding that the analysed parameters showed a significant ecotypic differentiation in biomass partitioning. A similar study was conducted by Hester et al. (1998), in order to highlight biomass and leaf morphological variations of different genotypes of *Panicum hemitomon* and *P. alternifolia* under salt stress. More recently, Aschenbach (2006) found a variation in growth rates under saline conditions of *Pascopyrum smithii* and *Distichlis spicata* collected from different source populations in Kansas and Nebraska.

In the Mediterranean basin, riparian and coastal vegetation has developed stress-adapted mechanisms to overcome changes in temperatures, water availability and salinity. In fact, the rivers of this regions are characterised by a scarce and irregular water flow both in time and space. The species that colonise these areas are likely to be well adapted to future conditions caused by the global warming effects (Thuiller et al., 2005). Moreover, their naturally survival capacity and genetic biodiversity can be harnessed to sequester a significant part of the high atmospheric CO_2 content by increasing their plantation in presently unutilized arid/flooded areas, where saline water or reused urban and industrial water waste might be present. A recent study (Han et al., 2007) has shown that forestation and reforestation constitute a low-cost option of carbon dioxide sequestration with significant economic and social benefits, such as forest products, improved soil and air quality, reduced erosion and improved ecosystem health. In areas of particular natural values, as reserves and parks, this option should be oriented to protect and preserve the natural habitats and species biodiversity. In these areas, the reforestation of over-exploited soils should be made with local species in mixed plantations. For these reasons, the characterisation of tolerant species and genotypes in the Mediterranean basin is becoming more and more important for the restoration of habitats which might be affected by drought, salinization and flooding with fresh and saline water as a consequence of climate change.

The genus *Tamarix*: taxonomy, distribution and ecology

The genus *Tamarix* is one of the four genera of *Tamaricaceae* and includes about 90 species of trees and shrubs (Zohary, 1972; Zhang et al., 2002). The taxonomy of the genus is quite complex, since its members exhibit very few external traits that are distinctive and easy to see. Diagnostic traits are usually related to the morphology of the small flowers, especially the androecium and the bracts that subtend the flowers, so it is difficult to classify individuals as belonging to a certain species unless they are flowering.

According to Aránzau Prada and Arizpe (2008), *Tamarix* spp. grow in arid and semi-arid climates, but require temporary edaphic humidity conditions from surface or ground water; 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.

Tamarix are either true trees with a well-developed trunk or shrubs (Baum, 1978). On average, they may grow up to 10 m tall (Frasier and Johnsen, 1991). Some species can reach large size and live for hundreds of years, although the typical life span has been reported to be 75 to 100 years (Horton, 1977).

Tamarix is usually deep and intensively rooted, with tap roots reaching 30 m in depth. According to Merkel and Hopkins (1957), root development depends on soil water status: when the plants grow 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). Superficial side roots are also capable of producing adventitious buds (Baum, 1978). 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 (Merkel and Hopkins, 1957; Baum, 1978; Zhang et al., 2002) or are under flooding conditions (Ginzburg, 1967; Zhang et al., 2002), usually during the second week after flooding (Merkel and Hopkins, 1957).

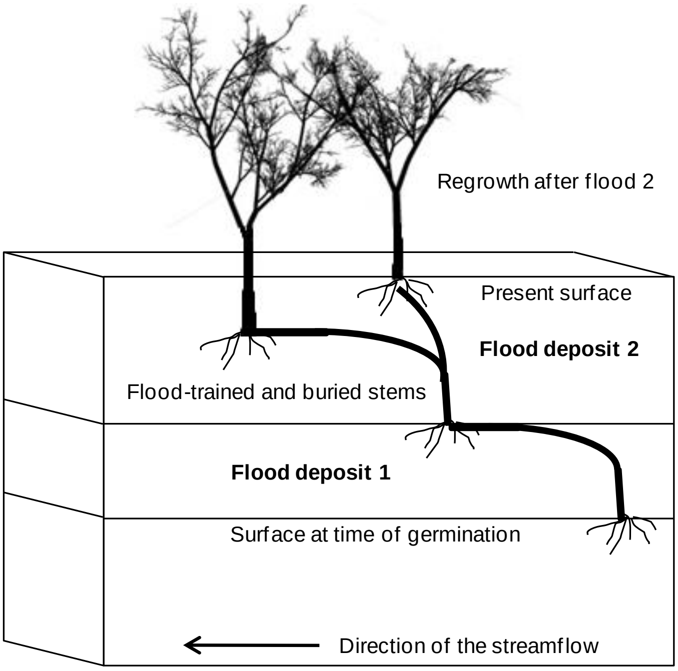


Fig. 8 Vegetative reproduction of *Tamarix* spp. through adventitious buds as a consequence of the natural layering of water sediments (modified from Everitt, 1980).

Resprouts from the roots are also visible after the top of the plant has been removed (Frasier and Johnsen, 1991). This phenomenon is probably an adaptive advantage in the vegetative propagation of *Tamarix* species in their natural habitats (Ginzburg, 1967). The vegetative reproduction of *Tamarix* spp. through adventitious buds is observed as a consequence of the natural layering of water sediments (Everitt, 1980) (Fig. 8) or, near the shoreline, after deposition or removal of material at the base of the stem due to the waves action (Merkel and Hopkins, 1957). Moreover, adventitious roots formation also enables these plants to be propagated through cuttings.

Woods of *Tamarix* species growing in the Sahara and in the Sahel were analyses by Neumann et al. (2001). The authors reported the presence of indistinct growth ring boundaries, and few and solitary large vessels. However, Abbate Edlmann et al. (1994), in a study on the characteristics of Mediterranean maquis wood anatomy, reported distinct tree rings in *Tamarix gallica*.

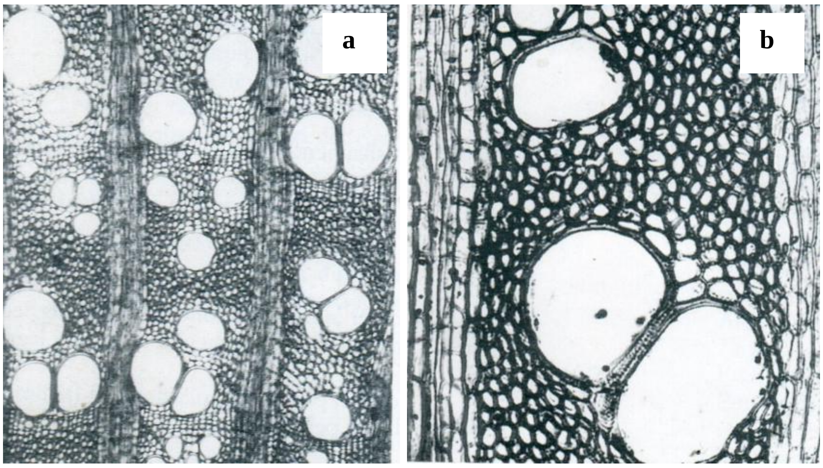


Fig. 9 Wood anatomy of *Tamarix* spp. at a magnification of (a) 51× and (b) 128× (from Abbate Edlmann et al., 1994).

The tree rings were characterized by larger vessels at the beginning of the ring and by 2-3 rows of fibres with a pressed shape (in radial direction) at the end of the ring. Round vessels were present, lonely or in groups of 2-3, while radial parenchyma was characterized by 5-18 series of cells (Fig. 9).

The genus is characterised by herbaceous, small, deciduous or persistent, scale-like leaves (Baum, 1978). The presence 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 can play an active role in the secretion of solutions of mineral salts (Fahn, 1988). The salt glands ultrastructure of *Tamarix aphylla* was studied by Thomson and Liu in 1967, by Shimony and Fahn in 1968 and by Thomson, Berry and Liu in 1969. A summary of this works is given by Fahn (1988). The salt gland of *Tamarix aphylla* consists of eight cells: two vacuolated basal cells, called collecting cells, and six upper cells with dense cytoplasm, called secretory cells (Fig. 10).

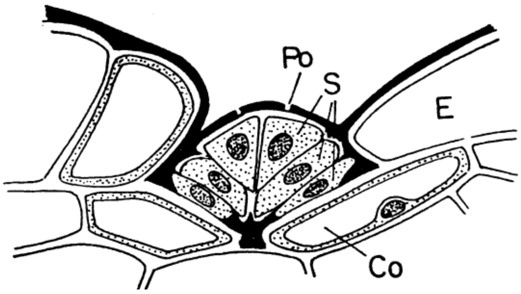


Fig. 10 Secretory gland of *Tamarix* spp. (Po: pore; Co: collecting cell; S: secretory cell; E: epidermal cell) (from Shimony and Fahn, 1968).

The secretory cells are enclosed by a cuticle except for portions of the walls between the two lowest secretory and collecting cells; these wall portions, which are penetrated by numerous plasmodesmata, are called transfusion areas. On the top of the gland, several distinct pores traverse the cuticle and many mitochondria and plastids occur in the uppermost pair of secretory cells, indicating that a highly active process is involved in the functioning of these glands.

The plastids contain osmiophilic droplets. Electron-dense material, apparently of pectic nature, occurs above the cuticle on the top of the gland, between this portion of the cuticle and the cell walls, and in the cuticle pores. This material forms a continuous channel system with the cell walls protuberances, and would appear to be the route by which the secreted salt solution reaches the outside of the plants. Salt glands not only enable *Tamarix* spp. to tolerate high salt salinity, but they also contribute to plants rapid recovery after fire, as ash deposits subsequent to fire are high in salinity and have elevated concentrations of phytotoxic boron (Di Tomaso, 1998). Although salt glands principal activity is salt secretion, Waisel (1991) proposed another mechanisms involved in the concentration of atmospheric carbon dioxide, which could be used during photosynthesis.

Though their centre of origin seems to be Central Asia, *Tamarix* species are also naturally distributed in the Mediterranean region and in the African deserts (Ginzburg, 1967). Nowadays, the distribution of some species (e.g. *Tamarix ramosissima*, *T. chinensis*) is extended to all continents (Brock, 1994). Particularly, in the United States, these species are considered invasive (Frasier and Johnsen, 1991; Hughes, 1993; Brock, 1994; Di Tomaso, 1998). *Tamarix* species are highly plastic and tolerant to adversity (Zhang *et al.* 2002) being able to occupy different habitats, thus 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 while others psammophytes. Some species are thermophilic, and vegetate in the warm region of the Dead Sea while others tolerate lower temperatures, extending their range to Europe. Some clones of *Tamarix* withstand strong winds and salt and are therefore suitable to live near the coasts. In some areas (e.g. in the region of the Aral Sea, in the Dead Sea, as well as in the Aral Valley) many *Tamarix* species coexist; it was reported that at least 13 species are present on the coasts of the Dead Sea. Stress-induced substances have been found in specific ecotypes of the genus *Tamarix*. The accumulation of these substances appeared to be correlated with a high productivity under salt stress conditions. Thus, plants of the genus *Tamarix* may be good candidates for being used in afforestation of arid and saline areas (haloforestry). Flooding tolerance is also documented in *Tamarix* spp. (Ginzburg, 1967; Brotherson and Field, 1987; Di Tomaso, 1998; 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). However, as plants are reproduced by cuttings, plantations of this species are uniform from a genetic point of view. Hence, 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.

Tamarix spp. responses to abiotic stresses

Tamarix species are able to grow in high saline conditions. According to Kleinkopf and Wallace (1974), since much smaller quantities of sodium are present in roots and stems than in leaves, the tolerance mechanism for *Tamarix* species is probably located in the leaves. *Tamarix* spp. are characterised by the presence of leaf salt glands. This salt-concentrating mechanism effectively removes the high salt stresses from internal cellular tissue (Kleinkopf and Wallace, 1974). Although salt secreted by the glands of most halophytes is predominantly sodium chloride, *Tamarix* revealed that there is a great variation in the cation composition (carbonate, potassium, bromine, calcium, nitrate, magnesium and sulphate) (Waisel, 1961; Berry, 1970), which depends on the composition of the root environment (Thomson et al., 1969). Thus, *Tamarix* glands are non-selective (Arndt et al., 2004). Particularly, Waisel (1961) found that *T. aphylla* excreted large amounts of calcium carbonate when growing on non-saline soils, whereas those reaching saline soil layers excrete mainly sodium chloride. Campbell and Thomson (1975) in localising chloride ions in leaves of *Tamarix aphylla* found that the ions moved to the gland predominantly via apoplast that probably served as the major pathway and shunt for the movement of all the ions to the glands. However, under saline conditions, *Tamarix* spp. are able to easily extract water from the soil compared to other riparian species (Vandersande et al., 2001), indicating that an ionic balance inside the mesophyll cells might have occurred. Although *Tamarix* species are considered tolerant to salinity, a reduction in growth rates under concentration of 100 mM NaCl or more has been reported by many authors (Waisel, 1961; Kleinkopf and Wallace, 1974).

Tamarix spp. are usually part of the riparian vegetation and may thus be frequently subjected to submergence. However, contrasting results are reported concerning their tolerance to flooding. Sprenger et al. (2001) declare that *Tamarix* spp. susceptibility to inundation varies depending on a number of factor, such as the level of submergence, the duration of flooding, plant age and plant size. Horton et al. (1960) observed a high rate of mortality of *Tamarix* spp. seedlings after 8-10 weeks of complete submergence. Tallent-Halsell and Walker (2002) also found a mortality of 85% in *Tamarix* subjected to complete submergence and plants survived only if they grew above the water level. *Tamarix* growth was found to decrease after 58 days of flooding conditions compared to other riparian species (Vandersande et al., 2001). In this study, *Tamarix* was reported to be the only species that did not develop an adventitious root apparatus under flooding conditions. On the other hand, Sprenger et al. (2001) found a high survival of *Tamarix* seedlings under flooding conditions comparable to that of control. According to the same authors, survival rates may be related to plant size rather than plant age, as larger plants may have higher levels of stored reserves. Moreover, González et al. (2010) reported a higher nutrient (N and P) resorption of *Tamarix* spp. under flooding conditions compared to *Populus* spp., minimising nutrients loss from leaf senescence. This ability determines a high plasticity in *Tamarix* genus which might be more adaptable to natural and human-induced nutrients reduction. Furthermore, short-term flooding events (8 h) were found to significantly increase plants productivity in desert zones (Gries et al., 2005).

Many researches emphasized *Tamarix* tolerance to very negative soil water potentials, enabling these species to grow under drought conditions and to endure water table depth variations (Cleverly et al. 1997; Devitt et al., 1997; Horton et al. 2001b; Gries et al. 2003). In many species, this tolerance is linked to their ability to reach the groundwater table. Sala et al. (1996) affirm that, even under high evaporative demand, which is characteristic of desert environments, *Tamarix* water uptake was sufficient to compensate for water loss at the leaf level. Moreover, Xu and Li (2006) found no variation in photosynthetic responses in *Tamarix ramosissima* to sustained drought, but also to heavy rain events. According to these authors, *T. ramosissima* does not rely on summer rain as part of its water use strategy but on groundwater and, therefore, does not respond to rain pulse events in any aspect of plant-water relation. However, a switch from primary dependence on groundwater to vadose-zone groundwater was also found (Nippert et al., 2010). The authors suggested that this shift primarily occurs when the water table decreases under an accessible root level. Pockman and Sperry (2000) found high transpiration rates in *T. ramosissima*, and a greater resistance to cavitation compared to other desert species; particularly, *Tamarix* spp. become 50% cavitated at a water potential of -4.5 MPa and completely cavitated at -7.0 MPa. Gries et al. (2003) argued that tolerance of lower leaf water potentials is due to higher concentrations of leaf osmotica. *Tamarix* spp. are also low sensitive to VPD changes (Horton et al., 2001). Although Cleverly (1997) did not find any decrease in photosynthesis from the beginning to the end of the drought period in *Tamarix* grown in the Majove Desert, a decrease in stomatal conductance was observed. In this case, the reduced stomatal conductance can bring to a decrease in plant growth (Gries et al., 2003). In dry periods, some *Tamarix* spp. stop growth and tend to drop their leaves (Horton, 1977).

Tamarix spp. 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.

Tab. 3 Morphological characteristics of *T. gallica* and *T. africana* (from Aránzazu Prada and Arizpe, 2008).

Taxon	*T. gallica* L.	*T. africana* Poiret
Bark	• Brownish-black or deep purple	• Black or dark-purple
Leaves	• 1.3-2.5 mm long • Glaucous	• 1.5-4 mm long • Smooth or minutely papillose • Margin scarious
Inflorescences	• 10-50 × 3-5 mm • Usually on current year's branches • Rachis usually glabrous • Loosely compound	• (15)30-70(80) × (5)6-9 mm • Usually on previous year's branches • Rachis sometimes papillose • Margin scarious
Bracts	• Usually shorter than calyx • Narrowly triangular, acuminate • Margin denticulate	• Longer or shorter than calyx • Narrowly oblong, shortly acute, to triangular, acuminate • Margin usually papillose
Flowers	• Pentamerous	• Pentamerous
Sepals	• 1-1.8 mm long • Trullate to ovate, acute; inner somewhat longer and more obtuse • Margin not very denticulate	• 1-1.8 mm long • Trullate, acute; outer slightly longer, narrower and more acute • Margin subentire
Petals	• (1.6)1.7-2 × 0.8-1 mm • Elliptic to ovate	• 2-3.3 × 1-2 mm • Trullate to ovate
Anthers	• Slightly apiculate	• Muticous or slightly apiculate
Nectariferous disc	• Synlophic • Not very fleshy	• Synlophic

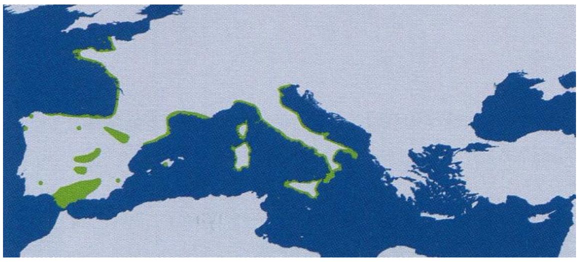


Fig. 11 Distribution of *Tamarix gallica* L. (from Aránzau Prada and Arizpe, 2008).

Fig. 12 Racemes of *T. gallica* on the branches of the current year.

Tamarix gallica L.

Tamarix gallica is distributed in South-western and South-eastern Europe, and Macronesia. In the Mediterranean region it is usually found in Portugal, Spain, France and Italy (Aránzazu Prada and Arizpe, 2008) (Fig. 11). *T. gallica* is a tree, often shrubby, up to 8 m height, with brackish-brown to deep purple bark; the stem diameter can reach up to 25 cm and the crown has an irregular shape (Pedrotti and Gafta, 1996). The flowers are grouped in racemes, blooming in early spring; the racemes are situated on growing branches of the current year (Fig. 12).

This species is heliophilus, very commonly distributed in coastal areas, salt marshes, on the riverbanks and on saline soils. It is very tolerant to salinity and drought. However, it also grows in exceedingly wet places (McAtee, 1914). *Tamarix gallica* is widespread in the Mediterranean basin. In Italy it is found in all the coastal regions (Conti et al., 2005).

Tamarix africana Poiret

Fig. 13 Distribution of *Tamarix africana* Poiret (from Aránzau Prada and Arizpe, 2008).

Tamarix africana is distributed in South-western and South-eastern Europe, Northern Africa and Macronesia. In the Mediterranean region it is usually found in Portugal, Spain, France, Italy, Tunisia, Algeria and Morocco (Aránzazu Prada and Arizpe, 2008) (Fig. 13).

It is a small tree, up to 6 m height, with dark bark. The flowers are grouped in racemes, blooming in spring (April-June); the racemes are situated on growing branches of the previous year (Fig. 14). This species is commonly along the riversides and on saline soils. In is distributed central and southern Italy (Conti et al., 2005).

Fig. 14 Racemes of *Tamarix africana* Poiret growing on previous year’s branches.

In Italy, *Tamarix* populations belong to the vegetation order *Tamaricetalia africanae*. This order includes pioneer shrubby associations present on the riverbanks of temporary or perennial streams. This riparian and marshy thermophile vegetation is determined by particular water conditions due to water table height variations and to water stagnation but does not follow a specific climatic zonation (Pedrotti and Gafta, 1996).

The order *Tamaricetalia africanae* includes shrubby, pioneer associations of permanent and temporary watercourses (called *fiumare*). This vegetation has a clear thermophilic character, being limited only to the Mediterranean Region. The species of this order show a high tolerance to water stress during summer; the main species in this order of vegetation 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):

- Association *Rubo-Nerietum oleandri*

This association is in the form of a tall shrubland, composed of *Nerium oleander*, *Tamarix africana* and *Vitex agnus-castus*, the lower shrub layer is dominated by *Rubus ulmifolius*, sometimes accompanied by *Spartium junceum* and *Calicotome infesta*, which are characteristics of xeric environments. The herbaceous layer consists of xerophilous species from other associations, such as *Scrophulario-Helichrysetalia*. *Rubo-Nerietum oleandri* is found on flood-rich pebbles and sands, which are only rarely subject to submergence during flood events. This association has been identified along the river Trionto, in Calabria, the rivers Pollina, Euriano, Rosmarino and Alcantara in north-eastern Sicily.

- Association *Tamaricetum gallicae*

It is a shrubland dominated by *Tamarix gallica* and *Tamarix africana*, which are sometimes accompanied by *Nerium oleander* and several species of willow (*Salix alba*, *Salix purpurea* and *Salix gussonei*). Again, the herbaceous layer is composed of ubiquitous species, with no particular phytosociological significance. This *Tamarix* stand usually spread over flood-rich silt and clay, on the lower and middle parts of rivers, which indicates its presence only in the thermo-meso-Mediterranean plan. The association has been reported in Sicily in the basin of rivers Simeto and Imera Meridionale, but also in Calabria, Basilicata and Sardinia.

- Association *Tamaricetum africanae*

This shrubby community is mainly composed of *Tamarix africana* and seems a vicarious of *Tamaricetum gallicae* in central and south-western Sicily. It is also present in the region Basilicata, along the riverbanks of rivers Cavone, Bradano, Basento. The *Tamaricetum africanae sensu lato* has been reported in the area of Butera and in the basin of river Platani.

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General objectives and hypotheses

The growth, the development and the reproductive potential of a plant are closely related to the environmental conditions in which it lives. In a certain environment, natural selection favours those individuals which are best adapted to certain biotic and abiotic growth conditions. In fact, although plants of the same species occupy ecologically similar environments, genetic variation exists within each population, determining inter and intraspecific differences in the response to the growth conditions to whom they are subjected. The identification of highly tolerant genotypes under saline water and soil conditions, and under flooding with both fresh and saline water, is of particular importance for the selection of material which could be suitable for ecosystems restoration, biomass production and climate change mitigation in degraded, submerged, saline and/or desert areas. Thus, the definition of the ecology and the ecophysiology of plants with different genetic characteristics may allow the selection of genotypes particularly suitable to grow in extreme environments.

For these reasons, the main objectives of this thesis were:

1. The identification of natural *Tamarix* spp. populations in Italy and the characterisation of stands structure and plants dendrometric characteristics. The hypothesis of this first chapter is that stand structure may explain plants responses to the major growth limiting factors. Moreover, basal area, diameter class distribution and stem age are intrinsic characteristics of a given plant population and, consequently, they are useful tools to compare vegetation characteristics among different sites.
2. The analysis of the physiological responses of two *Tamarix* spp. provenances to continuous flooding with fresh and saline water. As many habitats occupied by *Tamarix* spp. will suffer climate change effects in the future, such as flooding with fresh and saline water, an experiment was designed to assess the effects of these two stresses on plants photosynthetic activity and plant growth. Moreover, two provenances were analysed; the hypothesis is that tolerance of *Tamarix* species also depends on their origin.
3. The investigation of photosynthetic and secondary growth responses of *Tamarix* spp. to water level reduction after continuous flooding with fresh and saline water. This experiment was design to assess the effect of water level reduction after flooding on photosynthesis and on some anatomical wood characteristics. The hypothesis is that *Tamarix* flooded with fresh water may recover from this condition and be comparable to plants grown under control conditions, while those flooded with saline water may suffer salt accumulation in the soil solution.
4. The study of salinity effects on stomatal and salt glands functioning in *Tamarix gallica* and *T. africana*. The ecological characterisation of *Tamarix* stands has highlighted the higher presence of *Tamarix gallica* in the dune sites compared to *T. africana*. The hypothesis is that the first species is more tolerant to salinity than the second one. Moreover, many studies underline the fact that *Tamarix* is an important water consumer. The hypothesis is that salinity may increase leaf water loss during salt extrusion from salt glands.
5. The analysis of wood anatomical responses to environmental constraints in *Tamarix gallica*. Xylem hydraulic architecture may reflect trees diversity adaptations or adjustments to environmental variability. Thus, wood anatomy represents an important tool for the identification of growth responses to the variation of environmental factors other than climate. The hypothesis is that plant growth not only responds to climate, but also to other environmental factors such as salinity.

Part I

Italian *Tamarix* spp. populations: distribution, structure and ecology

Chapter 1

In situ comparative traits of *Tamarix* spp. Italian provenances

Abstract

Wetlands and coastal Mediterranean areas are increasingly at risk, as they are particularly exposed to a range of hazards connected to climate change. Consequently, the selection of local tolerant species and genotypes may be important for the future restoration of these environments. *Tamarix* spp. are tolerant to a range of abiotic stresses (drought, flooding, salinity) and have been used in desert areas for biomass production under saline soil conditions. In Italy, *Tamarix* populations are present on the riverbanks of temporary or perennial streams and on coastal areas of Southern Italy. Although the floristic composition and plant community diversity of these formations have already been studied, an account of the general stands structure is lacking. In this work, the dendrometric characteristics and the structure of six natural Italian *Tamarix* spp. populations have been analysed, considering three different environments: the dune, the river mouth and the riverside.

Tamarix spp. stands are shrublands, characterised by structures which differed among the three analysed environments. Particularly, the plants grown on the dune were characterized by a lower diameter, basal area, height and age compared to the riverside and the mouth ones, probably as a result of the higher salinity observed in this environment, which can limit plants photosynthesis and energy production. Although a high salinity was observed at the river mouth, this environment was composed of *Tamarix* plants characterised by high structural dimensions, sometimes higher than those observed in plants grown on the riverside. These characteristics are probably the result of a higher fresh water availability compared to the dune, and to the riverside, as the water discharge is higher at the mouth compared to the upriver stations. Among the populations located in the same environment, plants growth and age were higher under low soil NaCl salinity and/or adequate and constant fresh water availability. Moreover, *Tamarix gallica* seems to be more tolerant to salinity, or less flooding tolerant, compared to *T. africana*, as it is more frequently distributed in dune sites compared to the mouth and the riverside ones.

Key words: *Tamarix* spp. dendrometric analysis, population structure, salinity, flooding

Introduction

The anthropogenic greenhouse gases emission in the atmosphere since the second half of the last century is considered to be the main cause of the observed global warming; actually, the average global temperature has increased by 0.74 °C in the last one hundred years (IPCC 2007). Global average temperature increase is not homogeneously distributed on the earth surface. Moreover, its effects may be different among biomes. The Mediterranean countries are hot spots of climate change (Giorgi, 2006), and are expected to increasingly experience drought, warming and precipitation and temperature inter-annual variability (Giorgi and Lionello, 2008). Above all, in this region, wetlands and coastal areas are increasingly at risk, as they are particularly exposed to a range of hazards connected to climate change (inundation and storm flooding, erosion, salinization, seawater intrusion and increased sea surface temperature) (IPCC, 2007; Torresan et al., 2008; Solomon et al., 2009). In fact, while a pronounced warming is projected in the Mediterranean region, with a maximum increase in temperature in the summer season (Giorgi and Lionello, 2008), the changes in precipitation patterns may lead to contrasting trends: on one hand, an increase in the occurrence of heavy precipitation events is expected all over Europe (Semenov and Bengtsson, 2002; Kjellstrom, 2004). Heavy rain precipitation coupled with an increase in land ice melting due to climate warming may lead to the occurrence of pick river flows and an increasing risk of inundation (Parry et al., 2007), which might damage vegetation and soil structure (Knox, 2000; Nicholls, 2002; Christensen and Christensen, 2003). On the other hand, precipitation and water availability have declined since the 1970s, especially in the warm season, with increasing areas affected by drought (Giorgi and Lionello, 2006; IPCC, 2007). Thus, Mediterranean climate and semiarid zones that are adjacent to arid areas can be very sensitive to desertification under climate change (Laave et al., 1998). Furthermore, the rise of the sea level due to oceans expansion, and the melting of permafrost, may have detrimental effects on coastal areas, as a result of seawater submersion.

The IPCC (2007) provides that some adaptation strategies may be put into effect to contrast sea level rise and flooding, such as the creation of marshlands and wetland buffer areas. Consequently, the selection of tolerant species and genotypes may be important for their revegetation, avoiding the loss of wetland functions and enabling the restoration of environments which might have been affected by flooding with fresh and saline water as a consequence of climate change. The success of such practices not only depends on environmental physical resemblance of the created environment, but have to lead to functional replacement; thus, the introduction of appropriate biotic material should initiate natural colonisation processes which continue indefinitely for the wetland creation (Campbell et al., 2002). The use of regional mixtures deriving from collections of genotypes, moderately adapted to the general environment and within a broad geographic zone seems to be a good strategy in restoration projects (Knapp and Dyer, 1997). Thus, the wide genotypic variation in natural plant populations must be recognized and exploited. This will ensure that genotypes used on a site are best adapted to local conditions and have a greater probability of survivorship than arbitrarily chosen material.

Tamarix genus includes about 90 species, which are naturally distributed in Africa, Asia and Europe. These trees and small shrubs are high plastic and tolerant to adversity (Zhang et al., 2002). The presence of leaf glands specialized in salt extrusion is a characteristic feature of *Tamarix* species and confers tolerance to saline conditions (Bar-Nun and Poljiakoff-Mayber, 1974). Moreover, many researches emphasized *Tamarix* tolerance to very negative water potentials, enabling these species to grow under drought conditions and to endure water table depth variations (Cleverly et al., 1997; Horton et al., 2001; Gries et al., 2003; Xu and Li, 2006). Actually, this tolerance is link to their ability to reach the groundwater table. Flooding tolerance is also documented in *Tamarix* spp. (Ginzburg, 1967; Brotherson and Field, 1987; Di Tomaso, 1998; Tallent-Halsell and Walker, 2002). *Tamarix* spp. have already been used in desert areas for biomass production under saline soil conditions (Eshel et al., 2010), and can represent a tool for revegetation in environments subjected to abiotic stress conditions. In Italy, *Tamarix* populations belong to the vegetation order *Tamaricetalia africanae*. This order includes pioneer shrubby associations present on the riverbanks of temporary or perennial streams and on coastal areas. This riparian and marshy thermophile vegetation is characterised by particular water conditions due to water table height variations and to water stagnation (Pedrotti and Gafta, 1996). According to Conti et al. (2005), *Tamarix* species in Italy are ten and among them *T. gallica* and *T. africana* are the most widespread. The floristic composition and plant community diversity of these formations have already been studied (Brullo and Spampinato, 1990; Martinelli, 1998; Borgarello et al., 2000; D'Ambra et al., 2002; Maiorca et al, 2005). However, an account of the general structure of these populations is lacking.

Dendrometric characteristics are intrinsic features of a given plant population and they are often used to compare vegetation types among sites (Natta, 2003). Thus, the objectives of this study were:

(1) The analysis of the dendrometric characteristics of Italian natural *Tamarix* spp. populations and
(2) the investigation of the structure of these populations

under different environmental constraints and in different regions.

Tab. 1 Geographic characteristics of the six analysed sites (Alcantara, Simeto, Imera Meridionale, Crati, Basento and Baratz) and environments (dune, mouth, riverside). [a] Aiuppa et al., 2003; [b] Ferrara and Pappalardo, 2004, Finocchiaro et al., 2009; [c] Bonanno and Lo Giudice, 2010, Selvaggi et al., 2010; [d] Italiano et al., 2010; [e] Polemio et al., 2003; [f] Martinelli, 1998.

River/Lake	Region	Coordinates	Altitude	Environment	Water electrical conductivity	Distance from the sea
Alcantara	Sicily	37°54'10''N, 15°04'11''E	0 m, 480 m	Dune, Riverside	1.5-2.3 dS m^{-1} [a]	0 km, 19 km
Simeto	Sicily	37°24'01''N, 15°04'04''E	0 m, 1 m, 7 m	Dune, Mouth, Riverside	4 dS m^{-1}, 6 dS m^{-1}, 1 dS m^{-1} [b]	0 km, 2 km, 6 km
Imera Meridionale	Sicily	37°28'49''N, 14°09'05''E	258 m	Riverside	7 dS m^{-1} [c]	42 km
Crati	Calabria	39°43'17''N, 16°31'43''E	0 m, 8 m	Dune, Riverside	1.2 dS m^{-1}, 1.2 dS m^{-1} [d]	0 km, 6 km
Basento	Basilicata	40°20'46''N, 16°48'46''E	0 m, 1 m, 39 m	Dune, Mouth, Riverside	4 dS m^{-1}, 2-4 dS m^{-1}, 1 dS m^{-1} [e]	0 km, 1 km, 28 km
Baratz	Sardinia	40°40'48''N, 8°13'34''E	24 m	Dune	5 dS m^{-1} [f]	1.7 km

Materials and Methods

Description of the study areas

The research involved six natural *Tamarix* spp. populations of Southern Italy, three of which were situated in Sicily, one in Calabria, one in Basilicata and one in Sardinia. The experimental areas were located in river basins; therefore, the geological substrate was made of alluvial and marshy deposits. *Tamarix* spp. were collected in the basin of rivers Alcantara, Simeto, Imera Meridionale (East, Southeast and Central Sicily respectively), Crati (Southeast Calabria) and

Basento (South Basilicata). Sardinian *Tamarix* spp. population was sampled on the shores of the salty lake Baratz (Northeast Sardinia) (Tab. 1).

All sites climate is Mediterranean semiarid, with annual average temperatures ranging from 15 to 18 °C (Fig. 1). Minimum temperatures are recorded in January-February and maximum temperatures in July-August. Annual precipitation is about 500 mm with more than 50% of rainfall occurring during autumn and winter; a long dry period is usually recorded from May to September.

In each basin, *Tamarix* spp. individuals were collected in environments having contrasting characteristics of salinity and water availability, from the sand dune to upriver. Three environments were investigated in this study: the dune, situated in front of the sea, which was characterised by a high salinity and a low fresh water availability, especially during the dry period; the mouth of the river, which extended along two kilometres from the sea and was characterised by a constant water availability whose salinity depended on the season (but was usually high); the riverside, upriver, which was characterised by a seasonal (from autumn to spring) water availability and a moderate salinity (Tab. 1).

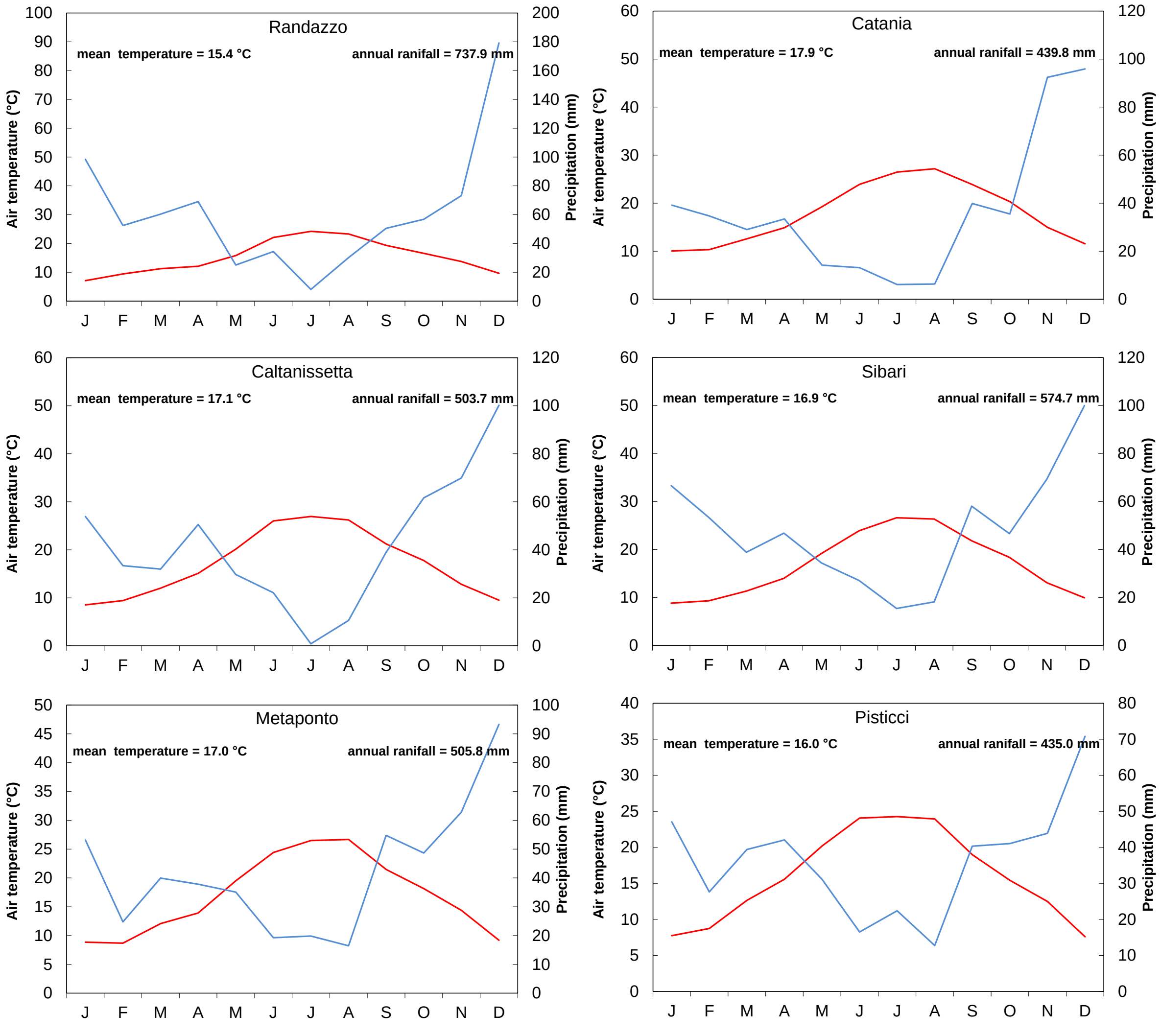
Tamarix spp. were differently associated with other species in the three environments. On the dunes, *Tamarix* spp. were associated with *Pancratium maritimum*, *Eryngium maritimum*, *Echinophora spinosa*, *Ammophila arenaria*, *Salicornia fruticosa*, *Suaeda maritima*, *Ephedra distachya*, *Phragmites australis*, *Juncus acutus* and *J. litoralis* in the herbaceous layer, and to *Pistacia lentiscus* and *Juniperus macrocarpa* in the dominant layer. At the mouth, *Tamarix* spp. were associated with *Phragmites autralis*, *Arundo donax* and *Arundo pliniana*, but also with *Schoenoplectus palustris* and *Typha angustifolia*. On the riverside, *Nerium oleander*, *Vitex gnus-castus*, *Rubus ulmifolius* and *Salix* spp. were found in the dominant layer, while the herbaceous layer was mostly composed of xerophytes.

Tamarix spp. populations were collected on the dunes of rivers Basento, Crati and Simeto, and at the mouth of the rivers Basento and Simeto. *Tamarix* spp. populations were also found upriver, in Basilicata and Calabria. Concerning rivers Alcantara and Imera Meridionale, *Tamarix* spp. populations were only investigated on upriver sites. As lake Baratz has a marine origin, it was considered as a dune site (Tab. 1).

Dendrometric characteristics of Italian populations

54 *Tamarix* spp. genotypes were selected on river Basento, 35 on Imera Meridionale, 24 on lake Baratz, 23 in Crati; 24 *Tamarix* spp. genotypes were also collected on river Alcantara and 43 on river Simeto. Species identification was performed according to the configuration of the staminal disc (Baum, 1978; see Abbruzzese, 2010). Moreover, a set of 14 SSRs markers and a Bayesian assignment method were used establishing the correspondence with the previously described *taxa* on the unidentified individuals (see Terzoli, 2010).

For each individual, the number of stems was considered. The basal diameter for stems >2 cm was measured with a calliper; smaller stems were only marked. For each plant, the diameter (D_d) of the stem with the dominant height (H_d) was measured with a stadiometer or with a Blume-Laiss ipsometer. The average plant diameter (D_m) of all polycormic trees was calculated. The individual basal area (G_i) was calculated as the sum of all plant stems. 102 stems were harvested from different populations; the total aboveground plant biomass (wood and leaves) was measured and allometric equations were built.



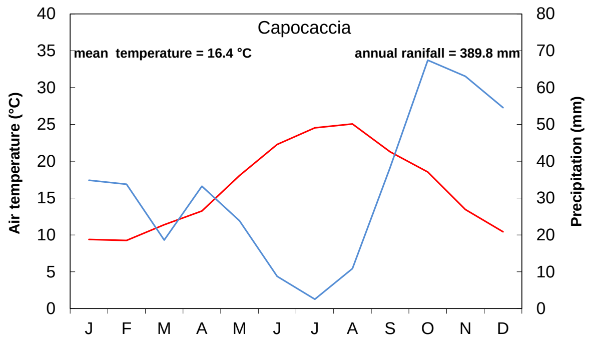


Fig. 1 Walter and Lieth climatic diagrams obtained from data collected during the period 1999-2008 in the meteorological stations of Randazzo (river Alcantara), Catania (river Simeto), Caltanissetta (river Imera Meridionale), Sibari (river Crati), Metaponto (mouth of river Basento), Pisticci (riverside of river Basento) and Capocaccia (lake Baratz). Data were taken from ALSIA, APAT, Agrometeo SIAS, ARSSA.

Wood cores were also collected from the harvested stems in order to determine plants age. The age was determined by cutting wood cores surface and by counting annual rings with a stereo microscope (Leica MZ16A, Leica Microsystems AG, Wetzlar, Germany).

Transects were made to describe plant distribution. The stand structure was analysed in the different environments selecting three model-transects from different populations. In each transect, the number of tree species was reported. The relative density of each species was calculated as well as the percentage of crown coverage. The nearest neighbour method was used to calculate the mean distance between plants.

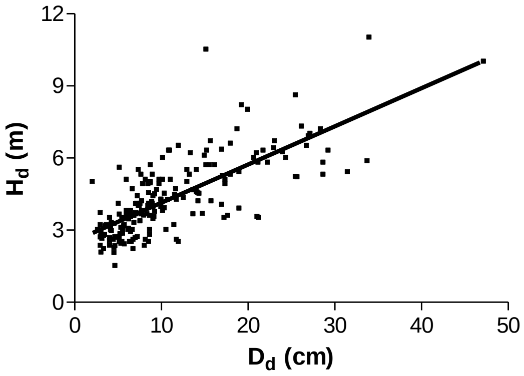


Fig. 2 Linear regression between dominant diameters and heights measured from each collected *Tamarix* spp. plant (n=201).

Statistical analysis

Statistical differences in dimensional characteristics among populations occupying the same environment were tested by ANOVA, considering the analysed parameters as dependent variables and the population as factor. Furthermore, the ANOVA was performed on the average dimensional parameters measured and calculated in the two populations where all the environments were represented (Basento and Simeto), and on the spatial parameters, in order to test differences among the three environments. A post-hoc Bonferroni test was applied to verify the significance of the differences revealed by the ANOVA. Correlations between the dendrometric parameters of the dominant sprout were built considering together all the individuals and populations. The statistical tests were performed with SYSTAT 12.0, while for descriptive statistics the software Prism 4.0 (GraphPad) was used. Statistical significance was considered for *p* values <0.05.

Results

Average characteristics of *Tamarix* spp. stands

The diameter of the dominant sprouts (D_d) ranged between 2.1 and 47.2 cm. The mean diameter (D_m) of *Tamarix* plants was 11.0 cm, while the average plant height was 4.3 m, ranging between 1.5 and 11.0 m. The mean plant basal area was equal to 345 cm^2, ranging from 3.1 to 2806 cm^2. The average number of sprouts per plant was equal to 9.1. The maximum number of sprouts was 146 although some monocormic plants were also observed. A significant correlation was found between the dominant diameter and the dominant height of the collected *Tamarix* spp. plants ($y=0.1589x+2.5$; $R^2=0.558$, $p<0.001$) (Fig. 2).

The measured sprouts diameters also well fitted their biomass through a power function ($y=0.0919x^{2.51}$; $R^2=0.940$) (Fig. 3).

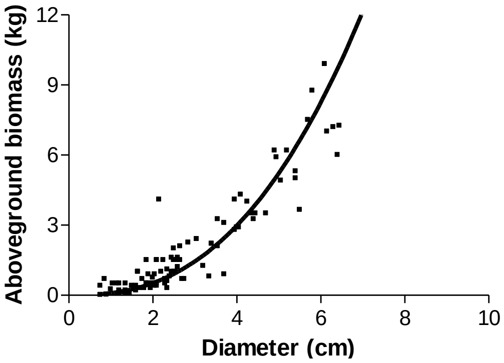


Fig. 3 Power regression between diameters and aboveground biomass measured for some selected sprouts (n=102).

Comparison among environments

The average values of plants dendrometric characteristics in the three analysed environments (dune, mouth, riverside) are shown on Tab. 2. The mean (D_m) and dominant diameters (D_d) were significantly lower in plants grown on the dune compared to those grown on the mouth ($p<0.001$; $p<0.01$ respectively) and on the riverside ($p<0.001$; $p<0.01$ respectively). Moreover, D_m and D_d measured on the riverside were lower compared to those measured on the mouth ($p<0.01$ and $p<0.001$ respectively). A lower plant dominant height (H_d) and a higher number of sprouts were observed on the dune compared to the mouth ($p<0.001$ and 0.01 respectively) and the riverside ($p<0.001$ and $p<0.01$ respectively). On the other hand, a higher individual basal area (G_i) was found on the mouth compared to plants grown in the other two environments ($p<0.001$ in both cases), which were characterized by similar G_i values. Furthermore, the maximum age was also found in plants situated near the river mouth.

Tab. 2 Average values of the analysed plant dendrometric characteristics (mean plant diameter (D_m), dominant plant diameter (D_d), individual basal area (G_i), dominant plant height (H_d), number of sprouts per plant) and maximum ages found in each of the three investigated environments (dune, mouth, riverside). Lowercase letters refer to the comparison among the environments. The values are means ± s.e. (n=512 in the dune; n= 187 in the mouth and n=136 in the riverside).

		D_m	**D_d**	**G_i**	**H_d**	**N of sprouts**	**Max age**
Dune	Mean	5.09 **a**	6.98 a	97.99 **a**	3.19 **a**	17.03 **a**	21
	s.e.	0.46	0.54	26.87	0.20	5.20	
Mouth	Mean	14.29 **b**	18.75 b	785.65 **b**	5.64 **b**	5.67 **b**	34
	s.e.	1.64	1.66	118.53	0.33	0.86	
Riverside	Mean	10.09 **c**	12.10 c	190.52 **a**	4.92 **b**	3.97 **b**	25
	s.e.	1.12	1.00	18.44	0.28	0.54	

Stem diameters distributions in the three environments also differed (Fig. 4). Particularly, the dune was characterised by an hyperbolic distribution of diameters, with a high number of stems belonging to the smaller diameter classes (2-6 cm) and a small number of stems of higher dimensions. Moreover, the maximum class reached in the dune was 12 cm. On the contrary, both mouth and riverside environments were characterised by different normally distributed groups of diameters. Moreover, the maximum diameter class reached was 48 cm on the mouth and 28 cm on the riverside.

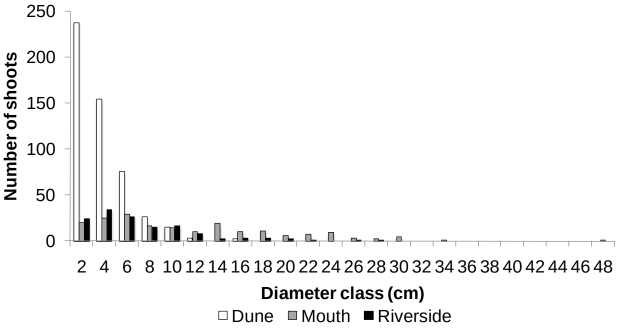


Fig. 4 Stems diameter distributions in 2 cm classes in the three environments (dune, mouth, riverside).

Tamarix gallica and *T. africana* species distribution was not similar in the three environments (Tab. 3). Particularly, *Tamarix gallica* was more widely distributed on dune sites compared to the mouth and to the riverside sites, while *T. africana* was more frequent along the riverside banks than in the other two environments.

Tab. 3 Distribution of the two most widely distributed collected species (*T. gallica* and *T. africana*) in the three environments (dune, mouth, riverside).

	T. gallica **(%)**	***T. africana*** **(%)**
Dune	42.0	14.3
Mouth	29.5	13.4
Riverside	28.5	72.3
	100	100

Species composition also differed among the three environments (Fig. 5). Particularly, *T. gallica* was less frequent on the riverside compared to *T. africana*, while on the dune and on the mouth, the two species were almost equally present.

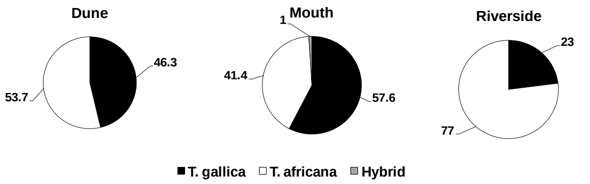


Fig. 5 Species distribution in the three investigated environments (dune, mouth, riverside).

Also stand structure and woody species composition differed among environments (Fig. 6).

Particularly, on the dune *Tamarix* spp. were accompanied by *Pinus halepensis*, *Pistacia lentiscus* and *Juniperus oxycedrus*. In this environment, the total percentage of coverage by tree species was equal to 12.6%, *Tamarix* spp. covering almost 50% of this value. The relative frequency of *Tamarix* spp. was 41.6% and the mean distance between plants was equal to 9 m. Differently on the mouth site, *Tamarix* spp. were associated with *Ricinus communis* and *Ficus carica*. The total percentage of coverage by tree species was 15% (14% by *Tamarix* spp. and 1% by the other species). *Tamarix* spp. relative frequency was equal to 70.6%, while the mean distance between plants was 6 m. On the riverside and with the only exception of river Alcantara where they were associated with *Salix gussoneii* and *Nerium oleander*, *Tamarix* spp. usually formed monospecific stands. Their relative coverage was on average equal to 10.7% and the mean distance between plants was 4.4 m.

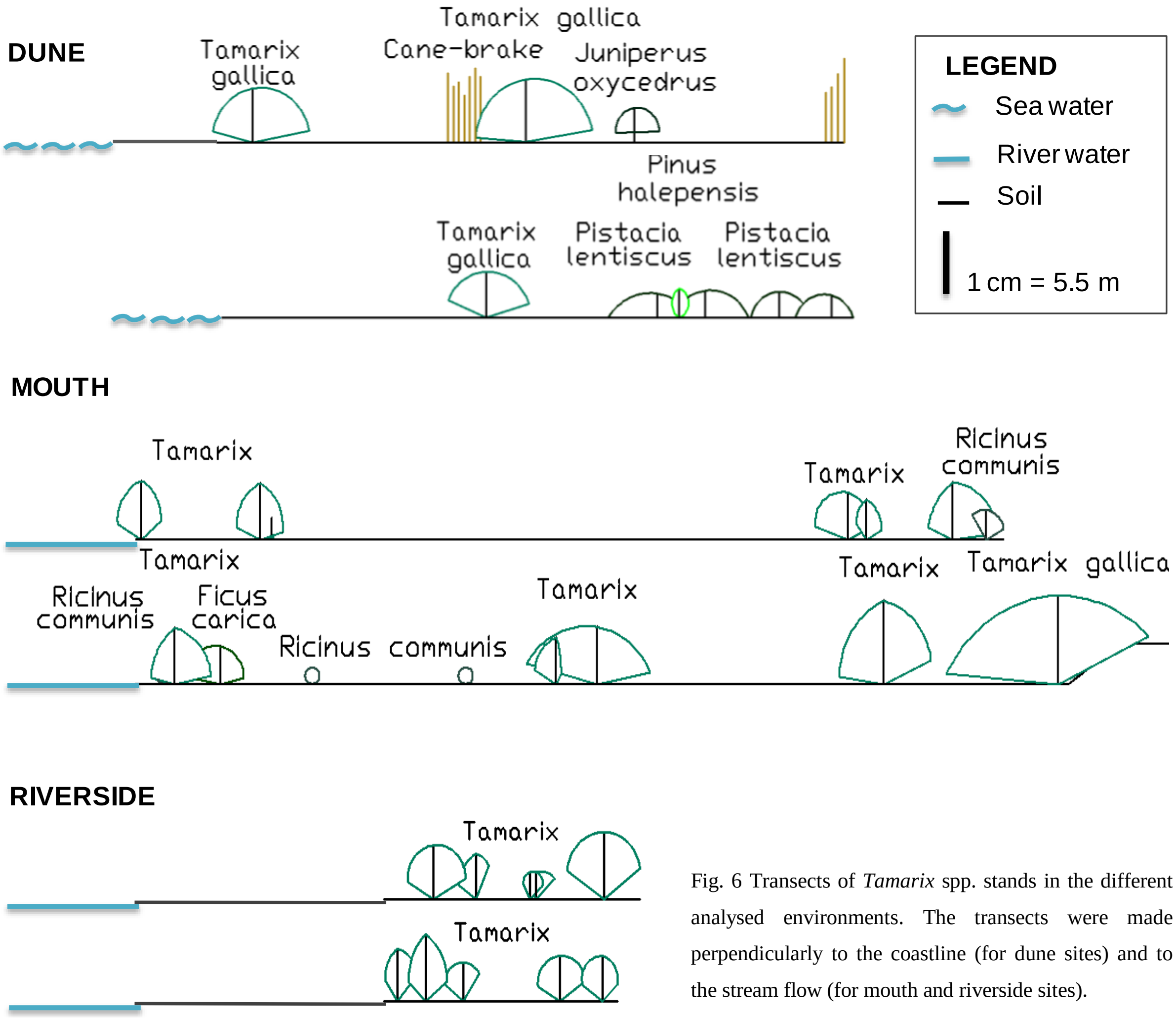


Fig. 6 Transects of *Tamarix* spp. stands in the different analysed environments. The transects were made perpendicularly to the coastline (for dune sites) and to the stream flow (for mouth and riverside sites).

Comparison among populations

The average values of plants dendrometric characteristics in the different analysed populations (Alcantara, Basento, Baratz, Crati, Imera and Simeto) are shown on Fig. 7. No differences were observed in the dendrometric parameters between the two mouth populations (Basento and Simeto). The mean diameter (D_m) was similar among the riverside populations, while a significant difference was observed among the dune ones ($p<0.05$). Particularly, lower D_m values were measured in Basento stand (4.5 cm) compared to Alcantara (9.1 cm) ($p<0.05$). The dominant diameter (D_d) was significantly higher in plants grown on the dune of Crati population compared to Basento ($p<0.01$) and Baratz ($p<0.01$). Moreover, among the riverside populations, D_d in Basento was higher compared to Alcantara. The dominant height (H_d) was on average higher in plants collected on the Crati, compared to Basento ($p<0.01$), Baratz ($p<0.01$) and Simeto ($p<0.001$) dune populations. Among the riverside populations, H_d was on average higher in Basento compared to both Alcantara ($p<0.001$) and Imera Meridionale ($p<0.01$). Individual basal area (G_i) was higher on Crati compared to Simeto ($p<0.01$), Basento ($p<0.01$), Alcantara ($p<0.001$) and Baratz ($p<0.001$) dune populations. A similar trend was observed among the riverside populations, Imera Meridionale being characterised by higher G_i compared to Alcantara ($p<0.05$), Basento ($p=0.01$), Crati ($p<0.05$) and Simeto ($p<0.05$). The number of sprouts per plant was similar in all dune populations; however, a difference was observed among riverside ones. Particularly, Basento was characterised by a lower number of sprouts compared to both Alcantara ($p=0.001$) and Imera Meridionale ($p<0.05$).

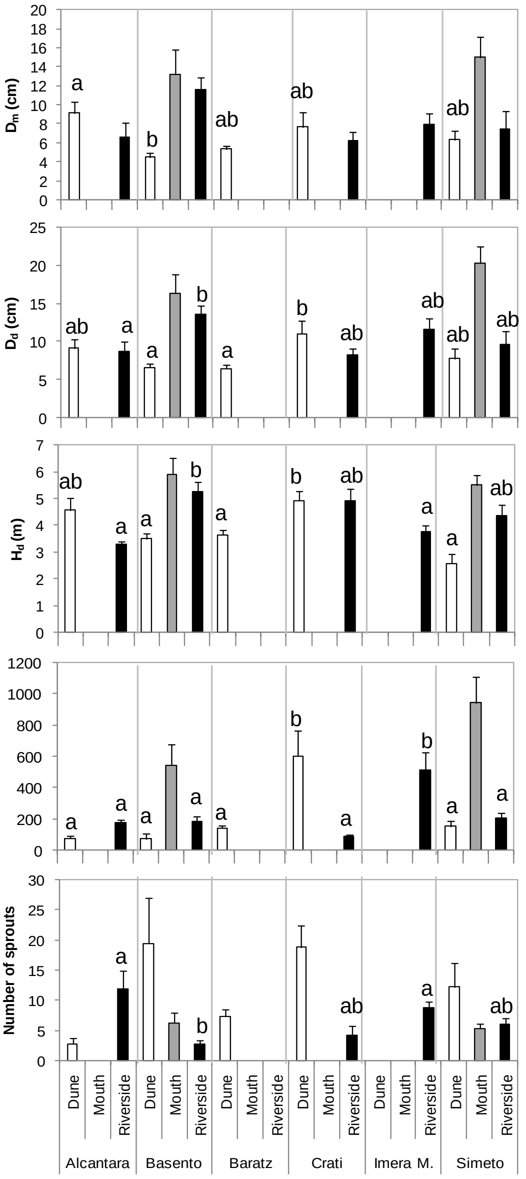


Fig. 7 The mean plant diameter (D_m), the dominant plant diameter (D_d), the dominant plant height (H_d), the individual basal area (G_i) and the number of sprouts per plant measured in the different populations and in the three analysed environments (dune, mouth, riverside). Lowercase letters refer to the comparison among the populations occupying the same environments. The values are means ± s.e.

Discussion

At a stand level, basal area, stem density, diameter class distribution, stem age, are intrinsic characteristic of a given plant population (Natta, 2003); for this reason, they are often used to compare vegetation types among sites. *Tamarix* spp. stands were characterised by a shrubby rather than an arboreous structure. Dewine and Copper (2008) report an average height of 4-5 m in early successional sites of *Tamarix ramosissima*, *T. chinensis* and *T. gallica*, which is similar to the average value found in this study (4.3 m); the basal area of living plants found by the same authors in the understorey (2612 cm^2) and in the dominant layer (1164 cm^2) is much higher compared to the average value found in this study (345 cm^2), although 17 plants with a basal area higher than 1000 cm^2 were also found. These differences may be the result of the environmental variability which significantly affects plants structural parameters.

In fact, the structure of *Tamarix* spp. populations significantly differed between the dune, the mouth and the riverside. Particularly, the plants grown on the dune were characterised by a lower diameter, basal area and height compared to the riverside and the mouth ones. Moreover, the maximum age found was 21 years, the lowest among the three environments (34 years in the mouth, 25 years on the riverside stands), indicating a decreased sprouts vitality in this environment. The plants grown on the dune were also characterized by a higher number of sprouts compared to the other environments. Moreover, the diameter frequency distribution suggests a high sprouts turnover, with a large number of them belonging to the first diameter classes, and no sprouts with a diameter bigger than 14 cm. On the mouth and on the riverside, a more stable stand structure was found, which was characterised by the presence of sprouts belonging to bigger diameter classes and an overall high dimension of the analysed parameters. According to Tyrrell and Crow (1994), this flatter distribution, characterised by few diameters in most diameter classes can be found in old growth stands, although the maximum age found in this study was 34 years, which is the same reported by Sexton et al. (2006) in *Tamarix* spp. stands of Central Eastern Montana. The difference in diameter distributions in the three environments may be due to plants age. However, under drought or salinity, which frequently occur on coastal environments, plant vitality may be affected, and severe stresses may lead to plants death. Under such environmental conditions, *Tamarix* spp. are able to maintain their vitality by replacing dead sprouts with new ones, as a consequence of adventitious buds production from superficial side roots (Baum, 1978), so that plant vitality does not only depend on one stem, but on the continuous emission of new sprouts. This phenomenon is probably an adaptive advantage in the vegetative propagation of *Tamarix* species in their natural habitats (Ginzburg, 1967). The dune site is characterised by a higher soil/water salinity compared to the riverside (*see Materials and methods*). In this environment, other shrubby species accompanied *Tamarix* plants; however, *Tamarix* were always placed in front of the sea, while the other species occupied backwards positions, indicating that (1) a gradient exists in water salinity from the sea to the dunes and that (2) *Tamarix* spp. are more tolerant to salinity compared to the other species. Although a high salinity is also observed in the mouth sites, this environment was composed of *Tamarix* plants characterised by high structural dimensions, sometimes higher than those observed in plants grown on the riverside (e.g. the mean and dominant diameter, the basal area) and a higher age. These characteristics are probably the result of a constant fresh water availability compared to the dune, but also to the riverside, as the water discharge is higher at the mouth compared to the upriver stations. *Tamarix* spp. were the most common species on the mouth and on the riverside stands. Vandersande et al. (2001) suggest that *Tamarix* spp. have a competitive advantage over other riparian species (*Populus*, *Salix* and *Baccharis*) with respect to salt tolerance. The authors reported a reduced tolerance to flooding in *Tamarix* spp., although contrasting results present in the scarce available literature (Horton et al., 1960; Sprenger et al., 2001; Tallent-Halsell and Walker, 2002; Gries et al., 2005) (*see chapter 3* and *4*). This higher salt tolerance among other riparian species, such as *Populus* spp., which are frequently found on riverside banks, especially in Central and Northern Italy, may explain the success of *Tamarix* spp. in these environments.

Tamarix spp. did not only differ in their structure among environments, but also among populations occupying the same environment. Among the dune populations, Crati had the highest dendrometric parameters. This difference may be due to stands age. However, plant age was higher in Basento and Baratz dune populations compared to Crati (data not shown), indicating that other factors are responsible for such differences. The results can thus be explained by the lower salinity observed in this site (*see Materials and methods*), suggesting that, although well tolerated by *Tamarix* spp., salinity is the main factor affecting growth in this environment. Salinity detrimental effects are related to (1) a reduced ability of roots to extract water due to a lower plant capacity to adjust osmotically to water potentials that are commonly in the range of 2-3 kPa (Flowers et al., 2010) and (2) the high salts concentration within the plants which causes toxic effects and nutrient ions imbalance (Gorham et al., 1985; Marschner, 1995; Mansour, 2000; Zhu, 2001; Munns and Tester, 2008). As already observed in *Tamarix* spp., salinity may reduce photosynthesis by increasing non stomatal limitation (*see chapter 2, 3 and 4*) and growth (*see chapter 5*). Moreover, the lower dendrometric parameters were measured on the dune populations of river Basento and lake Baratz, which were the sites characterised by the highest water electrical conductivity values (4-5 dS m^{-1}).

While no differences were observed among mouth populations, the Imera Meridionale showed the highest individual basal area among the riverside populations. This population probably consists of plants characterised by a high number of big sprouts compared to the other populations. In fact, the Alcantara riverside population was composed of plants characterised by a high number of sprouts; however, these were on average of small dimensions compared to Imera Meridionale, probably as a result of the site elevation (frost damages) or of plants position inside the riverbed which is frequently characterised by a high discharge during Etna snow melting period or during precipitation events (Randazzo is the site characterised by the highest annual rainfall among the analysed sites). Moreover, plants grown on Imera Meridionale riverbanks are probably less subjected to flooding events, as river discharge is on average low (5 m^3 s^{-1}) (Bonanno and Lo Giudice, 2010). On the other hand, the Basento riverside population was composed of sprouts of high dimensions; however, the number of sprouts was the lowest among the ones observed. The Imera Meridionale is characterised by a high water electrical conductivity (*see Materials and methods*); however, this high salinity is not only due to NaCl, but to the major presence of other salts (magnesium and calcium sulphates and bicarbonates; Selvaggi et al., 2010). Thus, the presence of salts other than sodium chloride may be less detrimental to plant photosynthesis and growth, as the main harmful effects of soil salinity are due to the passive entry of Na^+ in the cells through K^+ uptake mechanism, these two ions being characterised by a same radius size and by the same ion hydration energy (Blumwald et al., 2000).

Species distribution among the three environments differed between *T. gallica* and *T. africana*. The first species was more widely distributed on the dune sites, while the second along riverside banks. Although no study exist on salinity tolerance between the analysed *Tamarix* species, *T. gallica* is probably more tolerant to salinity (*see also chapter 5*) or less tolerant to flooding compared to *T. africana*.

Conclusions

Tamarix spp. stands structure significantly differed among the environments they occupied. Particularly, plants growth and dimensions seemed to be lower in dune stands, where a high soil or water NaCl concentration can limit plants photosynthesis and energy production. Among the populations distributed in the same environment, the highest plants growth and age were related to lower soil NaCl salinity and/or to adequate and constant fresh water availability. Moreover, *Tamarix gallica* seems to be more tolerant to salinity, or less flooding tolerant, as it is more frequently distributed in dune sites compared to mouth and riverside environments.

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Part II

Tamarix spp. photosynthetic and growth responses to abiotic stresses

Chapter 2

Photosynthetic and growth responses of two Italian provenances of *Tamarix* spp. to 45 days of continuous flooding with fresh and saline water

Abstract

The rise sea level and the increase in heavy rain events are expected to occur as a consequence of global warming, leading to the inundation of coastal regions and river plains in many areas of the world. Flooding with fresh water triggers a series of biological, chemical and physical mechanisms on habitats that, altogether, alter soil capacity to support plants growth. Together with waterlogging, salinity can cause severe damage to plants, assembling harmful effects of both salt and anoxic stresses. The characterization of tolerant species and genotypes is becoming more and more important for the restoration of environments which will be affected by flooding with fresh and saline water. *Tamarix* spp. are known for their tolerance to salinity; however, contrasting information is reported concerning their tolerance to flooding with fresh water. Moreover, *Tamarix* tolerance to flooding with saline water has never been studied. Thus, the objective of this work was the identification of the physiological processes sensitive to continuous flooding (45 days) with salt (NaCl concentration 200 mM) and fresh water in two Italian *Tamarix* spp. provenances (SIM and BAR), by analysing plant growth and the photosynthetic responses in leaves formed before (young leaves) and after (old leaves) the onset of the treatments.

In both provenances, gas exchanges and plants growth were not reduced by flooding with fresh water conditions. On the other hand, a different response was observed in the two provenances concerning gas exchanges measured under flooding with saline water conditions. Particularly, while no effects of the treatment were observed in SIM, gas exchanges were significantly reduced under flooding with saline water in young BAR leaves. This reduction was a consequence of non-stomatal limitations, probably due to a greater root translocation of Na to the rest of the plant, which might have caused an inhibition of RuBisCO activity. No effects of flooding with saline water were observed on gas exchanges of old leaves and on the relative growth rates of both provenances, suggesting an overall moderate tolerance to the treatment.

Key words: *Tamarix* spp., flooding, saline water, leaf gas exchanges, leaf ageing, growth

Introduction

Changes in the precipitation patterns as a consequence of climate change, such as the increase of heavy rain events and consequently, of the frequency and the intensity of flooding with fresh water events, might damage vegetation and soil structure (Knox, 2000; Nichols, 2002; Christensen and Christensen, 2003). Moreover, the increase in the global average air and ocean temperatures is leading to a widespread melting of snow and ice, and to the thermal expansion of water, resulting in the rise of global average sea level, which could cause the salinization of irrigation water, estuaries and freshwater systems (IPCC, 2007).

Based on the FAO (1997) Soil Map of the World, the total area of saline soils is 397 million ha while sodic soils cover 434 million ha. Soil salinity may lead to a decrease of water potential in the root medium, a toxicity caused by excessive Na^+ and Cl^- uptake and accumulation, and a nutrient ion imbalance, especially for K^+, due to the disturbance of essential intracellular ion concentrations (Mansour, 2000), which cause an overall reduction of plant growth. Plant response depends on the salt concentration of the water available for root uptake, on the duration of the stress and on plant salt tolerance (Neumann, 1997; Munns, 2002). According to their tolerance to salt stress, plants are classified as glycophytes or halophytes. Halophytes constitute about 1% of the world's flora, and are defined as plants that can complete their life cycle in a salt concentrations above 200 mM (Flowers and Colmer, 2008). To cope with salinity, halophytes should preserve water uptake under very low soil water potentials and avoid the detrimental effect of high intracellular salt levels. Plants achieve this condition through salt exclusion from root absorption, ions cellular compartmentalisation, compatible solutes synthesis, or extrusion of excess stress-inducing ions by salt glands (Waisel et al., 1986; Ramadan, 1998). Usually, salt is accumulated in the cell vacuoles being an energetically cheap osmoticum (Cram et al., 2002), while ion transport, cellular compartmentalisation, compatible solutes synthesis as well as salt extrusion are energy consuming processes (Robinson et al., 1997; Flowers and Colmer, 2008). Consequently, halophytes tolerance depends on the capacity they have to assure the energy required by these processes, especially under high salinities; in such conditions, plants might also reduce their ability to adjust osmotically as a result of saturation of solute uptake systems (Munns et al., 1983). If lowering water potential is not achieved, high cellular salt concentration might have a detrimental effect on stomatal guard cells, reducing stomatal conductance as well as mesophyll conductance (Bongi and Loreto, 1989; Delfine et al., 1998; Nandy (Datta) et al., 2007). Furthermore, under high salinity, leaf photosynthetic capacity could be limited by the electron transport and by the activity of RuBisCO (Bongi and Loreto, 1989; Delfine et al., 1998; Lovelock and Ball, 2002). Leaf sensitivity also depends on leaf age: in old leaves, high salinity increases the costs of maintaining because leaf aging and salt stress both reduce the photosynthetic rates and the salt accumulation capacity (Suárez and Medina, 2005).

Flooding is a common environmental condition in many habitats occupied by halophytes (Colmer and Flowers, 2008). Flooding events trigger a series of biological, chemical and physical mechanisms on habitats that, altogether, alter soil capacity to support plant growth by: (1) reducing O_2 diffusion and supply to the roots (2) increasing mineral solubilisation, (3) promoting anaerobic metabolism of roots and microbes, which leads to the formation of toxic compounds, and (4) causing aggregates breakdown, clays deflocculation and destruction of cementing agents (Blom and Voesenek, 1996; Kozlowski, 1997). According to Kozlowski (1997), flooding can give rise to detrimental effects also at leaf level, by inducing stomatal closure and, consequently, limiting gas exchange and plant growth (Chen et al., 2005; Rengifo et al., 2005; Fernandez, 2006). In plants growing in waterlogged soils, stomatal closure is induced by a hormonal signal transmitted from the roots to the shoots (ABA and cytokinin) rather than by a reduction in leaf water potential or a loss of leaf turgor (Kozlowski, 1997). As two third of the earth’s land mass is flooded at least occasionally, many species have developed different strategies to survive hypoxia (Schulze et al., 2005). Under scarce or none O_2 soil availability, the increase in ethylene synthesis in tolerant species and its accumulation in roots trigger the formation of a tissue characterised by a high proportion of gas-filled spaces or lacunae – the aerenchyma (Drew et al., 2000); this tissue provides an interconnected system of air channels, enabling gases to diffuse or ventilate between plant organs (Blom and Voesenek, 1996), and to remove gases (carbon dioxide, ethylene, methane) from roots and soil (Colmer, 2003). The oxygen supplied may come from photosynthesis or from the atmosphere; oxygenation of the rhizosphere around the growing 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). Moreover, many tolerant species are able to form adventitious roots when their primary root system cannot function properly (Visser et al., 1995). These roots usually grow on the base of the shoot, on the upper part of the tap root and on the stem nodes, mostly exploring the better aerated soil layers (Blom and Voesenek, 1996) or floating on water surface (Kuzovkina et al., 2004). The re-establishment of a contact between the root apparatus and the air enables stomatal opening and, thus, leaf gas exchange and growth. Together with waterlogging, salinity can cause severe damage to plants (Barrett-Lennard, 2003), assembling harmful effects of both stresses.

The characterization of tolerant species and genotypes is becoming more and more important for the restoration of environments which might be affected by flooding with fresh and saline water as a consequence of climate change effects, such as riverine and coastal areas. In fact, the responses to these stresses might be different among species and, within the same species, among genotypes (Kozlowski, 1997). *Tamarix* genus includes about 90 species, which are naturally distributed in Africa, Asia and Europe. These trees and small shrubs show a high adaptability to different environments and high endurance of adversity (Zhang et al., 2002). *Tamarix* species are characterized by leaf glands specialized in salt extrusion; therefore, their growth is not inhibited by high salt soil concentration (Bar-Nun and Poljiakoff-Meyer, 1974). Many researches were carried out to identify the response of water use during drought events and after water table depth variations (Cleverly et al., 1997; Horton et al., 2001; Gries et al., 2003; Xu and Li, 2006), and to determine gas exchange and growth in saline environments (Glenn et al., 1998). *Tamarix* spp. responses to flooding conditions have also been analysed (Ginzburg, 1967; Brotherson and Field, 1987; Di Tomaso, 1998; Tallent-Halsell and Walker, 2002); however, contrasting information is reported concerning their tolerance under such stress. Moreover, *Tamarix* spp. tolerance to flooding with saline water has never been studied.

The objective of this work was the identification of the physiological processes sensitive to continuous flooding (45 days) with saline (NaCl concentration 200 mM) and fresh water in two Italian *Tamarix* spp. provenances, by analysing plant growth and the photosynthetic responses in leaves formed before and after the onset of the treatments. The hypothesis were that

(1) *Tamarix* spp. tolerance to flooding with fresh and saline water could vary depending on plants origin. Particularly, a higher tolerance to flooding with fresh water is expected in plants collected on the riverbanks of perennial streams, whereas plants collected on marine deposits are supposed to be more tolerant to salt stress;

(2) leaf tolerance is expected to be higher in young leaves, as old leaves has been transpiring, accumulating and extruding salt for a longer period of time.

Material and Methods

Two provenances of *Tamarix* spp. were collected in November 2008 from two sites of Southern Italy. Three genotypes were randomly selected on the shores of lake Baratz (BAR) (40°40’48’’ N, 8°13’34’’ E; Northwest Sardinia, Italy) and of river Simeto (SIM) (37°24’01’’ N, 15°04’04’’ E, East Sicily, Italy). The genotypes were replicated six times by cuttings, which were planted in 1.6 dm^3 pots (10.5×10.5×22 cm) containing sand (35%) and loamy soil (65%). The cuttings were grown in a greenhouse for six weeks and then were transplanted in 2.6 dm^3 pots (17.5×17.5×25 cm) containing the same soil mixture. The pots were inserted into nine plastic boxes (60×40×40 cm) in groups of four (two provenances per two randomly selected genotype). The plants were grown for two months in a growth-chamber under a photosynthetic photon flux density (PPFD) of approximately 550 $\mu mol\ m^{-2}\ s^{-1}$, 60% relative humidity, a photoperiod of twelve hours and a day/night temperature of 25/15 °C. The plants were watered three times a week with fresh water. Two weeks before the beginning of the experiment, a modified Hoagland solution at 0.5× concentration was supplied. The nine boxes were divided into three blocks, each composed of three treatments. The treatments provided: (1) Daily irrigation with potable tap water (Control - C), (2) Flooding with fresh water (F) and (3) Flooding with fresh water added with NaCl 200 mM (FS). Flooding levels were maintained 5 cm above the soil surface and kept constant for 45 days by adding water once a week. Moreover, a proportional amount of Hoagland solution (0.5× concentration) was provided to all treatments (C, F, and FS) once a week, according to plant nitrogen needs.

To estimate this amount, the tenth youngest leaf[1] was selected from the highest (dominant) sprout and weekly leaf length growth was determined. The leaf was cut and its dry weight was determined by placing it in oven at 70 °C, until no further weight change occurred. To estimate weekly leaf growth, considering a constant leaf water content, the initial leaf dry weight was calculated as:

$$DW_i = DW_f / LL_f * LL_i \qquad \text{(Eq. 1)}$$

Where DW_i is the initial leaf dry weight in g, DW_f is the final leaf dry weight in g, LL_i is the initial leaf length in cm and LL_f is the final leaf length in cm. Thus, weekly leaf growth was estimated as follow:

$$DWG = DW_f - DW_i \qquad \text{(Eq. 2)}$$

Where DWG is the leaf dry weight growth in g.

Weekly plant growth was estimated by multiplying leaf growth by the total number of leaves of the dominant sprout, by the total number of sprouts per plant and by a correction factor of 0.5 (not no overestimate the nitrogen needs). Total dry weight growth was then multiplied by leaf nitrogen content (on average 1.5%, determined as reported below).

Gas exchanges and chlorophyll fluorescence measurements

Two plants per treatment and per block (one SIM and one BAR) were analysed by gas exchange and fluorescence measurements. Gas exchanges were measured three days before the beginning of the experiment on one young green leaf (YL0; the tenth leaf from the apical bud); on day 45 after the onset of the experiment, gas exchanges were measured on one young leaf (YL45; the tenth leaf from the apical bud, formed after the beginning of the treatment) and on one pre-existent old leaf (OL45; an YL0 neighbour’s shoot at time 0). All leaves were selected from the dominant sprout.

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[1] *Shoots composed of small scale-like leaves will be called **leaves** all over the thesis*

Gas exchanges were measured using a portable infrared gas analyser (LI-6400, LI-COR Biosciences, Inc., Lincoln, NE) equipped with a conifer chamber (LI-6400-05). The air flow was set at 400 µmol m^{-2} s^{-1} and the cuvette temperature at 25 °C. An artificial light was generated by a halogen incandescent lamp and transmitted to the cuvette by a fibre-optic (FL-400 with 400-F; Walz, Germany). The light brought a saturating photon flux (1800-2000 µmol $m^{-2}s^{-1}$). Net assimilation rate (A) changes in response to intercellular CO_2 concentration (C_i) was determined for ambient CO_2 concentration values (C_a) of 400, 300, 250, 200, 150, 50, 400, 500, 650, 750, 850, 1000 µmol mol^{-1} as suggested by Long and Bernacchi (2003); the assimilation values were recorded as soon as C_a was stable (cv<0.7%) (Ainsworth et al., 2002). Net assimilation rates (A_{400}) and stomatal conductance (g_s) measured at C_a values of 400 µmol mol^{-1} were considered as the assimilation and the conductance at growth chamber CO_2 concentration. Intrinsic water use efficiency (A/g_s) was calculated as the ratio between A_{400} and g_s. The maximum carboxylation rate (Vc_{max}) and the maximum rate of electron transport (J_{max}) were also estimated according to Farquhar et al. (1980). Dark respiration (R_d) was measured at ambient C_a (400 µmol mol^{-1}), after 5 minutes of adaptation. After gas exchange measurements, the leaves were cut off and their fresh weight was determined. The leaves were then scanned, and the images analysed with the software Skyroot (Llandrindod Wells, Powys, UK) in order to obtain the total leaf length. Leaf area was estimated by multiplying leaf length by the mean leaf diameter (0.7 mm). A small leaf portion was dried at 70 °C for dry weight estimation while the rest of the leaf was dark-adapted for 15 minutes for chlorophyll fluorescence measurements.

Chlorophyll fluorescence was measured with a PAM 2000 fluorimeter (Heinz Walz, Effeltrich, Germany) on the same samples used for gas exchange measurements. The photochemical efficiency was estimated by the quantum yield of PSII in dark adapted leaves (F_v/F_m). Additional far red light (735 nm) was used to estimate the ground state fluorescence (F_0). The fluorescence yield Φ_{PSII} (i.e. the quantum yield of PSII in the light) was measured by using a saturating pulse of white light.

Leaf and roots characteristics

After drying, the young and old leaves measured on day 45 (YL45 and OL45) were inserted into a plastic tube added with 50 ml of deionised water in order to estimate the electrical conductivity of the liquid phase, which is proportional to the amount of the dissolved salt extruded by the salt glands. The tubes were shaken at 500 rpm for 20 minutes. The liquid phase was then separated from the leaves with filter paper. Leaf dry weight without salt was determined after drying in oven at 70 °C until constant weight. Electrical conductivity (EC) of the liquid phase was measured using a conductimeter (HI9811, Hanna instruments INC, USA) equipped with an electrode probe (HI1285, Hanna instruments INC, USA). The EC value was expressed as dS m^{-1} g^{-1} of leaf dry weight without salt. The extruded salt mass, calculated as the difference in dry weight before and after leaf washing, and EC were linearly related (R^2=0.896).

Leaf mass per area (LMA) was calculated as the ratio between leaf dry mass without salt and leaf area. Leaves were then ground for nitrogen (N) and carbon (C) determination, using an elemental analyser (N.C. Soil Analyser, FlashEA 1112 series, Thermo Electron Corporation, USA).

Leaves (YL45 and OL45) and belowground roots (see below) were oven dried at 80 °C for 48 hours, then ground to obtain a fine homogeneous powder. The plant material was prepared according to the wet ash method using HNO_3/H_2SO_4 followed by a microwave digestion. The concentration of Na was determined using the AAS-flame technique (C_2H_2/Air).

Growth measurements

The number of living and dead sprouts as well as the number of green leaves and the percentage of yellow and dead leaves on total leaves number, were determined before the beginning of the treatments and on day 45. Sprout mortality was calculated as the percentage of dead sprouts from the beginning of the experiment. On day 45, plants were cut and divided into belowground roots (BGR; diameter >2mm), adventitious roots (ADV; the roots formed after the beginning of the flooding experiment and situated at the base of the stem, outside the soil), wood (all the lignified material) and leaves (without distinction between young and old leaves), and dried in an oven at 70 °C to constant weight, in order to determine the biomass of the different plant fractions. The leaf biomass was estimated after leaf salt extrusion evaluation. Final aboveground biomass (AGB_f) and total root biomass (TRB) were calculated as the sum of wood and leaves biomasses, and as the sum of belowground and adventitious root biomasses respectively. Adventitious roots biomass and belowground roots biomass were expressed as percentage of total roots biomass. Adventitious roots to aboveground biomass (ADV/AGB_f), adventitious roots to belowground roots biomass (ADV/BGR) and total root biomass to aboveground biomass (TRB/AGB_f), were also calculated.

A power relation was found between sprout biomass (SB) and sprout length (SL) ($SB=0.0012SL^{2.028}$; $R^2=0.818$). This equation was used to estimate initial aboveground biomass (AGB_i), being the range of variation of the final sprout length similar to that of the initial sprout length. Relative plant growth rate (RGR) was then calculated as mean of the 45 days, as:

$RGR= (Log_e (AGB_f) - Log_e (AGB_i))\ days^{-1}$ (Eq. 2)

Statistical analysis

A repeated analysis of variance (ANOVA) was performed using the statistical software Systat 12.0 (SPSS Inc.) on absolute YL0 and OL45 values to test the time effect on leaf development and on leaf photosynthetic responses. The relative change (which represents the percentage of a parameter value at time 45 with respect to the value at time 0) was analysed by ANOVA, to evaluate the main effects of provenances, treatments and their interactions. The post hoc analysis was performed using Fisher's LSD test.

ANOVA was also performed on all photosynthetic and growth parameters to test the treatments effect, leaf development and their interaction after 45 days of treatment. The parameters expressed as percentage were transformed using the arcsine of the square root of the analysed parameter divided by 100. Post hoc was performed using Fisher's LSD test.

The software Prism 4 (GraphPad software, Inc) was used to test the correlations between Vc_{max} and N and between Vc_{max} and J_{max}, and to evaluate whether the slope and the intercept of Vc_{max} and J_{max} linear equations for both provenances and leaf types were different from each other.

Statistical significance was considered for *p* values <0.05.

Tab. 1 Absolute values and relative changes of the analysed photosynthetic parameters: net assimilation rates (A_{400}), maximum carboxylation rate (Vc_{max}), dark respiration (R_d), stomatal conductance (g_s), intrinsic water use efficiency (A/g_s), maximum rate of electron transport (J_{max}), quantum yield of PSII in the dark (F_v/F_m) and in the light (Φ_{PSII}), ground state fluorescence (F_0) and leaf mass per area (LMA) measured in the two provenances (SIM and BAR) before the beginning of the experiment on the tenth leaf from the apical bud (YL0) and after 45 days from the beginning of the treatment on a YL0 neighbour's leaf (OL45). A repeated ANOVA was performed to test time effect on leaf photosynthetic responses. The relative changes were analysed by ANOVA to evaluate the main effects of provenances, treatments and their interactions. Values are means ± s.e. (n=3).

			A_{400}		**Vc_{max}**		**R_d**		**g_s**		**A/g_s**		**J_{max}**		**F_v/F_m**		**Φ_{PSII}**		**F_0**		**LMA**	
			(μmol CO_2 m^{-2} s^{-1})		(μmol CO_2 m^{-2} s^{-1})		(μmol CO_2 m^{-2} s^{-1})		(mol H_2O m^{-2} s^{-1})		(μmol CO_2/mol H_2O)		(μmol photon m^{-2} s^{-1})								g m^{-2}	
			Mean	s.e.	Mean	s.e.	Mean	s.e.	Mean	s.e.	Mean	s.e.	Mean	s.e.	Mean	s.e.	Mean	s.e.	Mean	s.e.	Mean	s.e.
SIMETO	**Control**	**Young Leaves 0**	17.1	*3.2*	100.1	*17.5*	4.4	*0.5*	0.39	*0.07*	44.2	*2.0*	235.0	*50.6*	0.77	*0.01*	0.35	*0.02*	0.032	*0.009*	170.8	*26.6*
		Old Leaves 45	15.4	*3.9*	73.7	*12.2*	1.8	*0.9*	0.33	*0.12*	63.2	*23.2*	161.7	*32.8*	0.78	*0.02*	0.30	*0.03*	0.027	*0.002*	120.4	*18.6*
		Relative change (%)	*-9.7*		*-26.4*		*-60.0*		*-15.0*		*43.0*		*-31.2*		*0.3*		*-14.0*		*-16.3*		*-29.5*	~~a~~
	Flooding	**Young Leaves 0**	17.0	*3.7*	78.0	*16.8*	3.5	*0.9*	0.47	*0.10*	36.6	*4.5*	180.5	*37.5*	0.77	*0.01*	0.26	*0.04*	0.056	*0.004*	171.3	*6.9*
		Old Leaves 45	12.9	*3.6*	58.1	*14.4*	1.1	*0.1*	0.31	*0.09*	42.7	*6.3*	117.1	*29.9*	0.81	*0.00*	0.42	*0.10*	0.032	*0.006*	143.7	*13.1*
		Relative change (%)	*-24.0*		*-25.5*		*-67.6*		*-33.6*		*16.6*		*-35.1*		*5.5*		*65.6*		*-42.9*		*-16.1*	**ab**
	Flooding + Salt	**Young Leaves 0**	17.4	*4.7*	101.5	*37.3*	3.7	*1.2*	0.29	*0.03*	58.3	*10.5*	256.7	*108.5*	0.79	*0.01*	0.25	*0.00*	0.045	*0.002*	147.9	*30.6*
		Old Leaves 45	12.0	*2.8*	75.0	*10.4*	2.2	*0.9*	0.16	*0.04*	78.0	*9.2*	145.2	*37.2*	0.77	*0.05*	0.35	*0.02*	0.020	*0.003*	164.8	*14*
		Relative change (%)	*-31.2*		*-26.1*		*-41.9*		*-45.1*		*33.6*		*-43.4*		*-3.0*		*40.3*		*-55.2*		*11.4*	**b**
BARATZ	**Control**	**Young Leaves 0**	18.4	*1.9*	104.0	*10.4*	2.9	*0.8*	0.48	*0.02*	37.2	*5.0*	228.3	*12.3*	0.76	*0.01*	0.31	*0.09*	0.047	*0.001*	124.83	*22.2*
		Old Leaves 45	13.5	*1.2*	59.9	*4.1*	1.8	*0.5*	0.36	*0.08*	40.9	*8.0*	130.4	*5.7*	0.81	*0.00*	0.31	*0.01*	0.033	*0.009*	74.9	*5.6*
		Relative change (%)	*-26.8*		*-42.4*		*-39.8*		*-25.4*		*9.9*		*-42.9*		*6.6*		*-2.1*		*-31.0*		*-40.0*	***a***
	Flooding	**Young Leaves 0**	23.7	*2.6*	148.0	*7.3*	5.9	*1.4*	0.37	*0.08*	68.0	*10.8*	363.0	*29.8*	0.78	*0.02*	0.36	*0.02*	0.047	*0.010*	152.8	*13.3*
		Old Leaves 45	16.0	*0.6*	78.7	*0.6*	2.6	*0.2*	0.30	*0.02*	53.0	*5.7*	191.5	*4.2*	0.76	*0.06*	0.29	*0.14*	0.023	*0.007*	112.1	*20.6*
		Relative change (%)	*-32.4*		*-46.8*		*-55.8*		*-17.9*		*-22.1*		*-47.3*		*-2.1*		*-19.1*		*-51.8*		*-26.6*	***ab***
	Flooding + Salt	**Young Leaves 0**	15.7	*3.5*	104.7	*23.6*	6.1	*0.4*	0.22	*0.01*	70.0	*15.2*	253.5	*64.9*	0.76	*0.02*	0.43	*0.05*	0.049	*0.001*	161.5	*39.4*
		Old Leaves 45	13.9	*0.9*	77.6	*9.4*	3.5	*0.7*	0.30	*0.08*	56.6	*18.4*	156.4	*17.6*	0.81	*0.03*	0.30	*0.09*	0.021	*0.008*	173.2	*25.2*
		Relative change (%)	*-11.6*		*-25.9*		*-42.6*		*32.1*		*-19.1*		*-38.3*		*6.9*		*-30.1*		*-56.2*		*7.2*	**b**
		Repeated ANOVA (Time)	**		**		***		*		n.s.		***		n.s.		n.s.		***		*	
		ANOVA on absolute changes																				
		Provenance	n.s.		n.s.		n.s.		n.s.		n.s.		n.s.		n.s.		n.s.		n.s.		n.s.	
		Treatment	n.s.		n.s.		n.s.		n.s.		n.s.		n.s.		n.s.		n.s.		n.s.		**	
		Treatment x Provenance	n.s.		n.s.		n.s.		n.s.		n.s.		n.s.		n.s.		n.s.		n.s.		n.s.	

Results

Leaf age effect on the photosynthetic activity

Net assimilation rate at growth CO_2 concentration (A_{400}), maximum rate of carboxylation (Vc_{max}), maximum rate of electron transport (J_{max}), stomatal conductance (g_s), ground state fluorescence (F_0) and leaf mass per area (LMA) were significantly lower after 45 days from the beginning of the experiment (in OL45), in all treatments, including leaves grown under control conditions (Tab. 1). However, water use efficiency (A/g_s), as well as quantum yield of PSII in the dark (F_v/F_m) and in the light (Φ_{PSII}) did not change over time. Treatments effect was significant only on leaf mass per area (LMA), whose relative change increased after 45 days of flooding with saline water (Tab. 1), in both provenances.

Flooding with fresh water

After 45 days of flooding with fresh water, A_{400} was not affected by the treatment, being on average similar in the two provenances (20.31 μmol CO_2 m^{-2} s^{-1} in YL45 and 14.29 μmol CO_2 m^{-2} s^{-1} in OL45; Fig. 1 a). Similarly to A_{400}, Vc_{max} was similar in plants grown under control and under flooding conditions (91.25 μmol CO_2 m^{-2} s^{-1} in YL45 and 66.34 μmol CO_2 m^{-2} s^{-1} in OL45 of both provenances; Fig. 1 b). Flooding did not alter R_d, which was similar to the control in both provenances (3.12 μmol CO_2 m^{-2} s^{-1}). Both g_s and A/g_s were not affected by the treatment in SIM and BAR (Fig. 1 d, e). On average, g_s and A/g_s were 0.40 mol H_2O m^{-2} s^{-1} and 42.9 μmol CO_2/mol H_2O respectively. Similarly, J_{max} was not influenced by flooding (228.0 μmol photons m^{-2} s^{-1} in YL45 and 146.8 μmol photons m^{-2} s^{-1} in OL45) in both provenances (Fig. 2 a). Fluorescence did not change in response to flooding in the two provenances. F_v/F_m (Fig. 2 b), Φ_{PSII} (Fig. 2 c) and F_0 (Fig. 2 d) were respectively equal to 0.77, 0.35 and 0.025.

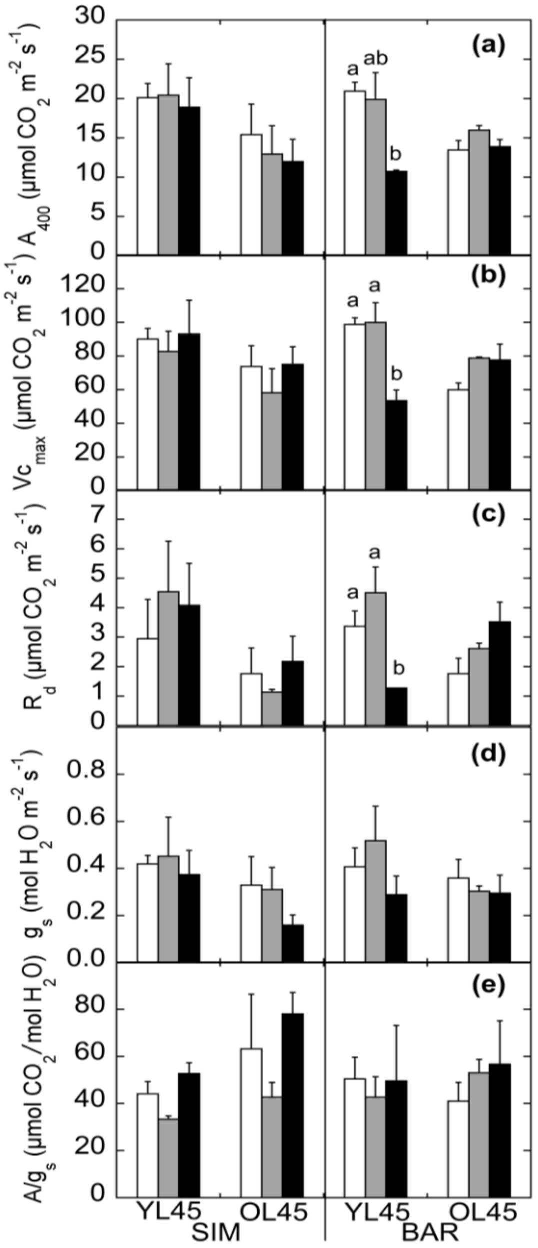

Fig. 1 Net assimilation rates (A_{400}) (a), maximum carboxylation rate (Vc_{max}) (b), dark respiration (R_d) (c), stomatal conductance (g_s) (d) and intrinsic water use efficiency (A/g_s) (e) measured after 45 days from the beginning of the experiment on the tenth leaf from the apical bud, formed after the beginning of the treatment (young leaf, YL45) and on a pre-existent leaf (the tenth leaf from the apical bud at time 0, formed before the beginning of the experiment; old leaf, OL45) in the two provenances (SIM and BAR) under control (white bars), flooding with fresh water (grey bars) and flooding with saline water (black bars). Values are means ± s.e. (n=3).

Leaf structure was not affected by 45 days of flooding with fresh water. LMA under flooding conditions (126.7 g m^{-2}) was comparable to the control (93.1 g m^{-2}) in YL45 and OL45 (147.5 g m^{-2} and 131.1 g m^{-2} respectively) of both provenances (Tab. 2). Leaf carbon (C) was 47.1% in flooded YL45, being on average higher than control (45.8%; $p<0.01$), and 45.9% in OL45; this difference was only observed in BAR provenance ($p<0.01$) (Tab. 2). No differences were found in leaf nitrogen (N) content after the treatment. N was 1.20% and 0.72% in YL45 and OL45 respectively, and was similar in both provenances (Tab. 2).

Tab. 2 Leaf mass per area (LMA), carbon content (C), and nitrogen content (N) measured in the two provenances (SIM and BAR) after 45 days from the beginning of the experiment on the tenth leaf from the apical bud, formed after the beginning of the treatment (young leaf, YL45) and on a pre-existent leaf (the tenth leaf from the apical bud at time 0, formed before the beginning of the experiment; old leaf, OL45) under control, flooding with fresh water and flooding with saline water. Values are means ± s.e. (n=3).

				Young Leaves 45			**Old Leaves 45**		
				Control	**Flooding**	**Flooding+Salt**	**Control**	**Flooding**	**Flooding+Salt**
Simeto	**LMA**	(g m^{-2})	Mean	143.0	176.7	167.5	120.4	143.7	164.8
			s.e.	*23.8*	*15.5*	*8.5*	*18.6*	*13.1*	*14.0*
	Carbon	%	Mean	47.2	48.3	48.9	45.6	47.1	46.6
			s.e.	*0.5*	*0.4*	*0.6*	*0.4*	*0.3*	*0.5*
	Nitrogen	%	Mean	0.90	1.14	0.76	0.47	0.27	0.76
			s.e.	*0.31*	*0.43*	*0.18*	*0.26*	*0.07*	*0.17*
Baratz	**LMA**	(g m^{-2})	Mean	110.3 **a**	118.3 **a**	171.6 **b**	74.9 **a**	112.1 **ab**	173.2 **b**
			s.e.	*10.6*	*14.8*	*34.5*	*5.6*	*20.6*	*25.2*
	Carbon	%	Mean	44.7 **a**	47.9 **b**	47.4 **b**	45.7	45.2	46.0
			s.e.	*0.6*	*0.1*	*1.3*	*0.2*	*1.0*	*1.4*
	Nitrogen	%	Mean	0.97	1.76	1.67	0.72	1.49	1.38
			s.e.	*0.19*	*0.70*	*0.89*	*0.37*	*0.52*	*0.46*

The amount of CO_2 fixed by photosynthesis per unit of leaf N (A_{400}/N, data not shown) was not influenced by flooding treatment or by provenance, and was on average equal to 7.45 μmol CO_2 m^{-2} s^{-1} g^{-1}. Flooding treatment did not modify the quantity of salt extruded by old and young leaves. The average electrical conductivity (EC) was on average equal to 1.3 dS m^{-1} g^{-1}, with no difference between SIM and BAR (Fig. 3). Sodium (Na) leaf and roots contents under flooding with fresh water were similar to control, being on average equal to 3.5 mg g^{-1} and 5.1 g mg^{-1} respectively, with no difference between the provenances (Fig. 4).

Flooding with saline water

A_{400} was on average 15.6 μmol CO_2 m^{-2} s^{-1} and 12.9 μmol CO_2 m^{-2} s^{-1} in YL45 and OL45 respectively. No effects were found in SIM in response to the treatment. However, A_{400} in BAR YL45 was reduced by 48.8% ($p<0.05$) in response to flooding with saline water (Fig. 1 a). This reduction was coupled with a decrease in Vc_{max}, which was reduced by 45.8% ($p<0.05$) (Fig. 1 b). On the other hand, Vc_{max} did not vary in response to flooding with saline water in OL45 of both provenances (75.0 μmol CO_2 m^{-2} s^{-1} in SIM and 77.6 μmol CO_2 m^{-2} s^{-1} in BAR) and in SIM YL45 (93.1 μmol CO_2 m^{-2} s^{-1}). R_d in treated plants (2.9 μmol CO_2 m^{-2} s^{-1}) was on average similar to the control (2.4 μmol CO_2 m^{-2} s^{-1}) in both young and old leaves (Fig. 1 c); however, R_d in BAR YL45 was strongly reduced by the treatment (-62.2%; $p<0.1$). g_s after 45 days of flooding with saline water (0.28 mol H_2O m^{-2} s^{-1}) was on average similar to that of control (0.38 mol H_2O m^{-2} s^{-1}).

Although flooding with saline water reduced A_{400}, Vc_{max} and R_d in BAR YL45, g_s showed a not significant reduction (Fig. 1 d). Water use efficiency (A/g_s) after 45 days of treatment was comparable to the control (59.2 μmol CO_2/mol H_2O), in young and old leaves of both provenances (Fig. 1 e).

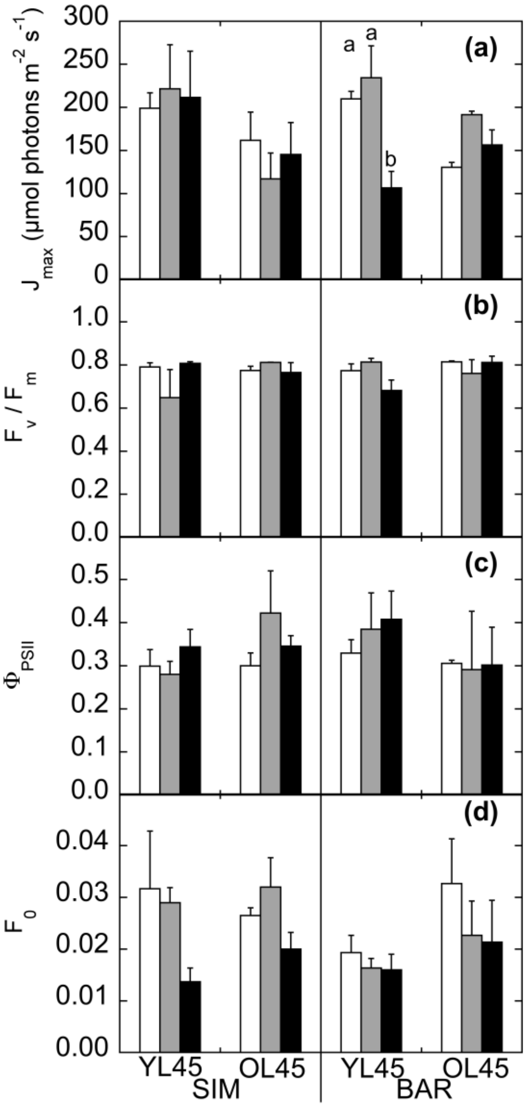

Fig. 2 Maximum rate of electron transport (J_{max}) (a), quantum yield of PSII in the dark (F_v/F_m) (b) and in the light (Φ_{PSII}) (c) and ground state fluorescence (F_0) (d) measured in the two provenances (SIM and BAR) after 45 days from the beginning of the experiment on the tenth leaf from the apical bud, formed after the beginning of the treatment (young leaf, YL45) and on a pre-existent leaf (the tenth leaf from the apical bud at time 0, formed before the beginning of the experiment; old leaf, OL45) under control (white bars), flooding with fresh water (grey bars) and flooding with saline water (black bars). Values are means ± s.e. (n=3).

J_{max} under FS was on average similar to control (167.7 μmol photons m^{-2} s^{-1}) in SIM, in both young and old leaves, and in BAR OL45. However, as observed for A_{400}, R_d and Vc_{max}, J_{max} in BAR YL45 was reduced by the treatment (Fig. 2 a). As in flooded with fresh water plants, chlorophyll fluorescence was not altered by treatment, in both provenances and leaf types. F_v/F_m was on average equal to 0.76 (Fig. 2 b), Φ_{PSII} to 0.35 (Fig. 2 c), while F_0 to 0.018 (Fig. 2 d).

On average, LMA was higher in flooded with saline water plants in both young (169.5 g m^{-2}; $p<0.05$) and old leaves (169.0 g m^{-2}; $p<0.001$) (Tab. 2). This difference was significant in BAR provenance, in both YL45 (171.5 g m^{-2}; $p=0.05$) and OL45 (173.2 g m^{-2}; $p<0.01$). Leaf C content was on average similar in control (45.8%) and treated plants (47.1%). However, in YL45, C content was significantly higher under flooding with saline water ($p<0.05$), especially in BAR, where leaf C content was 47.4%, while in control it was equal to 44.7% ($p<0.05$) (Tab. 2).

N content did not vary in response to the treatment in young and old leaves of both provenances (Tab. 1), being on average equal to 1.0%. The amount of CO_2 fixed by photosynthesis per unit of leaf N (A_{400}/N, data not shown) was significantly higher in control than after 45 days of flooding with saline water ($p<0.01$), especially in young leaves. However, this trend was only observed in BAR, were YL45 were characterized by a lower CO_2 fixation per unit of leaf N (3.8 μmol CO_2 m^{-2} s^{-1} g^{-1} with respect to control 8.8 μmol CO_2 m^{-2} s^{-1} g^{-1}).

Salt extrusion increased significantly after 45 days of flooding with saline water ($p<0.001$), being electrical conductivity equal to 5.9 dS m^{-1} g^{-1} under flooding with saline water and to 1.7 dS m^{-1} g^{-1} in control. On average, old leaves extruded more salt than young leaves (7.7 and 4.2 dS m^{-1} g^{-1} respectively; $p<0.001$), although BAR YL45 and OL45 extruded the same amount of salt (Fig. 3).

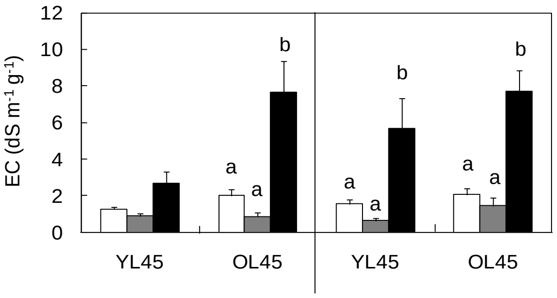

Fig. 3 Electrical conductivity of the water where leaf extruded salts were dissolved, measured in the two provenances (SIM and BAR) after 45 days from the beginning of the experiment on the tenth leaf from the apical bud, formed after the beginning of the treatment (young leaf, YL45) and on a pre-existent leaf (the tenth leaf from the apical bud at time 0, formed before the beginning of the experiment; old leaf, OL45) under control (white bars), flooding with fresh water (grey bars) and flooding with saline water (black bars). The data were normalised per unit of leaf mass. Values are means ± s.e. (n=3).

Moreover, the quantity of salt extruded by young BAR leaves was 52.8% higher than that extruded by young SIM leaves ($p<0.05$) (Fig. 3). Leaf Na content was affected by the treatment, being significantly higher under flooding with saline water conditions (14.1 mg g^{-1}; $p<0.001$).

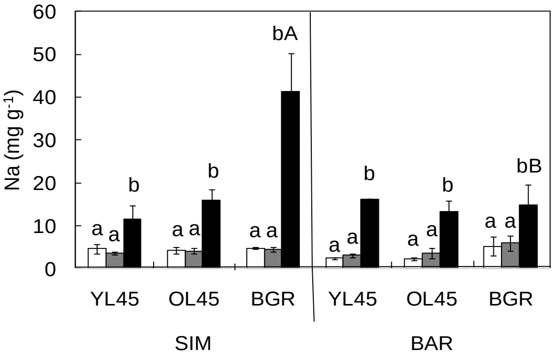

Fig. 4 Sodium (Na) content of the tenth leaf from the apical bud, formed after the beginning of the treatment (young leaf, YL45), of a pre-existent leaf (the tenth leaf from the apical bud at time 0, formed before the beginning of the experiment; old leaf, OL45) and of belowground roots biomass (BGR) measured under control (white bars), flooding with fresh water (grey bars) and flooding with saline water (black bars). Values are means ± s.e. (n=3).

Leaf Na content of treated plants was similar in young and old leaves of both provenances (Fig. 4), although BAR YL45 Na content was slightly higher than SIM YL45 ($p=0.07$). Na content of belowground roots was significantly higher under flooding with saline water ($p<0.001$) in both provenances. However, Na content in SIM roots under flooding with saline water (41.3 mg g^{-1}) was significantly higher than in BAR (14.8 mg g^{-1}; $p<0.001$) (Fig. 4).

Vc_{max} and J_{max} were linearly related in SIM YL45 ($R^2=0.8084$) and OL45 ($R^2=0.9268$) as well as in BAR leaves ($R^2=0.9218$ and $R^2=0.7080$ respectively for YL45 and OL45). Furthermore, slopes and intercepts of the four linear regressions were similar, and one equation was used for all the dataset ($R^2=0.882$) (Fig. 5). On the contrary, Vc_{max} and leaf N were not correlated in both SIM and BAR YL45 and OL45 leaves (data not shown). A_{400} varied in accordance to N only in SIM provenance, in YL45 ($R^2=0.6822$; $p<0.05$) and OL45 ($R^2=0.7919$; $p<0.01$) while in BAR these two parameters were not significantly correlated ($R^2=0.3869$ in young leaves and $R^2=0.4473$ in old leaves).

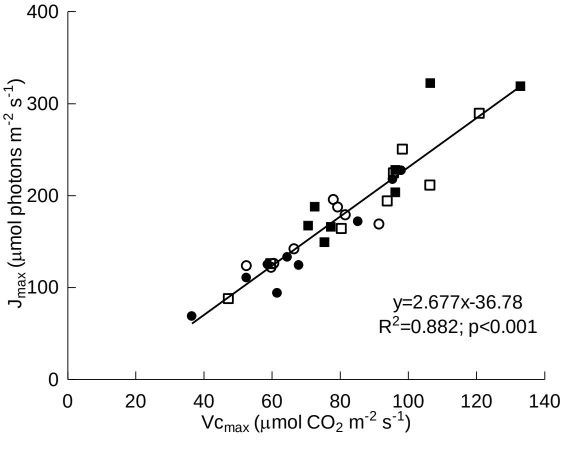

Fig. 5 Correlation between maximum carboxylation rate (Vc_{max}) and maximum rate of electron transport (J_{max}) obtained from net assimilation rate (A) versus intercellular CO_2 concentration (C_i) curves of both SIM (black symbols) and BAR (white symbols) provenances in young (squares) and old (circles) leaves, under control, flooding with fresh water and flooding with saline water. Equation, R^2 and p value are referred to the global fit.

Plant responses to flooding with fresh and saline water

Plants characteristics after 45 days of treatments are shown in Tab. 3. Aboveground biomass (AGB) of treated plants was similar to control (6.49 g), although it was significantly higher in SIM (10.3 g) than in BAR (2.6 g; p<0.001). Particularly, AGB was higher in flooded SIM than in control SIM (p<0.05). The average number of living sprouts (2.7) and sprout mortality (26.0%) was similar to control in both provenances and treatments. The number of green leaves was also similar among treatments and between provenances, being on average equal to 34.2. The percentage of yellow and dead leaves in F and FS did not differ from control (13.3%), and was similar in both provenances (16.5% in SIM and 10.1% in BAR). However, the percentage of dead leaves in SIM was higher than in BAR (p<0.01). Treatments and provenance effects on total root biomass (TRB) were similar to those on belowground roots, with a reduction in root biomass (1.8 g in F, p<0.05; 1.6 g in FS p<0.05) with respect to control (3.8 g), particularly in SIM (p<0.01 in FS). Lower TRB values were observed in BAR (p<0.01). Belowground roots biomass (BGB) was on average higher in control (3.8 g) than in flooded with fresh (1.72 g; p<0.05) and saline water plants (1.53 g; p=0.01) of both provenances. Moreover, SIM was characterised by a higher BGB (3.3 g) than BAR (1.4 g; p<0.01).

Adventitious roots were only produced under flooding conditions; consequently, adventitious roots biomass (ADV) was significantly higher under flooding treatments of both provenances, with no difference between fresh and saline water. ADV/AGB ratio was not influenced by treatments or by provenances, being on average equal to 0.023. However, BAR provenance was characterised by a higher ratio under both flooding treatments. ADV/BGR ratio was significantly influenced by treatments (p<0.05), as adventitious roots were only produced under flooding conditions. The ratio was higher under flooding with fresh (0.36) and saline water (0.28) compared to control. Particularly in BAR provenance, the ratio was significantly higher under flooding with fresh water (0.46) than in control (p<0.05). TRB/AGB was reduced by flooding (0.11; p<0.05) and flooding with saline water (0.12; p<0.05), being higher in control (0.28); particularly in SIM, flooded with fresh water plants had lower roots per shoot values than control (p<0.05).

Tab. 3 Above ground biomass (AGB), number of living sprouts, sprouts mortality, relative growth rate of the above ground biomass (RGR), number of green leaves, percentage of yellow and dead leaves, total roots biomass (TRB), percentage of belowground roots biomass (BGR), percentage of adventitious roots biomass (ADV), adventitious roots to aboveground biomass ratio (ADV/AGB), adventitious roots to belowground roots biomass ratio (ADV/BGR) and total roots biomass to aboveground biomass (root/shoot; TRB/AGB) measured and calculated in the two provenances and in the three treatments. Values are means ± s.e. (n=3).

			AGB	**Living sprouts**	**Sprout mortality**	**RGR**	**Green leaves**	**Yellow leaves**	**Dead leaves**	**TRB**	**BGR**	**ADV**	**ADV/AGB**	**ADV/BGR**	**TRB/AGB**
			(g)	no.	(%)	(g g^{-1} day^{-1})	no.	(%)	(%)	(g)	(%)	(%)	(%)	(%)	(%)
Simeto	**Control**	**Mean**	19.2 **a**	2.7	11.1	0.023	36.7	16.0	15.1	5.51 **a**	100 **a**	0.00 **a**	0.0	0.0	33.6 **a**
		s.e.	*4.5*	*0.7*	*11.1*	*0.010*	*4.4*	*3.0*	*1.6*	*1.15*	*0.0*	*0.0*	*0.0*	*0.0*	*12.6*
	Flooded	**Mean**	30.6 **b**	2.3	33.3	0.021	39.0	19.0	11.6	3.32 **a**	80.2 **b**	19.80 **b**	2.2	26.7	10.8 **b**
		s.e.	*3.8*	*0.7*	*8.3*	*0.090*	*14.2*	*6.7*	*2.9*	*0.64*	*6.8*	*6.8*	*0.8*	*11.7*	*1.7*
	Flooded+Salt	**Mean**	15.7 **a**	2.0	16.7	0.019	31.3	14.5	26.9	2.39 **b**	77.7 **b**	22.30 **b**	3.4	29.0	15.0 **ab**
		s.e.	*3.6*	*0.6*	*16.7*	*0.006*	*5.8*	*6.2*	*7.6*	*1.00*	*2.7*	*2.7*	*1.1*	*4.6*	*4.2*
Baratz	**Control**	**Mean**	10.0	2.7	24.4	0.028	43.0	12.9	7.7	2.06	100 **a**	0.00 **a**	0.0 **a**	0.0 **a**	22.3
		s.e.	*1.0*	*0.3*	*12.4*	*0.040*	*2.0*	*2.5*	*2.0*	*0.52*	*0.0*	*0.0*	*0.0*	*0.0*	*7.9*
	Flooded	**Mean**	5.9	2.7	27.8	0.022	36.7	12.8	3.9	1.12	70.4 **b**	29.60 **b**	5.0 **b**	45.5 **b**	18.2
		s.e.	*1.0*	*0.3*	*2.8*	*0.005*	*2.9*	*4.5*	*2.7*	*0.62*	*7.3*	*7.3*	*1.8*	*16.9*	*8.4*
	Flooded+Salt	**Mean**	8.7	4.0	42.5	0.024	33.3	4.7	3.6	1.37	80.7 **b**	19.30 **b**	3.5 **b**	28.0 **ab**	15.3
		s.e.	*3.8*	*1.6*	*3.82*	*0.008*	*1.7*	*2.6*	*2.3*	*0.71*	*9.8*	*9.8*	*2.7*	*16.9*	*5.0*

The sum of above and belowground biomasses (total biomass) after 45 days of treatment was similar to control (17.4 g) in both provenances (data not shown); total biomass was higher in SIM provenance (25.2 g) than in BAR (9.6 g; $p<0.001$). Moreover, SIM plants were characterised by a higher total biomass under flooding with fresh than under flooding with saline water ($p<0.01$). Although differences in SIM and BAR biomasses were found, the relative AGB growth rate (RGR) was similar in both provenances, being on average equal to 0.023 g g^{-1} day^{-1}.

Discussion

Gradual loss of photosynthetic activity is usually observed in ageing foliage (Ethier et al., 2006; Katahaka et al., 2007), and it is frequently associated to lower dark respiration rates (Urban et al., 2008). In this study, the photosynthetic capacity strongly decreased after 45 days from the beginning of the experiment. In fact, old leaves (OL45) showed lower A_{400}, g_s, Vc_{max}, J_{max} and F_0 values and lower dark respiration rates (R_d) compared to young leaves (YL0). However, these reductions were not induced by the treatments, as the loss of photosynthetic capacity also occurred under control conditions. The only treatment effect was observed on LMA values which were significantly higher under 45 days of flooding with saline water. This increase was probably due to leaf thickening, as a result of changes in mesophyll anatomy under salt stress (Delfine et al. 1998).

A high tolerance of both *Tamarix* provenances to continuous flooding with salt and fresh water was observed in this study. In fact, at the end of the experiment, all plants were still alive and the relative growth rate was similar to control in both treatments. Particularly, photosynthetic capacity and growth were not affected by flooding with fresh water in both provenances. Seedlings tolerance to flooding with fresh water was already observed in *Tamarix ramosissima* (Sprenger et al., 2001). High photosynthetic rates, maximum rate of carboxylation and electron transport were found in young leaves of both provenances, reaching values similar to those reported by Urban et al. (2008) in young *Populus alba* leaves of plants not subjected to any treatment. However, our photosynthetic rates values were lower than those measured by Horton et al. (2001) in *T. chinensis* under well watered conditions (35.4 µmol CO_2 m^{-2} s^{-1}), but comparable to those reported by Moore et al. (2008) in the same species. Stomatal closure is a common effect observed in shoots of flooding intolerant species (Pezeshki et al., 1996; Braendle and Crawford, 1999; Rengifo et al., 2005) and it is induced by the increase in phytohormones concentration (especially ABA) in response to anoxic root conditions (Kozlowski, 1997). However, in our study, as observed by (Pezeshki et al., 1996) in *Taxodium disticum*, flooding did not inhibit stomatal conductance. Moreover, g_s was higher in *Tamarix* spp. than in *Melaleuca cuticularis* and *Casuarina obesa*, two wetland trees, under flooding conditions (Carter et al., 2006). Alleviation of anoxic stress is usually achieved by aerenchyma and adventitious roots formation (Visser et al., 1995; Blom and Voesenek, 1996; Drew et al., 2000; Colmer et al., 2003; Evans et al., 2004). Although we did not investigate the timing of aerenchyma formation, adventitious roots development was observed after fourteen days from the beginning of the treatment, allowing the re-establishment of a contact between the air and the root apparatus, enabling stomatal opening and gas exchange. Consequently, aboveground biomass growth was not inhibited by the treatment in both provenances. On the other hand, belowground roots biomass was lower under flooding conditions, independently of water salinity. This effect may be the result of (1) increased roots mortality due to anoxic conditions

(Megonigal and Day 1992; Kozlowski 1997; van Bodegom et al. 2008), at least before adventitious roots formation (Batzli and Dawson 1997) (2) reduced root growth and increased C allocation to aboveground growth as a consequence of increasing water availability (thus, a decrease in root/shoot ratio, as observed in this study) (Tilman 1988; Kercher and Zedler, 2004) (3) plant strategy to reduce the respiratory demand during the replacement of the belowground roots by more efficient aerenchymatous roots (Batzli and Dawson 1997). Adventitious roots development was already observed by (Ginzburg, 1967) in *Tamarix aphylla* where roots developed in the lenticels, which underwent characteristic changes after branches submergence. However, Vandersande et al. (2001) showed that *T. ramosissima* did not develop a prolific adventitious rooting system after flooding with fresh water. Thus, this ability in *Tamarix* genus probably differs among species.

Continuous flooding with saline water was well tolerated by SIM provenance. Both SIM young and old leaves showed high photosynthetic capacity; all the analysed parameters were similar to control. Flooding with saline water was also well tolerated by old BAR leaves. High tolerance to flooding with saline water was already observed in species that occupy estuarine environments and is usually associated with the regulation of foliar sodium, chloride and potassium concentration (Carter et al., 2006). Differently, gas exchanges in young BAR leaves were strongly reduced after flooding with saline water. Moreover, a decline in maximum rate of carboxylation and maximum rate of electron transport were also observed. Lovelock and Ball (2002) reported that biochemical limitations can occur under saline stress as a result of the deactivation of RuBisCO, due to a reduced RuBP regeneration resulting from the accumulation of carbohydrates induced by the lower consumption of photosynthetic products for growth. Moreover, the reduction in maximum rate of electron transport was not accompanied by a reduction in fluorescence parameters, suggesting a down regulation of leaf photochemistry to match the reduction in C acquisition (Delfine et al., 1998) or an involvement of the xanthophyll cycle (non-photochemical quenching was lower in young FS SIM leaves ($p<0.1$), data not shown). A decrease in plant photosynthetic capacity and growth was reported in both glycophytes (Bongi and Loreto, 1989; Mc Load et al., 1999; Centritto et al., 2003; Loreto et al., 2003) and halophytes (Ueda et al., 2003; Parida et al., 2004; Nandy (Datta) et al., 2007). In most cases, the decline in the photosynthetic capacity is linked to a reduced stomatal conductance. In our study, no decline in stomatal conductance was observed under flooding with saline water. However, salt extrusion and deposition on leaf surface, which were higher in young BAR leaves under flooding with salt conditions, could play an important osmotic role in the leakage of leaf internal water through the salt glands, altering (increasing) the measured g_s values. Nevertheless, any difference in the ratio between photosynthetic rate and stomatal conductance was observed among treatments in both leaf types and provenances, suggesting a similar contribution of stomatal and non stomatal (salt glands) water loss in all provenances and leaf types (*see also chapter 4*). Moreover, Vc_{max} and J_{max} were linearly related which indicates a co-ordination between photochemistry and Calvin cycle to match each other, thus the absence of stomatal limitations (Centritto et al., 2003). The mean leaf nitrogen concentration measured in this study (1%) was lower than that reported by Horton et al (2001) (2.6%), by Busch and Smith (1995) and by Cleverly et al (1997). However, the photosynthetic reduction was not due to a decrease in nitrogen availability, being N leaf content similar to control, although N might be allocated in the production of compatible solutes, as reported in *Tamarix jordanis* (Jones et al., 2006), reducing N availability to photosynthesis. Net assimilation rate reduction was not associated to an increase in dark respiration, as also observed by Loreto et al. (2003) in salt stressed olive leaves, although leaf mass per area, and consequently leaf thickness, did increase significantly. We can conclude that flooding with saline water did not reduce stomatal conductance, allowing salt to be transported up to the leaves. Stomatal conductance was on average lower in old leaves, so that the additional salt accumulation in these leaves was much slower than the initial uptake into new leaves (Cram et al., 2002). Marcar et al. (2002) also reported that O_2 deficiency in the root zone leads to higher concentration of Na^+ and Cl^- in the foliage of plants under flooding with saline water conditions. In this study, roots Na content was higher in SIM provenance than in BAR, suggesting a greater translocation of Na to the rest of the plant in the latter provenance. A slightly higher leaf Na content was observed in BAR YL45, compared to SIM YL45. Moreover, the quantity of salt extruded by young BAR leaves was significantly higher than that extruded by young SIB leaves and comparable to the quantity extruded by old leaves of both provenances. As hypothesized by Delfine et al. (1999), it is possible that a threshold exists in leaf salt accumulation and that photosynthetic capacity (especially RuBisCO activity, see the reduction in Vc_{max}) is not inhibited only when salt accumulation is maintained under this threshold. An increase over this threshold might have occurred in young BAR leaves, obliging these to extrude a large quantity of salt with a subsequent increase in energetic costs and a consequent reduction in their photosynthetic capacity. However, leaf respiration had reached low values on day 45 from the beginning of the experiment, indicating that this stress already occurred during the previous days. Meyer and Boyer (1972) reported that salt stress can limit cellular expansion, which is more sensitive to low water potentials than cellular division. Smaller cells tightly arranged in hypersaline conditions (Sobrado, 1999) increases mesophyll density that is inversely correlated with the diffusion through the liquid phase (Syverten et al., 1995). Under salt stress, changes in mesophyll structure (Bongi and Loreto, 1989; Delfine et al., 1998) and a decrease in osmotic potential of the liquid phase (Delfine et al., 1999) might also occur. Thus, the photosynthetic capacity in young BAR leaves under flooding with saline water might have also been reduced by higher mesophyll resistance.

Conclusions

This study demonstrates that *Tamarix* seems to be a suitable genus for the restoration of environments which might be affected by flooding, where these species are already naturally distributed. However, more information is needed concerning salt tolerance variability among species, provenances and genotypes. In fact, young BAR leaves decreased their photosynthetic capacity under flooding with saline water conditions, probably as a result of the increase in leaf salt accumulation over a tolerated threshold. Further investigations are also required to find out how mesophyll conductance may change in response to flooding with saline water in *Tamarix* spp., to characterize leaf morphology under such conditions and to determine salt effects on water loss through salt glands and water conductance. Moreover, an analysis of plant responses to water level fluctuations is essential, especially considering estuaries and coastal habitats where the decrease of water level may lead to increasing salt concentration.

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Chapter 3

Photosynthetic and growth responses of *Tamarix* spp. to water level reduction after continuous flooding with fresh and saline water

Abstract

Salinity and flood frequency are important factors affecting plant growth and structure in estuarine environments. In Mediterranean countries, climate change effects and human activities may alter water level and flooding frequency, subjecting such habitats and their vegetation to different kind of stresses. Particularly for saline and coastal areas, water level decrease after flooding may lead to the accumulation of salt in the soil solution, which might cause severe damage to plants photosynthetic capacity and growth. Of course, plants tolerance under such conditions varies among species. *Tamarix* spp. constitute important populations along river streams, in dry and in coastal areas, and might therefore be primarily affected by climate change effects. For these reasons, a study was made to assess *Tamarix* tolerance to water level decrease by evapotranspiration after continuous flooding with fresh and saline water (200 mM).

Under saline conditions, the decrease of water level caused an increase in salt extrusion through salt glands. In plants which were previously flooded with fresh water, water level reduction significantly increased stomatal conductance, inducing higher rates of water reduction compared to plants flooded with saline water. Under saline conditions, water level decrease was lower, as salt significantly influenced both evaporation and transpiration processes. In fact, a decrease in stomatal conductance was observed as a probable consequence of (1) a direct salt effect on stomatal cell guards, which provoked stomatal closure inducing lower photosynthetic assimilation rates, (2) a drought-like induced stress, which caused the formation of smaller vessels, characterised by higher cavitation safety but lower water transport efficiency. The decrease in stomatal conductance coupled with photosystem damage, decreased the energy production through light reactions and, thus, reduced CO_2 carboxylation rates. Moreover, the possible accumulation of Na^+ in the cytoplasm due to a decrease in leaf salt extrusion, could have detrimentally affected the activity of RuBisCO, severely reducing plants photosynthetic performances. These results highlight *Tamarix* spp. survival risk in those habitats where, as a consequence of climate change effects or human activities, water level under saline conditions could be frequently altered.

Key words: *Tamarix* spp., water level decrease, saline water, fresh water, photosynthetic responses, wood anatomy

Introduction

Mediterranean countries are considered hot spots of climate change. In these regions, the predicted changes in precipitation, mean surface air temperature, and their inter-annual variability may be detrimental for entire ecosystems, especially wetlands and coastal areas, as they are particularly exposed to a range of hazards connected to climate change (IPCC, 2007; Torresan et al., 2008; Solomon et al., 2009). Particularly, estuarine ecosystems represent sensitive sites for plants existence as salinity and flooding, which frequently occur in such areas, can be important stressing factors for plants growth (Blom and Voesenek, 1996; Kozlowski, 1997; Mansour, 2000; Chen et al., 2005; Rengifo et al., 2005; Fernandez, 2006; Flowers and Colmer, 2008). Plants ability in maintaining their environment depends on a number of factors including the rate of sea level rise, tide range and flooding frequency (Reed 1995).

Salinity detrimental effects on plant growth concern with the decrease of water potential in the root medium, due to an osmotic effect, the toxicity caused by excessive Na^+ and Cl^- uptake and accumulation, and the nutrient ion imbalance, especially K^+, due to the disturbance of essential intracellular ion concentrations (Marschner, 1995; Mansour, 2000; Zhu, 2001). Of course, plant responses to these stresses also depend on their intrinsic tolerance and adaptability, and on the strength of the stressing factor. Moreover, the response may be different among species and, within the same species, among genotypes (Kozlowski, 1997). Some plants, called halophytes, are particularly capable of tolerating high salt concentrations and are thus able to complete their life cycle in environments where the salt concentration is around 200 mM NaCl or more (Flowers and Colmer, 2008). In these plants, some strategies are adopted in order to guarantee their osmotic adjustment, such as ions compartmentalisation in the vacuole, compatible solutes synthesis, extrusion of excess stress-inducing ions from salt glands (Waisel et al., 1986; Ramadan, 1998; Blumwald et al., 2000). These processes are energy consuming, consequently they divert most of the energy otherwise available for plant growth, although low growth rates may determine a low sodium transport strategy, thus an increased salt tolerance in some species (Tattini et al., 2002). Salinity also shares some similarities with drought stress such as the increasing risk of vessels cavitation; as a result, vessels should be adapted to reduce the impact of cavitation on sap flow by preventing gas expansion and by minimizing the loss of conductive area upon embolization (Schmitz et al., 2007). Under very high salinity, plants might not be able to reduce their ability to adjust osmotically, as a result of the saturation of solute uptake systems (Munns et al., 1983). In these cases, detrimental effects are observed, especially at stomatal level, in both glycophytes (non-tolerant) and halophytes (Bongi and Loreto, 1989; Delfine et al., 1999; Nandy (Datta) et al., 2007).

Many of the habitats occupied by terrestrial halophytes are not only saline, but are also prone to flooding (e.g. coastal mangroves and coastal and inland salt marshes) (Colmer and Flowers, 2008). Flooding reduces O_2 diffusion and supply to the roots (Blom and Voesenek, 1996; Kozlowski, 1997), decreasing water absorption and stomatal conductance, and causing sensitive plants to wilt in a similar way to drought (Jackson and Drew, 1984). Among the processes generated by soil submergence, the one that most limits plant growth is the lack of oxygen. According to Blom and Voesenek (1996), the immediate consequence of a lack of oxygen in higher plants, flooding tolerant or not, is the decrease of roots aerobic respiration and, consequently, of ATP generation. This causes a decrease in the energy available for root growth and, consequently, a reduction in the vegetative growth (Liao and Lin, 2001). Flooding can give rise to detrimental effects also 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). As two third of the earth's land mass is flooded at least occasionally, many species have developed different strategies to survive hypoxia (Schulze et al., 2005). These species are capable of producing, in response to an increase in the concentration of ethylene and auxin, numerous adventitious roots characterised by a highly developed aerenchyma (Blom and Voesenek, 1996). These roots usually grow on the base of the shoot, on the upper part of the tap root and on stem nodes, mostly exploring the upper better aerated soil layers (Blom and Voesenek, 1996) or floating on water surface (Kuzovkina et al., 2004). The re-establishment of a contact between the root apparatus and the air enables stomatal opening and, thus, leaf gas exchange and growth. Together with waterlogging, salinity can cause severe damage to plants (Barrett-Lennard, 2003), assembling harmful effects of both stresses. In fact, besides the stress induced by salinity and flooding as single factors, it has been demonstrated that salinity compromises flood tolerance mechanisms (Salter et al., 2010), preventing 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). However, 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).

Water level reduction after fresh water flooding, as a result of water evapotranspiration and/or drainage, may bring to the re-establishment of the initial soil water condition; contrarily, in the case of saline water flooding, water height decrease implies an accumulation of salts in the soil, with a resulting damage to the vegetation occupying such habitats.

In the Mediterranean basin, riparian and coastal vegetations have developed stress-adapted mechanisms to overcome changes in temperatures, water availability and salinity, and are likely to be well adapted to future conditions caused by the global warming effects (Thuiller et al., 2005). *Tamarix* genus includes about 90 species, which show a high adaptability to different environments and a high endurance of adversity (Ginzburg, 1967; Bar-Nun and Poljakoff-Mayber, 1974; Brotherson and Field, 1987; Cleverly et al., 1997; Di Tomaso, 1998; Glenn et al., 1998; Horton et al., 2001; Tallent-Halsell and Walker, 2002; Zhang et al., 2002; Gries et al., 2003; Xu and Li, 2006). In the Mediterranean Basin, *Tamarix* spp. inhabit coastal and riparian areas and salt marshes. As these habitats may be severely damaged by climate change effects, a study was made to assess their tolerance to water level decrease after 45 days of flooding with fresh and saline water (200 mM).

Our hypothesis were that plants submerged with fresh water would recover from the treatment, being their final water condition comparable to the control, while plants grown under submergence with saline water would suffer from the accumulation of NaCl in the soil solution after water evapotranspiration. Thus, the objectives of this work were the identification of the physiological processes sensitive to the treatments, by analysing (1) leaf photosynthetic performances, (2) leaf salt extrusion and (3) wood anatomical variations in response to water level reduction under saline and non-saline conditions.

Material and Methods

Four *Tamarix* spp. genotypes were collected in November 2008 from Southern Italy and were replicated four times by cuttings, which were planted in 1.6 dm^3 pots (10.5×10.5×22 cm) containing sand (35%) and loamy soil (65%).

Cuttings were grown in a greenhouse for six weeks and then were transplanted in 2.6 dm^3 pots (17.5×17.5×25 cm) containing the same soil mixture. Plants were grown for two months in a growth-chamber under a photosynthetic photon flux density (PPFD) of approximately 550 $\mu mol\ m^{-2}\ s^{-1}$, 60% relative humidity, a photoperiod of twelve hours and a day/night temperature of 25/15 °C. The pots were watered three times a week with fresh water and were then divided into three groups. The plants belonging to each group were inserted into plastic boxes (60×40×40 cm). Three treatments were provided to each group: (1) daily irrigation with potable tap water and Hoagland solution (0.5× concentration, once a week) (Control - C), (2) flooding with fresh water (F) and (3) flooding with fresh water added with NaCl 200 mM (FS). Flooding levels were maintained 5 cm above soil surface and kept constant for 44 days by pouring respectively fresh water (F) and saline water (FS), and a modified Hoagland solution (0.5× concentration) in the boxes once a week. After 14 days of continuous flooding, adventitious roots were formed at the shoots base of all plants grown under flooding conditions, independently of water salinity (*see below*). After 44 days, no more water was added to the F and FS treatments and then the water level in each box decreased till poor water (2-5 cm height) was observed in the bottom of the boxes (day 89).

Environmental variables

Water salinity was measured using a conductimeter (HI9811, Hanna instruments INC, USA) equipped with an electrode probe (HI1285, Hanna instruments INC, USA), were monitored from the beginning of the experiment on days 3, 18, 33, 44, 60, 73 and 89 under both F and FS treatments. On the same days, the water level was also recorded.

Gas exchange and chlorophyll fluorescence measurements

Leaf gas exchanges were measured on the tenth leaf from the apical bud on days 44, 60, 73 and 89 after the onset of the experiment, in order to follow the dynamic of leaf functioning in response to the water decrease. All leaves were selected from the dominant sprout. Gas exchanges were measured using a portable infrared gas analyser (LI-6400, LI-COR Biosciences, Inc., Lincoln, NE) equipped with a conifer chamber (LI-6400-05). The air flow was set at 400 $\mu mol\ m^{-2}\ s^{-1}$ and the cuvette temperature at 25 °C. An artificial light was generated by a halogen incandescent lamp and transmitted to the cuvette by a fibre-optic (FL-400 with 400-F; Walz, Germany). The light brought a saturating photon flux (1800-2000 $\mu mol\ m^{-2}s^{-1}$). Net assimilation rate (A) versus intercellular CO_2 concentration (C_i) was determined for ambient CO_2 concentration values (C_a) of 400, 300, 250, 200, 150, 50, 400, 500, 650, 750, 850, 1000 $\mu mol\ mol^{-1}$ as suggested by Long and Bernacchi (2003); the assimilation values were recorded as soon as C_a was stable (cv<0.7%) (Ainsworth et al., 2002). Net assimilation rates (A_{400}) and leaf conductance (gl_{400}) measured at C_a=400 $\mu mol\ mol^{-1}$ were considered as the assimilation and the conductance at the ambient (growth chamber) CO_2 concentration. Intrinsic water use efficiency (A/gl) was calculated as the ratio between A_{400} and gl_{400}. Maximum carboxylation rate (Vc_{max}) and maximum rate of electron transport (J_{max}) were also estimated according to Farquhar et al. (1980). Dark respiration (Rd_{400}) was measured at ambient C_a (400 $\mu mol\ mol^{-1}$), after 5 minutes of adaptation. After gas exchange measurements, leaves were cut off and fresh weight was determined. Leaves were then scanned, and their images analysed with the software Skyroot (Llandrindod Wells, Powys, UK) in order to obtain the total leaf length. Leaf area was estimated by multiplying the total leaf length by the mean leaf diameter (0.7 mm). A small leaf portion was dried at 70 °C for dry weight estimation while the rest of the leaf was dark-adapted for 15 minutes for chlorophyll fluorescence measurements.

Chlorophyll fluorescence was measured on the same leaves used for gas exchange measurements with a PAM 2000 fluorimeter (Heinz Walz, Effeltrich, Germany). Photochemical efficiency was estimated by measuring the quantum yield of PSII in dark adapted leaves (F_v/F_m).

Leaf characteristics

After drying, leaves were inserted into a plastic tube added with 25 ml of deionised water in order to estimate the electrical conductivity of the liquid phase, which is proportional to the amount of the salt extruded by salt glands and accumulated on the leaf surface. The tubes were shaken at 500 rpm for 20 minutes. The liquid phase was then separated from the leaves with filter paper. Leaf dry weight without salt was determined after drying in oven at 70 °C until constant weight. Electrical conductivity of the liquid phase was measured using a conductimeter (HI9811, Hanna instruments INC, USA) equipped with an electrode probe (HI1285, Hanna instruments INC, USA). Electrical conductivity values were converted in order to obtain the amount of salt accumulated outside the leaf, which was expressed as g NaCl g^{-1} leaf dry weight.

Secondary growth analysis

At the end of the experiment, the plants were cut and wood samples were taken from the base of the dominant shoot of each plant. Transverse wood microsections were made with a GLS1 microtome (Schenkung Dapples, Zürich, Switzerland). The thickness of the sections varied between 30 and 50 µm. According to Schweingruber et al. (2008), wood cross sections were stained with safranine and astra-blue mixed in equal proportions, then dehydrated with alcohol at 50, 75, 95 and 100% and embedded in Canada balsam. The sections were observed with a magnification of 50× and 100× using a microscope (Leica DM4000B, Leica Microsystems AG, Wetzlar, Germany) connected to a digital camera (Leica DFC420, Leica Microsystems AG, Wetzlar, Germany). Photographs of two radii were taken from each section and adjusted by the software Leica Application Suite (LAS, Leica Microsystems AG, Wetzlar, Germany). Image analysis was performed using an image analysis tool (IMAGEJ, National Institute of Health, Bethesda MD) that automatically recognizes and measures vessels. Images were examined in order to analyse vessels distribution along the radius; five 200×200 µm areas were selected from three parts of each of the two radii per plant: (1) a wood portion located near the pith (Pith), which corresponded to plant secondary growth before the beginning of the submergence, (2) a second wood portion located in the middle of the radius (Centre), representing the period under continuous flooding conditions and (3) a third area near the bark (Bark), representing wood growth under water level reduction. Inside each area, the mean vessel area (MVA) and the total vessel area (TVA) of vessels >200 μm^2 were measured.

Statistical analysis

Analysis of variance (ANOVA) was performed on the environmental variables and all the photosynthetic and leaf parameters using the statistical software Systat 12.0 (SPSS Inc.) to evaluate the main effects of the treatments for each date of investigation. Post hoc was performed using Fisher's LSD test. Repeated ANOVA was performed for each treatment in order to analyse the variation of the environmental variables (salinity and water height) and photosynthetic and leaf responses over time, using the statistical software Systat 12.0 (SPSS Inc.). The post hoc was performed using Bonferroni's test.

The software Prism 4 (GraphPad software, Inc) was used to test the correlations between Vc_{max} and J_{max}. As after 30 days from the beginning of the treatments one *Tamarix* plant grown under FS conditions died, the number of replicates was 3 for FS. Under C and F, statistical analysis was made on 4 replicates. Significance was considered for *p* values <0.1.

Results

Water level variation and its salinity

Water level and salinity variations under (from day 0 to day 44) and after (from day 44 to day 89) flooding (F) and flooding with saline water (FS) conditions are shown in Fig. 1.

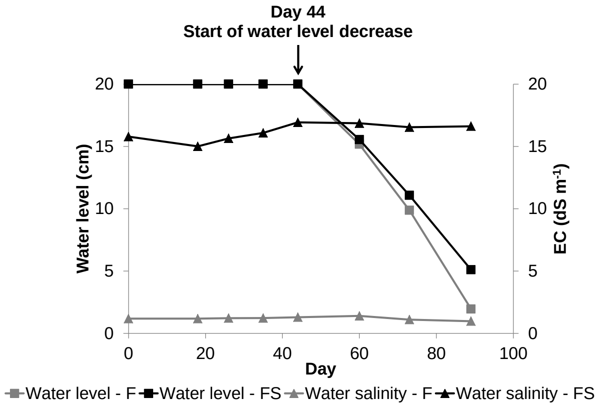

Fig. 1 Water level and salinity dynamics under flooding (F) and flooding with saline water (FS) conditions (from day 0 to day 44) and during water level decrease (from day 44 to day 89) in both treatments. The values are means ± s.e. (n = 4 under F; n = 3 under FS).

Water level significantly decreased over time ($p<0.001$) after no more water was added to the boxes, in both F and FS treatments. In fact, water level was significantly higher during the first 44 days of continuous flooding (on average 20 cm height) compared to days 60 (15.4 cm; $p<0.001$), 73 (10.5 cm; $p<0.001$) and 89 (3.5 cm; $p<0.001$). Fresh water and saline water levels did not decrease at the same rate: water level under FS was significantly higher on days 73 (11.1 cm; $p<0.001$) and 89 (5.1 cm; $p<0.001$) compared to water level under F conditions (9.9 and 2.0 cm respectively).

Furthermore, water salinity changed over time ($p<0.05$ and $p<0.001$ respectively under flooding with fresh and saline water). Under F conditions, a slight increase in water salinity was observed until day 60, when water conductivity was equal to 1.4 dS m^{-1}. Then, salinity decreased reaching values similar to those observed at the beginning of the experiment (1.1 dS m^{-1}). Under FS, salinity increased significantly till day 44, then remained constant during water level decrease, reaching values of 16.6 dS m^{-1}. Water salinity was always significantly higher under flooding with saline water than under flooding with fresh water conditions.

Leaf photosynthetic activity

Leaf photosynthetic performances are shown in Tab. 1. Net assimilation rates measured at a CO_2 concentration of 400 μmol mol^{-1} (A_{400}) were similar in all the treatments on days 44, 60 and 73. However, a significant decrease of A_{400} (-52.2%; $p<0.05$) was observed on day 89 in plants that were flooded with saline water (FS), while in plants that were flooded with fresh water (F), A_{400} did not decrease compared to control (Tab. 1). Furthermore, A_{400} significantly changed over time under FS, being intermediate on day 44, and higher on days 60 ($p<0.05$) and 73 ($p<0.1$) compared to day 89. A_{400} did not change over time in plants under F. The maximum rate of carboxylation (Vc_{max}) was lower under FS than under control on days 44 and 89 ($p<0.1$ and $p<0.05$ respectively) (Tab. 1), whereas on days 60 and 73, no variations of Vc_{max} were observed among the treatments. Vc_{max} did not change over time under both treatments and was linearly correlated with maximum rates of electron transport, independently of the treatment (Fig. 2).

Tab. 1 Net assimilation rates (A_{400}), maximum carboxylation rate (Vc_{max}), dark respiration (Rd_{400}), stomatal conductance (gl_{400}), intrinsic water use efficiency (A/gl), quantum yield of PSII in the dark (F_v/F_m) measured in the four dates after water level decrease on the tenth leaf from the apical bud. Lowercase letters refer to the comparison among the treatments. Capital letters refer to the comparison among dates. The values are means ± s.e. (n = 4 under C and F; n = 3 under FS).

				DAY					
		44		**60**		**73**		**89**	
		Mean	s.e.	Mean	s.e.	Mean	s.e.	Mean	s.e.
A_{400}	**C**	19.8	1.2	19.7	1.3	18.7	2.4	**a** 20.7	4.1
(μmol CO_2 m^{-2} s^{-1})	**F**	16.7	3.7	22.0	1.2	24.7	6.3	**ab** 14.5	1.6
	FS	**AB** 13.6	1.5	**A** 19.8	2.5	**A** 20.0	2.4	**B b** 9.9	2.5
Vc_{max}	**C**	**a** 93.9	6.0	116.5	12.0	104.7	8.4	**a** 110.1	19.3
(μmol CO_2 m^{-2} s^{-1})	**F**	**ab** 90.1	12.2	109.7	11.4	112.1	28.6	**ab** 77.1	4.3
	FS	**b** 68.6	4.7	87.9	28.3	99.3	16.8	**b** 67.5	5.6
Rd_{400}	**C**	2.0	0.5	3.3	0.7	2.7	0.5	2.5	0.6
(μmol CO_2 m^{-2} s^{-1})	**F**	4.0	1.0	3.4	0.8	2.4	0.8	2.3	0.5
	FS	**A** 2.7	1.5	**AB** 2.8	0.7	**AB** 3.6	0.5	**B** 2.4	0.0
E_{400}	**C**	5.7	1.1	**a** 3.9	0.4	**ab** 4.2	0.3	4.4	1.4
(mmol H_2O m^{-2} s^{-1})	**F**	5.0	1.3	**b** 6.0	0.3	**a** 7.7	2.6	3.9	0.9
	FS	5.8	2.4	**ab** 4.6	1.3	**b** 4.9	1.2	1.7	0.5
gl_{400}	**C**	0.39	0.03	**a** 0.31	0.04	**ab** 0.37	0.07	**ab** 0.36	0.11
(mol H_2O m^{-2} s^{-1})	**F**	**A** 0.42	0.14	**A b** 0.49	0.04	**AB a** 0.64	0.18	**B a** 0.28	0.07
	FS	0.23	0.04	**c** 0.14	0.09	**b** 0.29	0.05	**b** 0.09	0.03
A/gl	**C**	49.3	6.0	57.3	7.6	**a** 51.8	3.7	63.5	12.4
(μmol CO_2/mol H_2O)	**F**	**AB** 47.0	7.8	**AB** 56.6	6.9	**A b** 39.3	1.9	**B** 83.8	13.0
	FS	61.8	6.3	60.5	14.3	**c** 71.1	6.3	86.6	29.5
	C	0.78	0.03	0.80	0.01	**ab** 0.82	0.02	**a** 0.79	0.01
Fv/Fm	**F**	0.80	0.02	0.82	0.02	**a** 0.84	0.01	**a** 0.80	0.03
	FS	**AB** 0.80	0.01	**A** 0.81	0.00	**AB b** 0.80	0.01	**B b** 0.75	0.01

Dark respiration (Rd_{400}) was on average equal to 3.1 µmol CO_2 m^{-2} s^{-1} (Tab. 1). While no variations among the treatments and over time were observed in Rd_{400} under F treatments, a lower dark respiration was observed on day 89 in plants which were flooded with saline water. Transpiration rates (E_{400}) were similar on day 44 in all treatments (Tab. 1). E_{400} measured in plants grown under F was 35.2% higher on day 60 ($p<0.05$) compared to the control. On day 73, E_{400} was significantly higher under F than under FS (+36.3%; $p<0.1$). Transpiration rates did not vary over time in all treatments. Under F treatment, gl_{400} significantly decreased with decreasing water level ($p<0.1$). Intrinsic water use efficiency (A/gl) was similar in all treatments on days 44, 60 and 89, while after 29 days from the beginning of water level decrease (day 73), A/gl was significantly higher under FS treatment than under control ($p<0.05$) and under F ($p<0.01$) (Tab. 1), A/gl being also significantly higher under control than under F ($p<0.1$). Intrinsic water use efficiency was significantly higher after 45 days of water level decrease than after 29 days (days 89 and 73 respectively; $p<0.05$). Quantum yield of PSII measured in the dark (F_v/F_m) was similar under all treatments on days 44 and 60, while it significantly decreased under FS conditions in the last two dates of investigation

(Tab. 1). On day 73, F_v/F_m significantly differ from F treatment ($p<0.1$) while on day 89, it significantly decreased compared to both control and F conditions ($p<0.1$ in both cases).

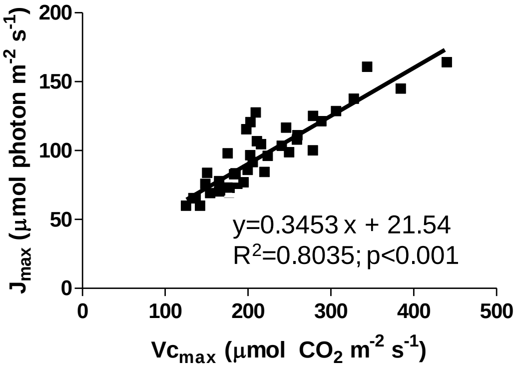

Fig. 2 Correlation between maximum carboxylation rate (Vc_{max}) and maximum rate of electron transport (J_{max}) obtained from net assimilation rate (A) versus intercellular CO_2 concentration (C_i) curves measured on day 44, 60, 73 and 89, under control, F and FS. Equation, R^2 and p value are referred to the global fit.

Salt accumulation outside the leaf

The salt accumulation dynamic is shown on Fig. 3.

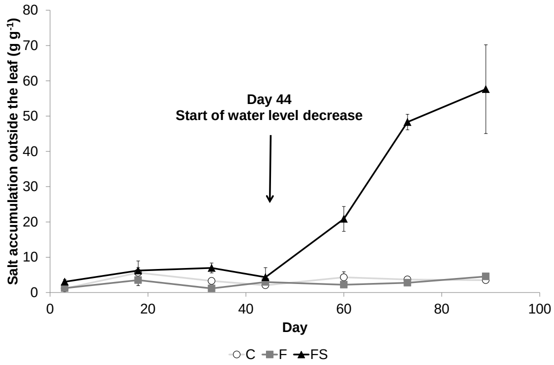

Fig. 3 Salt accumulation outside the leaf under flooding (F) and flooding with saline water (FS) conditions and during water level decrease in all treatments. The values are means ± s.e. (n = 4 under C and F; n = 3 under FS).

With the exception of days 18 and 44, when no significant differences were observed concerning the quantity of salt accumulated outside the leaf in the different treatments, a higher amount of salt was observed on days 3, 33, 60, 73, 89 in plants grown under FS compared to plants grown under control ($p<0.05$ for days 3 and 33, $p<0.001$ for all the other dates) and F conditions ($p<0.1$ for day 3, $p<0.01$ for day 33, $p<0.001$ for all the other dates). Salt accumulation measured on days 3, 18, 33, 44 (on average equal to 5.15 g NaCl g^{-1} leaf) under FS was significantly different from that measured on days 73 (48.3 g NaCl g^{-1} leaf; $p<0.05$ in all cases) and 89 (57.6 g NaCl g^{-1} leaf; $p<0.01$, in all cases) under the same treatment. Moreover, under FS conditions, the quantity of salt accumulated outside the leaf after 45 days from the beginning of water level reduction (day 89) was significantly higher than that measured on day 60 (20.9 g NaCl g^{-1} leaf). Under the same treatment, salt accumulation rate was not constant along the period of investigation. Particularly, it seemed to be higher after the beginning of water level decrease (higher slopes of the lines connecting day 44 to day 60 values, and day 60 to day 73 values), while the rate decreased from day 73 to day 89, although it was higher than those observed before day 44 (Fig. 3).

Wood anatomical characteristics

The observation of wood microsections revealed the presence of a band of tiny cells near the pith of all plants that were flooded with both fresh and saline water (Fig. 4).

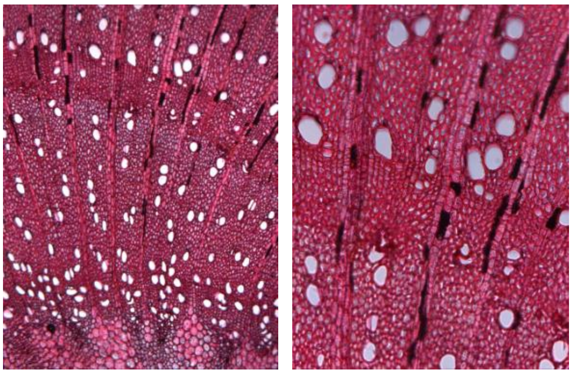

Fig. 4 Wood transverse sections at a magnification of (a) 50× and (b) 100× of a plant grown under F conditions. A band of smaller cells has been produced near the pith as an immediate response to flooding.

These bands were never observed under control conditions. Mean vessel area (MVA) significantly differed among treatments. Particularly, while no variations were observed in the wood portions located near the pith and at the centre of the section, MVA was significantly lower near the bark of plants grown under FS conditions than those grown under control (-51.3%; $p<0.01$) (Fig. 5). Accordingly, total vessel area (TVA) was lower in the wood portion located near the bark of plants grown under FS conditions compared to those grown under control (-55.1%; $p<0.01$). Moreover, in plants grown under FS conditions, a significant decrease of TVA was observed in the wood portion close to the bark, compared to that measured near the pith ($p<0.05$) and at the centre of the wood section ($p<0.01$) (Fig. 5).

Discussion

Water level reduction and water salinity

Water level significantly decreased after no more water was added to the boxes, independently of water salinity. However, a higher rate of water level reduction was observed under flooding with fresh water conditions, indicating an effect of salt on evapotranspiration processes. The presence of salt in the solution may affect both evaporation and transpiration processes. In fact, while evaporation from a freshwater reservoir directly depends on its temperature, evaporation from a saltwater reservoir is also affected by the presence of electrolytes, which have been demonstrated to reduce evaporation rates (Panin and Brezgunov, 2007). The effect of salt presence in the solution not only affected evaporation, as transpiration rates also decreased, particularly on day 73 after the beginning of the experiment. Moreover, stomatal conductance was also reduced under flooding with saline water conditions (days 60, 73 and 89) (*see below*), hence inducing a decline in water loss through stomata. On the contrary, stomatal conductance and transpiration rates were higher under flooding with fresh water conditions, which could explain the lower water levels measured on days 73 and 89 under non-saline conditions compared to the saline treatment. Under control and flooding with fresh water conditions, stomatal conductance was similar to the value reported by Horton et al. (2001) in *Tamarix chinensis* grown in the Sonoran desert.

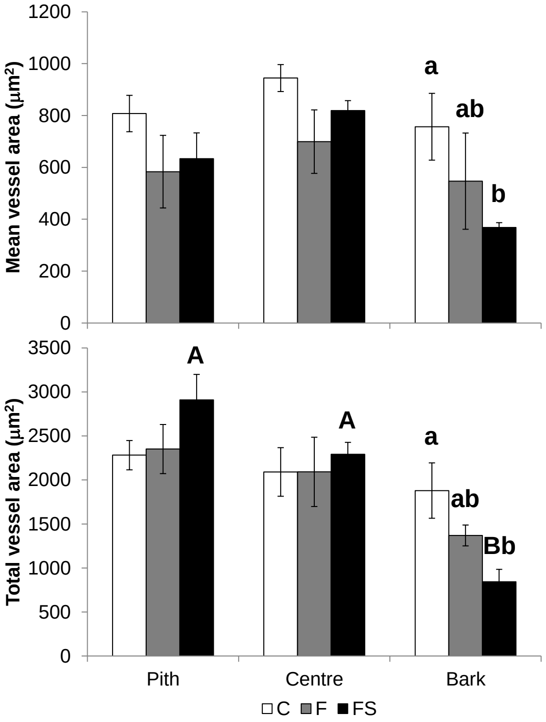

Fig. 5 Mean vessel area (a) and total vessel area (b) measured under control, F and FS in the wood portions (near the pith, on the centre of the microsection, near the bark). Lowercase letters refer to the comparison among the treatments. Capital letters refer to the comparison among wood portions. The values are means ± s.e. (n=4).

Under flooding with saline water conditions, water salinity remained constant after the beginning of water level decrease. According to our estimates, the quantity of salt present in the volume of water on day 44 was on average equal to 303 g. During the following days of investigation, the quantity of salt present in the respective volumes of water was equal to 234 g, 164 g, and 76 g, respectively for days 60, 73 and 89. Thus, at the end of the experiment, a missing sink of 227 g of salt was found. The quantity of salt accumulated outside the leaves of *Tamarix* plants grown under flooding with saline water conditions was equal to 56.7 g per g of leaf biomass. At the end of the experiment, leaf biomass was on average equal to 4.3 g under flooding with saline water conditions (data not shown). According to our estimates, the quantity of salt extruded by this leaf biomass was equal to 204 g, which is similar to the quantity of our salt missing sink. These results bring us to two conclusions: (1) *Tamarix* spp. are able to absorb, translocate and extrude large amounts of salt, and may then contribute to the desalinization of areas affected by such stress; (2) the salt absorbed by roots is not stored in other plants organs but is almost all extruded by leaf salt glands and accumulated on leaf surface.

Salt extrusion significantly increased after the beginning of water level reduction. Under flooding with saline water, reduced extrusion was probably due to the presence of adventitious roots (*see chapter 2*), which may exclude Na^+ (Saqib et al, 2005), or to adventitious roots potential as reservoir for this ion, as already observed by Boursier and Läuchli (1990), reducing the quantity of salt present in photosynthetic organs.

Tamarix responses to fresh water level decrease

Stomatal closure, induced by the increase in phytohormones concentration (especially ABA) in response to anoxic root conditions (Kozlowski, 1997), is a common effect observed in shoots of flooding intolerant species (Pezeshki et al., 1996; Braendle and Crawford, 1999; Rengifo et al., 2005). After 44 days of continuous soil flooding with fresh water, no decrease of stomatal conductance was observed. Alleviation of anoxic stress is usually achieved by aerenchyma and adventitious roots formation (Visser et al., 1995; Blom and Voesenek, 1996; Drew et al., 2000; Colmer, 2003; Evans, 2004). Although the formation of aerenchyma was not investigated, adventitious roots development was observed after fourteen days from the beginning of the treatment (*see chapter 2*), allowing the re-establishment of a contact between the air and the root apparatus, enabling stomatal opening and gas exchanges. Adventitious roots were formed at the base of the shoots, floating on water surface. Thus, their ability to absorb water decreased simultaneously with water level decrease. On day 60, when water level was reduced by 5 cm compared to day 44, adventitious roots were no more able to achieve their function. Thus, water absorption during water level decrease was probably carried out by belowground roots. An increase in stomatal conductance and transpiration rates was observed on days 60 and 73, indicating higher water availability probably due to an increased water transport through belowground roots and to a beneficial effect of the increased presence of oxygen in the soil after water level decrease. On day 89, stomatal conductance significantly decreased and water use efficiency increased compared to the previous days, although they were not different from the controls. The decrease in stomatal conductance may be a consequence of a lower water availability in soil surface layers and an insufficient root uptake capacity which might have caused a drought-like stress.

Tamarix responses to saline water level decrease

Stomatal conductance significantly decreased after 15 days (day 60) from the beginning of saline water level reduction. This decrease did not cause any variation in the assimilation rates, as the maximum rate of carboxylation increased compared to day 44 from the beginning of the experiment. Stomatal conductance also decreased on day 73 compared to non-saline flooding, and on day 89 compared to control conditions. On day 89, gas exchanges were strongly reduced and a decrease in the photosynthetic rates was observed at the end of the experiment. Moreover, a decline in the maximum rate of carboxylation was also observed and was coupled with a reduction in electron transport, as these two parameters were linearly related. A decrease in plant photosynthetic capacity and growth was reported in both glycophytes (Bongi and Loreto, 1989; Mc Leod et al., 1999 Centritto et al., 2003; Loreto et al., 2003) and halophytes (Ueda et al., 2003;

Parida et al., 2004; Nandy (Datta) et al., 2007). In most cases, the decline in the photosynthetic capacity is linked to a reduced stomatal conductance. Particularly, Lovelock and Ball (2002) reported that a control of stomata could be the result of feed-back controls in response to the accumulation of intermediates or products of the Calvin cycle that accumulate because of reduced growth under saline conditions. Moreover, once no more flooded, belowground roots may have been more efficient in ions absorption compared to adventitious roots (Saqib et al., 2005), inducing a higher salt assimilation inside the plant. In *Tamarix* spp., both the symplast and the apoplast routes are available for salt transport towards the glands (Thomson and Liu, 1967). Thus, a higher Na^+ cytosolic concentration may probably have activated a cell guard signal transduction pathway, as observed by Véry et al. (1998) in halophytic *Aster* spp.. According to these authors, an increase in the cytosolic concentration of Ca^{2+} is part of this Na^+ signalling pathway, acting in a similar way to an increase in abscisic acid and leading to the inactivation of inward rectifying K^+ channels and, thus, to stomatal closure (Zhu, 2001). Moreover, stomatal conductance may also decrease as a result of the physical obstruction of stomata by salt particles accumulation (*see chapter 4*). The decrease in maximum rate of electron transport was also accompanied by a reduction in fluorescence parameters. Particularly, quantum yield of PSII measured in the dark significantly decreased on day 73 and 89, indicating a lower proportion of effectively functioning PSII units compared to control and to plants grown under flooding with fresh water conditions. A decrease in the quantum yield of PSII has already been observed under saline conditions, and it was usually associated to photosystems damage induced by an excess of energy which overexcites the reaction centres, leading to the generation of activated oxygen species (Foyer and Noctor, 2005, Müller et al., 2001). Maximum rates of carboxylation decrease may be due to the direct effect of salt on the activity of RuBisCO (Bongi and Loreto, 1989) or to the decreased energy production during the light reactions and, thus, to a lower energy availability for RuBP regeneration. Net assimilation rate reduction was not associated to an increase in dark respiration as it significantly decreased on day 89 with respect to the other dates of measuring. Moreover, the rate of salt accumulation outside the leaf also decreased (from day 73 to day 89). This decrease may be (1) the consequence of the reduced stomatal conductance, thus, to the reduced water and salt transport to the leaf, and (2) the cause of an increase in intercellular concentration of Na^+ (less salt extruded, thus more salt retained into the leaf), which might have triggered photosystems, RuBisCO and cell guards damages, inducing the decrease in net assimilation rates. In fact, as hypothesized by Delfine et al. (1999), it is possible that a threshold exists in leaf salt accumulation, and that photosynthetic capacity (RuBisCO activity and photosystems integrity) is not inhibited only when salt accumulation is maintained under this threshold.

Meyer and Boyer (1972) reported that salt stress can limit cellular expansion, which is more sensitive to low water potentials than cellular division. Moreover, excess salinity causes osmotic stress and thus shared some similarities with drought stress, as the increasing risk of vessels cavitation. As a result, vessel characters should be adapted to reduce the impact of cavitation on sap flow by preventing gas expansion and by minimizing the loss of conductive area upon embolism (Schmitz et al., 2007). Save hydraulic structures concern with the construction of vessels characterised by a small diameter (Junghans et al., 2006; Sobrado, 2007), a small wall area and thus, a relatively low pit area per vessel (Hacke et al., 2006). In fact, as the cavitation resistance is determined by the maximum and not by the average pore diameter of the pit membrane, the larger is the pit area, the bigger is the chance on an exceptional large pore increasing cavitation vulnerability (Hacke et al., 2006). Thus, the decrease in mean vessel area and total vessel area in the wood portion formed during saline water level decrease may be the result of salt or (salt induced) drought stress and wood structure responses to increasing risk of cavitation. Although these vessels are characterised by a higher cavitation safety, they are also less efficient in water transport (Sperry et al., 2006). Consequently, the observed stomatal closure may also be the result of an increased hydraulic resistance rather than the response to a direct effect of the increased Na^+ cytosolic concentration.

Conclusions

Water level decrease effects on the photosynthetic activity and on wood anatomy after flooding with fresh and saline water were analysed in *Tamarix* spp.. Under saline conditions, the decrease of water level caused an increase in salt extrusion through the leaf salt glands. Thus, the use of *Tamarix* spp. for the desalinisation of saline areas by restoration ecology's projects could be more rapid under non-flooded conditions, as flooding significantly reduced salt extrusion through salt glands. In plants which were previously flooded with fresh water, water level decrease caused an increase in stomatal conductance, inducing higher rates of water reduction compared to plants flooded with saline water. This result suggests a positive role of *Tamarix* spp. in the restoration of areas affected by flooding. Under saline conditions, water level decrease was lower, as salt significantly influenced both evaporation and transpiration processes. In fact, although *Tamarix* spp. tolerated flooding with saline water (*also see chapter 2*), a decrease in saline water level caused a reduced stomatal conductance due to (1) a direct salt effect on stomatal cell guards, which provoked stomatal closure inducing lower photosynthetic assimilation rates, (2) a drought induced stress, which also caused the formation of smaller vessels, characterised by a high cavitation safety, but a lower water transport efficiency. The decrease in stomatal conductance coupled with photosystems damage caused by salinity, decreased energy production through light reactions and, thus, decreased CO_2 carboxylation rates. Moreover, Na^+ may also have negatively affected the activity of RuBisCO, severely reducing plants photosynthetic performances. These results highlight *Tamarix* spp. survival risk in estuarine and coastal areas where climate change effects or human activities may provoke an alteration of the water levels.

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Chapter 4

Salinity effects on stomatal and salt glands functioning in *Tamarix gallica* and *T. africana*

Abstract

Salinity affects 397 million of ha worldwide and it is recognised as one of the main causes leading to desertification in arid and semiarid lands. Under high saline concentrations, plants undergo an osmotic stress and a toxic effect due to the accumulation of salt within the plant. Halophytes are well adapted to survive under high saline conditions. One of the adaptations they have developed is salt extrusion through leaf salt glands, although these structures are believe to contribute to water loss, increasing the risk of plants dehydration. As other secreting species, *Tamarix gallica* and *T. africana* also extrude salts from leaf salt gland. In this work, we hypothesised that the presence of salt glands may increase leaf water losses during salt extrusion. Moreover, a short term photosynthetic response to increasing salinity was analysed in detached shoots of the two species.

Increasing salinity significantly decreased leaf gas exchange in both *Tamarix* spp.. Photosynthetic rates in *T. gallica* only significantly decreased under a salt concentration of 400 mM, while net CO_2 assimilation was already reduced at 100 mM in *T. africana*. The higher tolerance observed in *T. gallica* was probably due to its capacity to extrude more salt at increasing NaCl concentration. The observed photosynthetic reductions were the result first of non stomatal limitations and, consequently of a reduced stomatal conductance. Particularly in *T. gallica*, salinity first decreased maximum rates of carboxylation, while stomatal conductance only decreased under 400 mM. Differently in *T. africana* stomatal conductance was not affected by salinity. Both species were characterised by a high dark transpiration compared to light conditions. This water loss occurred through opened or partially opened stomata. Transpiration rates did not increase in response to salinity. Moreover, stomatal conductance measured in the light and in the dark decreased with increasing leaf salt extrusion as a consequence of salt hygroscopic effect on water vapour losses through stomata or of the physical obstruction of stomata by salt particles.

Key words: *Tamarix gallica*, *Tamarix africana*, salinity, stomatal conductance, transpiration, salt extrusion

Introduction

Saline environments are defined as those lands whose soil contains high percentages of soluble salts, and where one or more of these is normally in excess compared to the others (Poljakoff-Mayber and Lerner, 1994). Soil salinization is the result of two distinct processes: a natural geological process and a human-induced process. Geo-historically originated saline soils are situated along the seacoast, at lakesides, or in dry lands that were previously under marine or lacustrine conditions, while man-made saline soils are mostly found in (semi) arid lands, and are the result of salt accumulation after irrigation (Oosterbaan, 2003). Based on the FAO (1997) Soil Map of the World, the total area of saline soils is 397 million ha while sodic soils cover 434 million ha representing, as a whole, more than 6% of the world's total land area (Munns and Tester, 2008).

For plants living in saline environments, the soil Na^+ concentration can range from 100 to 1380 mM Na (Flowers, 1985). Under such conditions, plants growth can be strongly decreased, as a result of (1) a reduced ability of roots to extract water due to a lower plant capacity to adjust osmotically to water potentials that are commonly in the range of 2-3 kPa (Flowers et al., 2010) and of (2) the high salts concentration within the plants which causes toxic effects and nutrient ions imbalance (Gorham et al., 1985; Marschner, 1995; Mansour, 2000; Zhu, 2001; Munns and Tester, 2008). Particularly, K^+ absorption is strongly reduced under saline conditions, as Na^+ and K^+ similar physicochemical structures mean that Na^+ competition at transport sites for K^+ entry into the symplast may result in K^+ deficiency (Maathius and Amtmann, 1999). The stress caused by high salinity conditions 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 an excessive cellular concentration of Na^+ and Cl^- (Blumwald et al., 2000).

One of the greatest challenge for plants inhabiting saline habitats is the ability to regulate transpiration in the presence of high concentrations of Na^+ and Cl^-. In fact, although plants water potential should be at least as low as that of soil in which they live to guarantee water extraction, the transpiration stream would carry and concentrate toxic ions to the leaves (Flowers et al., 2010). Stomata represent the main pathway for water loss in the leaf, and their functioning may be altered by a high salt concentration. Moreover, stomata also regulate the CO_2 exchange between the inside of the leaf and the atmosphere. Thus, plants require a sufficient quantity of CO_2 to enter the leaf for photosynthesis while conserving water to avoid dehydration (Lawson et al., 2009). According to Véry et al. (1998) and to Zhu (2001), an increase in the cytosolic concentration of Ca^{2+} is part of this Na^+ signalling pathway, leading to the inactivation of inward rectifying K^+ channels and, thus, to stomatal closure and to a reduced plant growth. On the other hand, stomatal opening was also reported in the presence of Na^+ ions, but a reduced stomatal closure was then observed in response to darkness, CO_2 and abscisic acid (Jarvis and Mansfield, 1980; Robinson et al., 1997), which might lead to plant dehydration. The inhibition of photosynthesis caused by salt accumulation in the mesophyll (Bongi and Loreto, 1989; Mc Leod et al., 1999; Centritto et al., 2003; Loreto et al., 2003; Ueda et al., 2003; Parida et al., 2004; Nandy (Datta) et al., 2007) may determine the increase of intercellular CO_2 concentration and, consequently, stomatal closure (Mansfield et al., 1990). According to Robinson et al. (1997), the inability of guard cells to exclude Na^+ and their tendency to accumulate it in preference to K^+ may significantly contribute to sodium toxic effect in non-halophytes.

In halophytes, which are defined as plants that can complete their life cycle in soils with salt concentrations above 200 mM (Flowers and Colmer, 2008), the mechanisms of salt tolerance fall into three categories (Munns and Tester, 2008): the tolerance to osmotic stress, Na^+ exclusion from leaf blades and tissue tolerance to Na^+ accumulation. Leaf succulence and ions excretion by salt glands or bladders represent two mechanisms of tissue tolerance to sodium ions. Salt glands are specialised epidermal cells or trichomes which actively eliminates salt from internal cellular tissues (Fahn, 1988; Kleinkopf and Wallace, 1974). The *Tamarix* genus includes about 90 species, which show a high adaptability to different environments and a high endurance of adversity (Zhang et al., 2002). According to Conti et al. (2005), the most widespread *Tamarix* species in Italy are *T. gallica* and *T. africana*. The first is mostly present on the seaside dunes, while *T. africana* is more frequent on the riverside banks. *Tamarix* spp. are characterised by a leaf glands specialised in salt extrusion; therefore, their growth is not inhibited by high salt soil concentration (Bar-Nun and Poljakoff-Mayber, 1974). However, they are also considered water consuming species (Brotherson et al., 1984) and are characterised by a lower water use efficiency compared to other riparian species (Vandersande et al., 2001). Moore et al. (2008) measured a high nocturnal transpiration in riparian *Tamarix chinensis* thickets, which could explain *Tamarix* high water consumption; according to the authors, these water losses were mainly due to stomatal opening rather than to cuticular transpiration. However, according to Rozema and Gude (1981), a quantity of water may also be lost through salt glands, increasing the risk of plant dehydration. Moreover, in halophytes which accumulate salt in the apoplast, a nocturnal transpiration rate could act as a mechanism for maintaining internal osmotic and tensile pressure potentials (Donovan et al. 2001).

In this work, we hypothesised that the presence of salt glands may increase leaf water losses during salt extrusion. Moreover, the two species distribution also suggests a higher salinity tolerance in *T. gallica* than in *T. africana*. In order to verify these hypothesis, gas exchanges and salt extrusion were measured on *T. gallica* and *T. africana* green detached shoots inserted in containers filled with NaCl solutions at different concentrations (0 mM, 100 mM, 200 mM and 400 mM). The objectives of this study were: (1) to evaluate the direct role of water salinity on leaf gas exchanges in the two *Tamarix* spp. and (2) to analyse leaf water losses in the light and in the dark under saline and non-saline conditions.

Material and Methods

Cuttings were collected from three *Tamarix gallica* and three *Tamarix africana* genotypes respectively grown on the mouth (40°20’46’’ N, 16°48’46’’ E; Southern Italy) and on the riverside (40°27’00’’ N, 16°31’44’’ E; Southern Italy) of river Basento. The six cuttings (2 species × 3 genotypes) were planted in 1.6 dm^3 pots (10.5×10.5×22 cm) containing sand (35%) and loamy soil (65%). The plants were first grown in a greenhouse and, afterwards, they were transplanted in 2.6 dm^3 pots (17.5×17.5×25 cm) and moved in a growth-chamber under a photosynthetic photon flux density (PPFD) of approximately 550 $\mu mol\ m^{-2}\ s^{-1}$, 60% relative humidity, a photoperiod of twelve hours and a day/night temperature of 25/15 °C. The pots were watered every evening for two months and, two weeks before the beginning of the experiment, a modified Hoagland solution (0.5× concentration) was supplied.

Gas exchange measurements

After plants watering, 4 green shoots were collected from the upper part of the crown of each plant. The shoots were cut in water to avoid xylem cavitation. The twenty-four shoots (4 treatments × 2 species × 3 genotypes) were respectively inserted into a plastic tube containing different water solutions and left for 12 hours under dark conditions. The solutions were constituted of: (1) deionised water, (2) deionised water added with NaCl 100 mM, (3) deionised water added with NaCl 200 mM, and (4) deionised water added with NaCl 400 mM. Leaf gas exchanges were measured using a portable infrared gas analyser (LI-6400, LI-

COR Biosciences, Inc., Lincoln, NE) equipped with a conifer chamber (LI-6400-05). The air flow was set at 400 µmol m^{-2} s^{-1} and the cuvette temperature at 25 °C. Leaf conductance (gl), transpiration rates (E) and respiration (Rd) were measured in the dark under an ambient [CO_2] of 400 µmol mol^{-1} and a VPD of 1.5 kPa. Leaf gas exchanges were then measured in the light, under the same VPD and a saturating photon flux of 1700 µmol $m^{-2}s^{-1}$. Net assimilation rate versus intercellular CO_2 concentration (C_i) was determined for ambient CO_2 concentration values (C_a) of 400, 300, 250, 200, 150, 50, 400, 500, 650, 750, 850, 1000 µmol mol^{-1} as suggested by Long and Bernacchi (2003); the assimilation values were recorded as soon as C_a was stable (cv<0.7%) (Ainsworth et al., 2002). Net assimilation rates (A) and leaf conductance measured under an ambient [CO_2] of 400 µmol mol^{-1} were considered as the assimilation and the conductance at growth chamber CO_2 concentration. The ratio between C_i and C_a (C_i/C_a) was also considered. The maximum carboxylation rate (Vc_{max}) was estimated according to Farquhar et al. (1980). After gas exchange measurements, shoots fresh weight was determined. Shoots were then scanned, and their images analysed with the software Skyroot (Llandrindod Wells, Powys, UK) in order to obtain leaf length. Leaf area was estimated by multiplying leaf length by the mean leaf diameter (0.7 mm). A small leaf portion was dried at 70 °C for dry weight estimation. After salt extrusion estimation (*see below*), the shoot dry weight without salt was determined.

Salt extrusion

After drying, the shoots were inserted into a plastic tube added with 25 ml of deionised water in order to wash them from the salt extruded through the salt glands. The tubes were shaken at 500 rpm for 20 minutes. The liquid phase was then separated from the leaves with filter paper. Leaf dry weight without salt was determined after drying in oven at 70 °C until constant weight. After washing, electrical conductivity of the liquid phase (EC), which is proportional to the amount of the salt extruded by salt glands and accumulated on the leaf surface, was measured using a conductimeter (HI9811, Hanna instruments INC, USA) equipped with an electrode probe (HI1285, Hanna instruments INC, USA).

Leaf epidermal characteristics

Leaf portions were used to analyse stomatal opening in the dark and in the light. Under these two conditions, the leaf surface was coated with a thin layer of clear nail polish. The polish was allowed to dry completely and was then peeled by pulling on a corner of a transparent tape. The tape was stuck on a microscope slide and the leaf impressions were analysed under a microscope (Leica DM4000B, Leica Microsystems AG, Wetzlar, Germany) connected to a digital camera (Leica DFC420, Leica Microsystems AG, Wetzlar, Germany). Photographs were taken and adjusted by the software Leica Application Suite (LAS, Leica Microsystems AG, Wetzlar, Germany).

Statistical analysis

Analysis of variance (ANOVA) was performed on the photosynthetic and leaf parameters using the statistical software Systat 12.0 (SPSS Inc.) to evaluate the main effects of the treatments. Post hoc was performed using Fisher’s LSD test. The software Prism 4 (GraphPad software, Inc) was used to test the correlations between gl measured in the light and in the dark and EC. Significance was considered for *p* values <0.1.

Results

Net assimilation rates (A) and dark respiration (Rd) measured at a CO_2 concentration of 400 µmol mol^{-1} in the two species and under the four salt concentrations are shown in Fig. 1. Net assimilation rates were on average equal to 12.8 µmol CO_2 m^{-2} s^{-1} under control in both species. Increasing NaCl concentration significantly reduced A ($p<0.001$). Particularly, A was on average equal to 9.1 µmol CO_2 m^{-2} s^{-1} under a concentration of 100 mM, *T. gallica* having a higher rate of CO_2 assimilation (12.5 µmol CO_2 m^{-2} s^{-1}) compared to *T. africana* (5.7 µmol CO_2 m^{-2} s^{-1}) ($p<0.1$; Fig. 1). A was equal to 5.0 µmol CO_2 m^{-2} s^{-1} under 200 mM in both species, and was significantly lower compared to the control ($p<0.05$). Under the maximum saline concentration (400 mM), the leaves were no more able to photosynthesise, especially in *T. africana* (Fig. 1), and the assimilation values significantly differed from those measured under control ($p<0.001$), under 100 mM ($p<0.01$) and under 200 mM ($p<0.1$). Dark respiration was on average equal to 2.4 µmol CO_2 m^{-2} s^{-1} and did not differ between the two species and among the treatments (Fig. 1).

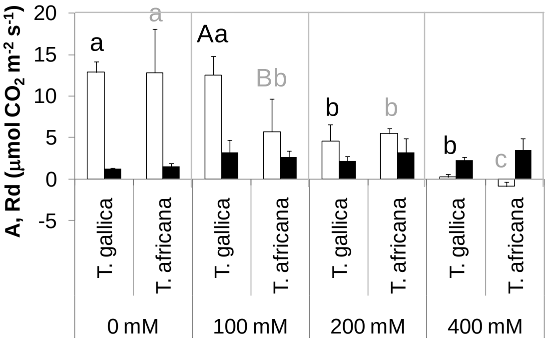

Fig. 1 Net assimilation (white bars) and dark respiration (black bars) rates measured in the two species (*T. gallica* and *T. africana*) and under the four NaCl concentrations (0, 100, 200, 400 mM). Lowercase letters of the same colour refer to the comparison among the treatments in the same species. Capital letters refer to the comparison between species under the same NaCl concentration. The values are means ± s.e. (n=3).

The maximum rate of carboxylation (Vc_{max}) was on average equal to 102 µmol CO_2 m^{-2} s^{-1} under control conditions. Vc_{max} did not change under 100 mM in *T. gallica* (118.1 µmol CO_2 m^{-2} s^{-1}), but was significantly reduced in *T. africana* (79.6 µmol CO_2 m^{-2} s^{-1}; $p<0.1$). Under 200 mM, Vc_{max} decreased (56.5 µmol CO_2 m^{-2} s^{-1}) compared to the control, but not significantly. A significant decrease was observed under 400 mM in both species (11.7 µmol CO_2 m^{-2} s^{-1}; $p<0.01$) (Fig. 2).

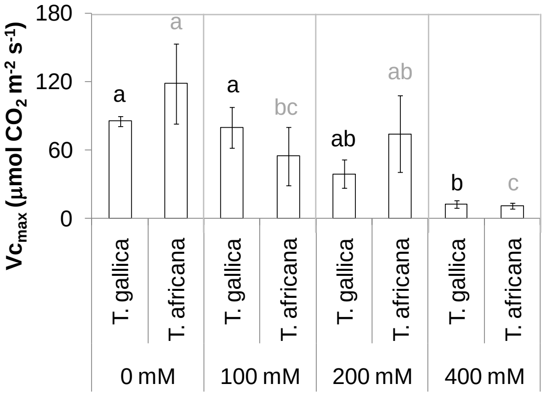

Fig. 2 Maximum rate of carboxylation (Vc_{max}) measured in the two species (*T. gallica* and *T. africana*) and under the four NaCl concentrations (0, 100, 200, 400 mM). Lowercase letters of the same colour refer to the comparison among the treatments in the same species. The values are means ± s.e. (n=3).

The ratio between C_i and C_a (C_i/C_a) was on average equal to 0.5 in both species under control conditions (Fig. 3).

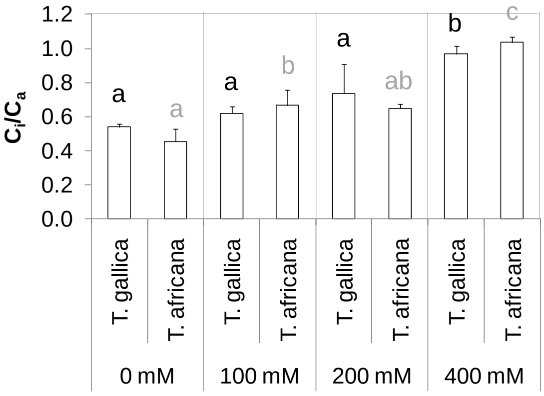

Fig. 3 Ratio between C_i and C_a (C_i/C_a) measured in the two species (*T. gallica* and *T. africana*) and under the four NaCl concentrations (0, 100, 200, 400 mM). Lowercase letters of the same colour refer to the comparison among the treatments in the same species. The values are means ± s.e. (n=3).

This ratio was significantly affected by salinity ($p<0.001$). Particularly, in *Tamarix gallica*, C_i/C_a significantly increased under 400 mM ($p=0.001$), while in *T. africana* an increase in the ratio was already observed under 100 mM ($p<0.1$), and under 400 mM ($p<0.01$) compared to control.

Leaf conductance (gl) and transpiration (E) values measured in the light and in the dark in the two species and under the four salinity treatments are shown in Tab. 1. Leaf conductance in the light was on average higher in *T. gallica*, compared to *T. africana* ($p<0.1$).

Tab. 1 Leaf stomatal conductance (gl) and transpiration (E), measured in the light and in the dark, under the four NaCl concentrations and in the two species. Lowercase letters of the same colour refer to the comparison among the treatments in the same species. Capital letters refer to the comparison between species under the same NaCl concentration. The values are means ± s.e. (n=3).

		Light				**Dark**			
		gl		**E**		**gl**		**E**	
		mol H_2O m^{-2} s^{-1}		mmol H_2O m^{-2} s^{-1}		mol H_2O m^{-2} s^{-1}		mmol H_2O m^{-2} s^{-1}	
		Mean	s.e.	Mean	s.e.	Mean	s.e.	Mean	s.e.
0 mM	***T. gallica***	0.13 a	0.02	2.1 a	0.4	0.06 a	0.01	0.9 a	0.1
	T. africana	0.10	0.03	1.6	0.4	0.07	0.02	1.1	0.4
100 mM	***T. gallica***	0.15 Aa	0.01	2.1 Aa	0.1	0.04 ab	0.01	0.7 ab	0.2
	T. africana	0.06 B	0.04	1.0 B	0.6	0.05	0.02	0.7	0.2
200 mM	***T. gallica***	0.12 a	0.05	1.9 a	0.9	0.03 b	0.00	0.4 ab	0.1
	T. africana	0.07	0.00	1.1	0.0	0.07	0.01	1.1	0.2
400 mM	***T. gallica***	0.03 b	0.02	0.5 b	0.3	0.03 b	0.01	0.4 b	0.2
	T. africana	0.05	0.01	0.8	0.2	0.05	0.01	0.7	0.1

Moreover, gl was significantly affected by the treatments. Particularly, gl was on average lower under 400 mM than under all other concentrations ($p<0.05$ for NaCl concentration of 0 and 100 mM; $p<0.1$ under 200 mM). However, this decrease was only observed in *T. gallica*, as leaf conductance in the light and in the dark, as well as transpiration under both light conditions did not significantly decrease with increasing salinity in *T. africana*. Moreover, gl and E measured in the light under a concentration of 100 mM were higher in *T. gallica*, compared to *T. africana* while the opposite was observed in the dark, where gl and E were lower in *T. gallica* compared to *T. africana* ($p<0.1$ in both cases). Furthermore, both gl and E were reduced in *T. gallica* as a consequence of increasing salinity: leaf conductance in the dark decreased by 30% under 100 mM, and by 50% under 200 and 400 mM ($p<0.05$ under all salinity concentrations), while E was reduced by 60% under 400 mM ($p<0.1$) compared to the control.

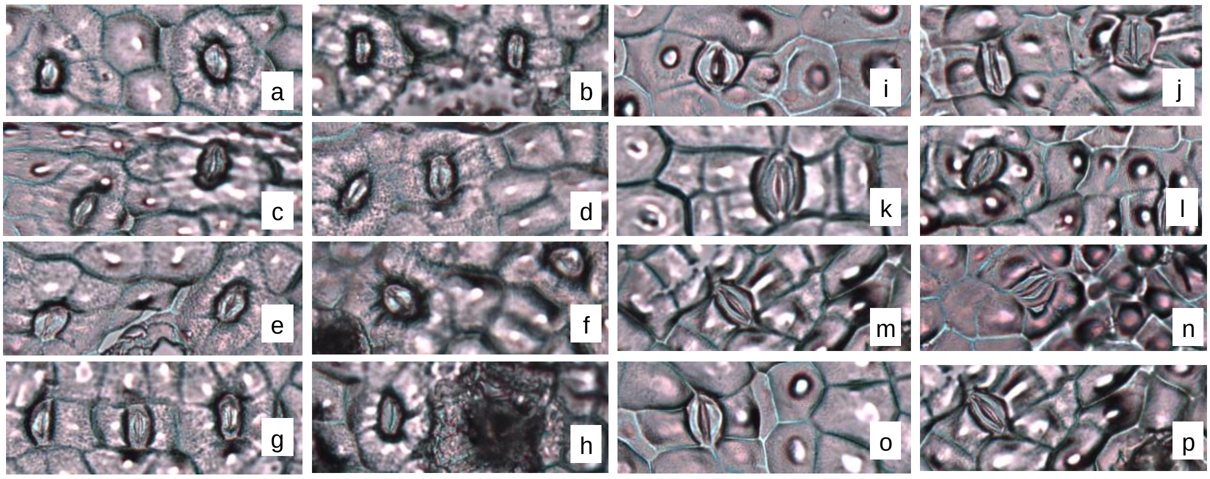

Fig. 4 Stomata observed at a magnification of 200× in the two species (*T. gallica*: from *a* to *h*; *T. africana*: from *i* to *p*), under the four NaCl concentrations (0 mM: *a, e, i, m*; 100 mM: *b, j, f, n*; 200 mM: *c, k, g, o*; 400 mM: *d, l, h, p*), in the dark (from *a* to *d*, and from *i* to *l*) and in the light (from *e* to *h*, and from *m* to *p*).

Open or partially opened stomata were observed under both light and dark conditions, in both species and under all salinity treatments (Fig. 4).

Water electrical conductivity (EC) after leaf wash (Fig. 5) was on average equal to 3.1 dS m^{-1} in *T. gallica* and 1.4 dS m^{-1} in *T. africana*. EC did not change with increasing salinity in *T. africana*, whereas it significantly increased in *T. gallica* under 400 mM compared to 100 mM ($p<0.05$). Moreover, under an NaCl concentration of 400 mM, *T. gallica* extruded 60% more salt than *T. africana* ($p<0.1$).

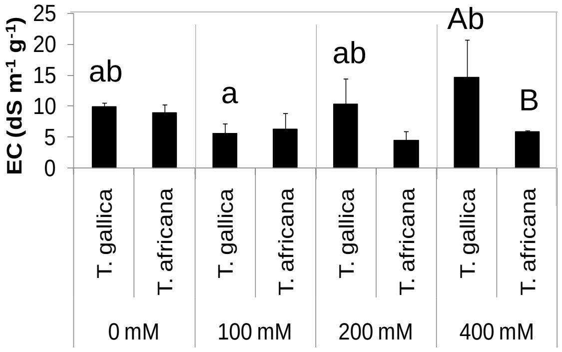

Fig. 5 Water conductivity after leaf wash (EC) in the two species (*T. gallica* and *T. africana*) and under the four NaCl concentrations (0, 100, 200, 400 mM). Lowercase letters refer to the comparison among the treatments in the same species. Capital letters refer to the comparison between species under the same NaCl concentration. The values are means ± s.e. (n=3).

A negative correlation was found between gl and EC under light and dark conditions (Fig. 6).

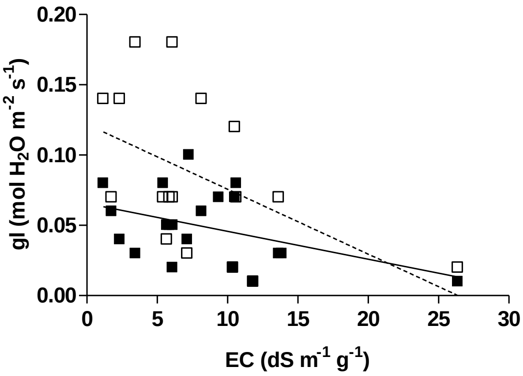

Fig. 6 Correlations between leaf conductance (gl) and water conductivity after leaf wash (EC) measured in the light (white squares) and in the dark (black squares) in the two *Tamarix* spp. and under the four NaCl concentrations (0, 100, 200, 400 mM). For the correlations in the light, the equations were $y=-0.0019x+0.065$ in the dark ($R^2=0.188$; $p=0.05$), and $y=-0.0046x+0.12$ in the light ($R^2=0.248$; $p<0.05$).

Discussion

Tamarix spp. tolerance to salinity is well documented in the literature (Bar-Nun and Poljakoff-Mayber, 1974; Carman and Brotherson, 1982; Di Tomaso, 1998; Vandersande et al., 2001; Eshel et al., 2010). However, no studies have focused on comparing salinity tolerance in different *Tamarix* spp.. As other halophytes, e.g. some species of *Spartina*, *Limonium, Avicennia* and *Halimione* genera, *T. gallica* and *T. africana* are characterised by leaf salt glands, which accumulate and actively extrude as far as 90% of the salt picked up by roots (*see chapter 4*). In many halophytic and non-halophytic species, salt exclusion by root membranes through selective ions uptake (Albert, 1975; Munns, 2002; Läuchli et al., 2008; Li et al., 2008) or through a reduced salt translocation from the belowground biomass to the leaves (*see chapter 3*; Chen et al., 2002) are also important salt regulating factors. Moreover, the first changes in plants metabolism and growth exposed to high salinity are thought to be a result of water relation effects, which are regulated by hormonal signals coming from the roots (Munns, 2002). Thus, excluding root induced control on leaf salt concentration, the rate of accumulation of salt in the shoots of halophytes is generally determined by (1) the rate of transpiration, (2) the concentration of Na^+ in the xylem sap (which, in this study, is probably equal to the salt concentration in the water solution) and (3) salt extrusion through salt glands (Robinson et al., 1997). Consequently, the direct salt short term effects on the leaf physiology analysed in this experiment were the result of these three factors only, excluding any interference caused by roots presence.

A different short term photosynthetic response was observed in the two analysed species. Particularly, *T. gallica* net photosynthetic rates were only reduced under 400 mM (and partially reduced under 200 mM), while salinity directly affected photosynthesis in *T. africana*, reducing CO_2 assimilation already at 100 mM. These reductions were not the result of increasing respiration. Flexas et al. (2004) reported that salt, as well as drought stress, predominantly decrease stomatal and mesophyll conductance, reducing CO_2 diffusion in the leaves and its consequent assimilation. Stomatal conductance reduction in plants grown under saline conditions was already observed in other experiments, in both halophytes and glycophytes (Bongi and Loreto, 1989; Delfine et al., 1998; Nandy (Datta) et al., 2007). Changes in the mesophyll conductance are probably related to variations in the structure of the mesophyll tissue and to a rearrangement and reduction of the intercellular spaces (Delfine et al., 1998; Bongi and Loreto, 1989); however, these leaf structural modifications are not immediate, and appeared after several days of salt treatment. In *T. gallica*, non stomatal limitations were first observed at 200 mM (partial Vc_{max} reduction), while leaf conductance measured in the light only decreased at 400 mM. Differently in *T. africana*, the decrease in CO_2 assimilation rates was only due to non stomatal limitations, as leaf conductance did not change in response to increasing salinity. Moreover, the carboxylation efficiency in *Tamarix africana* seems to be more affected by salinity compared to *T. gallica* as it was already reduced by a NaCl concentrations of 100 mM. Salinity can decrease RuBisCO activity and content (Delfine et al., 1998), inducing a reduction in maximum rates of carboxylation and an increase in C_i/C_a values as observed in this experiment. Stomatal opening may be inhibited by (1) a direct harmful effect of Na^+ on guard cells functioning (Robinson et al., 1997; Véry et al., 1998; Zhu et al., 2001), (2) a top-down regulation mechanism: according to Robinson et al. (1997), when the concentration of Na^+ increases in the vicinity of guard cells, stomata close to prevent additional salt to be transport through the transpiration stream, (3) a signal transduction pathway which causes an increase in the cytosolic concentration of Ca^{2+}, acting in a similar way to an increase in abscisic acid (Véry et al., 1998), (4) the feed-back controls on stomata in response to the accumulation of intermediates or products of the Calvin cycle (Lovelock and Ball, 2002), and (5) an increase in intercellular CO_2 concentration as a result of photosynthesis inhibition. Although all these mechanisms act under light conditions, leaf conductance and transpiration under increasing salinity in *T. gallica* were also reduced in dark conditions.

The quantity of salt extruded by *T. gallica* leaves increased with increasing NaCl concentration in the water solution, while *T. africana* extruded the same quantity of salt under the different salt water concentrations. These findings could be explained as the result of (1) lower transpiration rates in *T. africana*, which causes a reduction in the quantity of salt transported to the leaves, but also (2) *T. gallica* higher capacity of extruding the accumulated salt, as this species is characterised by a higher salt glands density compared to *T. africana* (Abbruzzese, 2010). An accumulation of salt in *T. africana* cytoplasm might have occurred, leading to non stomatal limitations. On the other hand, leaf conductance in *Tamarix africana* under dark conditions was not significantly different from that measured in the light. Donovan et al. (2001) suggested that nocturnal transpiration is a mechanism for maintaining a high osmotic potential under saline conditions. Moreover, Jarvis and Mansfield (1980) found that salinity did not inhibit stomatal opening in the light in *Commelina communis*, but that stomata were no more able to close under dark conditions. In this study, however, leaf conductance measured in the dark was also high under control conditions. Moore et al. (2008) already reported high nocturnal transpiration rates in *T. chinensis* thickets grown along the Pecos River. The authors also demonstrated that the high nocturnal transpiration rates were caused by stomatal opening rather than by cuticular water losses. Our findings are in agreement with these results. According to

Burkhardt (2010), excessive hygroscopic particles (such as NaCl) accumulation on leaf surface may work as desiccants, making stomatal transpiration independent of stomatal opening. However, a negative correlation between the quantity of salt extruded outside the leaf and leaf conductance under light and dark conditions and in both species was found in this study. This reduction may be explained by (1) an hygroscopic salt effect which may trap the water vapour lost by transpiration, leading to an alteration of the measure, or (2) by a physical obstruction of stomata by salt particles accumulation, which reduces gas exchanges. In fact, Hirano et al. (1995) found that the presence of dust on leaf surface decreases stomatal conductance in the light by an effect other than shading, while it increases stomatal conductance in the dark. Particularly, the dust plugged the opened stomata in the light, and prevented stomata from closing in the dark. Thus, the presence of particles on the leaf surface may have affected stomatal conductance by covering the opened stomata, leading to a reduction in gas exchanges.

Conclusions

This study highlighted a different short term photosynthetic response to salinity in the two analysed species. Particularly, the carboxylation activity was reduced in *T. gallica* under an NaCl concentration of 200 mM, while it already decreased at 100 mM in *T. africana*. These results suggest the higher suitability of *T. gallica*, rather than *T. africana*, to be used for the restoration of saline areas. The higher tolerance observed in *T. gallica* was probably due to its capacity to extrude more salt at increasing NaCl concentration, and can explain the two species natural distribution. The observed reductions are principally the result non stomatal limitations, rather than stomatal ones. Particularly in *T. gallica*, salinity first decreased maximum rates of carboxylation, while stomatal conductance only decreased under 400 mM. Differently in *T. africana* stomatal conductance was not affected by salinity. Both species were characterised by a high dark transpiration compared to light conditions. This water loss occurred through opened or partially opened stomata. Differently from what was first hypothesised, transpiration rates did not increase in response to salinity. On the contrary, stomatal conductance measured in the light and in the dark decreased with increasing leaf salt extrusion as a consequence of salt hygroscopic effect on water vapour losses through stomata or of the physical obstruction of stomata by salt particles.

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Chapter 5

Growth anatomical traits of *Tamarix gallica* exposed to contrasting water availability and salinity

Abstract

In coastal Mediterranean areas, plants are frequently exposed to many environmental stresses, such as groundwater salinity and prolonged period of drought. These factors reduce water potential, subjecting the xylem vessels to an increased risk of cavitation. One of the strategies plants may use to safeguard their water transport is the construction of cavitation-resistant xylem, characterised by small vessels with a decreased hydraulic conductance and a high hydraulic safety of implosion by negative pressure. *Tamarix* species are well adapted to grow in desert and saline areas. Under these environmental conditions, *Tamarix* spp. are rarely associated to other tree species. Therefore, their wood may represent an invaluable source of information for dendrochronological studies in these areas. However, the hydraulic strategies that enable them to survive in such extreme environments are not well known. In this study, we aim at comparing plants growth and anatomical characteristics of the water conducting tissue in two contrasting sites of *Tamarix gallica* located close to a river estuary.

Plants structure significantly differed between the two analysed stands of *Tamarix gallica*. Particularly, plants grown on the dune, which was the more saline habitat, were characterised by an overall shrubby carriage compared to mouth plants, which presented higher dimensions and a reduced number of sprouts. Marked differences between the two sites were also found in the wood anatomical features and particularly in the first third part of the ring. In this wood portion, the plants grown on the dune were characterised by a lower mean vessel area, a lower total vessel area and a higher vessels density compared to the mouth plants. These findings likely represent *Tamarix gallica* adaptations to high salinity and low fresh water availability to prevent cavitation and embolism. Under such conditions, the mean vessel area seems to be a more suitable parameter for dendrochronological investigations compared to ring width.

Key words: *Tamarix gallica*, wood anatomical traits, cavitation, stand dendrometric characteristics

Introduction

The basic wood anatomical structure of any species is constant and determined by genetic factors, so that it is often utilised for species identification (Wimmer, 2002). However, vessels anatomical features are subjected to modifications through the environment, thus bear ecologically relevant information (Wimmer, 2002; Fonti and Garcìa-Gonzàlez, 2004) which can be used to identify trees diversity adaptation or adjustment to the environmental variability (Fonti et al., 2009). Earlywood anatomy has been successfully used as a dendroclimatic proxy for mesic (Garcìa-Gonzàlez and Eckstein, 2003; Fonti and Garcìa-Gonzàlez, 2008; Tardif and Conciatori, 2006) and

Mediterranean sites (Campelo et al, 2010). However, in dry coastal Mediterranean areas, plants are not only subjected to the direct effect of climate (prolonged summer drought, high temperatures), but are also exposed to a range of hazards which are directly connected to climate change (IPCC, 2007; Torresan et al., 2008; Solomon et al., 2009). Particularly, the increase in global average air and ocean temperatures is causing a widespread melting of snow and ice and seawater expansion, resulting in the rise of global average sea level, which could cause the salinization of irrigation water, estuaries and freshwater systems (IPCC, 2007). Moreover, the decrease in precipitation during summer can extend the drought period, also leading to an increase in groundwater salinity. Although plants inhabiting these areas are adapted to survive under strong stress conditions (Thuiller et al., 2005), the growing environmental pressure may lead to an invaluable loss of species diversity. In fact, salinity can severely reduce plants growth in both halophytes and glycophytes (Kozlowski, 1997; Mansour, 2000; Ben Hassine et al., 2008), as a consequence of the direct phytotoxic effect of Na^+ and Cl^- (Marschner, 1995; Mansour, 2000; Zhu, 2001). Salinity also causes a reduction of the water potential in the root medium as a consequence of an osmotic effect, subjecting the xylem vessels to the risk of cavitation in a similar way to drought (Kolb and Davis, 1994; Mansour, 2000; Hacke and Sperry, 2001; Allen et al., 2010). One of the strategies plants may use to safeguard their water transport is the construction of cavitation-resistant xylem (Tyree and Sperry, 1989; Tyree and Ewers, 1991; Reich et al., 2003); in particular, small vessel area decreases hydraulic conductance, inducing a high hydraulic safety against vessel implosion by negative pressure (Hacke et al., 2001) and cavitation (Hacke et al., 2006), so that the size of the vessels produced during the growing season represents a balance between water transportation efficiency and vulnerability to cavitation (Campelo et al., 2010). Of course, plant responses to these stresses depend on their intrinsic tolerance and adaptability, and on the strength of the stressing factor. Moreover, the responses may be different among species and, within the same species, among genotypes (Kozlowski, 1997; Chaves et al., 2003).

Tamarix genus includes about 90 species, which are naturally distributed in Africa, Asia and Europe. The presence of leaf glands specialized in salt extrusion is a characteristic feature of *Tamarix* species; therefore, their growth is not inhibited by high salt soil concentration (Bar-Nun and Poljakoff-Mayber, 1974). Moreover, many researches emphasized *Tamarix* tolerance to very negative water potentials, enabling these species to grow under drought conditions and to endure water table depth variations (Cleverly et al., 1997; Horton et al., 2001; Gries et al., 2003; Xu and Li, 2006). The high plasticity and tolerance to adversity allows the plants belonging to the *Tamarix* genus to survive in desert and saline areas. Under these environmental conditions, *Tamarix* spp. are rarely associated to other tree species. Therefore, their wood may represent an invaluable source of information for dendrochronological studies in these areas. In Italy, *Tamarix* species are ten and, among them, *T. gallica* is the most widespread species (Conti et al. 2005) occupying coastal saline areas. Analysing *Tamarix* growth traits would contribute to better identify which are the most suitable conducting water structures and strategies that enable plants to survive under such extreme stress conditions. In this study, we aim at comparing plants structure and the anatomical characteristics of the water conducting tissue in two sites of *Tamarix gallica*. The sites are located close to a river estuary and they contrast in term of water availability and degree of salinity.

Materials and Methods

Description of the areas and study sites

The study area is located near the estuary of river Basento (Metaponto; 40°20’46’’ N, 16°48’46’’ E; Southern Italy) (*see chapter 1*). The climate of this site is typically Mediterranean semiarid, with a mean annual temperatures of 17.0 °C (1999-2008; the data were taken from the meteorological station of Metaponto: 40°22’26 N, 16°48’26’’ E) (Fig. 1). Minimum temperatures are recorded in January (8.7 °C) and maximum temperatures in July (26.7 °C). The annual precipitation is about 500 mm, with more than 50% of rainfall occurring during autumn and winter; a long dry period is usually recorded from May to September. The geological substrate is composed of quaternary silica-carbonates, with silt and clay deposits being present near the river mouth.

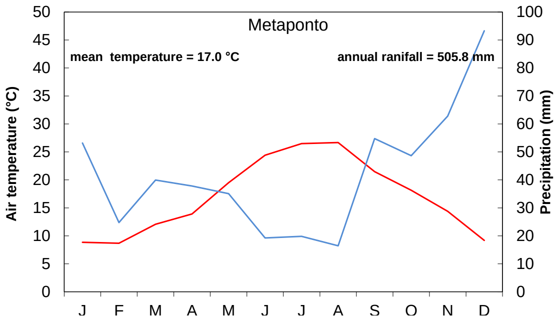

Fig. 1 Walter and Lieth climatic diagram obtained from data collected during the period 1999-2008 in the nearby meteorological station of Metaponto

Two adjacent *Tamarix gallica* stands were considered in this study; the first was located on the seashore (dune) and was characterised by a groundwater salinity of 4 dS m^{-1} (Polemio et al., 2003), while the second was situated along the river shores of Basento mouth (mouth), at about 100 meters far from the dune site. In this environment, soil salinity ranged between 2 and 4 dS m^{-1} (Polemio et al., 2003). The mean river water height ranged from a maximum of 1.6 m in the winter period (December-February) to a minimum of 0.6 m during the summer months (June-August) (the data are referred to the mean water height values measured at the hydrometric station SS106, at Metaponto, in the years 1999-2008). *Tamarix* species identification was performed according to the configuration of the staminal disc (Baum, 1978; Abbruzzese, 2010). Moreover, a set of 14 Simple Sequence Repeats (SSR) markers and a Bayesian assignment method were used establishing the correspondence with the previously described *taxa* of the unidentified individuals (Terzoli, 2010). On the dune, *Tamarix* spp. were associated with *Pancratium maritimum*, *Salicornia fruticosa*, *Suaeda maritima*, *Ammophila arenaria*, and other psammophytes in the herbaceous layer, and to *Pistacia lentiscus* and *Juniperus macrocarpa* in the dominant layer. Near the mouth, *Tamarix* spp. were only associated with herbaceous wetland species, such as *Phragmites autralis*, *Arundo* spp., *Carex* spp. and *Juncus* spp.. The dune stand was characterised by a maximum age of 21 years, while the mouth stand 34 years.

Dendrometric measurements

Twenty and thirteen *Tamarix gallica* plants were respectively selected from the dune and the mouth site. The number of plants was proportional to the stand extension. For each monocormic and polycormic plant, the sprouts were counted; the stem base diameter of sprouts >2 cm and the diameter (D_d) of the sprout characterised by the dominant height (H_d) were measured. The individual mean diameter (D_m) and basal area (G_i) were then calculated. Diameter frequency distributions were obtained by plotting the number of individuals belonging to each diameter class (the class width was equal to 2 cm).

Tree rings and vessels anatomical characteristics

Similarly to many Mediterranean species, *Tamarix* growth rings are sometimes hard to distinguish; this is mostly due to the presence of indistinct ring boundaries (Neumann et al., 2001; Friedman et al., 2005). In November 2008, wood cores were collected from the stem base of plants living in the dune and in the mouth stands. The wood surface was sanded and then cleaned with high pressure water to remove both tyloses and wood dust. Among the collected samples, five of them were selected according to the presence of a distinct ring boundary, which clearly separated the latewood formed during the year n-1 from the earlywood formed in the year n (Fig. 2). The number of rings considered in this study was ten, which correspond to the growing period 1999-2008.

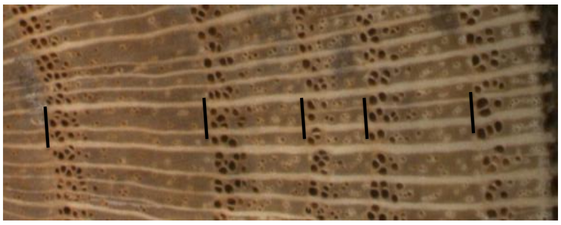

Fig. 2 *Tamarix gallica* transverse wood cross section with visible and distinct ring boundaries

One cross sections per plant was stained black with printer ink, and lumina were filled with white chalk powder to improve the contrast between the vessel lumina and the wood matrix. The sections were observed at a magnification of 40× using a stereo microscope (Leica MZ16A, Leica Microsystems AG, Wetzlar, Germany), connected to a digital camera (Leica DFC420, Leica Microsystems AG, Wetzlar, Germany), and photographs were taken ring by ring, and adjusted by the software Leica Application Suite (Leica Microsystems AG, Wetzlar, Germany). Image analysis was performed using an image analysis tool (IMAGE PRO PLUS, v4.5, Media Cybernetics, Bethesda, MD, USA) that automatically recognizes and measures vessels >500 μm^2. To exclude objects of irregular shape, a filter removing objects whose length was at least twice their width was used. The accuracy of vessel identification was visually confirmed by observation under a stereomicroscope and when necessary vessels outlines were manually corrected. For each tree ring, and for the two sites, vessel lumen area (VA) and the coordinates of its centroid were recorded. This allowed the determination of the relative radial position (in percentage) of each vessel within a ring. Moreover, the absolute vessel area and the number of vessels (NV) distributions across the ring were averaged for ring width classes of 0.05 mm. The ring width (RW), the number of vessels, the mean vessel area (MVA), the total vessel area (TVA) and the vessels density (VD) of each tree ring were measured and calculated. Moreover, NV, MVA, TVA and VD were also analysed in the first third portion of the tree ring. Ten-year chronologies of RW and MVA were established for individual time series (data not shown). The quality of the obtained chronologies was assessed using correlation between trees (R_{bt}), that represents the mean Pearson correlation coefficient obtained from the singles Pearson correlation coefficients between samples chronologies taken in pairs.

Results

Plants dendrometric features

The structure of *Tamarix gallica* stands was significantly different in the two environments. Particularly, the mean diameter (D_m), the individual basal area (G_i), the dominant diameter (D_d) and the height of the dominant shoots (H_d) were lower in plants grown on the dune than in those present on the mouth ($p<0.001$ in all cases), while the number of sprouts was significantly higher in the dune compared to the mouth ($p<0.05$) (Tab. 1).

Tab. 1 The mean diameter (D_m), the mean basal area (G_i), the mean diameter (D_d) of the sprout characterised by the dominant height, the mean height of the dominant shoot (H_d) and the mean number of sprouts measured and calculated in *Tamarix gallica* plants present in the dune and in the mouth stands. The comparison between the two environments was analysed by an unpaired t-test and the statistical significance was considered for *p* values <0.05. Lowercase letters refer to the difference between the two environments. The values are means ± s.e. (n=20 for the dune; n=13 for the mouth)

			D_m		**G_i**		**D_d**		**H_d**		**N of sprouts**
			cm		cm^2		cm		m		
Dune	Mean	**a**	5.1	**a**	98.0	**a**	7.0	**a**	3.2	**a**	17.0
	s.e.		0.5		26.9		0.5		0.2		5.2
Mouth	Mean	**b**	14.3	**b**	785.6	**b**	18.8	**b**	5.6	**b**	5.7
	s.e.		1.6		118.5		1.7		0.3		0.9

Moreover, the sprouts diameter frequency distributions differed between the two sites. In fact, the dune was characterised by a stand composed of plants with a large number of small sprouts, and no sprouts with a large diameter were observed (Fig. 3). Conversely, the mouth environment was characterised by plants composed of few sprouts with larger diameters.

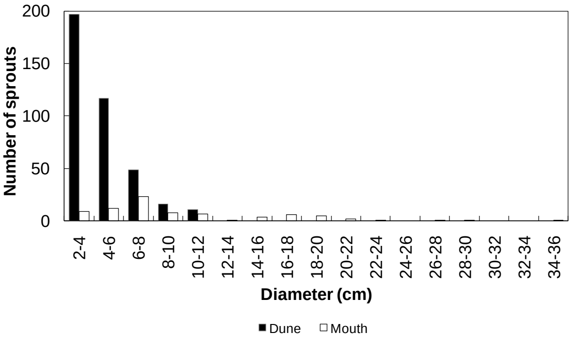

Fig. 3 Diameter frequency distributions of the two environments (n=20 for the dune; n=13 for the mouth)

Vessels characteristics

The vessel lumen area (VA) frequency distribution measured in the ten years of growth in plants grown in both environment was skewed to the left; however, a higher number of small vessels was found in plants present on the dune site, where vessels with a lumen area higher than 4000 μm^2 were only 15% of the total number of vessels, while in the mouth they represented more than 50% (Fig. 4).

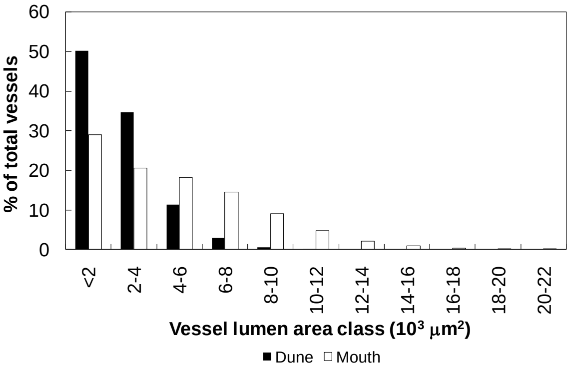

Fig. 4 Frequency distribution of vessel area classes in the two environments (n=2115 for the dune; n=2130 for the mouth)

Vessels lumen area distribution across the ring is shown in Figs. 5 and 6. Larger vessels were more frequent in the first portion of the ring in both environments. However, while in the mouth the mean vessel area was more than twofold higher in the first third of the ring compared to the rest of it, in the dune this difference was not so marked. In fact, the mean vessel area in plants grown on the dune was on average equal to 3000 μm^2 in the first third of the ring, and to 2000 μm^2 in the other two thirds (Fig. 5).

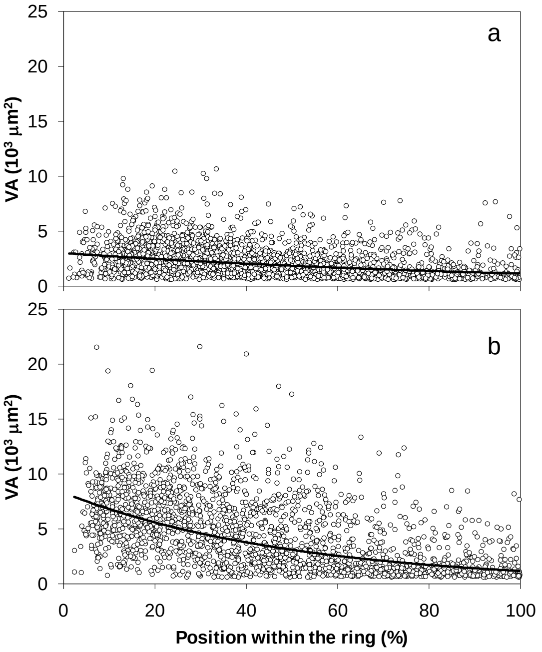

Fig. 5 Relative variation of the vessel lumen area of all vessels across the ring in (a) the dune (n=2115) and (b) the mouth (n=2130)

Moreover, the maximum ring width was lower in plants grown on the dune (2.2 mm) compared to those present on the river mouth (3 mm) (Fig. 6).

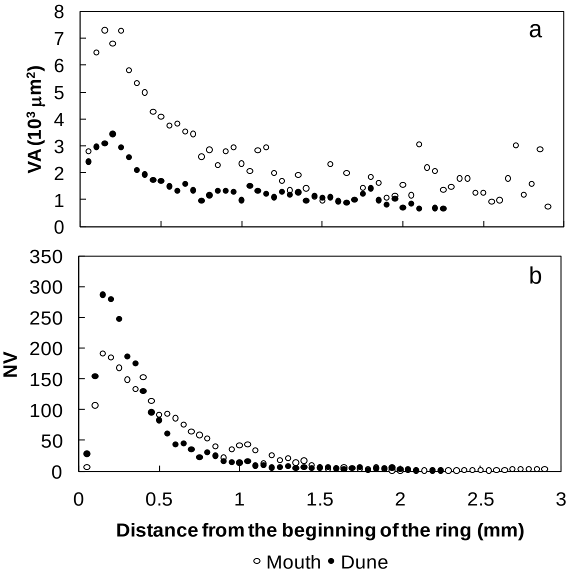

Fig. 6 Absolute vessel lumen area variation (VA) (a) and number of vessels (NV) (b) across the ring in the dune (closed circles) and in the mouth sites (open circles). The circles represent the mean value of vessels found in each 0.05 mm classes

Conversely to the vessel area, the number of vessels in the first third portion of the ring was higher in the dune compared to the mouth, reflecting a higher vessels density in the first site.

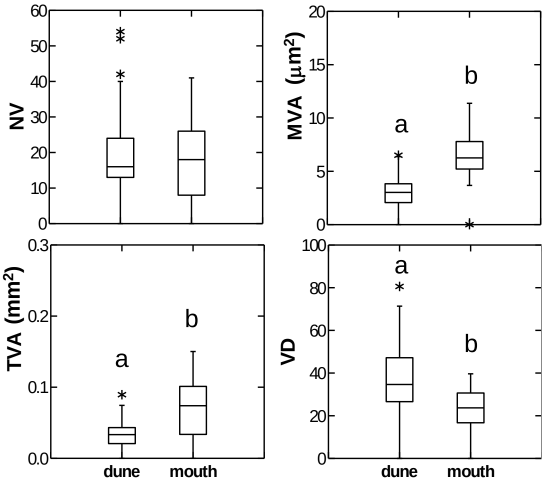

Fig. 7 Number of vessels (NV), mean vessel area (MVA), total vessel area (TVA) and vessels density (VD) calculated as mean of ten years for the analysed sections in the first third portion of the ring. The comparison between the two environments was analysed by an unpaired t-test and the statistical significance was considered for *p* values <0.05. Lowercase letters refer to the difference between the two environments. The values are means ± s.e. (n=48 for the dune; n=49 for the mouth)

As most of the differences between the two sites were observed in the first third portion of the ring, the number of vessels (NV), the mean vessel area (MVA), the total vessel area (TVA) and the vessels density (VD) were analysed in this wood area (Fig. 7). As mean of the ten years of investigation, NV present in each tree ring was similar in the two environments. On the other hand, the plants grown on the dune were characterised by a lower MVA ($p<0.001$), a lower TVA ($p<0.001$), and a higher VD ($p<0.001$) compared to those present near the river mouth. Moreover, MVA was characterised by a lower variability compared to the other analysed parameters.

The correlations referred to the parameters measured in the whole ring are reported in Tab. 3.

Tab. 3 Pearson coefficient values of the correlations (for $p<0.05$) among the number of vessels (NV), the mean vessel area (MVA), the total vessel area (TVA), the vessels density (VD) and the ring width (RW) and correlation between trees (R_{bt}) analysed in the two environments (n=50) and correlation between trees calculated for RW and MVA (n=10)

Dune

	NV	MVA	TVA	VD	RW
NV	-				
MVA	-0.45	-			
TVA	0.81	-	-		
VD	0.49	-	0.54	-	
RW	0.69	-0.34	0.41	-	-

Mouth

	NV	MVA	TVA	VD	RW
NV	-				
MVA	-	-			
TVA	0.737	0.299	-		
VD	-	-	-	-	
RW	0.573	-	0.67	-0.4	-

R_{bt}

	RW	MVA
Dune	-0.02	0.41
Mouth	0.34	0.17

In the dune, MVA was negatively correlated with both NV ($p=0.001$) and RW ($p<0.05$), while a positive correlation was found between NV and TVA ($p<0.001$), NV and VD ($p<0.001$) and NV and RW ($p<0.001$). RW also positively correlated with TVA ($p<0.01$). Concerning plants grown near the river mouth, NV was positively correlated with both TVA ($p<0.001$) and RW ($p<0.001$). Moreover, RW was positively correlated with TVA ($p<0.001$) and negatively with VD ($p<0.01$).

Contrarily to the mouth site, the ring width showed no common signal in plants grown on the dune, while a higher correlation between trees was observed considering the mean vessel area in this site (Tab. 3).

Discussion

At a stand level, basal area, stem density, diameter class distribution and stem age are intrinsic characteristic of a given plant population (Natta, 2003); for this reason, they are often used to compare vegetation types among sites. The structure of *Tamarix gallica* populations significantly differed between the two environments. The plants grown on the dune were characterised by a shrubby carriage, with lower diameters, heights and basal areas compared to those grown near the mouth of river Basento. Moreover, the maximum plant age found in the dune was lower compared to the mouth, indicating a decreased sprouts vitality in this environment: in fact, the diameter frequency distribution suggests a high sprouts turnover, with a large number of sprouts belonging to the first diameter classes, and no sprouts with a diameter bigger than 10 cm. Under stress conditions, *Tamarix* spp. are able to maintain their vitality by replacing dead sprouts with new ones, as a consequence of adventitious buds production from superficial side roots (Baum, 1978). This phenomenon is probably an adaptive advantage in the vegetative propagation of *Tamarix* species in their natural habitats (Ginzburg, 1967), which are frequently subject to a multitude of stresses such as drought, salinity and flooding. On the mouth, a more stable stand structure was found, which was characterised by the presence of sprouts belonging to bigger diameter classes.

Together with plants dendrometric features, also wood anatomical characteristics differed between the two environments. Particularly, the wood of plants grown on the dune was composed by 85% of vessels characterised by a lumen vessel area <4000 μm^2. The difference in vessels area between the two sites was especially marked in the first third portion of the ring, which likely represent the spring growing period, as samples collected in November were characterised by the absence of large vessels in the outermost part of the last ring (summer-autumnal growth) (*see Fig. 2*). In this wood portion, the mean vessel area as well as the total vessel area were on average significantly lower in plants grown on the dune compared to those grown on the river mouth, while vessels density was higher in the first site. As a consequence, while plants grown on the mouth showed a high variation in vessels size along the growth ring, this difference was not so marked in plants occupying the dune environment. According to Arnold and Mauseth (1999), changes in vessels density and/or size are likely due to changes in water availability. In fact, a low water availability causes a reduction in water potential, subjecting the xylem vessels to the risk of cavitation (Kolb and Davis, 1994; Mansour, 2000; Hacke and Sperry, 2001; Hacke et al., 2006; Allen et al., 2010). Schmitz et al. (2006) also found a positive correlation between vessels density and salinity. Moreover, these authors suggest that seasonal fluctuation in vessels density can be partly attributed to an increase in salinity from the rain to the dry season. Salinity represents a detrimental stressor for plant growth as a consequence of (a) the toxicity caused by excessive Na^+ and Cl^- uptake and accumulation, (b) the nutrient ion imbalance, especially K^+, due to the disturbance of essential intracellular ion concentrations, and (c) the decrease of water potential in the root medium, due to an osmotic effect (Marschner, 1995; Mansour, 2000; Zhu, 2001). Under such conditions, vessel characters should be adapted to reduce the impact of cavitation on sap flow by preventing gas expansion and by minimizing the loss of conductive area upon embolization (Schmitz et al., 2007). Safe hydraulic structures concern with the construction of numerous vessels (Verheyden et al., 2005) characterised by a small diameter (Verheyden et al., 2005; Junghans et al., 2006; Sobrado, 2007), a small wall area and thus, a relatively low pit area per vessel (Hacke et al., 2006). In fact, cavitation resistance is determined by the maximum and not by the average pore diameter of the pit membrane; thus, the larger is the pit area, the bigger is the chance on an exceptional large pore increasing cavitation vulnerability (Hacke et al., 2006). As under water stress vessels tend to become narrower to prevent collapse of vessels under high negative xylem pressures (Yang et al., 2010), hydraulic conductance is decreased. Actually, this parameter depends not only on the stem cross sectional area occupied by vessels, but also on the size and number of vessels (Poorter et al., 2010). Wider vessels guarantee a larger hydraulic conductance (Sperry et al., 2006) and a higher stomatal conductance, allowing a higher photosynthetic carbon gain (Santiago et al., 2004). As a consequence, smaller vessels cause a decrease in photosynthetic carbon gain, thus a lower growth rate as observed in the dune stand.

In both sites, ring width depended on the number and the total area of vessels produced during the growing period. However, the higher ring width observed in plants grown on the mouth is likely not due to a difference in the number of vessels produced, but rather to the higher mean vessel area observed in this site, especially during spring. In fact, in the mouth sites, the wood was characterized by a greater frequency of large vessels in the first third of the ring, gradually decreasing in the rest of it. In the dune stand, the mean vessel area was negatively correlated with the number of vessels and ring width. When the ring is narrow, few large vessels can occupy the major part of the ring, so that the ring is mainly composed of earlywood vessels characterised by a high mean vessel area. It is possible that under particular conditions (high soil water salinity induced by low precipitation, intense drought stress), ring growth stops in plants growing on the dune, while it continues in mouth plants probably as a consequence of their higher water availability due to the presence of a minimum but constant river discharge and to a lower soil water salinity.

According to Schmitz et al. (2006), a time-standardized measuring approach should be used when comparing wood anatomical features between different sites in any dendroecological investigation. In the mouth site, the ring width showed a stronger common signal compared to the mean vessel area. Although the collected plants were chosen for the presence of clear ring boundaries, ring width showed a weaker common signal compared to the mean vessel area, contrarily to what reported for the mouth site and by Campelo et al. (2010) in *Quercus* spp. grown under Mediterranean climate. Moreover, this parameter was characterised by a lower dispersion compared to the number of vessels, the total vessel area and the vessels density. It is possible that under particular stress conditions, parameters other than ring width may be more suitable to describe plants responses to microclimatic and edaphic conditions.

Conclusions

Plants structure significantly differed between the two analysed stands of *Tamarix gallica*. Particularly, plants grown on the dune were characterised by an overall shrubby carriage compared to mouth plants, which presented higher dimensions and a reduce number of sprouts. Marked differences between the two sites were also found in the wood anatomical features and particularly in the first third part of the ring. In this wood portion, the plants grown on the dune were characterised by a lower mean vessel area, a lower total vessel area and a higher vessels density compared to the mouth plants. These findings likely represent *Tamarix gallica* adaptations under high salinity and low fresh water availability to prevent cavitation and embolism. Under such conditions, the mean vessel area seems to be a more suitable parameter for dendrochronological investigations compared to ring width.

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Appendix

Ex situ conservation of *Tamarix* spp. Italian provenances

Background, objectives and main results

The coastal vegetation of the Mediterranean Basin has developed stress-adapted mechanisms to overcome changes in temperatures, water availability and salinity. The species which inhabit these areas are likely to be well adapted to future conditions caused by the global warming effects (Thuiller et al., 2005). Although particularly tolerant, these species may be damaged by climate change as a consequence of habitats disappearance and by the increase in stress strength. The conservation of this vegetation and its biodiversity is then needed. In areas of particular natural values, as reserves and parks, this option should be oriented to protect and preserve the natural habitats and species biodiversity.

A recent study (Han et al., 2007) has shown that forestation and reforestation constitute a low-cost option of carbon dioxide sequestration with significant economic and social benefits, such as forest products, improved soil and air quality, reduced erosion and improved ecosystem health. Moreover, Mediterranean species natural survival capacity and genetic biodiversity can be harnessed to sequester a significant part of the high atmospheric CO_2 content, by increasing their plantation in presently unutilised arid/flooded areas worldwide, were saline water or reused urban and industrial water waste might be present. In these areas, the reforestation of over-exploited soils should be made with local species in mixed plantations. Thus, the characterization of tolerant species and genotypes has become more and more important for the restoration of these habitats as the responses to the stresses might be different among species and, within the same species, among genotypes.

In order to analyse and conserve *Tamarix* species and their intraspecific biodiversity, and to make plant material available for the restoration of degraded areas, a collection of plant material was made in different natural populations of Southern Italy and among these, in different environments (dune, mouth of the river, riverside upriver). In fact, the understanding of plants diversity and distribution is crucial for conservation purposes (Tobler et al., 2007). *Tamarix* spp. populations were collected on the dunes of rivers Basento (Basilicata), Crati (Calabria) and Simeto (Sicily), and at the mouth of the rivers Basento and Simeto. *Tamarix* spp. populations were also found upriver, in Basilicata and Calabria. In rivers Alcantara (Sicily) and Imera Meridionale (Sicily), *Tamarix* spp. populations were only investigated on upriver sites. *Tamarix* spp. were also collected on the shores of lake Baratz (Sardinia) which was considered a dune site, as a result of its salinity and marine origin. Cuttings from 54 *Tamarix* spp. genotypes were selected on river Basento, 35 on Imera Meridionale, 24 on lake Baratz, 23 in Crati; 24 *Tamarix* spp. genotypes were also collected on river Alcantara and 43 on river Simeto. The number of collected individuals was variable and proportional to the population extension. Samplings were made on plants that were 25-30 m far from each other, in order to increase the genetic variability.

In each site and from each plant, we collected three living shoots, of about 2 meters length, with many secondary shoots. Plant material was then brought to Vivai Michelini Nursery in Viterbo and was replicated several times by green cuttings, which were planted on started plugs. After 4 month, plants were transplanted into small pots (7×7×7 cm). A high mortality was observed after the propagation with green cuttings: 24.1% for Basento genotypes, 91.7% for Baratz genotypes, 62.9% for Imera genotypes, 34.8% for Crati genotypes, 45.8% for Alcantara genotypes and 72.1% for Simeto genotypes. The mortality was reduced when woody cuttings were planted, and about 90% of the genotypes survived: on October-November 2008, a collection of 65 of the 106 dead genotypes was made, and new genotypes were also collected. The plant material (8 woody cuttings per genotype) was brought to the University of Tuscia's Farm "Nello Lupori" and was planted on 10.5×10.5×22 cm pots containing sand (35%) and loamy soil (65%). All the material planted in Vivai Michelini Nursery was brought to the University of Tuscia's Farm and 8 individuals per genotype were transplanted using 10.5×10.5×22 cm pots containing sand (35%) and loamy soil (65%) (Fig. 1).

Fig. 1 *Tamarix* spp. collection at the University of Tuscia's Farm "Nello Lupori"

One individual per genotype was selected; 200 genotypes were planted in the Regional Park of Burano Lake (Toscany, Italy) (Fig. 2) at the beginning of May 2009. The distance in the row is 1 meter, while between the 2 rows the distance is 5 meters.

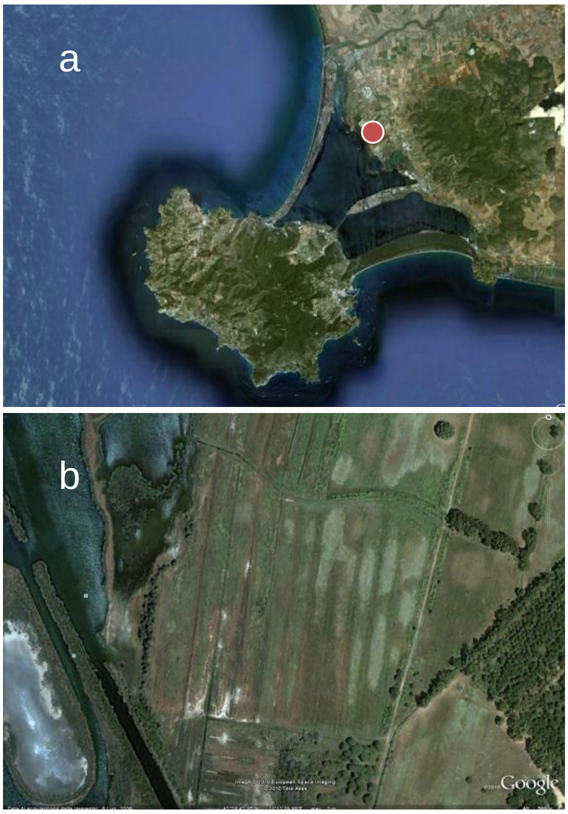

Fig. 2 (a) View of the Argentario, the location of the permanent *Tamarix* spp. collection in the Regional Park of Burano Lake (Toscany). (b) White spots at the bottom part of the photo indicate the presence of a salt crust on the ground surface

A station equipped with sensors (5TE, Decagon Devices, Inc.) measuring soil water content, soil temperature and water electrical conductivity, was installed in the two rows at two different depths (20 and 40 cm). The area was characterised by heterogeneous salinity in space and time. Particularly, the Eastern row was more salty compared to the Western one (Fig. 3). Peaks of salinity were observed in correspondence to rain events at shallow points of measurements (depth-1) in the Western row. Thus, water rain probably solubilises the salts present in the first few cm of soil. In the Eastern row, salinity increases in the more deep soil layer compared to the shallow one. In this case, sea water intrusion may have occurred.

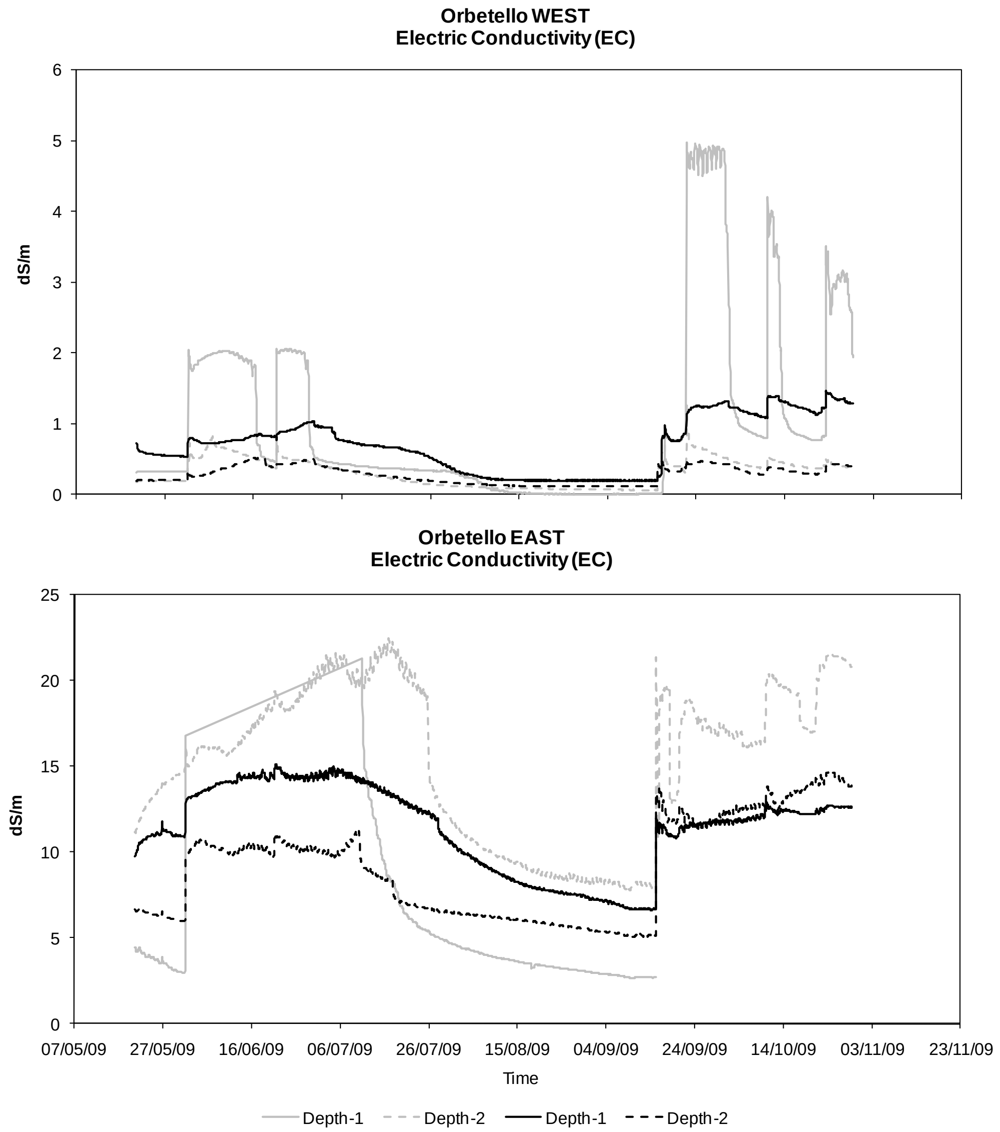

Fig. 3 Electrical conductivity (EC) measured at two depths (Depth-1: 20 cm and Depth-2: 40 cm), in the Western and Eastern rows, from the 20th of May to the 29th of October 2009

The percentage of plants in the collection belonging to the different populations (Alcantara, Baratz, Basento, Crati, Imera Meridionale and Simeto), environments (dune, mouth and riverside) and to the main *Tamarix* species (*Tamarix gallica* and *Tamarix africana*) are reported in Fig. 4.

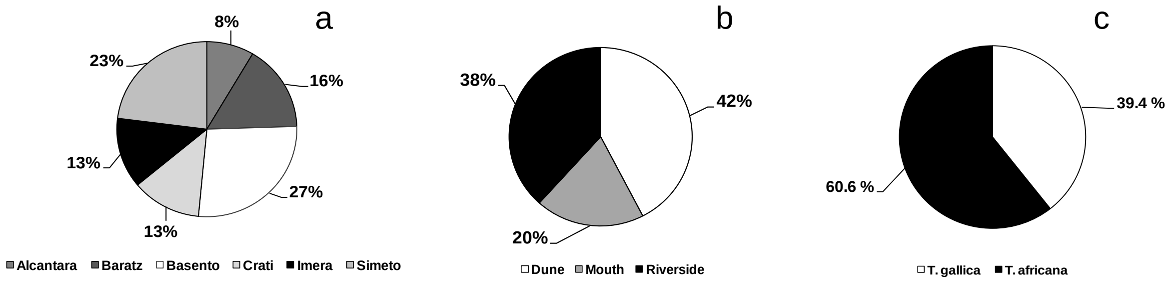

Fig. 4 Percentage of plants in the collection belonging to (a) the different populations, (b) the different environments and (c) the two main *Tamarix* species

Six months after the plantation, a census of the living plants was done (Fig. 5). The number of sprouts per plant, the dominant diameter and the dominant height were measured, and the dead plants were also marked. Baratz was the population with the highest percentage of dead plants, followed by Imera Meridionale and Alcantara. Particularly, mortality was higher in plants collected from the riversides, probably because most of them were *Tamarix africana* (*see Chapter 1*) which exhibited a higher mortality compared to *T. gallica*.

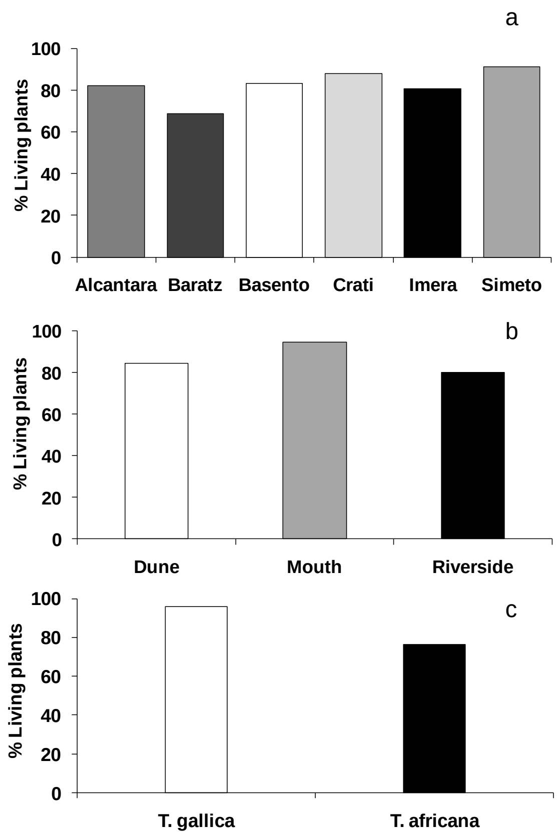

Fig. 5 Percentage on living plants found six months after the plantation belonging to (a) the different populations, (b) environments and (c) *Tamarix* species

The dead plants were replaced.

After one year from the first plantation, a second census was made. The number of sprouts per plant, the dominant diameter and the dominant height were measured. The formation of new sprouts was observed after one year from the plantation in plants collected from the dunes of rivers Basento, Crati and Simeto and from the mouths of rivers Basento and Simeto (Fig. 6). With the exception of *Tamarix* collected from Alcantara dune and Simeto riverside, all the plants were characterised by a higher dominant diameter (D_d) in 2010 (Fig. 6). Moreover, the dominant height was significantly higher in plants collected in the Basento three different environments, in the dune of Crati and in the riverside of Imera Meridionale, after one year from the first planting (Fig. 6).

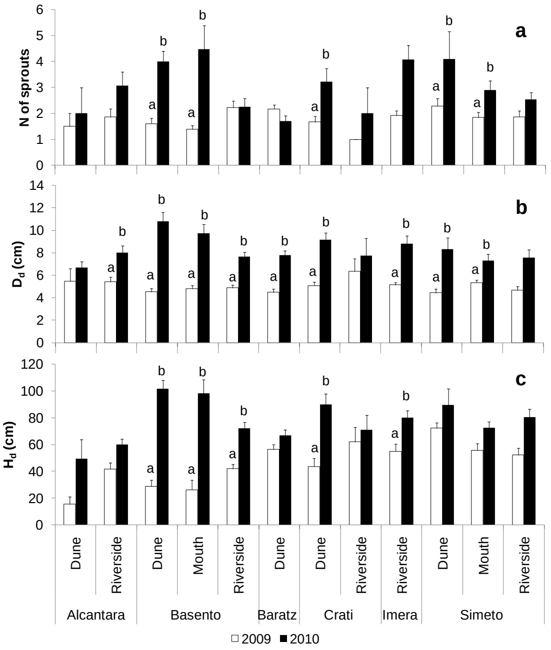

Fig. 6 Number of sprouts, dominant diameter (D_d) and dominant height (H_d) measured in 2009 after one month from the plantation and in 2010 after one year from the plantation, in plants collected from the different populations and environments

In November 2010, the plants present at the University of Tuscia’s Farm “Nello Lupori” were coppiced and new cuttings were produced. The cuttings, on average 8 per genotype, were planted in 10.5×10.5×22 cm pots containing sand (35%) and loamy soil (65%) and are now available for a field common garden plantation at Tuscania. The genotypes performances will be evaluated under the same climatic and edaphic conditions.

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General conclusions

Six natural populations of *Tamarix* spp. were found in river basins of Southern Italy. These populations were selected from three different environments: the dune, the mouth and the riverside. The three environments were chosen expecting an increasing salinity gradient from the riverside to the dune. However, this was not always true. Moreover, salinity differed among sites occupying the same environment. Accordingly, also water availability (precipitation, permeability of the substrate) did differ among sites and seasons, determining drought, normal, or flooding conditions, or an alternation of them. Populations and plants structure significantly differed according to these conditions. Particularly, plants growth and dimensions seemed to be lower in dune stands, where a high soil or water NaCl concentration can limit plants photosynthesis and energy production, as also demonstrated by the gas exchanges experiment. Among the populations distributed in the same environment, the highest plants growth and age were related to lower soil NaCl salinity and/or to adequate and constant fresh water availability. Moreover, although *Tamarix gallica* seems to be more frequent in the dune environment and more tolerant to salinity compared to *Tamarix africana*, probably as a result of its capacity to extrude more salt at increasing NaCl concentration, its gas exchanges capacity is also reduced under high saline conditions (200-400 mM), which could occur in particularly dry periods (summer) leading to an increased salt concentration in the soil solution. Salinity also seems to differently affect provenances, although the analysed plant material was collected from sites characterised by a similar salinity. Moreover, salinity tolerance depends on leaf age, older leaves being characterised by a higher tolerance compared to young leaves.

Gas exchanges reduction under saline conditions (200 mM) is primary due to non stomatal limitations, independently of flooding, and particularly to a decreased RuBisCO activity, probably caused by the increase in leaf salt accumulation over a tolerated threshold, which induces a reduction of the net assimilation rates. At higher salinity, photosystems damage is observed, coupled with an increase in salt extrusion, and with a reduction of stomatal conductance probably due to (1) a direct harmful effect of Na^+ on guard cells functioning, (2) a top-down regulation mechanism to prevent additional salt to be transport through the transpiration stream, (3) a signal transduction pathway which causes an increase in the cytosolic concentration of Ca^{2+}, acting in a similar way to an increase in abscisic acid, (4) the feed-back controls on stomata in response to the accumulation of intermediates or products of the Calvin cycle, (5) an increase in intercellular CO_2 concentration as a result of photosynthesis inhibition, (6) a lower efficient in water transport of the vessels characterised by a higher cavitation safety, (7) an hygroscopic salt effect which may trap the water vapour lost by transpiration, leading to an alteration of the measure, or (8) a physical obstruction of stomata by salt particles accumulation, which reduces gas exchanges.

Tamarix spp. are very tolerant to continuous flooding. Particularly, photosynthesis did not decrease in plants subjected to this treatment. This tolerance is the result of adventitious roots emissions, which enable gases (particularly oxygen) to be transport to/from the roots from/to the atmosphere. Thus, complete or partial continuous soil submergence expected in the future years could probably not affect *Tamarix* growth.

These results, coupled with the high rates of transpiration due to a constant stomatal aperture, even during the night, suggest a positive role of *Tamarix* spp. in the restoration of areas which could be affected by flooding, where these species are already naturally distributed. *Tamarix* spp. could also be used for the desalinisation of salinized areas, as about 90% of the salt present in the soil solution is extruded through the leaf salt glands. However, the desalinisation capacity of *Tamarix* spp. could be more rapid under non-flooded conditions, as flooding significantly reduced salt extrusion through salt glands, probably as a result of the reduced ions absorption by the adventitious roots; moreover, soil desiccation would also be reduced as salinity causes a decrease in stomatal conductance.