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CICLO XXIV

Adaptability of species of the genus *Atriplex* to different thermic regimes

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Abstract

A very effective tool to combat desertification is rivegetation. Promising species for this purpose are the evergreen shrubs of the genus *Atriplex*. The purpose of the research is to study the various physiological responses of *A. halimus* to thermic stress and to select clones resistant to low temperatures. The test was conducted in 4 sites in Sardinia characterized by different environmental conditions. In every site five clones of *A. halimus* were compared. After a period of acclimatization, the growth of plants in terms of linear growth of the biomass was measured. In the same periods, the fresh and dry biomass amount per plant were determined, as well as the content of the main macro and microelements. Correlations between the index of cumulated cold (ICC), which consists of the sum of the degrees of differences between the average daily air temperature and the thresholds of critical temperatures of 0, 5 and 10 °C, and the various parameters were analyzed. Differences among the five clones, with regard to the influence of low temperatures on plant growth, on the production of biomass, and on accumulation of macro and microelements were evaluated. Among the five clones tested, the clone GIO1 and SAN3 resulted more sensitive to temperature. The clones MAR1, PAL1 and FAN3 resulted less sensitive to low temperatures and also in the site characterized by the lowest minimum temperatures have shown greater adaptability, and thus a positive growth.

Riassunto

Uno dei mezzi di lotta più efficaci contro la desertificazione risulta essere la rivegetazione, a questo fine risultano essere molto promettenti gli arbusti sempreverdi del genere *Atriplex.* L'obiettivo della ricerca è stato quello di studiare le differenti risposte fisiologiche di alcuni cloni di *A. halimus* allo stress termico (in particolare alle basse temperature) e la selezione di cloni resistenti alle basse temperature.

La prova è stata condotta in 4 siti della Sardegna caratterizzati da condizioni ambientali differenti. In ogni sito sono stati posti a confronto 5 cloni di *A. halimus*. In particolare dopo un periodo di acclimatazione, sia all'inizio della stagione fredda che nel periodo primaverile è stato valutato l'accrescimento delle piante in termini di accrescimento lineare della biomassa. Nello stesso periodo sono state effettuate le analisi fisiche della biomassa fresca e secca e su quest'ultima è stata determinata la quantità dei principali macro e microelementi. Per la valutazione dell'influenza delle temperature sull'accrescimento e sullo stato nutrizionale dei diversi cloni sono state valutate le correlazioni esistenti tra l'indice di freddo cumulato (che consiste nella sommatoria dei gradi di differenza tra le soglie critiche di temperatura di 0, 5 e 10 °C e la temperatura media giornaliera dell'aria) e i diversi parametri analizzati.

Dai risultati sono emerse delle differenze tra i cinque cloni per quanto riguarda l'influenza delle basse temperature sull'accrescimento, sulla produzione di biomassa e sull'accumulo di macro e microelementi. Alcuni cloni sono risultati sensibili più di altri e questo conferma l'elevata variabilità intraspecifica della specie *A. halimus*. Nel clone GIO1, le basse temperature hanno portato a una riduzione e a una regressione nell'accrescimento della vegetazione. Tra i cinque cloni analizzati, il clone GIO1 e il clone SAN3 sono risultati i cloni più sensibili alla temperatura. I cloni MAR1, PAL1 e FAN3 sono risultati meno sensibili alle basse temperature e anche nel sito caratterizzato dalle temperature minime più basse hanno mostrato maggiore adattabilità e quindi una crescita mediamente positiva.

1. INTRODUCTION

1.1. The desertification

1.1.1. The desertification: definitions and diffusion

The desertification is one of the international environmental problems whose global importance has been recognized by the international community. This importance is clearly visible from the mass participation that the Nations have provided with the adoption in 1994 of the United Nations Convention to Combat Desertification (UNCCD, 1994) in countries experiencing serious drought and/or desertification, particularly in Africa.

The issue of desertification has received and continues to receive much attention. The United Nations General Assembly declared 2006 the International Year of Deserts and Desertification to spread the awareness of the world's deserts and the problem of desertification.

Over a hundred formal definitions of desertification have been proposed, covering many spatial and temporal scales and representing different viewpoints (Thomas, 1997; Reynolds and Stafford Smith, 2002).

Often the term desertification was associated with the advance of deserts, and then the indicators studied were able to measure the relative movements of climatic and vegetation zones using biophysical and socio-economic parameters (APAT, 2006). Later it became clear that desertification not did coincided with the advance of the desert but was a phenomena caused by very different processes, which may lead to reduction of the surface layer of the soil and the loss of its productive capacity.

The first United Nations Conference on Desertification held in Nairobi in 1977, adopted the definition of desertification as "the reduction of the biological potential of land that can lead to desert conditions" that regardless of the geographic location of the affected regions (polar, tropical), their climatic characteristics, the cause (natural or anthropogenic) and processes (salinization, erosion, deforestation, etc..), that originated the biological degradation potential of the soil.

Later, in Paris in 1994, the desertification was defined, by the UNCCD as "land degradation in arid, semiarid and dry sub-humid areas resulting from various factors, including climatic variations and human activities". Land degradation is in turn defined as the reduction or loss of the biological or economic productivity of drylands. The general concept of degradation of the lands, which is related to the decrease of one or more qualities of the soil, must be distinguished from that of desertification, which is a particular type of degradation of lands in a specific area and climate. Desertification involves essentially irreversible loss of the possibility of economically or ecologically sustainable agricultural and forestry production (Costantini *et al*., 2007).

In the context of a sustainable development the term drylands excludes hyper-arid areas (deserts). When land degradation occurs in the world's drylands, it often creates desert-like conditions. But what are dryland? In environmental terms, drylands are characterized by low, infrequent, irregular and unpredictable precipitations, by large variations between day and night-time temperatures, by soil containing little organic matter, and a lack of water; and plants and animals adapted to climatic variables (drought-resistant, salt-tolerant, heat-resistant, and resistant to lack of water).

The UNCCD, although excluding the hyper-arid dryland from its consideration, adopted the classification presented in the *World Atlas,* which is based on a global coverage of mean annual precipitation and temperature data collected between 1951 and 1980. The temperature data, together with the average number of daylight hours by month, were used to obtain a global coverage of corrected Thornthwaite's potential evapotranspiration values (Middleton and Thomas, 1997). Aridity index values lower than 1 indicate an annual moisture deficit, and the *World Atlas* defined drylands as areas whit $AI \leq 0.65$ that are areas in which annual mean potential evapotranspiration is at least ~ 1.5 greater than annual mean precipitations (MEA, 2005a).

Drylands cover about 41% of Earth's land surface and are inhabited by more than 2 billion people (about 33% of world population). Dryland populations on average lag far behind the rest of the world on human well-being and development indicators.

Desertification affects the livelihoods of rural populations of drylands in particular the people who depend on livestock, crops, water resources, and limited wood for fuel. These areas are characterized by the presence of ecosystems particularly vulnerable to inadequate land use and uncontrolled exploitation of water resources. Poverty, political

instability, irrational irrigation practices, deforestation, improper and excessive grazing can affect the productivity of these lands.

Existing water shortages in drylands are projected to increase over time due to population increase, land cover change, and global climate change.

From 1960 to 2000, global use of fresh water (drylands included) expanded at a mean rate of 25% per decade. This increased water stress will lead to reduced productivity of croplands and availability of fresh water, resulting in further adverse impacts on human wellbeing in drylands. There is a high degree of certainty that global climate change, land use developments, and land cover changes will lead to an accelerated decline in water availability and biological production in drylands (MEA, 2005b).

The high economic and social importance of natural resources, agriculture and animal husbandry means that in many countries the term "combating desertification" and "development" are the same (UNCCD).

Drylands support the 50% of livestock worldwide and are among the most important natural habitats. Because of unfavourable climatic conditions that characterize these areas, arid lands have given rise to an incredible variety of highly specialized species, and are very important for the conservation of biodiversity.

1.1.2. Causes of desertification

The concept of desertification has evolved over time in an attempt to define a process of global importance and the reasons for this. There is a great deal of debate among scientists as to whether the causes of desertification should be sought in the socio-economic or the biophysical sphere. These discrepancies were immediately evident and for this reason, to date, there are over a hundred different definitions of desertification.

However, most authors (Turner *et al*., 1995; Puigdefábregas, 1998; Geist and Lambin, 2004) agree that there is not one single factor that causes desertification or land degradation. Both biophysical and socio-economic factors should be considered, even jointly, as they interact and reinforce each other to induce transition trigger events (Turner *et al*., 1995; Puigdefábregas, 1998).

Le Houérou (1996a) says that desertification is mainly caused by the abuse of man in the area but that the adverse weather conditions can trigger or accelerate the phenomenon. The same conclusions have been confirmed by numerous other studies of

various authors in relation to different areas of the world. In fact, it was confirmed that desertification can also occur in the absence of drought and only by the soil abuse.

The causes of desertification can be divided into two groups: direct causes and indirect causes. The direct causes of land degradation and desertification resulting from the reduction and destruction of perennial vegetation cover, able to protect soil from the erosive action of atmospheric agents such as the action of the wind and the beating action of rain and runoff from this. In addition, the vegetation protects the soil from compaction caused by trampling of livestock. The absence of vegetation cover promotes evaporation of water from the soil and consequent accumulation of salts thus causing salinization. The causes are mainly (Le Houérou, 1996b):

- the reduction of the organic content of the soil due to a reduction of the biomass and a permanent reduction of waste products; following the reduction of the content of organic substance there is reduced the stability of the aggregates of the soil and therefore the structure of this becomes more fragile and prone to destruction;
- unstable and poorly developed structure translates into a greater bulk density of the soil (compaction), lower porosity, low permeability to air and water; lower water storage and reduced oxygenation;
- low permeability of the soil, recruitment and retention of water result in an increase of edaphic dryness and consequently a reduced productivity;
- low organic matter content and delicate texture of the soil leading to crusting of soil surface that may increase the flow of water by more than 30-50%;
- small amount of organic matter results in poor biological activity of the micro, meso and macroflora and wildlife, this reduces the turnover of items, and then the fertility and productivity of the soil.
- permeability of the soil could be reduced by the formation of a surface crust of cyan bacteria, lichens and mosses, this would lead to an increase in surface runoff and thus erosion (Verrecchia *et al*., 1995);
- destruction or reduction of perennial plant cover decreases the roughness of the landscape causing an increase in wind speed at the soil surface.

Indirect causes are constituted by human activities that reduce or destroy the vegetation cover resulting in the denudation of the land for long periods of time and thus triggering erosion both by water and wind. The causes are the same all over the world regardless of the type of climate and are generated by the increasing pressure on land due

to the exponential growth of population and livestock, without the adoption of appropriate management practices. The main causes are the overexploitation, overgrazing, the accumulation of salts in the soil due to poor drainage, excessive and uncontrolled harvesting of wood and deforestation. Land tenure, population density, cultural traditions and other socio-economic characteristics play a key role in determining the kind of cause. The collection of wood, for example, is very important in many parts of the dry lands in developing countries in Asia and Africa, where the firewood is often 90% of the energy consumption of the families of rural people (Le Houérou, 1996b).

In particular, from the point of view of natural phenomena (like climate) that characterize the soil degradation there are aridity, drought and rainfall erosion. Upon variation of one of these factors varies the intensity with which the factors of desertification occur (Gambarelli *et al.*, 2007). Arid climate is a characteristic determined by the simultaneous scarcity of rainfall and high evaporation (APAT, 2006).

Drought is a meteorological phenomenon complicated by several issues, including the duration of special importance events, its extension in various geographical areas, the entity, and its influence on human activities, in particular on agricultural practices (Morgillo *et al.*, 2002). It is a phenomenon that occurs when rainfall is below normal recorded levels, causing serious hydrological imbalances. It can affect areas not dry and affects land degradation bringing damage to agricultural and livestock production activities. Natural ecosystems have in fact, generally, the necessary resilience to overcome periods of drought. The drought in arid areas can break the delicate balance between environmental resources and productive activities leading food crises, abandonment of territories and even migration and conflict. The erosion by rain is due to the intensity of rainfall. When short and intense rain falls on sloping land without vegetation, runoff generated erodes the soil from the top layer richer in organic matter. Other natural factors that can trigger degradation are the morphology and topography of the area, in fact, slope and aspect of an area combine to determine the vulnerability of the area to erosion of hydro-meteors (APAT, 2006). As mentioned above the degree of vegetation cover is a natural factor critical to the protection of the soil. In the absence of vegetation cover there is the reduction of the system ability to maintain a sufficient quantity of water at the disposal of the biological activity, thus leading to an environmental stress that can trigger processes of desertification. Desertification can therefore be qualified as physical or chemical, depending on the processes involved.

Physical degradation occurs on slopes and is very diffuse. The dominant physical process is accelerated soil erosion that occurs on marginal lands that have lost more than 60% of the vegetation cover (Thornes, 1988) and which are located within the semi-arid and sub-humid regions.

Chemical desertification is the secondary salinization of soils due to the irrational management and use of water (Nicholas *et al.*, 2001).

The main effects of desertification recognized in the literature results in a decrease in the fertility of the soil, its water retention capacity, and productivity of vegetation, with a consequent reduction in crop agriculture, yield of livestock, forest biomass and biodiversity of vegetation. These effects, resulting in land use practices increasingly unsustainable, may in turn exacerbate the desertification process.

When strong erosion can lead to the onset of desertification, thise may be reversible or irreversible. Desertification is reversible when soil moisture falls below the tolerance level of the economic and environmental value of the plants, but the depth of cultivation of the soil has not been reduced below the critical thresholds. The phenomenon may be reversible when overgrazing has encouraged the spread in the territory of plants of low economic and environmental value.

The irreversibility is arbitrarily understood as the inability of a degraded system to recover its original state after a period of 25 years of total protection: a human generation (Floret and Pontanier, 1982). Desertification is an irreversible end-stage accelerated erosion, when this has reduced the cultivation layer and the water storage capacity of the soil below the tolerance level of the economic and environmental value of the plants (Nicholas *et al*., 2001). Irreversible situations exist and have been documented in various reports and publications.

The irreversibility is of course more frequent when the environment is dry and the soil is thin (Le Houerou, 1968; Floret *et al.*, 1986, 1995; Grainger, 1990).

1.1.3. Climate change and desertification

Climate change is one of the key challenges of our generation. The term climate change, using the IPCC (Intergovernmental Panel on Climate Change), refers to any change in climate over time, due both to natural variability and as a result of human activity. This is different from the definition of the United Nations Framework Convention on Climate Change (UNFCCC), where climate change means for every change of climate,

which is attributed directly or indirectly to human activity and that alters the composition of the global atmosphere, and which is in addition to the natural climate variability observed over a period of comparable time.

In 1980, the World Meteorological Organization organized the first conference on climate change in which early concerns about the changes to the Earth's atmosphere caused by increased emissions of certain gases, most notably carbon dioxide, originating from the combustion, methane, nitrogen oxides and chlorofluorocarbons, and the potential effect on the heat balance of the Earth were raised. The atmospheric concentration of carbon dioxide in 2005 greatly exceeded the natural range of the last 650.000 years (180 to 300 ppm) as determined from the analysis of ice cores representing many thousands of years. The global atmospheric concentrations of carbon dioxide, methane and nitrous oxide have substantially increased as a result of human activities since 1750 and now exceed pre-industrial levels (IPCC, 2007). The global increase in carbon dioxide concentration is mainly due to the use of fossil fuels and changes in land use, while increases in methane and nitrous oxide are primarily due to agriculture.

An experimental study from all continents and most oceans shows that many natural systems are affected by regional climate changes, particularly temperature increases. Currently, the air temperature at the surface of the planet has increased by 0.3-0.6 °C in the last century and the effects of global warming are increasingly evident as retreating glaciers in the Alpine regions, the reduction of the total mass of polar ice, water heating of sub-surface of the seas.

We can see, in fact, an enlargement and increase in the number of glacial lakes, increasing the instability of the ground in regions with permafrost, avalanches of rock types in the mountainous regions, changes in some Arctic and Antarctic ecosystems, including sea-ice biomes and predators in the upper levels of the food chain.

Additional effects are occurring on hydrological and terrestrial biological systems. With regard to the first there is an increased run-off and earlier spring peak discharge in many rivers fed by glaciers and snowfields, warming of lakes and rivers in many regions, with effects on thermal structure and water quality. According to projections, the average annual run-off of rivers and water availability, in mid-century, will increase of 10-40% at high latitudes and in some wet tropical areas, while they will decrease by 10-30% in some dry regions at medium altitudes and in dry tropics. Some of these areas are already subject to dryness.

In the course of the next century, water supplies stored in glaciers and snow cover will decrease, reducing the amount of water available in the regions that benefit from the melt water from major mountain ranges, regions where he now lives more than a sixth of the world population. The extent of the areas affected by drought is likely to increase.

As for terrestrial biological systems, global warming affects the advance of spring events, such as flowering, bird migration and egg-laying, movement toward the pole and into the high latitudes of plant and animal species.

The recent climate changes are beginning to have an effect on many other natural and human systems. Projections indicate that in many African countries and regions agricultural production, including access to food, can be seriously affected by climate change and variability. It is expected a decrease in the available areas for agriculture, in the length of the growing season and the harvest potential, especially in marginal areas of arid or semi-arid territories. This would adversely affect food security and worsen malnutrition in Africa. In some countries, agricultural yields are highly dependent on rainfall and could be reduced by up to 50% in 2020.

There is a strong and clear links between desertification and climate change, these in fact share common causes and have points in common in terms of adaptation strategies. Whereas it has been studied a correlation between the development of industrialized countries and climate change, a synergistic relationship has also been observed in developing countries between poverty and desertification. The populations in these areas are forced to invade fragile ecosystems in order to meet their basic needs (OECD, 2002). We can therefore say that desertification is exacerbated by climate change and *vice-versa*. Climate change threatens marginal lands, increasing the risk of degradation and desertification. In addition, land degradation, in particular that due to unsustainable management practices in agricultural land and deforestation, is a very important factor as it is responsible for the increase of greenhouse gas concentrations in the atmosphere that is responsible for climate change induced by man. An increase in extreme weather events such as droughts and heavy rains, caused by global warming, leads to further degradation of the soil and the process of desertification finally affects the climate. Some experts predict that by 2100, the climate zone of the regions at mid-latitudes will move 150-500 km at the poles and because of melting glaciers and thermal expansion of sea, moreover sea level rise of 65 cm or more threatening coastal areas (GTZ, 2002).

In the third IPCC report (2001), was put forth that the desertification involves impacts on climate, by increasing the surface temperature through changes in vegetation, or because of the effects of the change in the power of carbon absorption or emission of methane in the desertified areas. Therefore, the desertification was seen more as a result of climate change than an effect.

The IPCC fourth Assessment Report (4AR) finally indicates that climate change and human pressure related to the use of the land will likely have a synergistic impacts on ecosystems and on the species of desert areas that may be at least partially offset by benefits in terms of productivity of vegetation and carbon sequestration due to increased CO_2 in the atmosphere.

The arid zones, in fact, can play an important role in mitigation through carbon sequestration in the soil. Although the arid zones have a low potential for sequestration of carbon per unit area, their great extension makes them important. This creates risks and opportunities for climate change. While soil degradation favours the emission of greenhouse gases, soil restoration prevents such emissions and even creates storage capacity due to the greenhouse gases already in the atmosphere.

1.1.4. Desertification in Italy

Italy and European countries are not immune to the phenomenon of desertification, the widespread presence of physical factors predisposing with excessive human pressure on natural resources caused the onset of degradation. In the Atlas of National areas at risk of desertification it can be seen as 20% of the area is at risk of desertification. The arid, semi-arid and dry sub-humid areas, which may turn on degraded areas, currently affected 47% of Sicily, 31.2% of Sardinia, 60% of Puglia, and 54% of Basilicata (Ferrara, 2003). Furthermore, a recent worsening of the risk profile in almost all Italian regions was observed. Soil degradation mostly encountered in these areas is represented by erosion and salinization of the land, unsustainable management of water resources and forests, frequency and extent of forest fires and urbanization.

Guidelines of the National Action Programme (PAN) to combat drought and desertification indicates that the causes of desertification in Italy are mainly attributable to human activities and only a small part to the phenomena of natural origin.

Looking at the climate and hydro geological data of the last 200 years, the National Research Council identifies several factors and areas that already show variations that can

be attributed to climate change. According to these data, the average temperature in Italy increased by 1.7 °C above pre-industrial levels: increase more concentrated over the last 50 years. The growth rate of average temperatures in Italy is almost twice the rate recorded for the global average. Over the past 50 years, the maximum temperatures have increased more than the minimum thus generating a greater daily temperature range. A summer heat wave significantly increased, as well as cold spells in winter decreased (Ferrara and Farruggia, 2007).

The Convention to Combat Drought and/or Desertification was ratified by Italy with Law no. 170 of 4 June 1997. The Convention provides that countries affected by desertification put in place and implement National and Regional Action Plans aimed at sustainable development, with the aim of reducing losses productivity of soils caused by climate change and human activities. For the implementation of the Convention, Italian government established the National Committee to combat desertification chaired and coordinated by the Ministry of Environment, in particular with the aim of following the preparation of the National Action Plan in the context of the Mediterranean basin.

Even the Autonomous Region of Sardinia has been active preparing the regional Programme for the fight against desertification. The Technical Regional Secretariat, in 2000, drew up a preliminary programme to define the main priorities with a preliminary individuation on the land of the epicentres of desertification risk based on information provided by technical unities of local administration. The ERSAT (Regional Agency for Development and Technical Assistance in Agriculture), now replaced by LAORE, has developed a programme of action and monitoring in collaboration with the meteorological services of the Region of Sardinia. This action is particularly aimed at "Realization of geographic information system for the detection and monitoring of sensitive areas to desertification in Sardinia".

1.1.5. Combat desertification

Desertification can be prevented and mitigated by appropriate policies to reduce vulnerability, mitigation of the causes, adaptation and implementation of measures affecting its causes and its effects. The knowledge of the environmental, social and economic causes is a prerequisite to the adoption of the best strategies to reduce impacts and remove the effects promoting actions based on environmental, social and economic strategies.

Prevention is the most effective weapon against desertification, in fact many attempts to rehabilitate drylands are very expensive and tend to deliver limited results. The assessment of future scenarios shows that in order to overcome the challenges of desertification will require action and changes in ecosystem management, and such interventions must be implemented both on a local and global scale (MEA, 2005b).

The fight against desertification is essential to reduce or eliminate extreme poverty and hunger, in fact, 50% of the world's people living below the poverty line lives in arid areas. The creation of a culture of prevention can reduce the onset of desertification. The integrated management of water and land are the main methods of prevention. We can say that all measures that protect the soil from the various forms of degradation, erosion and salinization, prevent desertification.

As mentioned previously the United Nations Convention to Combat Desertification is a universal agreement to promote a comprehensive response to desertification. The main strategic objectives that will guide the actions of all UNCCD partners are 4:

- to improve the living conditions of affected populations;
- improving the conditions of the ecosystems concerned;
- to generate global benefits through effective implementation of the UNCCD;
- to mobilize resources to support the implementation of the Convention through building effective partnerships.

1.2. The importance of plant resources to combat desertification

1.2.1. The role of plants

Plants play a vital role in environmental restoration of degraded areas. These in fact perform many functions. The most important are the stabilization of surfaces with consequent minimization of water and wind erosion. Proper maintenance of vegetation cover can prevent, during dry periods, the loss of ecosystem services.

The loss of vegetation cover due to overgrazing or as a result of mining and over-consumption of timber can cause a decrease in precipitation. The reduction in the amount of precipitation is associated with a lower evapotranspiration due to the absence of the plants and to a consequent increase of the albedo (MEA, 2005b). In some studies (Charney, 1975) it is assumed that the variations of albedo due to an excessive grazing and the consequent stripping of the soil are reflected on a reduction in precipitation. Charney (1975) in his study suggested an increase in albedo in arid regions. The result of this increase would be a reduction in rainfall and a southward shift of the ITCZ (The Intertropical Convergence Zone). Other studies have concluded that there is a positive feedback between albedo and desertification.

The degradation of the vegetation cover decreases the ability of carbon sequestration in arid environments, thus increasing emissions of carbon dioxide in the atmosphere. Unfortunately, there are few studies that document the storage capacity of the carbon in arid zones, indeed it seems that this is an underestimate regarding to the capacity of the typical species of the arid zones to develop a high amount of biomass below the surface of soil. In the Sahel, for example, it has been shown that the trees were developed mainly below the soil surface. The quantity of biomass underground was largely higher than that of canopy, in fact, the roots may extend to a distance of 70 m from the trunk and 30 m deep (Jonsson, 1995).

1.2.2. Restoration and environmental rehabilitation

Among the numerous measures to combat desertification ecological restoration plays an important role. Ecological restoration of degraded areas is one of the objectives of the Convention to Combat Desertification. The ecologist attention to restoration actions to

combat desertification has been relatively limited. In fact, as reported in the database of SERI (Society for Ecological Restoration International) projects involving ecological restoration of arid and semi-arid Mediterranean areas amounted to 22 out of a total of 226 projects. Ecological restoration can be defined in different ways (Cortina *et al*., 2011). The definition given by the SER defines restoration as "the intentional alteration of a site to establish a defined indigenous, historic ecosystem". The objective is therefore to emulate the structure, operation and dynamics of a specific ecosystem. The restoration in the strict sense made attempts to restore the ecosystem through the introduction of species from the indigenous communities of the previously existing ecosystem. Through the restoration "in a broad sense" instead we try to stop the degradation of the ecosystem and to redirect it to a trajectory similar to the one that presumably would have prevailed before the onset of the disorder. In any case, the goal of the two types of restoration is the conservation of biodiversity, structure and dynamics of ecosystems (Aronson *et al.*, 1993). According to Le Houèrou (2000) restoration includes two different processes: exclusion and afforestation - reafforestation. Exclusion, means that the area under consideration is protected from humans and livestock intrusion, usually by fencing. Alternatively to the exclusion there is another procedure of defence is the controlled access, which in many cases can bring more benefits than exclusion. Reafforestation is the planting of native species on lands that shortly before were forests. Afforestation is the planting of trees and shrubs, regardless of whether they are native or exotic, in soils that recently were not occupied by forest.

Another type of intervention of recovery is the rehabilitation, which concerns the artificial creation of a new type of vegetation, different from native, which was degraded or destroyed (Le Houerou, 2000). Rehabilitation attempts to repair the damaged ecosystem functions with the aim to improve productivity for the benefit of the local population. Such changes are made in the shortest time as possible (Aronson *et al*., 1993).

This is usually achieved through the planting of exotic or sub-native species: trees, shrubs, and herbs. The species most commonly used are the Eucalyptus trees, *Pinus radiata*, Acacia, some salt-tolerant shrubs such as *Atriplex nummularia*, *A. canescens*, *A. lentiformis*, *A. Amnicola*, *A. undulata*, some Cactus as *Opuntia ficus-indica* f. *inermis* and *O. amyclaea*, *Agave americana*, and many other species. What we call rehabilitation was often referred to the term reclamation. But reclamation was also used as a synonym for both types of restoration and for some examples of what we call reallocation (Aronson *et al*., 1993).

The latter is a general term and need to describe what happens when a part of a landscape, in any state, is given to a new use. The large plantations of fodder shrubs in North Africa, such as *Atriplex*, *Acacia* and *Opuntia* spp., are also examples of reallocation. In contrast to the restoration and rehabilitation, reassignment or reallocation plays a permanent role, needs managerial people, and usually requires ongoing subsidies, in the form of energy, water and fertilizer.

The use of agroforestry techniques, such as the planting of trees or shrubs fodder, can provide a certain amount of feed to be used in periods of drought, and therefore more unfavourable, and has a high influence on pastoralism and in particular on nomadism. In fact, these plantations are planted in strategic points, and farmers can use, in the period of high drought, the forage produced by such plantations, without leaving their home country. This strategy is a kind of insurance against drought (Le Houerou, 1994).

The success of ecological restoration projects often is uncertain and depends on many factors. The failure of many actions of recovery of degraded areas, in part, is due to the failure and failure of monitoring that was not considered fundamental parts of management programs to combat desertification. Often, the various actions and their objectives were defined on the basis of one or a few objectives (establishment of tree cover, increased biodiversity, introduction of a species), regardless of their impact on the supply of other goods or services. Their goals were usually defined on the basis of one or a few targets, ignoring their impact on the provision of other goods and services (Cortina *et al*., 2011).

For example, information on the effects of conifer plantations on biodiversity, water and carbon balance or aesthetics is often poor, despite the significance of these plantations in arid areas of the world (Maestre and Cortina, 2004; Pausas *et al* ., 2004). In recent decades, this lack of information has fuelled fierce debate about the benefits of these interventions. In Sardinia, which according to many studies is an area at high risk of desertification (Costantini *et al*., 2004), from 1970 to present, more than 40,000 ha of land have been replanted with conifers generally Mediterranean, which required the use of significant human and financial resources, and induced changes in the landscape of entire areas, due to the elimination of natural vegetation and operations of land preparation sometimes triggering phenomena of soil degradation (Bianchi *et al*., 2005).

Often failed recovery surgery is due to wrong choices during the design or errors at the time of the plant species to be introduced for the recovery of an area. Other times the

failure is caused by the failure or mismanagement of the plantations immediately after implantation or during its course of production of this.

1.2.3. Biodiversity

Land degradation or desertification is regarded as a vector of reduced biodiversity (Jauffret, 2001). The United Nations Convention on Biological Diversity defines biodiversity as "the variability among living organisms from all sources including, terrestrial, marine and other aquatic ecosystems and the ecological complexes of which they are part, this includes diversity within species, between species and of ecosystems". Among the indicators of desertification and degradation of vegetation is therefore the diversity of species. According to the MEA (2005a), the components of biological diversity directly affect the ecosystem services of drylands.

The UNCBD recognizes the knowledge of indigenous communities as an important element for the conservation of biodiversity. Knowledge of biodiversity, of species and their ecological and social functions are threatened by the advance of desertification. In arid regions biodiversity is relatively unknown. In fact, drylands are more known for their genetic diversity within a species, the variety and number of their species. It is impossible to confirm the correlation between the rate of degradation of drylands and the rate of extinction of species due to lack of data on the distribution of endemic species (Bonkoungou, 2001). Many plant and animal species indigenous to many arid areas affected by desertification also contain chemical molecules with potential use for industry or healthcare.

The best way to ensure this biological capital for the benefit of humanity consists of *in situ* conservation projects. Most people would agree on this, but storage is expensive, especially in areas where the pressure on land is high due to the high population growth. The only solution seems to lie in partnerships and funding from industrialized countries (Le Houérou, 1996b).

In arid areas, different stress affects biodiversity and help to determine the selection processes of different species. Within each arid zone other factors such as topography, geology, presence of water and nutrients in the soil can also affect the distribution of living organisms.

The seasonal pattern of rainfall in dryland, highly variable in time and space, makes organisms (plant and animal) able to complete in a short time their life cycle. Plant species,

or more precisely the plant species used to combat desertification, may be used as soil protection and as a source of supply for livestock, and they play a fundamental role with regard to biodiversity. The plants in fact serve as home to many species of wildlife and habitats of many insects and micro organisms. Also the closeness of the plants, it retains more moisture, because the foliage of plants repairs the soil by direct solar radiation, thus minimizing the losses of water for direct evaporation from the soil. The retention of a minimum of moisture can promote the development of other herbaceous species native to the area. The presence of plants, depending on the species used, may also affect the allocation of nutrients in the soil. Many clovers containing, for example, bacteria in the roots can fix nitrogen.

1.2.4. Abiotic stress

In areas at risk of desertification, most species are subjected to different abiotic stresses. These limit the productivity of crops and play an important role in determining the distribution of plant species in different natural environments. The concept of stress is closely associated with that of resistance to stress that is the way to adapt to the environments unfavourable to plants. The main abiotic stresses that characterize arid and semi-arid areas are water scarcity, soil salinity and temperature. A solution for the use of such soils is the cultivation of species that tolerate high levels of dryness and salinity and are able to tolerate extreme temperatures that occur in these areas.

The resistance to abiotic stress, defined as "plant's ability to survive, grow and produce in the presence of unfavourable factor" (Levitt, 1980), involves the evolution of specific mechanisms, which can be distinguished according to the following classification:
- *escape* (avoid adversity): the plant reacts to stress by changing its life cycle so as to avoid adverse environmental conditions (for example, plants early escape to summer drought ending its life cycle before the occurrence of such a dry season);
- *avoidance* (avoid stress): the plant reacts to stress by creating morphological or physiological barriers (plants close their stomata to prevent excessive transpiration during periods of drought);
- *tolerant* (tolerate stress): the plant activates mechanisms aimed at the molecular level to resist and adapt to stress or to repair damages caused by the stress.

Plants adapted to arid environments are called xerophytes. The loss of water by evaporation-transpiration is a major problem for the maintenance of homeostasis in plants.

Ecosystems and species in arid areas have developed unique strategies to cope with sporadic and low rainfall. In addition, following a disturbance, such as a fire, excessive grazing pressure and drought, they are able to withstand and recover quickly. These adaptive characteristics have significance in the world especially as anticipation of the climate changes.

These species have some adaptations that reduce water loss by evapo-transpiration, or allowing storing water in the tissues. Some species have a particular chemical mechanism that allows it to carry out photosynthesis keeping the stomata closed. The reduction in size of the leaves is the most immediate solution to drought, because decreasing the surface of transpiration is reduced the loss of water through the stomata.

Another adaptation is the presence on the surface of the leaves of dense hairs, which retains moisture. The water deficit also stimulates the abscission of leaves and increased root growth. The relationship between root and stem is governed by a functional balance between root absorption and photosynthesis of the shoot. Then a shoot will grow until it will be so great that the water absorption by the roots will limit its further growth, *vice-versa* the roots will grow until their request of carbohydrates from sprouts will be equal to their availability. If water availability decreases this budget is changed. In fact, if plants cut down the leaf expansion and consequently the consumption of carbon and energy, therefore, a greater proportion of the compounds assimilated by the plant can be distributed to the root system. All these factors lead to the preferential growth of the roots in areas that remain wet (Taiz and Zeiger, 2009).

The adaptations are not only about leaves and roots, but also affect the reproductive strategies of the plant. Many plant species that live in deserts, for example, are annual plants. These plants produce seeds that remain dormant and able to withstand prolonged periods of drought. The seeds are produced and dispersed during humid periods. With the onset of the dry season, the plant dies. The seed is, however, able to exceed the period of drought and germinates when the rains (even a simple temporal) make available the necessary water.

The salinity may limit the functions of the plant, growth and development processes. The deleterious effects of salinity on plant growth are associated to several factors: the low osmotic potential of the circulating solution (water stress), in nutritional imbalances, and the effect of specific ions (salt stress) or the combination of these factors (Shannon, 1998).

Some plants, however, have evolved various protective mechanisms that allow them to survive and grow in the harsh environmental conditions and respond to water stress through multiple physiological mechanisms. Plants growing in soils with high salinity are called halophytes. Some halophytes are known as regulators of salinity. Some salinity regulators do not absorb the salts but actively extrude them from the roots, others absorb the salts but they can expel large quantities of them through specialized salt glands. The salt excreted crystallizes on the surface of the leaves, where it is no longer harmful.

The accumulators of salts use a high absorption of ions to maintain the cellular turgor in conditions of lower water potential than that of the ground. Some species of *Atriplex,* for example, have very low water potential in the leaves, down to -2 MPa, compared with -0.2, -0.3 MPa of a non-halophyte. The excess ions build up in the vacuole, while the cytoplasmic concentrations of Na^+ ions and Cl^- are kept low.

The salt stress can produce many damages. The dissolved solutes in the root zone generate a low osmotic potential, which decreased the water potential of the soil. Since the leaves need to develop a more negative water potential to maintain a gradient of water potential in the "down" between the ground and the leaves, the water balance of the plant resulting disrupted.

Plants growing in saline environments can adjust osmotically. This adjustment prevents the loss of turgor while generating a lower potential that allows plants to access water in the soil solution for growth. The solutes that contribute to adaptation in halophytes and under water stress are the same osmotic solutes, including proline, glycine, betaine and sorbitol. Other adverse effects are those caused by the toxicity of some specific ions, especially Na^+ and Cl^- when they accumulate in cells at harmful concentrations. The excess of Na^+ may cause problems with the membranes, inhibition of enzymes or a general metabolic dysfunction. The enzymes isolated from halophytes are sensitive to the presence of NaCl as those of glicofite. So the halophytes do not have a metabolism that tolerate salt, and use other mechanisms to reduce the damage and facilitate metabolic functions. At elevated concentrations the Na^+ can move the Ca^+ from the plasma membrane resulting in changes in permeability which can be measured as loss of K^+ from the cells.

1.3. Plants of the genus *Atriplex*

1.3.1. Origin, distribution and species

The genus *Atriplex* contains about 260 species that are largely distributed in the arid and semi-arid regions of Europe, Asia, America, and Australia (Sukhorukov and Danin, 2009). Arrigoni in the first volume of the "Flora dell'Isola di Sardegna" in 2006, ranked the genus *Atriplex* in the family *Chenopodiaceae*, as done by Camarda in 1992 (Camarda and Valsecchi, 1992; Arrigoni, 2006).

Based on the phylogeny, classification APG II (Association plant genome) of 2003 does not include the taxonomic identity of the family, and divides the genders in most subfamilies (*Chenopodioideae, Gomphrenoideae*, *Salicornioideae*, and *Salsoideae*) included in the family of *Amaranthaceae*.

The *Chenopodiaceae* is especially widespread in the coastal regions of the Mediterranean Sea, the Caspian Sea and the Red Sea, in the arid steppes of Central Asia and Eastern Europe, on the edge of the Sahara desert, alkaline plains of the United States, in the Karoo in South Africa, in Australia and in the Pampas of Argentina (Mulas and Mulas, 2004). In general, plants of the genus *Atriplex* grow in saline or alkaline soils, and in arid, desert or semidesert environments.

According on the shape of the embryo, the genera of *Chenopodiaceae* are divided into *Spirolobeae* and *Cyclobeae*. The genus *Atriplex* belongs to *Cyclobeae* and contains the greatest number of species of the family. The embryo, in fact, is horse-shaped or semi-circular and includes in part or completely the endosperm, unlike the tribe *Spirolobeae* where the embryo is twisted spiral and the endosperm is divided into 2 parts from the embryo.

The *Chenopodiaceae* are mostly annual or perennial herbaceous plants but also shrubs and trees are included. Plants from Chenopodiaceae have highly developed roots to absorb water from the deeper layers, the leaves are simple, without stipules, alternate or opposite, sometimes very small in size and covered with hair or powder, sometimes thorny. The flowers are hermaphrodite or unisexual, actinomorphic, inconspicuous and small, grouped in inflorescences or solitary. The perianth is sepaloid with 3-5 petals welded at least at the base, sometimes missing. Androecium with 1-5 stamens opposite the perianth

lobes (Arrigoni, 2006). The one-celled ovary has three carpels and is connected to two stigmas. It produces only one ovule that matures as an achen (Rosas, 1989).

The genus *Atriplex* is the genus of the family of the *Chenopodiaceae* which includes the highest number of species. Species have a high polymorphism, to recognize, the most observed character is the morphology of the flower. Leaves are alternate, stalked or sessile, showing paper consistence. Internodes are often stretched, sometimes so reduced as to leave the leaves grouped. The anatomy leaf is of type Kranz, i.e. has a sheath of clorenchimatic cells largely surrounding vascular tissues.

Kranz anatomy is associated with the four-carbon (C4) photosynthetic pathway, which is a metabolism of high photosynthetic efficiency. In this metabolism, carbon dioxide is linked to phosphoenolpyruvate to generate oxaloacetate, a compound of four carbon atoms that originated the name of this metabolic cycle. This reaction occurs in the mesophyll tissue, where the oxalate is then reduced to malate. Subsequently, malate reaches the bundle-sheath cells surrounding the vascular bundles of the leaf, where it is decarboxylated to yield carbon dioxide and pyruvate. The free carbon dioxide then enters the Calvin cycle, while the pyruvate returns to the mesophyll cells, where it reacts with adenosine triphosphate (ATP) to form more molecules of phosphoenolpyruvate, to restart a new cycle.

Flowers are monoecious, solitary or in clusters in the axils or terminals. Staminate flowers have a 3-5-parted calyx and no bracts. Pistillate flowers are hard or cartilaginous, and protected by two separated or joined (at least in the base) bracts. Calyx of pistillate flowers is usually absent. The fruit is enclosed by the bracts and the pericarp is membranous. The seed is usually free, erect, and rarely horizontal, the perisperm is powdery, and the primary root may be basal, lateral or apical (Rosas, 1989).

The genus *Atriplex* L., in Sardinia is represented by 7 species which, more precisely, are: *A. halimus* L., *A. portulacoides* L., *A. prostata* L., *A. littoralis* L., *A. patula* L., *A. tornabenei* L., and *A. rosea* L.

Almost all species of the genus *Atriplex* are dioecious, but there are also some examples of monoecious species (Mulas and Mulas, 2004).

Species most used and the most widespread popular are the *Atriplex nummularia* and *Atriplex halimus*.

Atriplex nummularia

The *A. nummularia* is a species native from Australia. It is a dioecious species with bushy *habitus* that can reach 1.30 m in height, has a high production potential, if irrigated can reach more than 30 t DM/ha per year, with a salinity of 15-20 mS/cm measured in EC (Le Houerou, 1994), has high tolerance to drought, due to high water use efficiency (15-20 kg DM/ha per year per mm of rain (Le Houérou, 1994), for this reason it can be used in environments where the rainfall is less than 200 mm.

It has a high tolerance to salinity, withstands to the submersion also for long periods, the regrowth after grazing is fast and very abundant, also thanks to the characteristic of this species, which is able to produce epicormic buds (both dolycoblasts and brachyblasts depending on the season); the roots are very deep, up to 10 m deep, so as to take advantage of surface aquifers; the limit more consisting in the use of the species consists in the poor resistance to overgrazing.

Atriplex halimus

The *Atriplex halimus* is a perennial wild species of arid and semi-arid areas of the Mediterranean. It is native to North Africa and after *A. nummularia* is the largest species. the *A. halimus* in Europe is widespread in France, Spain, Portugal, Italy, and Greece. In Italy it is present in Liguria, Tuscany, Marche, Lazio, Abruzzo, Campania, Puglia, Basilicata, Calabria, Sardinia, and Sicily.

The *Atriplex halimus* L. is a branched shrub, silvery white, 1-3 m high, with shortly petiolate leaves, fleshy-leathery, oval, oval-triangular or rhombic, of 1.5-4 x 1-4 cm, 6x4 cm up to large, sometimes hastate, the upper lanceolate (Arrigoni, 2006).

The inflorescences are located at the apex of the branches, are 5-15 cm long, without leaves (Camarda and Valsecchi, 1992).

The flowers can be of different types. Numerous studies have been performed to evaluate and identify the different flower types present in *Atriplex halimus*. The studies carried out showed that there are six different types of flowers (Talamai *et al*., 2001, 2003). Flowers are arranged in long terminal panicles, gathered in small clusters that bring the female flowers at the base and at the top the male ones (Camarda and Valsecchi, 1992; Arrigoni, 2006).

The *A. halimus* has often been cited as a highly polymorphic species (Kinet *et al*., 1998; Ferchichi *et al*., 1997; Chalbi *et al*., 1997), probably due to its ecology, spreading, and to the allogamous reproduction dominance (Haddioui and Baaziz, 2001). However, so far, few studies have focused on the evaluation of this polymorphism (Abbad *et al*., 2004a).

There are two subspecies, subsp. *halimus* and subsp. *schweinfurhii*.

The distribution of the subspecies *halimus* goes from semi-arid to humid regions, from Morocco to the English Channel and is quite common on the coasts of the Mediterranean Basin. It has upright habitus, very short bearing branches, about 20 cm, covered with leaves. The subsp. s*chweinfurhii* is widespread in arid and desert conditions, but only in depressions where groundwater is present. This subspecies has tangled branches, fruit-bearing branches about 50 cm long and free of leaves (Mulas and Mulas, 2004).

The characteristics of the species allow it to colonize strongly anthropized and degraded areas. It is spontaneous near to ponds, brackish and coastal windswept areas. It is a heliophilous and halophilous species, according to the phytoclimatic classification of Pavari, is a part of hot sub-zone of Lauretum. The highest productivity, about 15-20 t/ha per year, may be obtained under a saline concentrations not exceeding 300 mmol/L of NaCl-equivalents (Mulas and Mulas, 2004).

1.3.2. Use of species of the genus Atriplex

The genus *Atriplex*, as mentioned previously, includes a large number of species, about 260. The high specific and inter-specific variability permits the use of these species in a variety of environments and for different purposes (Mulas and Dessena, 2010). There are numerous uses in both naturalistic and landscape fields, as well as in the livestock sector. The species of the genus *Atriplex* play a key role in combating desertification. Preferably growing in arid and semiarid regions of the world and having a few requirements from the nutritional point of view, *Atriplex* shrubs can be a useful tool in areas at risk of desertification (Mulas *et al.*, 2010a). For this reason, many plantations have been planted in Africa, Mexico, Australia and other parts of the world (Fig. 1). *Atriplex nummularia* is the most used species in Africa.

Fodder crops are essential for the development of animal production necessary to meet the needs of the population. The animal nutrition through the use of species of the

genus *Atriplex* is a feasible solution to minimize the problem of water scarcity typical of these areas. In fact, these shrubs are able to grow at moderate speeds and produce a good amount of biomass also in soils characterized by high levels of salinity.

Figure 1. Plantation of *A. nummularia* in Morocco (from above).

Figure 2. Plant of *A. nummularia* growing in Morocco subjected to intensive grazing.

Many studies have highlighted the high nutritional value of the *A. nummularia* forage mainly characterized by protein content comparable to that of alfalfa hay (Chiriyaa and Boulanouar, 2000).

After the feeding of livestock exclusively with *A. nummularia* was noted a decrease in weight of the animals, this decrease was due to the lower energy content of the *Atriplex* forage. For this reason it is preferable to use the *A. nummularia* as protein supplementation of diets based on the use of straw or roughage (Mulas and Mulas, 2004).

The addition of *A. nummularia*, up to 20%, to alfalfa hay does not produce negative effects on the weight of the kids. Above this percentage increase in weight is reduced and this could be due to excessive assumption of K and Na (Meneses *et al.*, 2012).

The high content of salts present in the edible parts of the plant forced the animals to drink often and the quantity of water ingested can be as high as 11 l/head/day (Le Houerou 1991; Mirreh, 2000). The large amount of water consumed is due to the need to eliminate, through the urine, the high percentage of accumulated salts. It is estimated that for every gram of NaCl ingested are needed 70-74 ml of water (Wilson *et al.*, 1969; Hassan *et al*., 1979).

The second most commonly used species after *A. nummularia* is *A. halimus*, capable of producing an excellent biomass for grazing that can satisfy a part of the nutrient needs of small ruminants during the shortage of forage (Wilson, 1977).

The biomass produced is rich in crude protein, an important source of nitrogen for livestock especially during the most difficult period (El-Shatnawi and Mohawesh, 2000; Choukr-Allah, 1991). *A. nummularia* has very satisfactory palatability (Chalbi *et al*., 1997) although lower than that of the *A. nummularia*. This species is capable of producing more than 4 tons of dry matter per hectare per year (Ben Ahmed *et al*., 1996). If the shrub is not grazed it can reach 4 m high.

With regard to the composition of the biomass produced from *A. halimus*, many studies provide data different from each other. The results of a study conducted in Spain, analyzing the biomass produced from different accessions of the two sub-species *halimus* and *schweinfurhii* from different areas of the Mediterranean, demonstrate the existence of high variability within and between subspecies within the species *A. halimus*, even among the sampling seasons. This could allow the selection of shrubs with improved properties and with variable chemical composition according to the requirements and systems of exploitation used (Andueza *et al.*, 2005).

The biomass produced from plants of different species of *Atriplex* can be fed to animals directly in the field through the grazing, or it can be cut and administered at a later time. The survival of the plants can be ensured by the correct management of grazing, avoiding overgrazing in some periods of the year so that the plants have the ability to react and to produce new shoots to be used for the following season.

In the *A. nummularia*, after a total defoliation, it takes about 8-10 months of rest before the plant recovers. If not used for grazing this species reaches at most 12-15 years of life. Rejuvenation cuts are necessary, about 20 to 40 cm, to be carried out every 5 years. For correct management the first grazing should be carried out to the third year and the period of exploitation should go from late spring to autumn, while in winter it is good to leave the plantation at rest (Fig. 2).

The species of the genus *Atriplex* besides being interesting for livestock feeding also have an ecological interest. They play an important role in the conservation of soil and water, and are capable, through the well developed root system, to keep the surface layers of the soil thus limiting erosion of the major atmospheric agents. In addition, they also have improving effect on the soil characteristics and contribute to increased soil organic matter and restore the fertility of the ecosystem. *Atriplex* spp has a great ability to accumulate high amounts of salts, and may be used for the desalination of soils. Another important use of such species may be in the rehabilitation of lands polluted by heavy metals (Lefevre *et al.*, 2009), in fact it has been demonstrated that may accumulate up to 0.083% of Cd on the basis of the dry weight of shoots.

Atriplex plants planted in arid and semi-arid areas also provide an important habitat for animals and insects, thus favouring the maintenance of a certain degree of biodiversity. The canopy can help to keep some moisture in the soil, encouraging the development of herbaceous species that would not otherwise have developed. The presence of *A. canescens* often enhances the growth of herbs which benefit from the presence of nitrogen and other minerals concentrated under the canopy of the *A. canescens*. It is one of the most valuable forage shrubs of the dry fields because of its abundance, accessibility, attractiveness, size, font evergreen, nutritional value, growth rate, and the large volume of foliage product.

The species of the genus *Atriplex* can also be used as ornamental plants. The *A. halimus*, for example, lends itself very well to be cultivated in gardens as an ornamental plant. Use in gardens, parks and along the coast, is due to its aesthetic characteristics, high resistance to soil and climate and plant pathogens. It is a plant that responds very well to

pruning emitting numerous shoots, so it can be big and made thick hedges. These can serve as a fence in gardens and as a windbreak.

1.4. Thermic regimes of the species *Atriplex halimus*

It is believed that the temperature is one of the most important factors that limit the distribution of plants on Earth. The limits of distribution often reflect the thermal characteristics of the major metabolic processes, especially photosynthesis. The temperature range compatible with the growth of higher plants is generally between 0 °C and 45 °C, even though there are species that live beyond these extremes. As a general rule, temperatures are optimal for growth when reflect those characteristics of the geographic region in which the species originated (Hopkins e Huner, 2008).

In the earth the highest average temperature occurs at around 15° of latitude North, where it is 27 °C, hence decreases going towards the polar region of 0.67 °C per degree of latitude. The reduction is not constant due to the mitigating effect of lakes, seas and oceans. The temperature varies with altitude, and it is reduced by 1 °C every 184 m.

Plants that tolerate very wide variations in temperature are called eurithermics, while those which admit only minor fluctuations are called stenothermics.

We can consider the optimum temperature as that at which the net photosynthesis provides more than 90% of its maximum yield. For many C4 plants the optimum yield occurs at temperatures between 30 and 40 °C, in individual cases even at 50 °C. The C4 way of carbon assimilation defines so the genotypic mark and represents environmental benefits for the population of very hot areas. However, there are C4 plants with lower optimum temperature of the net photosynthesis, such as the varieties of corn grown in the temperate zones or certain species of *Spartina* and *Atriplex* adapted to lower temperatures (Larcher, 1993)

The temperature also affects the germination of numerous halophytes. Ajmal Khan *et al*. (2004) evaluated the seed germination of *Atriplex rosea* subjected to different regimes of temperature and salinity. In the seeds subjected to temperatures lower than optimum, the percentage of germination was reduced by 50%, then if they were treated with salt, the percentage was nearly zero.

Although numerous studies have been carried out on the effects of temperature using plants grown in greenhouse or in controlled environments, it is still difficult to conduct a research in the open field with a certain degree of accuracy. This is because the

leaves and roots of plants are commonly subject to a wide variety of fluctuations in the thermal regime, due to the presence of clouds, varying wind speed, depth and moisture of the soil, as well as his structure (Hopkins and Huner, 2008).

The temperature affects all biochemical reactions of photosynthesis, and that is the reasons because responses to temperature are very complex. The highest speed of photosynthesis adaptation observed in response to the temperature represents the so-called optimal response to temperature. When temperature is over this limit the photosynthetic rate decreased (Taiz and Zeiger, 2009).

At low temperatures, photosynthesis is often limited by the availability of phosphate in the chloroplast (Sage and Sharkey, 1987). Probably the first sensory process activated from the plant under stress is the activation of protein trans-membrane, which acts as channels for calcium transport. This leads to a flow of calcium (Ca^{2+}) into the cytosolic region. The channels for the release of Ca^{2+} ions are regulated by specific ligands. These ligands are substances that act as secondary messengers, as demonstrated in plant cells (and in particular in the guard cells of stomata), where their application determines the release of Ca^{2+} (Schroeder *et al*., 2001). Experiments in *Arabidopsis* have shown that the calcium concentration inside the cell increases if the plant is exposed to low temperatures (Plieth *et al*., 1999).

1.4.1. Damages from low temperatures

Cold injuries (chilling injury) occurs in species sensitive to low temperatures for normal growth but not low enough to form ice, usually these damages affecting tropical or subtropical species. Species which are generally considered sensitive to freezing show appreciable changes in response to cold temperatures, but the resistance increases if the plants are hardened by slow and gradual exposure to cold but not harmful temperatures. Sudden exposure to temperatures below 0 °C (freezing) cause an inhibition of photosynthesis, lower respiration rate, inhibition of protein synthesis and increased degradation of the proteins (Levitt, 1980).

These responses are mainly due to damages to the plasma membrane of chloroplasts subsequent to cooling. The damages to the membrane are largely due to dehydration associated with freezing. The membrane lipid mainly consists of two types of fatty acids: unsaturated fatty acids and saturated fatty acids. Unsaturated fatty acids have one or several double bonds between two carbon atoms, while saturated fatty acids are

fully saturated with hydrogen atoms. It is a well known fact that lipids containing saturated fatty acids solidify at temperatures higher than those containing unsaturated fatty acids. Therefore, the relative proportion of unsaturated fatty acids in the membrane strongly influences the fluidity of the membrane (Steponkus *et al*., 1993).

The temperature at which a membrane changes from a semi-fluid state to a semi-crystalline is known as the transition temperature. The plants sensitive to cold generally have a higher percentage of saturated fatty acids and, therefore, a higher transition temperature. The resistant species instead are characterized by a high proportion of unsaturated fatty acids and therefore a lower transition temperature (Mahajan and Tuteja, 2005).

The real cause of the lesions of membrane after cooling is the formation of ice crystals in the cells. When a tissue is cooled in natural conditions, the ice usually is formed first between the intercellular spaces and in the xylem vessels of the leaves and stalks, where it propagates quickly because of the large size of the vessels and the fact that the crude sap has a high freezing point. This ice formation is not lethal. If the exposure to low temperatures is prolonged, the water from the protoplast migrates to the ice formed in the apoplast causing dehydration of the cell and contributing to enlargement of existing ice crystals which cause a mechanical deformation on the cell wall and the breaking of the plasma membrane cell (McKersie and Bowley, 1997; Olien and Smith, 1997).

After the rupture of the cell membrane there is a loss of cellular compartmentalization (Gutierrez *et al*., 1992). In many studies it has been shown that the cold can cause the swelling and the consequent rupture of the plasma membrane (Tao *et al*., 1991), the destruction of the endoplasmic reticulum, the vesiculation of the membranes (Marangoni *et al*., 1990) and modifications of the Golgi apparatus (Yoshida *et al*., 1989). The cytoplasmic drying during freezing is due to the non-reversible changes in the level of the lipid composition of the plasma membrane. Analysis carried out with the electron microscope of unacclimated tissues have allowed the identification of regions of membranes which lack of intramembranose particles (proteins): their absence entails changes in the plasma membrane that assumes a conformation defined as "hexagonal phase II", with loss of the phospholipid bilayer. In plants acclimatized hexagonal phases were not formed and this may be due to changes in membrane lipid composition that occur upon exposure to low temperatures.

Remarkable changes also concern the mitochondria, in fact the cold causes its bulge and degeneration (Gutierrez *et al*., 1992). In addition, the cold disturbs the formation of prelamellar plastids (Ikeda and Toyama, 1987) and results in changes in the structure of chloroplasts.

Exposure to cold of sensitive plants can lead to disturbances in the physiological processes related to the hydrological regime of the plant, mineral nutrition, photosynthesis, respiration and total metabolism. In fact, as mentioned previously, as a result of exposure to cold you can have a withering or drying of the plants, for the reduction of the ability of the roots to absorb and transport water in the aerial part of the plant, as well for the inability to close their stomata in response to water deficit (Pardossi *et al.*, 1992; Wilkinson *et al.*, 2001; Bloom *et al*., 2004). The insufficient water supply causes the rapid decrease in the water potential of the leaves during the first hours of cooling.

It is well documented that low temperatures can contribute to the production of reactive oxygen species (ROS), which can form intercellular ice resulting in damages to the membrane. Moreover, low temperatures affect the mineral nutrition of plants. With exposure to low temperatures, the absorption of ions by roots and the movement of these in various organs of the plant are difficult, there is then a rapid decrease in the content of nutrients. The cooling of the plants leads to a decrease of the activity of nitrate reductase, to a reduction of nitrogen incorporation into aminoacids and proteins, to a decrease of the fraction of organic phosphorus and an increase in the content of inorganic P (Zia *et al* ., 1994.). The role of certain mineral elements is essential in reducing damages from exposure to low temperatures.

The administration of nitrogen in the form of nitric oxide (NO), highly reactive, can protect plants from stress by low or high temperatures, by acting as an antioxidant on reactive oxygen species (ROS) that are produced as a result of stress. Even the potassium plays a critical role in increasing the resistance of plants to low temperatures. Deficiencies of K can cause dysfunction in photosynthesis, in fact there is a reduction of CO_2 fixation and a lower distribution and utilization of carbohydrates. This causes an excess of electrons and the stimulation of the production of ROS by transferring electrons to oxygen (Waraich *et al*., 2011). In general the genotypes which tolerate stress from low temperatures are able to maintain high leaf water potential by closing the stomata and limiting the transpiration of water (Wilkinson *et al*., 2001).

Calcium is a key requirement, in tolerant genotypes, for the stomata closure after cooling. Increasing the Ca supply there is an induction of the stomata closure. Even the magnesium plays a fundamental role, in fact it is responsible for the root development with the consequent increase of the absorption of water and nutrients. Furthermore, in conditions of stress from low or high temperatures, magnesium, increases the translocation of carbohydrates and reduces the formation of ROS and photo-oxidative damage to load the chloroplast (Waraich *et al.*, 2012). Among the micronutrients, manganese plays a crucial role in reducing the production of oxygen free radicals and increasing in antioxidant compounds (Aloni *et al*., 2008).

1.4.2. Resistance and acclimatization

The plants may acclimatise to different levels of stress, developing a tolerance to the stress factor, which induced a change and often also to other factors. The majority of the species that survive to freezing temperatures tolerate ice formation within their tissues. There are several mechanisms of resistance. The development of resistance to freezing is a metabolic process that requires a source of energy: light and photosynthesis. Factors that induce faster growth, such as fertilizers and irrigation, inhibit acclimatization (Salisbury and Ross, 1994).

The frost resistance usually develops when the plants are subjected for several days at relatively low temperatures. Sometimes to obtain maximum acclimatization is necessary to reach temperatures from -3 to -10 °C (Larcher and Bauer, 1981; Weiser, 1970).

The main environmental factor responsible for the increase in freezing tolerance is the phenomenon known as "acclimatization to cold." That is the process in which some plants, as a result of prior exposure to low temperatures (above zero), increase their tolerance to freezing (Mahajan and Tuteja, 2005).

Primary function of acclimatization to cold is the stabilization of the membrane with respect to the damage caused by frost. One of the results of acclimatization is the increase of unsaturated fatty acids of the membrane with the consequent reduction of the transition temperature. Furthermore the cold acclimation induces a physical restructuring and biochemistry of cell membranes through changes in lipid composition and induction of other non-enzymatic proteins that alter the freezing point of water (Mahajan and Tuteja, 2005).

When the plants are subjected to stresses such as high salinity, drought and low temperatures, they accumulate highly soluble low molecular weight organic compounds called compatible solutes. These organic compounds, present in a stable form within the cells, are not easily metabolized, but at the same time had no effect on cell function even when we find them at high concentrations. These solutes may have an important role in preventing water loss from the cells by increasing the osmotic pressure within these. Typical compatible solutes include sugar alcohols, mannitol and others, amino acids such as proline, and some amino acid derivatives such as glycinebetaine. Some of these solutes such as proline are accumulated in all plants, others as for example glycinebetaine, only in plants tolerant to salt or to the cold (Robinson and Jones, 1986).

Recently it has been shown that a relationship exists between the habitat of *A. halimus* and the strategy for drought tolerance. The populations from a coastal saline area, accumulate in response to drought mainly osmoprotectans such as glycinebetaine, while the populations of a dry hinterland region preferentially accumulate proline (Ben Hassine *et al*., 2008). In both cases, however, even the total soluble sugars accumulated give an osmotic contribution, from the quantitative point of view, in both populations (Ben Hassine *et al*., 2008).

So we can say that the major metabolic changes related to the acclimatization consist of:

- an increase in the osmotic concentration due to the accumulation of sugars.
- in the loss of water, in fact the acclimatization simultaneously promotes the loss of water and the accumulation of starch. The loss of water reduces the amount of water available for the formation of ice.
- increased lipid and the degree of instauration of fatty acids.
- increase in the content of soluble proteins
- increase in the concentration of abscisic acid. In fact, the cold tolerance is closely associated with quiescence and dormancy (induced by ABA) and is hampered by promoting growth due to gibberellins.

Different studies demonstrate that plants adapted to regimes of low temperature, when submitted to high temperature, showed low biochemical activity as compared to plants adapted to grow at high temperature (Smrcka and Szarek, 1986). The protein turnover, defined as the rate of flow of aminoacids towards the biosynthesis of proteins, could play

an important role in the regulation and adaptation to the temperature (Smrcka and Szarek, 1986).

The temperature also affects the photosynthetic capacity of the species. Some experiments were carried out to compare two species of *Atriplex*. These were grown at low and at high temperatures. It was reported that both species had a high photosynthetic capacity between 4 and 10 °C, and this was higher in plants grown at high temperature (Caldwell *et al*., 1977).

There is evidence that some species of desert, active in the summer, have physiological adaptations that enable high rates of absorption of CO_2 even at high temperatures. In contrast to the species mainly active in the course of a season, evergreen desert species are active throughout the year and have a wide temperature tolerance, or are able to acclimate to seasonal changes in the temperature regime. Recent experiments have shown that many desert species that are active have a capacity to acclimate and to operate under the prevailing temperature regime.

Unacclimatized plants may show different ranges of tolerance to particular conditions. Thanks to their genotype, some species can tolerate, better than others, higher temperatures or dehydration (Adam 1990). Therefore, in order to survive, the vegetation and predominantly halophytic species are well adapted to face these conditions and their photosynthetic apparatus is acclimatized for a wide variety of environmental stress (Das Neves *et al*., 2008).

A. halimus exists both in the form of diploid (2n = 2x = 18) and tetraploid (2n = 4x = 36) populations, the diploid form spreads in Spain and France, and the second in North Africa and in the eastern parts of the Mediterranean basin (Walker *et al*., 2005). Other species of the genus *Atriplex* present different types of ploidy and this makes these species adaptable to different types of environment.

The species of the genus *Atriplex* grows well in deep soils with only 150-200 mm of rain per year, but can survive with 50 mm of rain. Furthermore, they resist to temperatures up to -10 °C and have a high resistance to frost (El Aich, 1987), but their biomass production is limited by below-zero temperatures (Aouissat *et al.*, 2009). Even Caldwell *et al*., (1977) states that the *Atriplex* species are C4 plants relatively tolerant to the cold.

Recently it has been shown that, under field conditions, tolerance of the populations of *A. halimus* to freezing seems to be related to the concentrations of Na and K in the

leaves and to the ploidy level (Walker *et al*., 2008). In fact, the diploid populations are more tolerant to cold. It seems that the high salinity of the soil, can improve the freezing tolerance of halophytic species such as *A. halimus* that are able to accumulate high concentrations of salt in the tissues. Since the concentration of cytoplasmic Na does not exceed 5-10 mM (Carden *et al*., 2003), the accumulation of Na in the vacuole may be the mechanism that has contributed to the freezing tolerance of plants of *Atriplex* on saline soils. However, since the lesions by freezing are mainly caused by cellular dehydration, due to loss of intracellular water and ice formation in the extracellular space, the accumulation of Na in the apoplast may be involved in lowering the temperature at which ice is formed (Xin and Browse, 2000; Uemura *et al*., 2003).

To evaluate the adaptability of the species *A. halimus* at low temperatures there are different methodologies in literature. For example Aouissat *et al.* (2009) determined the freezing tolerance of two species of *Atriplex* (*A. halimus* and *A. canescens*), by analyzing the content of electrolytes in the leaves and visually assessing the damage after exposure of plants to temperatures between -5 and -25 °C. Their study showed that there was a significant correlation between the tolerance to freezing and concentrations of Na and K in the sap of the leaves. The tolerance was favoured by soil salinization, but there was no relationship between frost tolerance and minimum winter temperatures or soil salinity of the places of origin of populations. Therefore, the main result of the research is that, for these halophytes species, the salinity of the soil (in sites characterized by low temperatures) is determinant to reducing the risks of freezing damage.

In the work of Walcher *et al*., (2005), resistance to freezing of the *A. halimus* was correlated with the accumulation of Na and K in the leaves. Furthermore, by analyzing the various compatible organic solutes authors have found a close correlation between the resistance to freezing and the concentration of soluble sugars in the leaves.

2. OBJECTIVES

The general objective of the research is to contribute to understand the mechanisms of resistance to stress in a species of the genus *Atriplex.*

The *A. halimus* is one of the many species used for the recovery of degraded areas and to stop the process of desertification.

In environmental restoration projects, which involve the use of plant species, the effectiveness of the intervention is influenced by many factors. The choice of species to be introduced is one of the most important factors. Depending on the objective of the intervention and depending on the climatic characteristics of the site, different species may be used. For this reason the studies and tests of adaptability of plants to various stresses that may occur are essential. In particular, in our research, attention has been paid to resistance to different thermal regimes typical of the areas affected by desertification, which showed not only the possibility of relatively high maximum temperatures, accompanied by dryness, but also the occurrence of very low minimum diurnal and seasonal.

Due to the high intraspecific variability of the species *A. halimus*, with our research, we sought to expand your knowledge of some selected clones in Sardinia, considering the different ability to adapt to different thermal regimes.

In particular, the specific objectives of the thesis are:

- to study the different physiological responses of some clones to thermal stress;
- to select clones resistant to low temperatures, for use in programs of environmental remediation in sites where these are a problem for vegetation cover development.

In particular, the clones on which we perform the test were taken from a field collection site in Oristano and were subjected to different thermal regimes, in particular to those of the sites of Oristano, Tempio, Sassari and Villasor. The influence of different temperature regimes on the plant growth rate was then evaluated. For each clone and for each site quantitative and qualitative analysis of the biomass produced was performed.

To do this, we evaluated the possible relationship between growth rate and temperature with reference to an index of chilling accumulated (ICC). The index of chilling accumulated was calculated from the average daily temperatures by stating three temperature thresholds (0, 5 and 10 °C), and considering only the differences below the temperature thresholds and average daily temperatures. One ICC unit was one degree of difference between the mean day temperature below the temperature threshold and the same value of the temperature threshold. The sum of such units gives the ICC for a defined period.

Also with regard to the chemical analysis of biomass, for each element and for each clone were evaluated possible correlations with index of chilling hours accumulated for each thermal threshold fixed. In fact, the accumulation of some elements may be the result of any stress to which the plants are subjected, and in our case this stress could be the low temperature.

Similarly the relationship between the temperature and the quantitative data of fresh and dry biomass was evaluated. In addition to the correlations for each parameter, the possible interactions between the various independent variables, as between localities and clones, between localities and growing period and among all the three main variables. At the end of the research was possible to identify the clones more resistant to low temperatures. This information can be useful in order to use the *Atriplex* species in environmental recovery projects, especially in areas where the temperatures are a problem for the survival of plants.

3. MATERIALS AND METHODS

The plant material necessary to conduct the research activity has been obtained from plants of A. *halimus* collected at the Experimental and Didactic Farm "Antonio Milella" of the Department Sciences of the Nature and Land of the University of Sassari located at Fenosu (OR). The field collection is the result of an accurate investigation carried out in 2005 on the entire coastal territory of Sardinia, which led to the collection and characterization of 30 accessions of *A. halimus*, mainly coming from the Southern, Western and Northern parts of the island *(*Mulas *et al.,* 2010b*)* (Tab. 1).

For each site where this species was present, one or more individuals were chosen, on which to perform the necessary measurements. The data of each mother plant were recorded in a preliminary descriptor list, which collected the following general and specific data: date of collection, the name of the location where grown the mother plant, the type of lithology matrix and the vegetation cover, and finally the identification code, the number of stems (if detectable) of which was made the plant, shape, vigour, plant health, average height, the largeness second exposure East-West and North-South, presence or absence of flowers and fruits, the type of propagation material collected. From each recorded plant, a sample of the vegetative material was also taken for the creation of the 30 clones that then contributed to the collection in the experimental field of the University of Sassari.

3.1. Propagation of vegetal material

As mentioned previously the plant material was taken from that collection field in the spring of 2009. More precisely were agamically propagated 7 clones of *Atriplex halimus*.

From portions of branch of field growing plants cuttings of 12 cm were obtained and placed in greenhouse to root on a frame of perlite substrate, where "mist" irrigation and basal heating at 28 °C were provided. Part of the cuttings was placed to root without any treatment, while others have been subjected to treatment with 0.5% 3-indolbutirric-acid (IBA) in powdery mixture with talc.

During the period of rooting the cuttings were subject to occasional inspections to observe the state of health and the dynamics of rooting. After a period of 30 days of rooting was evaluated the percentage of rooted cuttings subsequently transplanted in pots of about 2 L of volume containing a substrate consisting of 1/3 of perlite, 1/3 of organic loam and 1/3 of soil. The substrate had the following physical and chemical composition:

Gravel (> 2 mm)	193 ‰	pH H_2O	5.44
Sand (2 ÷ 1 mm)	152 ‰	Carbon	21.2 ‰
Sand (1 ÷ 0.5 mm)	118 ‰	Organic matter	36.5 ‰
Sand (0.5 ÷ 0.2 mm)	111 ‰	Total Nitrogen	1.71 ‰
Coarse sand	381 ‰	Carbon / Nitrogen	12
Fine sand	468 ‰	P assimilable	47.2 ppm
Total sand	849 ‰	Copper	2.3 ppm
Silt	64 ‰	Zinc	12.4 ppm
Clay	87 ‰	Iron	97.2 ppm
		Manganese	36 ppm
		Calcium	4430 ppm
		Magnesium	218 ppm
		Sodium	286 ppm
		Potassium	188 ppm

After transplant pots were placed under shading and properly irrigated. For the test of adaptability, among clones were chosen those showing the higher percentage of rooted cuttings. After a period of growth in nursery we have set up 4 experimental sites. Plants were then transferred to the various sites characterized by very different environmental conditions. The sites considered are Tempio (OT), Sassari (SS), Oristano (OR) and Villasor (CA).

3.2. Characteristics of clones

The clone MAR1 comes from the location of the locality Mari Pintau of Quartu S. Elena, in the province of Cagliari. The morphology of the plant type is characterized by a semi-prostrate *habitus*, a low vegetative vigour, flowering and fruit production of small quantities. The size of the plant is approximately 90 cm in length and 100 cm in width. The shape of the adult leaf is elliptical/oval and the average size of 1.85±0.15 cm in width and 2.82±0.12 cm in length. The average size of the leaf is 1.41±0.21 cm^2.

The phenology exhibits vegetative activity that stops in July to resume in late December. The flowering period is concentrated between the months of September and

October, the ripening of fruit occurs in October and ripen fruits remain on the plant until December. The leaf drop was observed from January to April (Fig. 3).

The clone GIO1 comes from the seaside locality called Giorgino, located a few km from Cagliari. The morphology of the plant type is characterized by a prostrate *habitus*, a low vegetative vigour, flowering and fruit production of average amount. The dimensions of the plant are approximately 70 cm in height and 80 cm in width.

The shape of the adult leaf is obovate and the average size of 2.40±0.10 cm in width and 2.76±0.09 cm in length. The average size of the leaf is 1.65±0.12 cm^2.

The phenology shows vegetative activity stop in August and resuming in November. The flowering period is concentrated mainly in the month of September, the fruit ripens in October and remains on the plant until late November. The leaf drop was observed from January to April and November to December.

The clone SAN 3 comes from the locality of Santa Giusta, located in the province of Oristano. The morphology of the plant type is characterized by a compact *habitus*, a high vegetative vigour, flowering and small quantities of fruit production. The size of the plant is approximately 110 cm in height and 160 cm in width. The shape of the adult leaf is sub-triangular and the average size of 2.68±0.06 cm in width and 4.21±0.14 cm in length. The average size of the leaf is 3.30±0.20 cm^2. The phenology shows vegetative activity that stops in July and then resume in November. The flowering period is concentrated in the months of September and October, fruit ripens in November and remains on the plant until December. The leaf drop was observed in the months of January and February.

The clone PAL 1 comes from the locality ”Su Pallosu”, located near to the town of Oristano. The morphology of the plant type is characterized by a semi-prostrate *habitus*, a medium vegetative vigour, flowering and abundance of fruit production. The dimensions of the plant are about 130 cm in height and 140 cm in width. The shape of the adult leaf is oval and the average size of 2.53±0.05 cm in width and 3.65±0.16 cm in length. The average leaf surface is 2.80±0.12 cm^2.

The phenology shows vegetative activity that stops in May and then resume in November. The blooming period occurs in August and continues to September. Fruit ripening occurs in mid-November and ripen fruits remains on the plant until December. The leaf drop was observed from February to April.

The clone FAN 3 was selected in the locality called Fangario, located few km from North of Cagliari. The morphology of the plant is characterized by upright *habitus*, a

medium vegetative vigour, flowering and production of small quantities of fruits. The size of the plants is about 120 cm in height and 140 cm in width. The shape of the adult leaf is obovate and the average size of 2.45±0.03 cm in width, and 2.96±0.07 cm in length. The average size is 4.17±0.99 leaf cm^2.

The phenology shows vegetative activity that stops in August and resume in November. The flowering period is concentrated mainly between September and October, fruit ripening in November and ripen fruits remain on the plant until late December. The leaf drop was observed from January to April.

Table 1. Origin of clones collected in the experimental field of Fenosu (OR).

Clone	Origin	Clone	Origin	Clone	Origin
1) *A. nummularia*	Morocco	12) FAN 3	Fangario di Cagliari	23) SOR 3	Marina di Sorso(SS)
2) PAL 1	Su Pallosu (OR)	13) FAN 2	Fangario di Cagliari	24) SOR 2	Marina di Sorso(SS)
3) MAC 1	Macchiareddu (CA)	14) FAN 1	Fangario di Cagliari	25) SOR 1	Marina di Sorso(SS)
4) MAR 1	Mari Pintau (CA)	15) BAC 2	Baccu Mandara(CA)	26) GIO 1	Località Giorgino (CA)
5) QUA 2	Quartu S.Elena (CA)	16) BAC 1	Baccu Mandara(CA)	27) STI 5	Saline di Stintino (SS)
6) QUA 1	Quartu S.Elena (CA)	17) SAN 3	Santa Giusta (OR)	28) STI 4	Saline di Stintino (SS)
7) PIS 2	Pischeredda (OR)	18) SAN 2	Santa Giusta (OR)	29) STI 3	Saline di Stintino (SS)
8) PIS 1	Pischeredda (OR)	19) SAN 1	Santa Giusta (OR)	30) STI 2	Saline di Stintino (SS)
9) MOR 3	Is Mortorius (CA)	20) PAU 2	Pauli e Sali (OR)	31) STI 1	Saline di Stintino (SS)
10) MOR 2	Is Mortorius (CA)	21) PAU 1	Pauli e Sali (OR)		
11) MOR 1	Is Mortorius (CA)	22) SOR 4	Marina di Sorso(SS)		

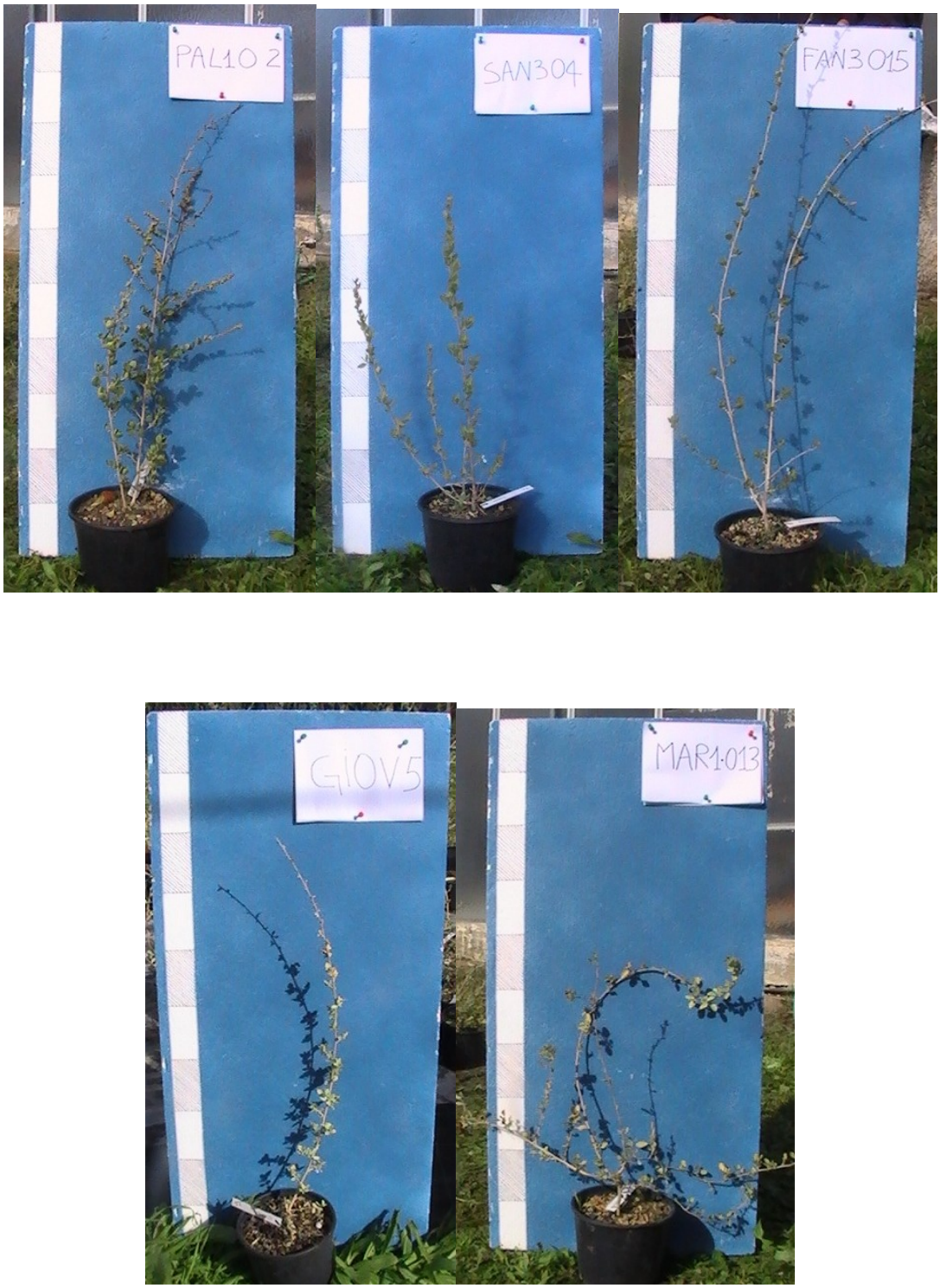


Figure 3. Clones of *A. halimus* subjected to the test of adaptability to temperatures.

3.3. Experimental sites

For the selection of experimental sites, we have taken into account, in addition to the availability of entities and host properties, especially environmental differences among the sites. We have tried, with every site, to represent some of the different climatic conditions in Sardinia (Table 2 and Fig. 4).

In all sites, pots containing plants of *Atriplex halimus* were placed outdoors in order to receive easily all the radiation, without being shaded by surrounding structures or vegetation. The pots were not in direct contact with the ground but over a double plastic layer in order to prevent the growth of weeds and of the roots of *Atriplex* toward the ground. In every site five clones of *Atriplex halimus* were compared. Forty plants per clone were arranged in every site: 30 were used for biometric measurements and sampling for quantitative and qualitative analysis of biomass, the remaining constituted the reserve. The clones compared in the various sites were: SAN3, PAL1, GIO1, FAN3, and MAR1. Plants of *Atriplex* over 3 years of research have not received mineral fertilization and any phytosanitary treatment. Shrubs were irrigated to keep the soil at values of humidity around field capacity in order to eliminate the possible effect of water stress on the growth of the plants.

The site of Oristano has been set up in the experimental station "Antonio Milella" of the Department of Sciences of the Nature and of the Land, of the University of Sassari, located at Fenosu (OR). The meteorological data of the area were recovered from a weather station located on the outskirts of Oristano (100 m far of the site). The station is a Davis Vantage Pro2 screen ventilated (Oristanometeo, 2012).

The site of Tempio was set up within the experimental station "La Naciola" of the Department of Sciences of the Nature and of the Land" of the University of Sassari, located near to the town of Tempio. The meteorological data of the area were recovered from a weather station located on a structure adjacent to the experimental site (Direttameteo, 2012).

The site of Sassari has been set up in the experimental station "Mauro Deidda"of the Department of Agriculture of the University of Sassari located in the locality of Ottava (SS). Weather data references are those of the weather station of Sassari (Direttameteo, 2012).

Table 2. Characteristics of the reference meteorological stations.

Locality	m s.l.m.	Lat. Nord	Long. Est
Oristano	5	39° 54' 89" N	8° 35' 35" E
Villasor	30	39° 12' 00" N	8° 30' 00" E
Tempio	540	40° 54' 31" N	9° 05' 44" E
Sassari	240	40° 43' 49" N	8° 34' 07" E

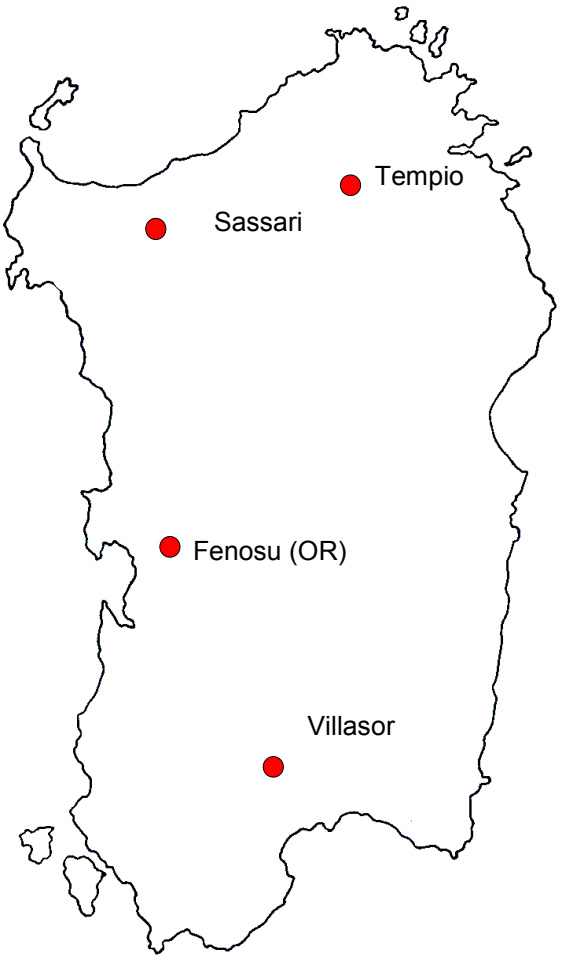


Figure 4. Distribution of experimental sites in the Sardinia region.

The site of Villasor is located in the experimental station of the Agency of Agricultural Research (AGRIS). The meteorological data were retrieved from the local weather station of Villasor (Direttameteo, 2012).

From all agrometeorological stations it was possible to find for every site data relating to rainfall, maximum, minimum and daily average temperature for the period January 2009 - May 2012.

3.4. Evaluation of plant growth

To evaluate the influence of different thermal regimes on growth of plants of five clones about 30 plants of each clone were examined in every site, for a total of 150 plants per site. Plants were examined before and after six months of growth by biometric measurements of the linear development of the canopy (stem and branches). The relative growth rate was calculated using the classical relationship:

$RGR = (M2-M1/M1) \times 100$

$T2 - T1 = 6$ months.

Where:

M1: linear development of canopy biomass at time T1; M2: linear development of canopy biomass at time T2.

To analyze the effect of low temperatures on the growth of different clones of *Atriplex*, for the period of canopy growth measured (every 6 months and all over the test), it was calculated the amount of degrees that the daily average temperature had fallen below a given temperature value, which was considered critical for the plant development. In order to calculate these sums three thresholds of critical temperatures were considered: 0, 5, and 10 °C. Daily values were added for the studied periods (semester or total test period) thus obtaining the sums like an indicator of chilling cumulate (ICC):

$ICC\ (0) = \Sigma\ (0 - Ta)$

$ICC\ (5) = \Sigma\ (5 - Ta)$

$ICC\ (10) = \Sigma\ (10 - Ta)$

Where ICC is the index of chilling cumulated (ICC) during the period and Ta indicates the average daily temperature of the air.

3.5. Quantitative and qualitative analysis of biomass

The quantitative and direct measure of the biomass produced by 5 different clones in 4 different sites was carried out on potted plants placed in different experimental sites. Plant sampling was carried out every six months, from November 2009 to May 2012. The first semester was comprised between 1 November 2009 and 30 April 2010, the second between 1 May 2010 and 30 October 2010, the third between 1 November 2010 and 30 April 2011, the fourth between 1 May 2011 and 30 October 2011, and the fifth between 1 November 2011 and 30 April 2012. In every experimental site, for every clone, were taken 3 whole plants, for a total of 15 plants per site. Plants were sampled and transferred to the laboratory of the Department of Sciences of the Nature and of the Land.

Leaves, branches and roots were separated and carefully cleaned from soil residues, limiting as possible the loss of roots. Subsequently, the roots were washed to remove the residue of substrate. The three fresh plant fractions were separately weighed using a laboratory balance to assess the fresh weight. After that, the plant material was placed in an oven for 2 days at 60 °C for the determination of the dry weight.

The evaluation of the mineral composition of the biomass produced was made on the ashes of samples of leaves, branches and roots of every sampled plant. To obtain the ashes of samples, 1 g of previously dried and ground biomass was placed within capsules of porcelain resistant to high temperatures and subsequently transferred in a muffle furnace for 24 hours at 500 °C.

The ashes were added of 5 mL of 4 N hydrochloric acid, the whole was transferred into 100 ml volumetric flasks by repeated washing of the capsules with deionised water to make sure that all the hydrochloric acid used was passed from the capsule flask. Subsequently, the flasks were brought to volume with deionised water, and in this way it was possible to obtain the stock solution of minerals of biomass samples. From the solution it was possible to further analyze and determine the content of the main macro- and microelements.

Phosphorus was determined from the mineral mother solution. This was mixed to 5 mL of 7.5 N sulphuric acid, 5 mL of ammonium molybdate and 1 mL of hydrazine, all in 100 mL volumetric flasks. To accelerate the reaction the flasks were placed in a water bath

at 80 °C for 30 minutes. In these conditions it is formed a complex of blue colour, the intensity increasing with increasing phosphorus concentration and dosable by reading through the UV-VIS spectrophotometer (Hitachi model 100-60) at a wavelength of 650 nm.

Nitrogen was determined by the Kjeldahl method, which consists in the oxidation of the hot sample of dry biomass (previously dried and finely ground) by means of sulphuric acid and subsequent distillation.

The other mineral elements were determined on mineral fraction by atomic absorption spectrophotometer reading (Perkin Elmer AAnalyst 100).

3.6. Plant response to pruning

On some plants not included in the sampling stock, a further test was carried out to evaluate the response of plants to pruning. The test was carried out at the site of the Tempio and of Oristano.

In the month of June 7 plants per clone were pruned up to 5 cm from the surface of soil substrate. Biomass resulting from the intervention of pruning was weighed. After 6 months the growth was assessed using biometric measurements. The results of this test may be useful to assess the ability of clones of *A. halimus* to tolerate grazing and plant response in terms of biomass production. We also evaluate the influence of different weather conditions on the plant recovery.

3.7. Statistical treatment of data

Data were submitted to ANOVA by the use of the software MSTAT-C. A factorial design was used to evaluate the influence of location, clones and different periods of observation on dependent variables related to plant growth and content of mineral elements. Mean separation was obtained by application of the Duncan's Multiple Range Test at $p \leq 0.01$ level of significance. Data have been also used to find linear regression. Using the same software data have also been analyzed for linear regression functions between dependent plant variables and indicators of chilling cumulated below the critical temperatures.

4. RESULTS

4.1. Climatic characterization of the experimental sites and micrometeorological trend during the three years of study

The following figures show the average thermic and rainfall trends of each area studied. Specifically, Figures 5 and 6 show the trends of average temperatures and monthly rainfall calculated on the basis of thirty years obseratons (1962-1992) (EAF, 1998). The analysis of the graphs shows a different thermic trend in the various sites of analysis. The peak of maximum temperatures is reached in the site of Villasor, in the month of July, with a temperature of 31.56 °C, while the minimum value is recorded in the site of Tempio, equally in the month of July, with a temperature of 28 °C. The sites of Oristano and Sassari reach maximum temperatures, both in July, respectively 30.59 °C and 29.5 °C. As regards the minimum year temperatures of thirty years, in the site of Tempio the lowest value of 5.03 °C was observed in the month of December, while the value of 8.54 °C was recorded in the site of Villasor, always in December, as highest minimum year temperature. The sites of Oristano and Sassari showed values of respectively minimum year temperature of 6.73°C and 7 °C.

As regards to the rainfall trend it is evident that in the site of the Tempio the maximum was in the month of December, while in the sites of Villasor, Sassari and Oristano this maximum is reached in the month of November and then a more or less marked decline in the month of December is shown. The site characterized by the highest value of average rainfall in the wettest month is Tempio with 120 mm, while the site less rainy was found in Sassari with 96.88 mm. Villasor and Oristano amounted respectively to 108.03 mm and 103.4 mm. The month more drought in all four sites was found to be July, with the extremely low value of (2.89 mm) at the site of Sassari, while in the site of the Tempio it was recorded the highest rainfall in the month of July with a value of 13.43 mm.

Figures 7-10 shows the thermo-pluviometric data related to the 4 locations considered (Oristano, Tempio, Sassari and Villasor) in the period 2009-2012, a period in which the test was carried out.

The trends recorded in all stations are typical of the Mediterranean climate. In particular the period from June to August appears to be the most arid, characterized by low rainfall and high temperatures. The rainfalls seem to be concentrated mainly in autumn and in winter. If we look at the rainfall data of the last three years, we see that the year 2010 was a special year. As we can see in the months of October and November rainfall peaks different from the seasonal average are observed.

We can say, in general, that on Sardinia, the cumulative precipitation of October 2010 was uneven, due to the very heavy rainfalls between 10 and 12 that affected the eastern and southern Sardinia (ARPAS, 2012), surpassing even the 300 mm/month, corresponding to more than 3 times the mean climatological, while over much of the western part of Sardinia, the cumulative ranged from 50 to 100 mm/month, corresponding respectively to 0.8 and 1.2 times the average climatology.

The rainfalls in November 2010 were abundant on the whole island, with the exception of the east coast. The cumulative monthly ranged from 60-70 mm/month for the latter part of the island, to 100-150 mm/month of much of central Sardinia, to 200 mm/month in the West Sardinia and nearly 300 mm/month of mountain areas. These values are well above the monthly average, up to more than double over the central part of the island and with peaks of more than 250% of the average in the area of Gallura and Alghero. If we look at the rainfall data for the month of October 2010 at sites subject of our studies, we note that the major peaks were reached at the site of Villasor (208 mm), the cumulative lowest were recorded in Sassari (78.2 mm) and Oristano (54.2 mm). At Tempio in the same month were recorded 138.9 mm of rain.

In November, the highest accumulated rainfall was recorded in Sassari and Tempio reaching respectively 261.4 and 252.7 mm of rain. More generally we can affirm that the wettest year was 2010 and this was confirmed in all 4 sites. Considering the fallen mm by year in the different sites, we have found that the site of the Tempio was the site in all 3 years that showed higher values of rainfall. The site of Oristano instead was found as the site characterized by the lowest annual values of rainfall, followed by the sites of Sassari and Villasor.

Regarding the temperature, in the Figures 7 - 10, the maximum, medium and minimum monthly average temperatures in the 4 experimental sites are reported.

The monthly average temperatures reached the maximum in Oristano, in the three-year period, ranged from a minimum of 11.8 °C (February 2012) to a maximum of 33.3 °C

(August 2011). In Tempio average monthly maximum temperatures reached the highest values in July 2010 (31 °C) and lowest values in February 2012 (7.6 °C). In Sassari temperatures were from a minimum of 9.9 °C in February 2012 to a maximum of 30.2 °C in August 2011. In Villasor the mean monthly maximum temperatures ranged from 12.5 °C (February 2012) to 32.7 °C (July 2010).

The average of monthly temperatures in Oristano was from 7.9 °C recorded in February 2012 to 25.2 °C in July 2010. In Tempio the average monthly temperatures ranged from 4.7 °C in February 2012 to 24.6 °C in July 2010. The site of Sassari showed average temperatures ranging from a minimum of 6.3 °C (February 2012) to a maximum of 24.6 °C (August 2011), while in Villasor was between 4.7 °C (February 2012) and 26.1 °C (July 2010).

The minimum temperatures in Oristano were between 1.16 °C (February 2012) and 18.5 °C (July 2010), while in Tempio between 2.4 and 19.7 ° C (July 2010).

The minimum monthly temperature of Oristano was between 1.16 °C (February 2012) and 18.5 °C (July 2010), while in Tempio ranged between 2.4 and 19.7 °C (July 2010). In Sassari monthly temperatures showed minimum values in February 2012 (2.3 °C) and maximum values in July 2010 (19.4 °C). Even in Villasor lowest monthly minimum temperatures were recorded in February 2012 and highest in July 2011, with average values of 2.7 °C and 20.1 °C respectively.

In general, the lowest monthly minimum temperatures, in all 4 sites were recorded in February of 2012. As we can see from the graphs the month of September 2011 was an exceptionally hot month, in fact, the average maximum temperature was higher than the seasonal average. The site with the highest maximum temperature was found in Oristano, in particular in summer 2011.

Regarding the index of cold, reported in Table 3, for each thermal threshold (<0, <5 and <10 °C), the indicator of chilling accumulated (ICC) relative to each location and each semester. The same was also calculated for the whole period of the test.

As we can see in the majority of cases the average minimum temperature is not frequently below zero, and only in the site of Tempio the temperatures are below freezing. For this reason, the sums of chilling index are positive. Also with regard to the other two temperature thresholds (5 and 10 °C) is the site of Tempio to present the chill accumulated index higher. The site Villasor does appear that for both thresholds has accumulated the lowest ICC.

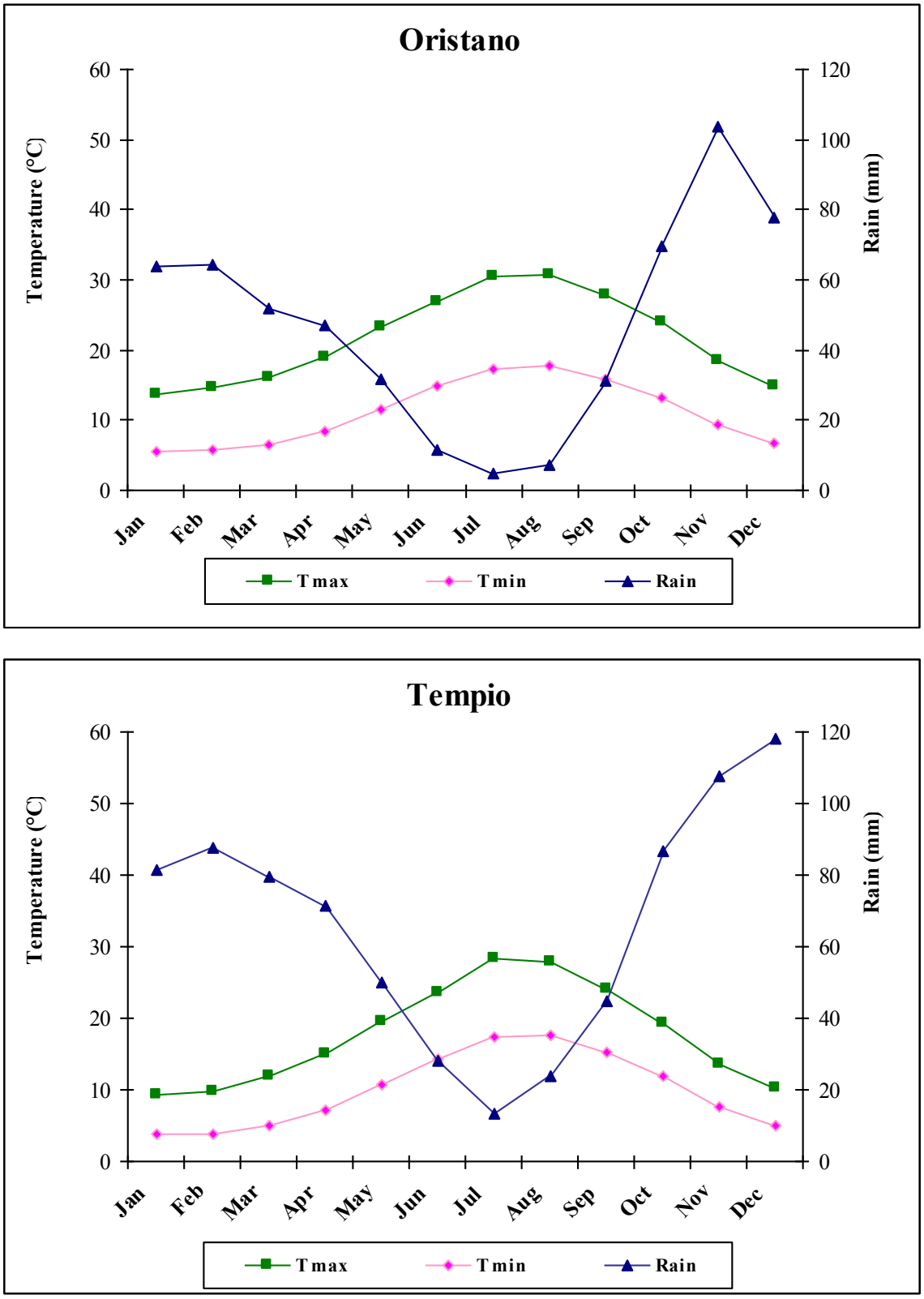
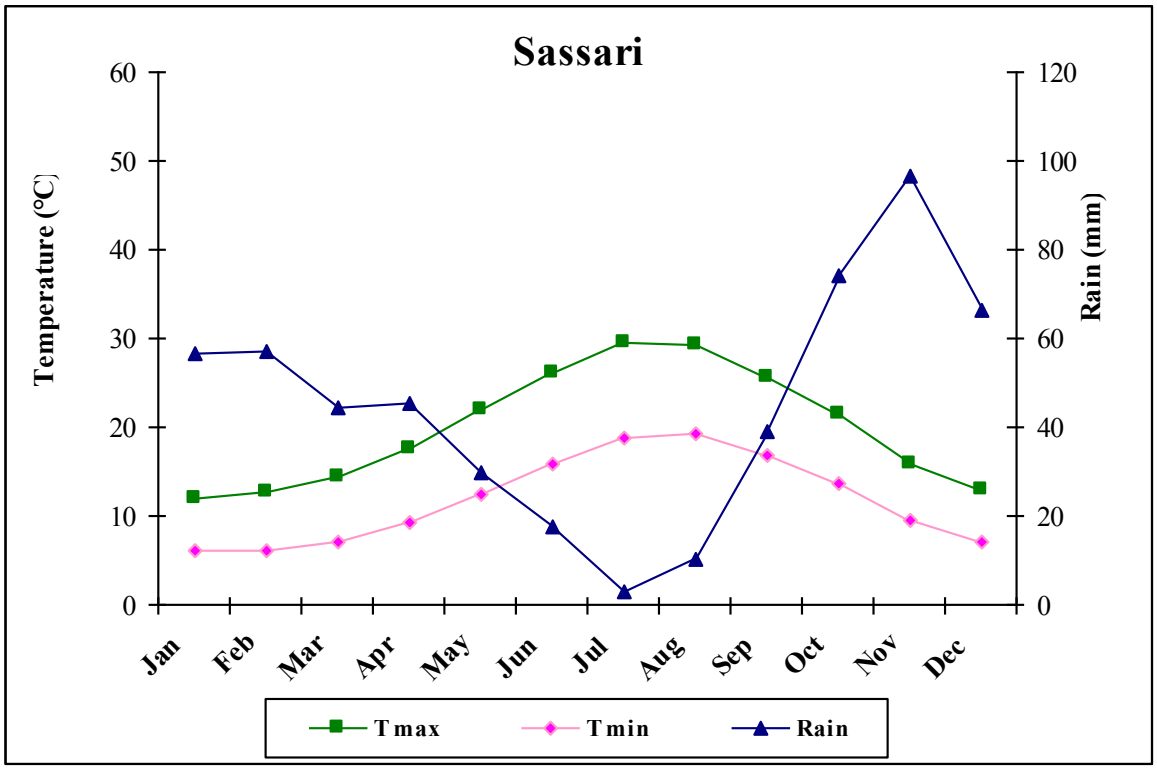


Figure 5. Rainfall and monthly temperature diagram of Oristano and Tempio. Every data is the mean of the 30 years observations.



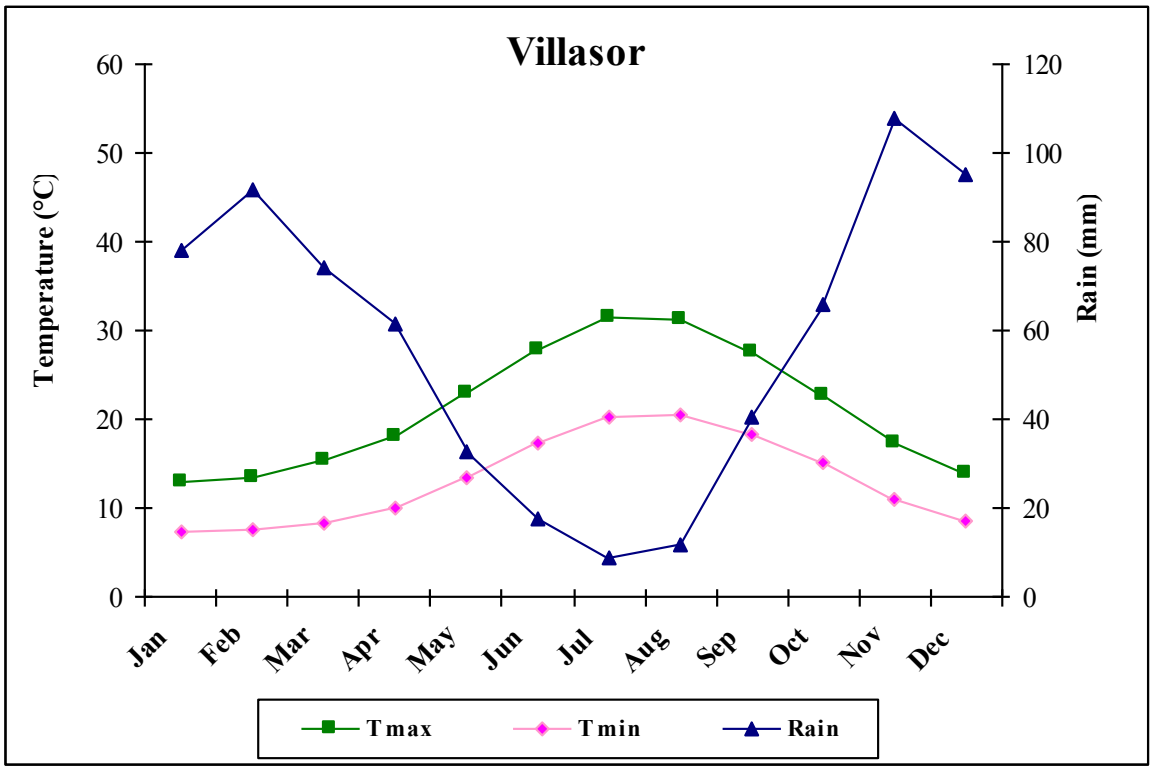


Figure 6. Rainfall and monthly temperature diagram of Sassari and Villasor. Every data is the mean of the 30 years observations.

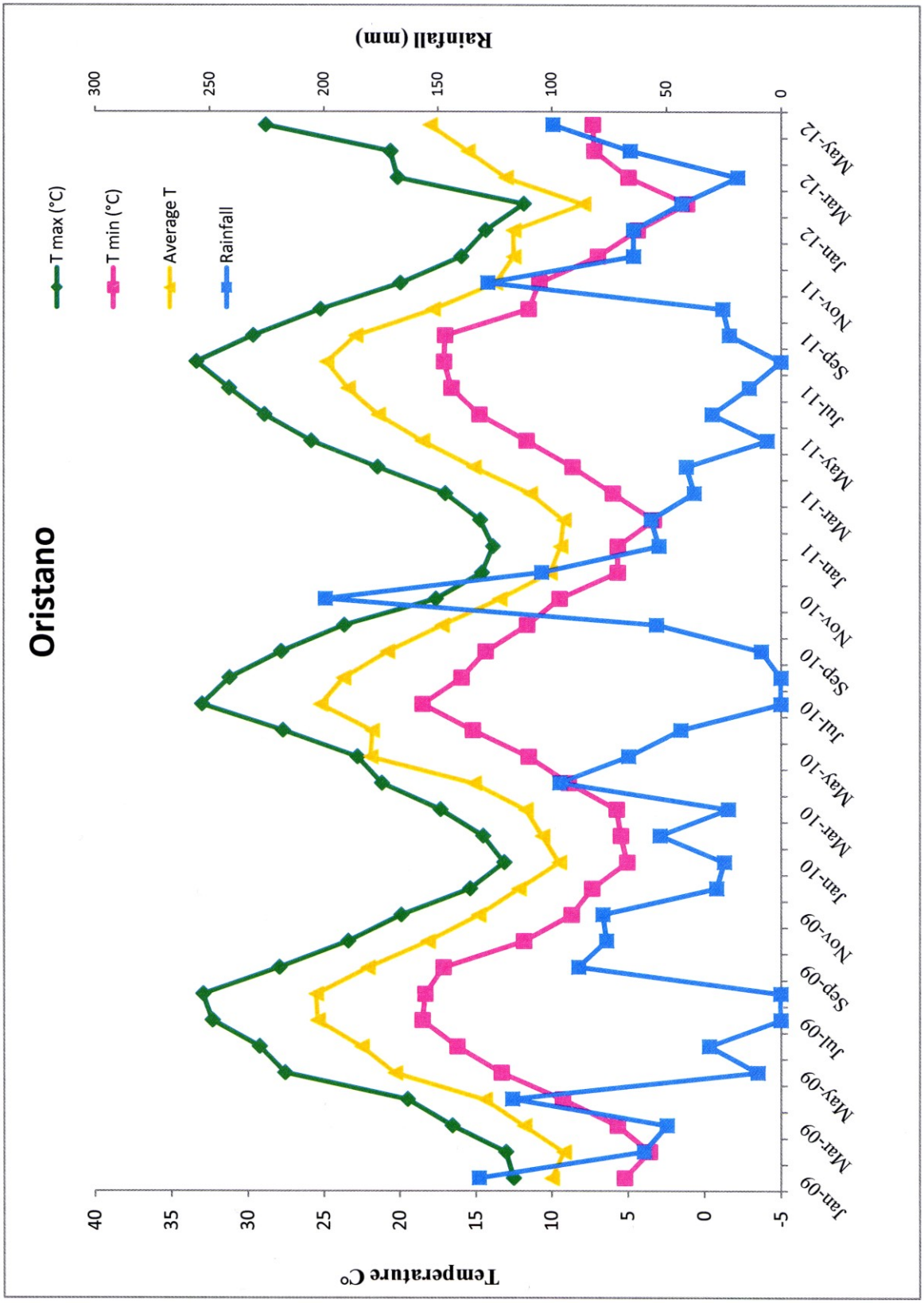


Figure 7. Monthly temperatures and rainfalls in the site of Oristano during 2009-2012 years.

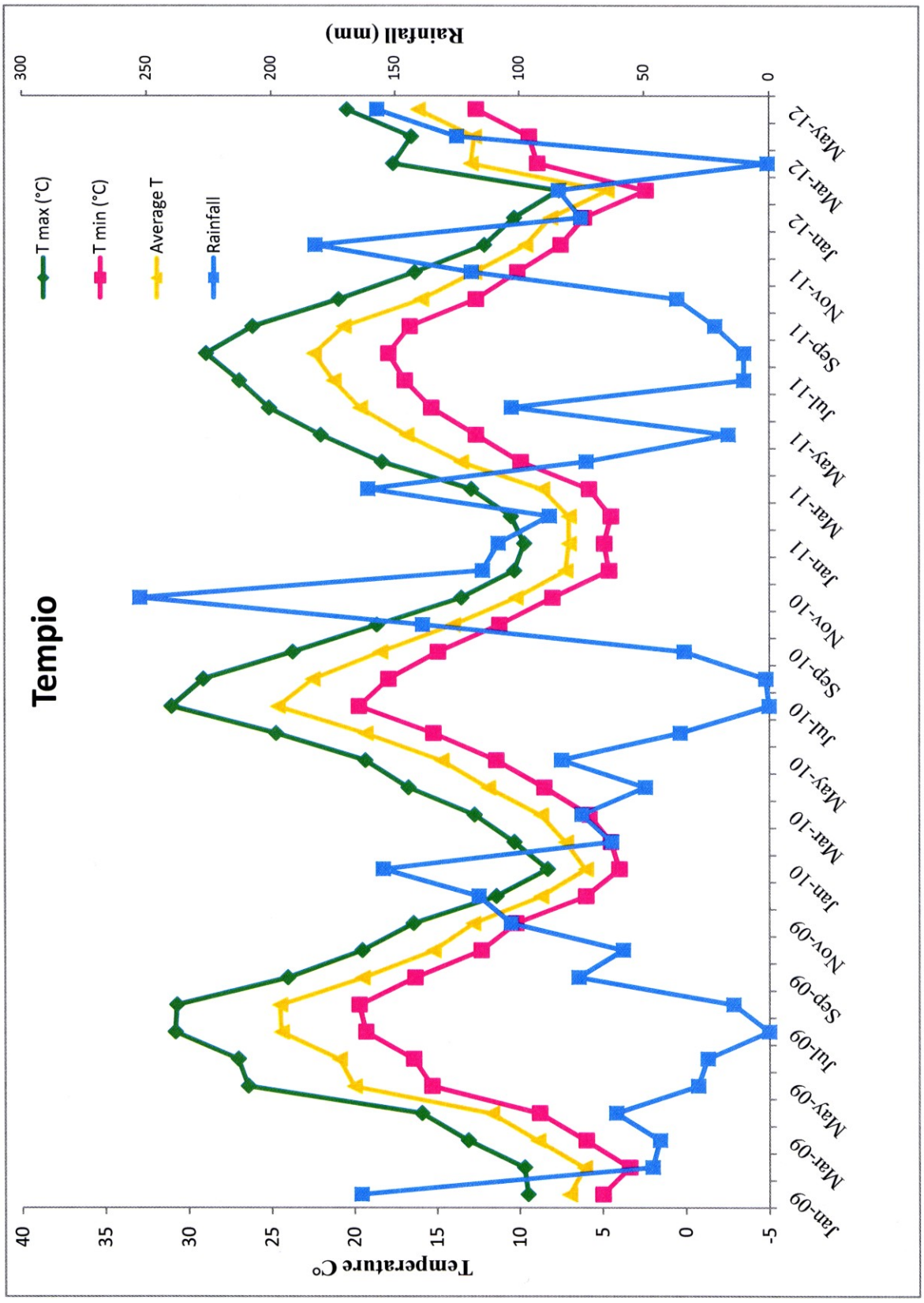


Figure 8. Monthly temperatures and rainfalls in the site of Tempio during 2009-2012 years.

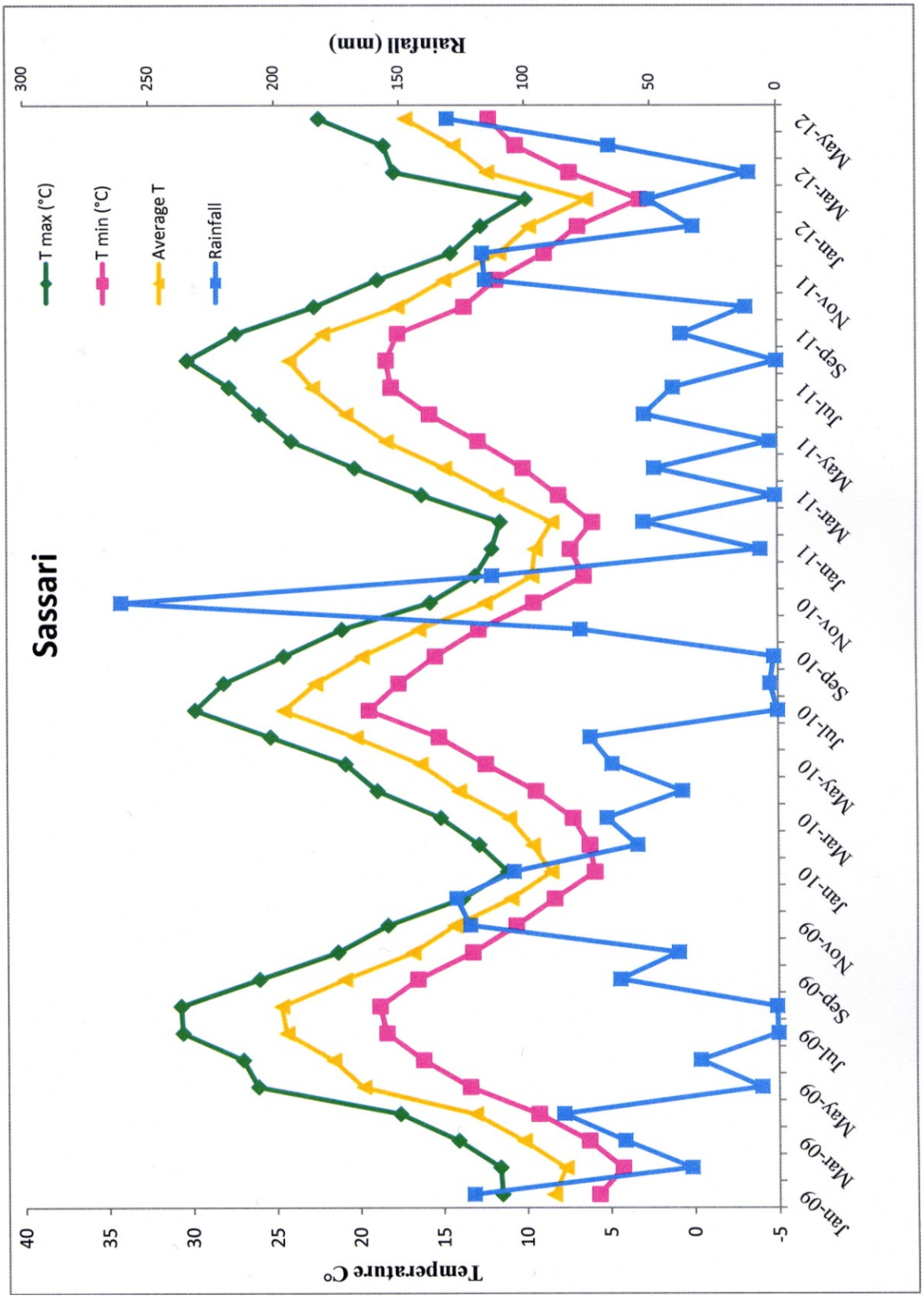


Figure 9. Monthly temperatures and rainfalls in the site of Sassari during 2009-2012 years.

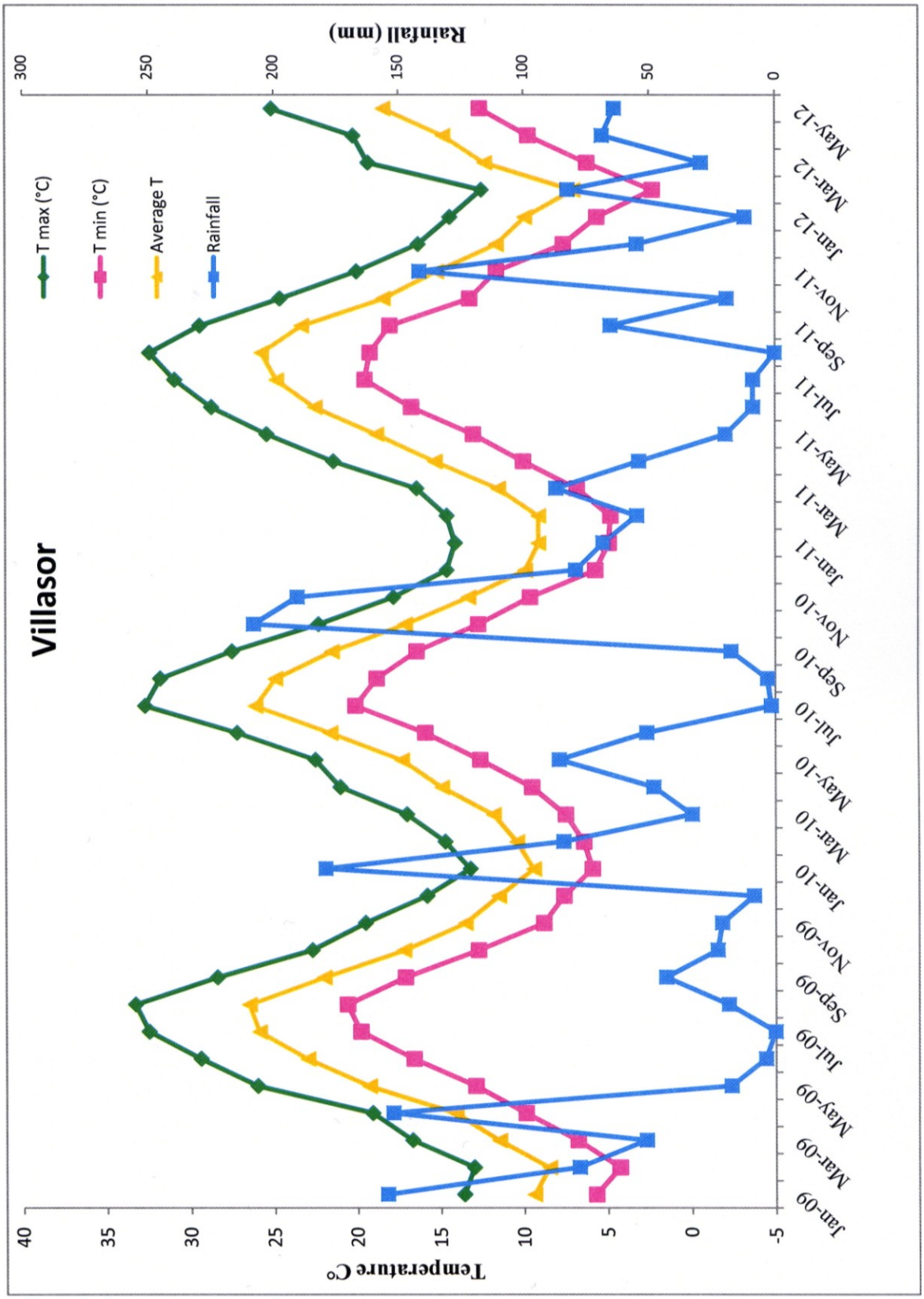


Figure 10. Monthly temperatures and rainfalls in the site of Villasor during 2009-2012 years.

Table 3. Indicator of cumulated chilling (ICC) for every critical threshold (0, 5 and 10 °C), in four localities for each semester and for total test period.

	critical T of 0 °C			
SEMESTER	ORISTANO	TEMPIO	SASSARI	VILLASOR
1	0	8	0	0
2	0	1	0	0
3	0	0	0	0
4	0	4	0	0
5	0	9	0	0
TOTAL	0	22	0	0
	critical T of 5 °C			
SEMESTER	ORISTANO	TEMPIO	SASSARI	VILLASOR
1	1.55	80	0.7	3
2	0	6	0	0
3	0.85	92	3	3
4	0	9	0	-
5	17.95	90	17	16
TOTAL	20.35	277	20.7	27
	critical T of 10 °C			
SEMESTER	ORISTANO	TEMPIO	SASSARI	VILLASOR
1	114.1	505	114.5	117
2	4.7	81	3.5	3
3	114.3	569	152	107
4	0	15	-	-
5	165.55	484	160	155
TOTAL	398.65	1654	430	392

4.2. Propagation

The agamic propagation of different clones of *Atriplex halimus* was performed in the spring of 2009 and 2011. In the Figure 11 percentages of rooting of cutting treated or not with IBA at 0.5% are showed as recorded in the spring of 2009 and 2011.

The clone PAL1, subjected to hormonal treatment, shown the highest percentage of rooting (40%). Among the untreated clones, the clone GIO1 showed the highest percentage of rooting (32%). The lowest rates were recorded in the clone GIO1 (4.44%) treated with IBA, we can say that in this case the hormone treatment has not stimulated rooting but on the contrary inhibited them. In general we can state that the hormone treatment has not given positive results, but on the contrary in most of the clones inhibited the rooting.

For this reason, the next test of rooting in spring 2011 was performed excluding the hormonal treatment. In general we can say that the rooting of the cuttings was good, although in the literature are indicated percentages of rooting even above 90%.

Regarding the second test of rooting, the results obtained are more positive than in the first test. In fact, maximum rooting percentage of 78% (SAN3) and 66% (PAL1) were reached. The clone showing the lowest percentage of rooting, also in this case, was the clone GIO1 (19.74%) (Fig. 12).

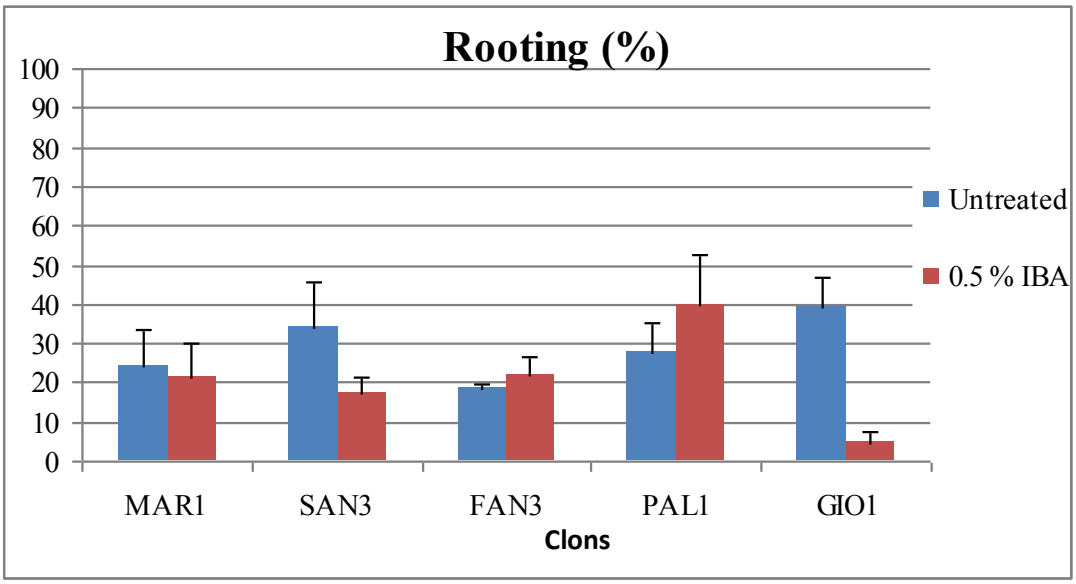


Figure 11. Percentage of rooting of cuttings of *A. halimus* subjected and not subjected to hormonal treatment.

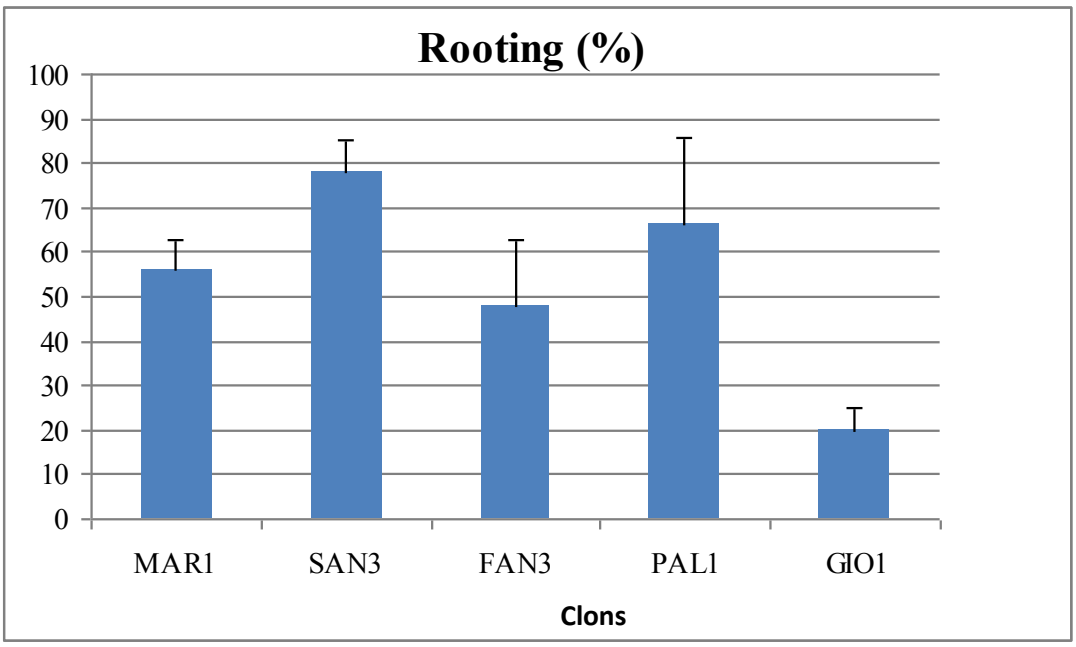


Figure 12. Percentage of rooting of cuttings of *A. halimus* not subjected to hormonal treatment.

4.3. Evaluation of plant growth

4.3.1. Experimental site of Oristano

The Figures 13 and 14 show the growth rates of the 5 clones observed. For every clone and semester is reported the related growth rate (RGR). The first semester corresponds to the period from November to May (2009-2010), the subsequent semester to the period from May to November (2010). The greater growth for all clones was recorded in the second semester. The growth of the first semester was lower because plants have had to adapt to the new environment. Plants have also been transferred in the month of October, so they should immediately tolerate the low winter temperatures, and this could be the cause of reduced growth (2009). The clone that has grown more in the second semester was the clone FAN3 (51.21%) (Fig. 13). In the second semester the lowest growth of all the clones was observed in the case of GIO1 (2.39%).

The third semester was unfavorable to growth, in fact, in three of five clones the growth was negative since there has been a loss of biomass. In this period, the MAR1 clone showed the highest biomass loss (-3.58%).

In the first and fourth semester the clone that showed the highest growth rate was the clone SAN3 with growth rates of respectively 13.89% and 30.44%. In the fourth semester was the clone PAL1 to record growth rates lower (-13.90%).

In general we can say that the clone with the lowest growth was the clone GIO1 that in all semesters presented growth rates very low, only the last semester was favorable to its growth (11.62%) (Fig. 14).

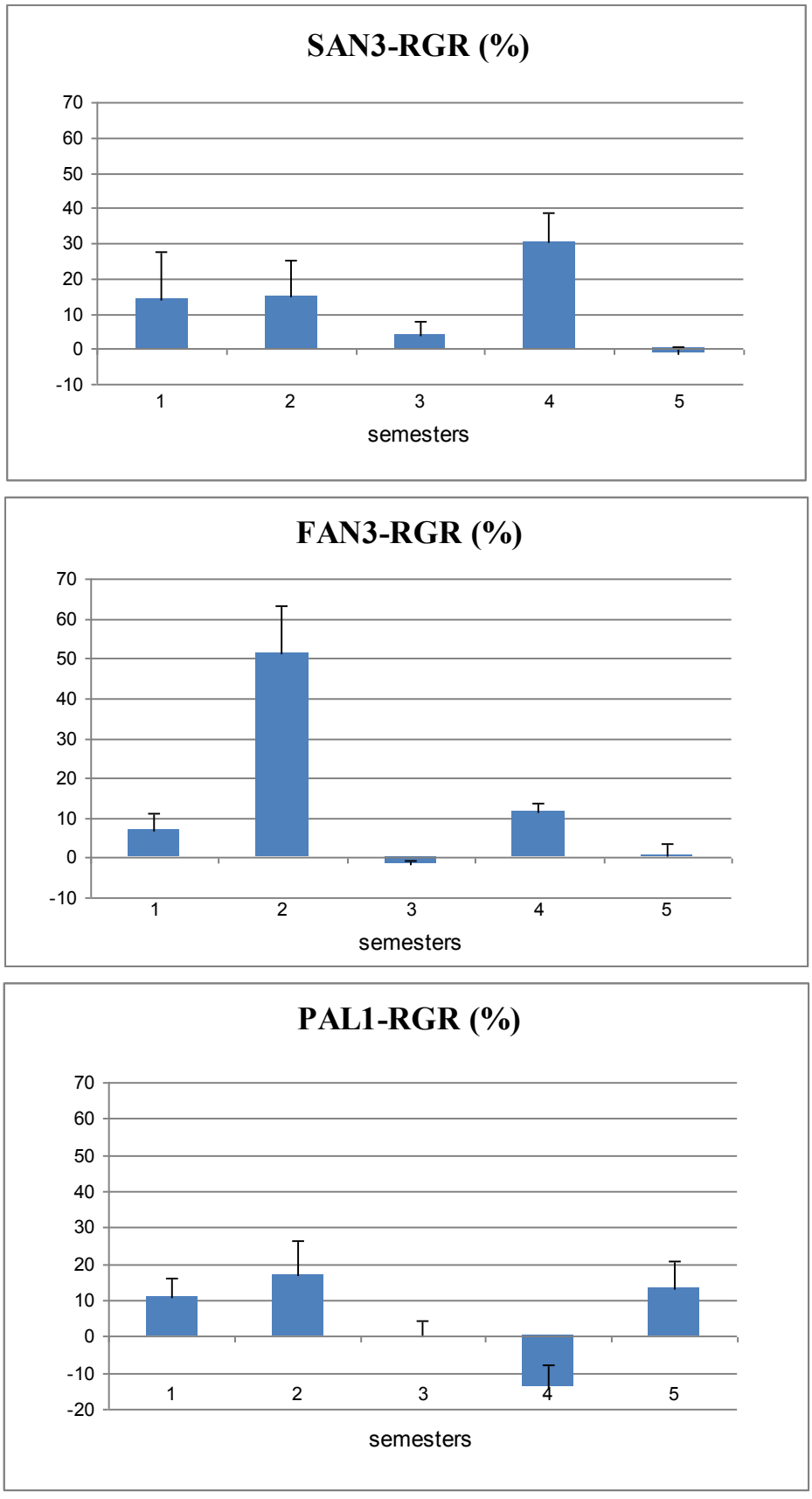


Figure 13. Relative Growth Rate (%) of clones SAN3, FAN3 and PAL1 of *A. halimus* in experimental site of Oristano for 5 semesters of observations.

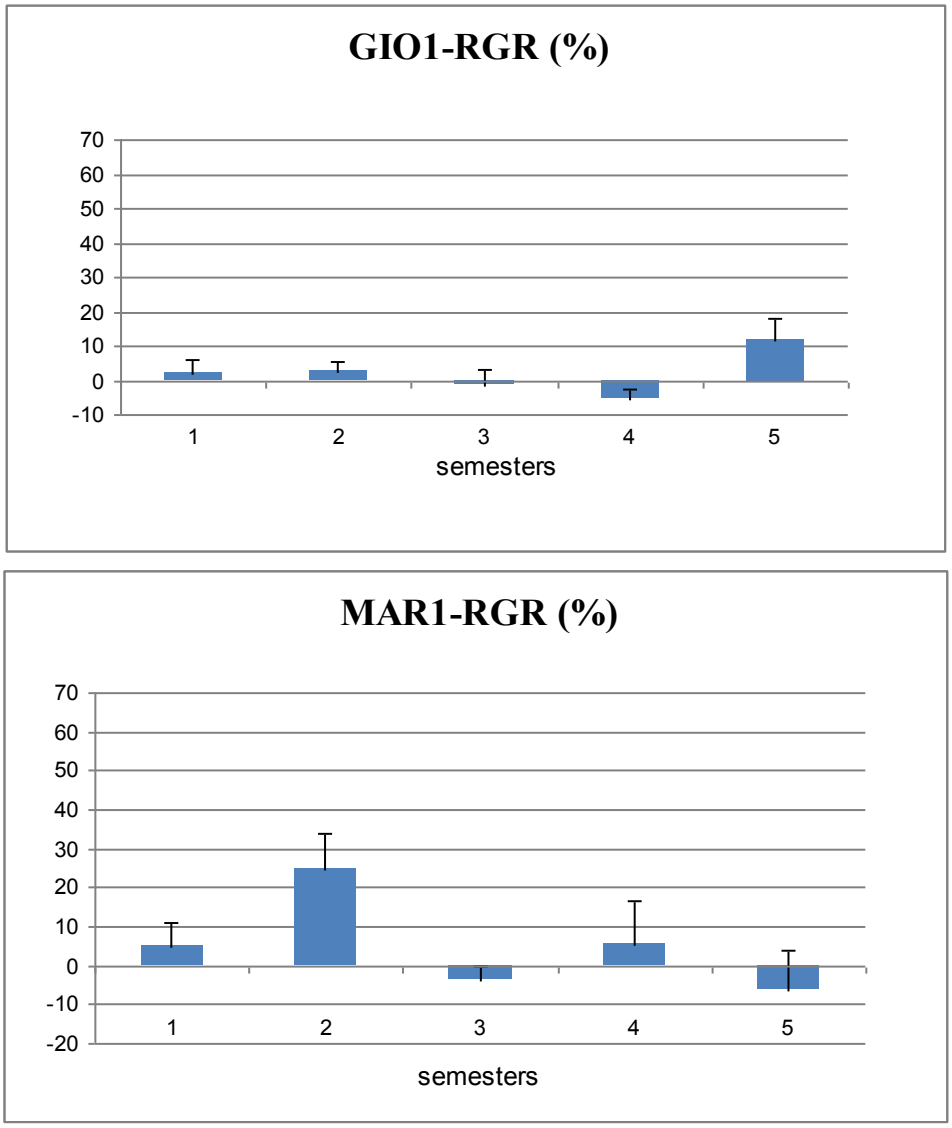


Figure 14. Relative Growth Rate (%) of clones GIO1 and MAR1 of *A. halimus* in experimental site of Oristano for 5 semesters of observations.

4.3.2. Experimental site of Tempio

The site of the Tempio was characterized by low average temperatures, where growth rates were lower than the site of Oristano.

The clone mostly affected by low temperature was SAN3 that in all semesters, except the first, presented negative growth rates, reaching at the third semester values of -17.21% (Fig. 15).

The clone GIO1, in four of five semesters presented negative rates, reaching a rate of -15.99% in the fourth semester. For this clone the only positive semester was the second semester (growth rate of 6.37%) (Fig. 16). For all clones, except clone SAN3, the second semester was favorable to growth.

The clones FAN3, MAR1 and PAL 1 were the more favored in this site, the clone FAN3 presented negative rates until the third semester (-0.65%), the clone MAR1 showed a decrease only in the third semester, while the clone PAL1 showed negative growth in the first semester (-1.49%). For the clone FAN3 was the fourth semester to be characterized by high growth (26.31%), for the clone MAR1 the highest rates were recorded in the second semester (16.19%), while the clone PAL1 was the fifth semester to be characterized by high growth.

As reported in Figure 15, the growth rate of the clone PAL1 increased from the first to third semester and then decreased to fourth semester and undergoes a further increase in the last semester. The clone FAN3 showed a trend of the rate of growth opposite to the clone PAL1, in fact, passing from the first to third semester there was a reduction in the rate.

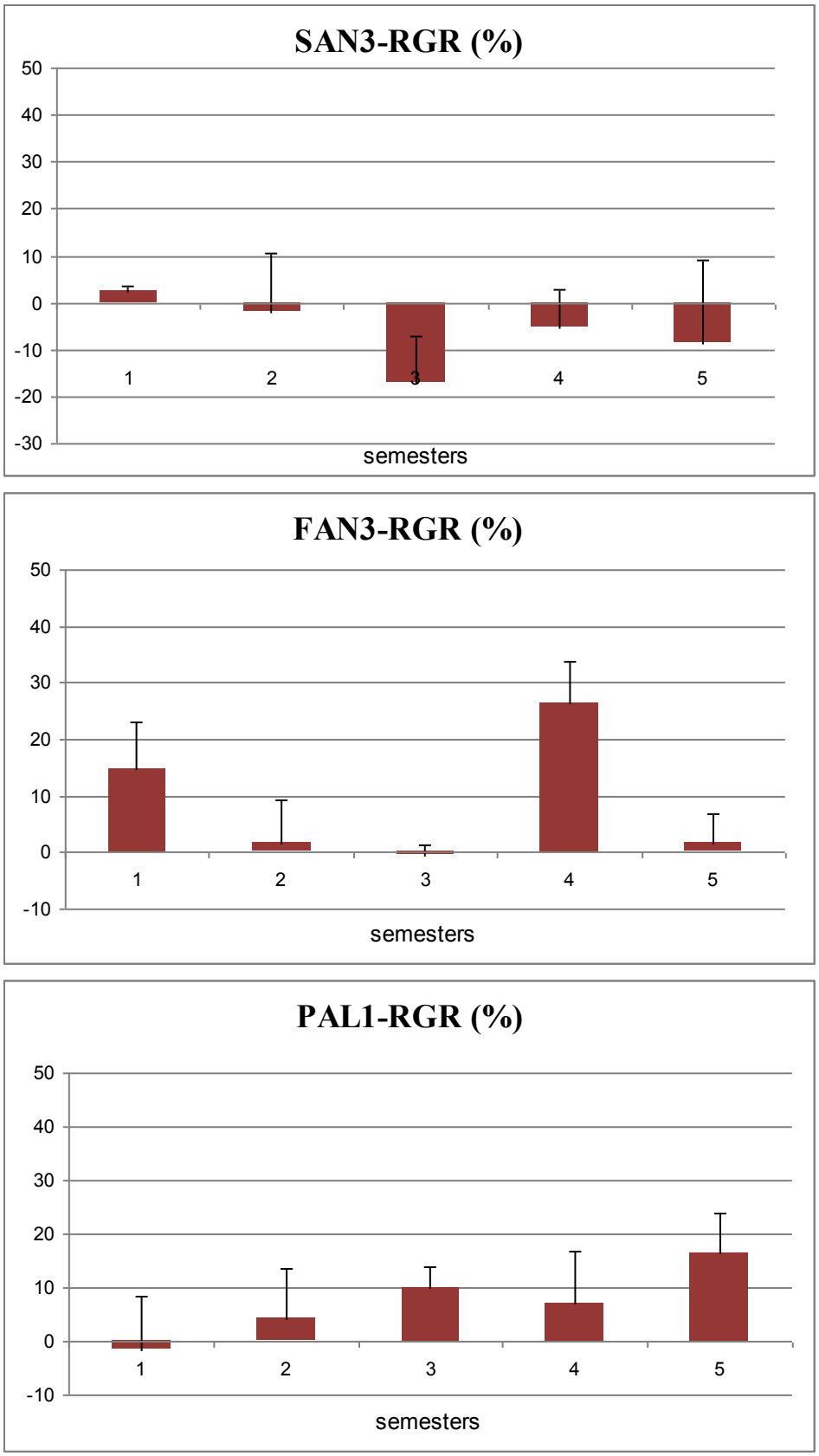


Figure 15. Relative Growth Rate (%) of clones SAN3, FAN3 and PAL1 of *A. halimus* in the experimental site of Tempio during 5 semesters of observations.

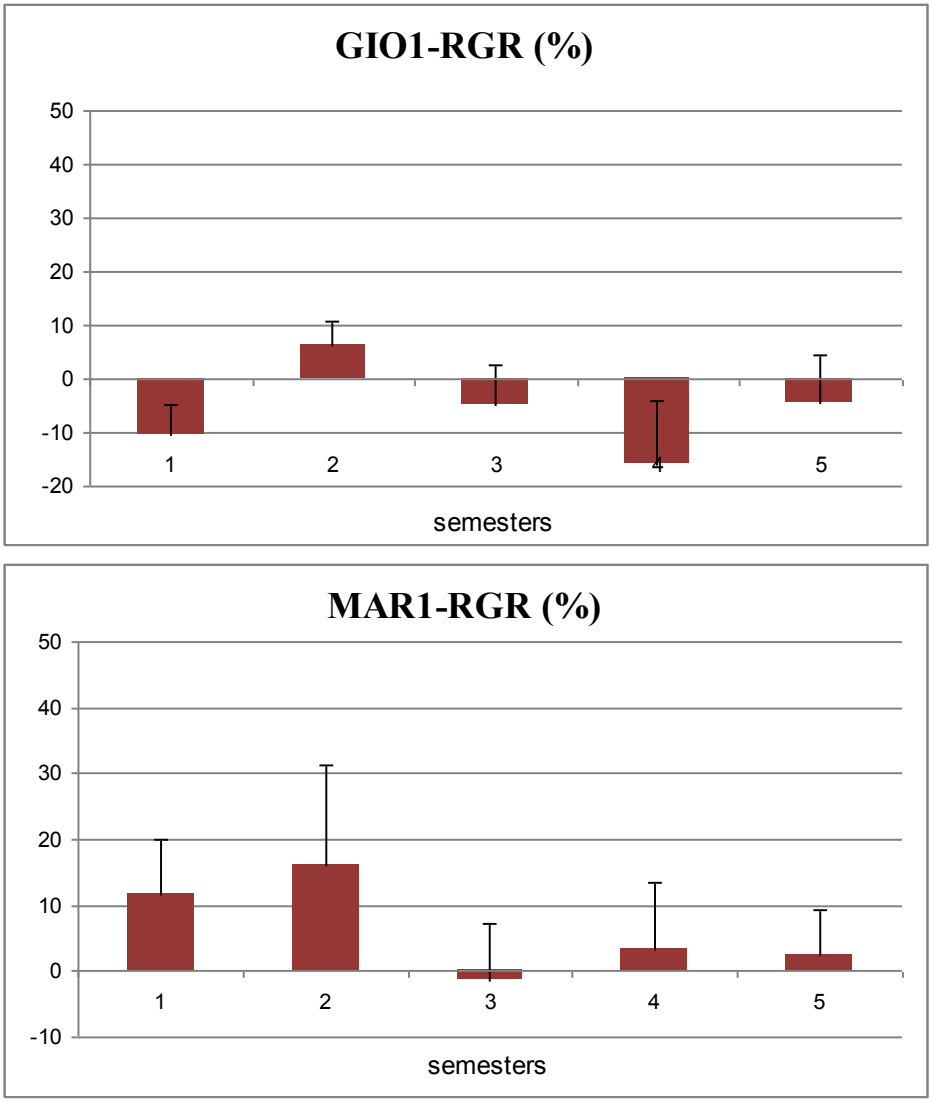


Figure 16. Relative Growth Rate (%) of clones GIO1 and MAR1 of *A. halimus* in the experimental site of Tempio during 5 semesters of observations.

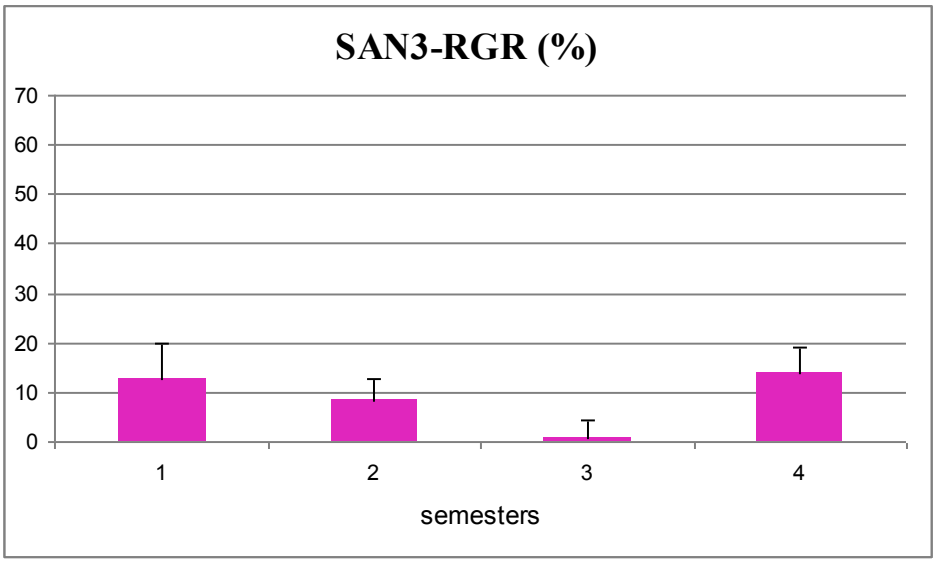
4.3.3. Experimental site of Sassari

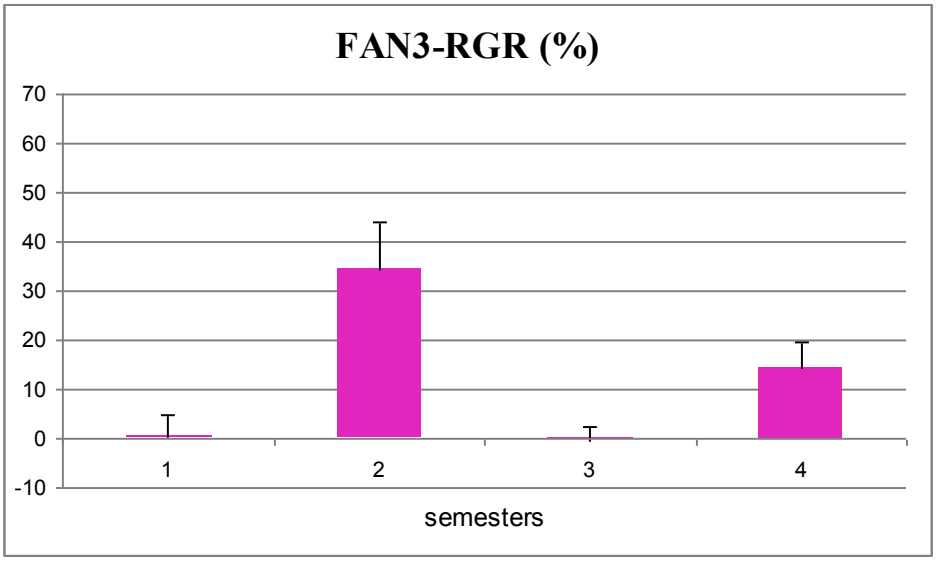
In this site the semesters of observation were 4 instead of 5 because this after the third semester there was a significant loss of plants for desiccation, this has led to the replacement of many of them and the loss of a semester of observation.

Observing the graphs in Figures 17 and 18 we can see that compared to the sites of Oristano and Temple the calculated rates are almost never negative. Only in the third semester and in particular in plants of the clone FAN3 and MAR1 was observed a slight decline in growth. The maximum rate achieved in this period was 2.27% in the clone PAL1. In that period, the clones FAN3 and MAR1 showed rates of respectively -0.49% and -0.08%.

The highest percentages of increase were observed in the second semester by clone FAN3 (34.56%) and MAR1 (42.67%). In the fourth semester the rates were positive for all clones, especially for the clone MAR1 and SAN3, which showed a growth of 28.66% and 14.51%.

In the site of Sassari also the clone GIO1 showed a positive growth rate in all semesters, however, the rate was not over 5.58%.





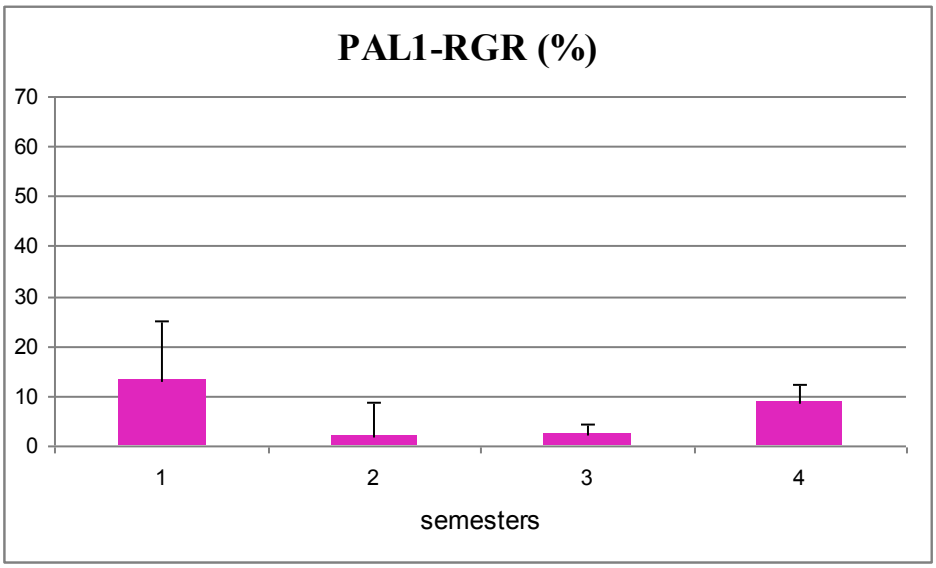
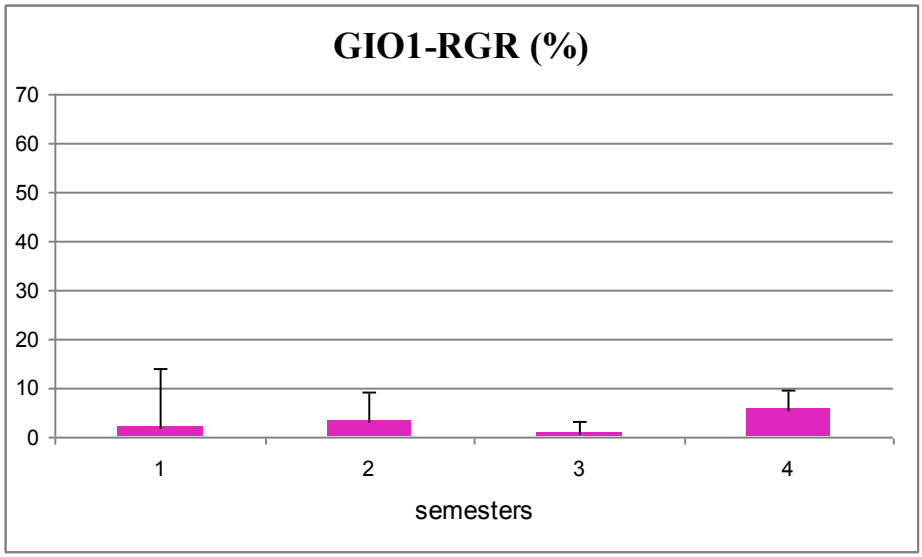


Figure 17. Relative Growth Rate of *A. halimus* clones SAN3, FAN3 and PAL1 in the experimental site of Sassari during 5 semesters of observations.



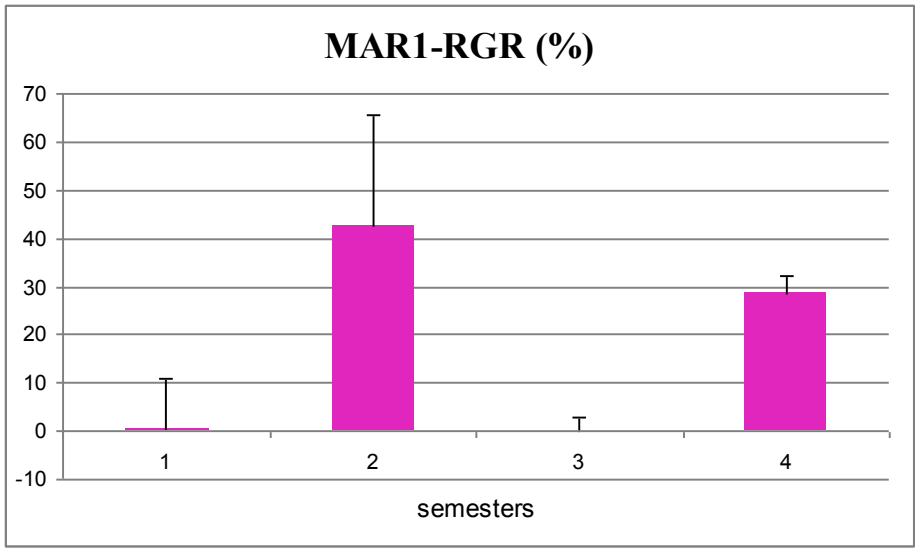


Figure 18. Relative Growth Rate of *A. halimus* clones GIO1 and MAR1 in the experimental site of Sassari during 5 semesters of observations.

4.3.4. Experimental site of Villasor

As for the site of Sassari also in this case the semesters of observations are 4 instead of 5. Even in the site of Villasor the third semester occurred a strong desiccation of plants, and as a result it became necessary to replace these.

As we can see from the graphs of Figures 19 and 20 growth rates were almost always positive even if not very high. Only the clone GIO1, in the first and in the second semester, showed a negative growth rate of -7.86% and -5% respectively. The maximum rate achieved by this clone was 8.87% in the fourth semester.

The fourth semester was a period favourable for clones FAN3 and MAR1 that showed their maximum values of growth, respectively of 15.62% and 17.11%.

The clone SAN3 showed its maximum growth in the second semester, with a growth rate of 12.45%. The maximum growth of the clone PAL1 was registered in the third semester with a rate of 17.57%.

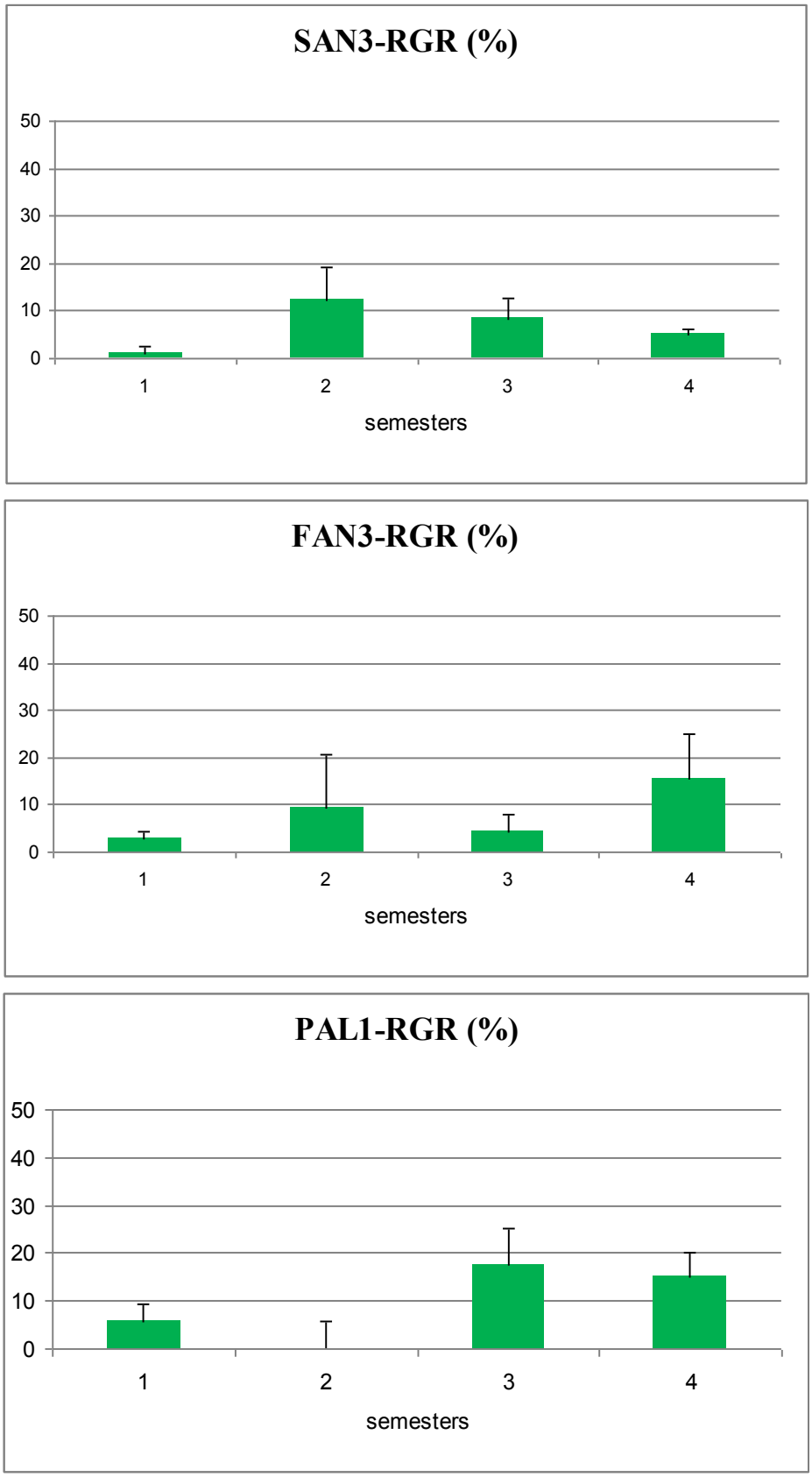


Figure 19. Relative Growth Rate of *A. halimus* clones SAN3, FAN3 and PAL1 in the experimental site of Villasor during 5 semesters of observations.

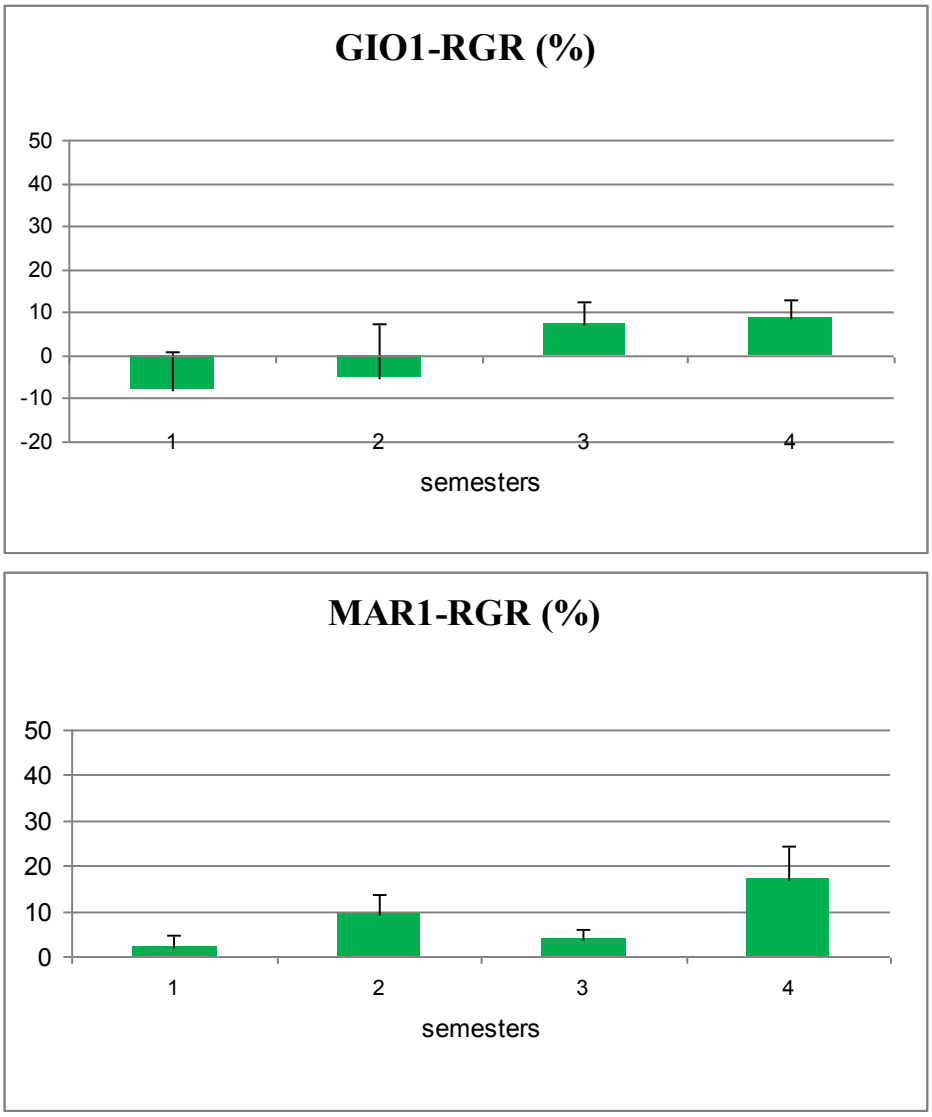


Figure 20. Relative Growth Rate of *A. halimus* clones GIO1 and MAR1 in the experimental site of Villasor during 5 semesters of observations.

4.3.5. Results of factorial analysis on the growth rate of plants.

Table 4 shows the averages of the growth rate and the analysis of variance. As we can see the differences between sites for growth rate (RGR) are statistically significant ($p < 0.01$). The higher growth rate was observed in the site of Sassari (7.92%) while the lowest increase was recorded in Tempio. Also differences among clones and among semesters were significant. Similarly, the interactions between localities and clones, between localities and semesters and among all three independent variables were statistically significant for $p < 0.01$.

Table 5 summarizes the data on the growth rate, by comparing the rates recorded in different sites and in different clones.

Table 4. Averages, *F* and *p* obtained from the analysis of variance with factorial design of the RGR parameter (Relative Growth Rate).

RGR (%)	
LOCALITY (A)	
Oristano	7.69
Tempio	2.112
Sassari	7.92
Villasor	6.997
F	7.6
p	0.007
CLONE (B)	
MAR1	8.297
GIO1	0.16
SAN3	5.16
PAL1	7.424
FAN3	9.859
F	23.15
p	0.001
LOCALITY x CLONE (AxB)	
F	7.42
P	0.001
SEMESTER (C)	
1	4.404
2	12.703
3	1.422
4	4.43
5	7.94
F	27.25
p	0.001
LOCALITY x SEMESTER (AxC)	
F	11.12
p	0.001
LOCALITY x CLONE x SEMESTER (AxBxC)	
F	5.7
p	0.002

Table 5. Relative Growth Rates (RGR %) of the 5 clones, in five semesters and 4 different sites. Data with letters in common are not statistically different for $p < 0.01$.

ORISTANO					
	MAR1	GIO1	SAN3	PAL1	FAN3
1 semester	4.86 h-u	1.72 h-v	13.90 e-n	10.99 f-r	6.90 h-u
2 semester	24.75 c-g	2.39 h-v	15.25 d-n	16.95 d-j	51.21 a
3 semester	-3.58 o-x	-1.26 l-x	3.75 h-u	0.15 h-w	-1.68 m-x
4 semester	5.41 h-u	-5.50 q-x	30.45 bcd	-13.90 vwx	11.58 f-q
5 semester	-6.30 r-x	11.62 f-q	-1.48 m-x	13.33 e-o	0.72 h-w
TEMPIO					
	MAR1	GIO1	SAN3	PAL1	FAN3
1 semester	11.73 f-q	-10.29 u-x	2.26 h-v	-1.50 m-x	14.92 d-n
2 semester	16.19 d-l	6.37 h-u	-2.15 n-x	4.14 h-u	1.61 h-v
3 semester	-1.32 m-x	-4.69 p-x	-17.21 x	10.15 f-r	-0.65 k-w
4 semester	3.38 h-u	-15.99 wx	-5.14 q-x	7.20 h-t	26.31 c-f
5 semester	2.48 h-v	-4.39 p-x	-8.55 t-x	16.38 d-k	1.52 h-v
SASSARI					
	MAR1	GIO1	SAN3	PAL1	FAN3
1 semester	0.34 h-w	2.15 h-v	12.54 e-p	13.23 e-o	0.33 h-w
2 semester	42.67 ab	3.35 h-u	8.51 g-t	1.87 h-v	34.56 bc
3 semester	-	-	-	-	-
4 semester	-0.08 i-w	0.82 h-v	0.89 h-v	2.27 h-v	-0.49 j-w
5 semester	28.66 b-e	5.58 h-u	14.04 e-n	8.85 g-t	14.51 d-n
VILLASOR					
	MAR1	GIO1	SAN3	PAL1	FAN3
1 semester	2.20 h-v	-7.86 s-x	0.91 h-v	5.89 h-u	2.84 h-v
2 semester	9.38 g-s	-5.00 q-x	12.45 e-p	0.06 i-w	9.49 g-s
3 semester	-	-	-	-	-
4 semester	4.06 h-u	7.23 h-t	8.33 g-t	17.57 d-h	4.18 h-u
5 semester	17.11 d-i	8.87 g-s	5.22 h-u	15.00 d-n	15.62 d-m

4.3.6. Influence of temperature on the rate of growth.

Tables 6 and 7 shows the correlation coefficients between the temperatures and the growth rate (RGR). In particular, Table 6 shows the results of the correlation between the index of cumulated cold (ICC) calculated every semester and the RGR, while in second table data are of the whole test period (average RGR of five semesters and ICC of all the whole test period). Examining the correlation values obtained in Tables 6 and 7 it can be noted a little level of significance of correlations. In fact, only for some clones and for some temperature thresholds significant correlation indexes were obtained. With regard to the Table 6 only the growth rate of the clone GIO1 appears significantly correlated with the index of cumulated cold below the thermal threshold of zero degrees.

The correlation was negative, and then we can say that in the clone GIO1 low temperatures lead to a reduction in growth and regression of vegetation. The ICC for the critical temperature of 5 and 10 °C, however, does not appear to be widely correlate with the plant growth. Only the growth rate of the clone SAN3, was significantly correlated (negatively) with the ICC for the critical temperatures of 5 and 10 ° C ($p < 0.01$).

The data in Table 7 (correlations calculated for all the test period) confirm the data of Table 6. In this case, the correlations are stronger, in fact r reaches values of -0.979 for $p < 0.05$.

Table 6. Correlations (r) between the semester ICC and the growth rate of every clone (ns = not significant, * = significant for $p < 0.05$, ** = significant for $p < 0.01$).

	ICC (0 °C)	ICC (5 °C)	ICC (10 °C)
RGR-MAR1	-0.086 n.s.	-0.176 n.s.	-0.282 n.s.
RGR-GIO1	-0.502 *	-0.308 n.s.	-0.207 n.s.
RGR-SAN3	-0.381 n.s.	-0.621 **	-0.635 **
RGR-PAL1	0.028 n.s.	0.153 n.s.	0.190 n.s.
RGR-FAN3	0.005 n.s.	-0.207 n.s.	-0.371 n.s.

Table 7. Correlations (r) between ICC for all the test period (5 semesters) and the average growth rate of every clone (ns = not significant, * = significant for $p < 0.05$, ** = significant for $p < 0.01$).

	ICC (0 °C)	ICC (5 °C)	ICC (10 °C)
RGR-MAR1	-0.334 n.s.	-0.339 n.s.	-0.311 n.s.
RGR-GIO1	-0.974 *	-0.979 *	-0.968 *
RGR-SAN3	-0.958 *	-0.964 *	-0.957 *
RGR-PAL1	0.011 n.s.	0.035 n.s.	0.000 n.s.
RGR-FAN3	-0.472 n.s.	-0.492 n.s.	-0.462 n.s.

4.4. Quantitative and qualitative analysis of plant biomass.

Shown below are the results for the quantitative analysis of the biomass produced per plant. Data on leaf, branches and roots will be presented separately.

4.4.1. Analysis of leaves

In Table 8 is shown the production of leaf biomass for plant in 4 the sites of Oristano, Tempio, Sassari and Villasor. For each dependent variable are presented the averages and the *F* values as observed in the analysis of variance. In addition, for every parameter, significance of differences among localities, clones, and semesters and interactions between locality and clone, locality and semester and among locality, clone and semester are also indicated.

The fresh weight of leaf biomass varies in the semester. As we can see from the analysis of variance and the data reported in Table 9, the greater fresh weight of leaves in all sites was relative to the first semester. The differences among semesters for leaf fresh biomass were statistically different ($p < 0.01$), as well as the differences among the clones ($p < 0.01$) and the interactions between localities and semesters ($p < 0.01$). Interactions between locality and clone are not significative.

The analysis of the biomass production in the various sites showed that in Oristano leaf fresh weight was highest in the first and in the second semester with the clone PAL1, which showed values of 8.73 g and 10.73 g respectively. The lowest fresh weight of leaf biomass is that relating to the clone MAR1 (1.43 g) in the fifth semester concerned. In the third and fourth semesters, lowest fresh weight was that of the clone SAN3 with values of fresh weight 2.12 g and 5.19 g, respectively.

In general we can state that for the clone MAR1, passing from the first to fifth semester, the fresh weight of the leaves decreased more and more. In Tempio the fresh weight of leaf ranges from a minimum of 0.30 g in the fifth semester (GIO1) to a maximum of 16.77 g in the first semester (MAR1).

Even in Tempio was the clone MAR1 to shows the highest values of fresh weight of the leaves, not only in the first but also in the third and fifth semester, while the clone GIO1 showed the lowest values in nearly all semesters (Table 9).

Even in Sassari in the last two semesters there was a contraction of leaf production. The clone that showed higher leaf fresh weight was the clone MAR1 in the first semester (12.92 g), while GIO1 in the first three semesters showed the lowest fresh and dry weight.

In Villasor the plant production of leaf biomass, was considerably reduced in all semesters than the other sites. The leaf fresh weight ranged from of 0.05 g on the third semester (GIO1) to 13.22 g in the first semester (PAL1). As mentioned before the third semester was a critical time for the plants of all four locations, but especially for plants in Villasor site to such an extent that in some clones (GIO1) the production of leaves was almost absent.

In general we can say that the clones more productive in terms of fresh leaf biomass were clones PAL1 and MAR1 while the clone GIO1 in all sites was the clone that showed the lowest production of leaf biomass. As we can see from the analysis of variance, the leaf dry weight follows the trend of fresh weight. Even in this case the greatest dry weight has been achieved by the plants placed in Sassari, while the lowest foliar dry weight regards the site of Villasor. The differences between the production of leaf dry weight, in the different sites were statistically significant ($p < 0.01$), as well as the production for semester and semester x locality interaction ($p < 0.01$). In the site of Oristano the highest leaf dry weight was that relating to the fourth semester and in particular to the clone FAN3. The lowest dry weight was instead registered in the fifth semester of the clone MAR1. As for the fresh weight, also in this case, the dry weight was reduced progressively over time (Table 10).

Even in Tempio leaf dry weight follows the trend of the fresh weight, with the recorded values ranging from a minimum of 0.05 g (GIO1) to a maximum of 4.16 g (FAN3). The clone FAN 3 showed, even in Sassari, the largest leaf dry weight (3.57 g). The clone GIO1 resulted as the clone that showed the lowest contents of both fresh and dry leaf biomass. Such behavior has also been found in Villasor especially in the third semester where the leaf dry weight per plant of clone GIO1 was equal to 0.02 g.

The percentage of dry matter in the leaves was highest in plants of Villasor, plants that had shown the lowest fresh weight. In Oristano the lowest percentage of dry matter was found in clone PAL1 that in all semesters except the fourth, showed the lowest values. The highest rates were those relating to the first semester of the clone GIO1 (64.25%) and the second, third and fourth semester of clone SAN3 (Table 11).

In Tempio the lowest percentage of leaf dry matter was that relating to fifth semester in the clone FAN3 (15.45%). The greatest percentage of dry matter was recorded in the first and second semester in the clone GIO1 with values of 31.26% and 30.52% respectively. In all cases we can say that in almost all clones highest values were achieved in the first semester to reach the minimum values in the fifth semester. This behavior has also been found at the site of Sassari, in fact the highest percentage of dry matter was found in the first semester in the clone SAN3 (39.67%), while the lowest one was on the clone MAR1 (19%).

In Villasor the percentage of leaf dry matter ranged from a minimum of 15.08% (PAL1 the third semester) to a maximum of 48.45% (MAR1 the first half). In general we can say that the highest percentage of leaf dry matter produced per plant is relating to site Villasor where the plants were more affected.

4.4.1.1. Influence of temperature on leaf biomass production

In Tables 12 and 13 are reported the correlation coefficients between the temperatures and the production of leaves for each clone. In particular, were evaluated relationship between temperature and the fresh weight, dry weight and percentage of dry matter. The Table 12 shows the results of the correlation between ICC of the semester and leaf biomass production in the same period, while in the Table 13 the data are related to the entire period of the test.

As we can see there was no any significant correlation between leaf production (fresh weight, dry weight and percentage of dry matter) and the three sums below the semi-critical temperature thresholds. Taking into consideration the ICC for the entire period of the test, we have been found significant correlations (p <0.01) between the percentage of dry matter of the leaves of the clone SAN3 and the three sums. The correlation was negative for all three temperature thresholds, with a value of r = -0.99.

Table 8. Averages, *F* and *p* values obtained from the analysis of variance of the dependent variable leaf fresh weight (f.w. leaves), dry weight of leaves (d.w. leaves), % dry matter (% d.m. of leaves).

	f.w. leaves (g)	d.w. leaves (g)	d.m. leaves (%)
LOCALITY (A)			
Oristano	5.696	1.469	27.793
Tempio	5.165	1.301	24.821
Sassari	6.109	1.533	25.168
Villasor	3.21	0.896	29.213
F	13.9	16.04	10.61
P	0.004	0.002	0.008
CLONE (B)			
MAR1	5.63	1.383	26.671
GIO1	3.532	0.972	28.539
SAN3	4.885	1.382	28.432
PAL1	5.905	1.348	23.864
FAN3	5.303	1.415	26.237
F	4.26	3.33	7.6
P	0.007	0.021	0.001
LOCALITY x CLONE (AxB)			
F	1.27	1.43	2.13
P	0.281	0.203	0.43
SEMESTER (C)			
1	8.749	2.469	32.247
2	5.15	1.28	25.731
3	3.578	0.847	25.886
4	4.392	1.14	25.506
5	3.356	0.764	24.372
F	24.71	41.34	20.1
P	0.001	0.001	0.001
LOCALITY x SEMESTER (AxC)			
F	4.94	5.41	4.97
P	0.0001	0.001	0.001
LOCALITY x CLONE x SEMESTER (AxBxC)			
F	0.94	0.78	3.23
P	n.s.	n.s.	0.001

Table 9. Quantitative analysis of fresh leaf biomass (g) produced per plant of *A. halimus* in the four sites and five semesters considered. Data with letters in common are not statistically different for $p < 0.01$.

ORISTANO					
	MAR1	GIO1	SAN3	PAL1	FAN3
1 semester	8.23 a-j	1.60 g-j	6.46 b-j	8.73 a-j	6.27 b-j
2 semester	8.17 a-j	5.27 b-j	4.89 c-j	10.73 a-j	4.43 c-j
3 semester	7.21 b-j	5.38 b-j	2.92 f-j	4.54 c-j	5.14 c-j
4 semester	5.86 b-j	7.68 b-j	5.19 c-j	7.41 b-j	8.63 a-j
5 semester	1.43 g-j	5.70 b-j	4.57 c-j	3.47 f-j	2.50 f-j
TEMPIO					
	MAR1	GIO1	SAN3	PAL1	FAN3
1 semester	16.77 A	6.67 b-j	10.33 a-h	12.93 a-d	14.37 ab
2 semester	5.69 b-j	2.78 f-j	7.53 b-j	6.81 b-j	4.70 c-j
3 semester	3.12 f-j	0.68 ij	0.47 ij	0.88 ij	1.85 g-j
4 semester	3.04 f-j	1.69 g-j	1.90 g-j	5.70 b-j	3.35 f-j
5 semester	6.77 b-j	0.30 j	0.97 ij	5.17 c-j	4.67 c-j
SASSARI					
	MAR1	GIO1	SAN3	PAL1	FAN3
1 semester	12.92 a-d	3.57 e-j	6.61 b-j	10.74 a-g	11.27 a-f
2 semester	6.68 b-j	2.74 f-j	5.19 c-j	6.43 b-j	4.84 c-j
3 semester	9.80 a-i	1.05 hij	12.77 a-e	4.76 c-j	7.74 b-j
4 semester	1.82 g-j	6.64 b-j	5.43 b-j	3.43 f-j	3.66 e-j
5 semester	4.03 c-j	5.93 b-j	6.07 b-j	4.67 c-j	3.93 d-j
VILLASOR					
	MAR1	GIO1	SAN3	PAL1	FAN3
1 semester	4.17 c-j	3.35 f-j	8.22 a-j	13.22 abc	8.57 a-j
2 semester	3.45 f-j	1.34 hij	2.61 f-j	3.56 e-j	5.16 c-j
3 semester	0.59 Ij	0.05 j	0.78 ij	0.77 ij	1.08 hij
4 semester	1.60 g-j	6.50 b-j	2.60 f-j	3.07 f-j	2.63 f-j
5 semester	1.27 hij	1.70 g-j	1.60 g-j	1.10 hij	1.27 hij

Table 10. Quantitative analysis of dry leaf biomass (g) produced per plants of *A. halimus* in the four sites and five semesters considered. Data with letters in common are not statistically different for $p < 0.01$.

ORISTANO					
	MAR1	GIO1	SAN3	PAL1	FAN3
1 semester	2.08 a-l	1.03 d-l	2.01 b-l	1.80 c-l	1.86 c-l
2 semester	1.84 c-l	1.24 d-l	1.32 d-l	2.27 a-l	1.13 d-l
3 semester	1.72 c-l	1.34 d-l	0.80 f-l	1.05 d-l	1.31 d-l
4 semester	1.39 c-l	2.16 a-l	1.82 c-l	1.96 c-l	2.41 a-k
5 semester	0.47 g-l	1.30 d-l	1.13 d-l	0.67 f-l	0.63 g-l
TEMPIO					
	MAR1	GIO1	SAN3	PAL1	FAN3
1 semester	4.09 ab	2.08 a-l	3.21 a-d	3.09 a-e	4.16 a
2 semester	1.46 c-l	0.80 f-l	2.04 b-l	1.66 c-l	1.16 d-l
3 semester	0.71 f-l	0.21 jkl	0.14 kl	0.19 jkl	0.43 g-l
4 semester	0.81 f-l	0.39 g-l	0.44 g-l	1.41 c-l	0.77 f-l
5 semester	1.23 d-l	0.05 l	0.17 jkl	1.05 d-l	0.77 f-l
SASSARI					
	MAR1	GIO1	SAN3	PAL1	FAN3
1 semester	3.26 a-d	1.18 d-l	2.58 a-g	2.50 a-i	3.57 abc
2 semester	1.70 c-l	0.57 g-l	1.46 c-l	1.26 d-l	1.20 d-l
3 semester	2.14 a-l	0.29 i-l	2.90 a-f	0.91 e-l	1.76 c-l
4 semester	0.42 g-l	1.76 c-l	1.49 c-l	0.75 f-l	0.96 e-l
5 semester	0.77 f-l	1.50 c-l	1.60 c-l	0.90 e-l	0.90 e-l
VILLASOR					
	MAR1	GIO1	SAN3	PAL1	FAN3
1 semester	1.74 c-l	1.06 d-l	2.57 a-h	3.08 a-e	2.43 a-j
2 semester	0.89 e-l	0.35 g-l	0.79 f-l	0.95 e-l	1.51 c-l
3 semester	0.19 jkl	0.02 l	0.31 g-l	0.23 jkl	0.31 h-l
4 semester	0.40 g-l	1.60 c-l	0.47 g-l	0.77 f-l	0.63 g-l
5 semester	0.37 g-l	0.50 g-l	0.40 g-l	0.47 g-l	0.40 g-l

Table 11. Percentage of dry matter in the leaves of the species *A. halimus* in the four sites and five semesters. Data with letters in common are not statistically different for $p < 0.01$.

ORISTANO					
	MAR1	GIO1	SAN3	PAL1	FAN3
1 semester	32.19 c-j	64.25 a	32.26 c-j	22.08 f-m	29.92 c-l
2 semester	22.43 f-m	23.43 f-m	26.90 d-m	21.68 g-m	25.12 d-m
3 semester	24.14 f-m	25.21 d-m	27.04 d-m	23.94 f-m	25.60 d-m
4 semester	22.72 f-m	27.48 d-m	34.66 c-h	26.53 d-m	27.85 d-m
5 semester	36.31 b-g	21.66 g-m	23.52 f-m	20.65 h-m	27.25 d-m
TEMPIO					
	MAR1	GIO1	SAN3	PAL1	FAN3
1 semester	24.96 e-m	31.26 c-k	30.85 c-k	23.88 f-m	29.03 d-m
2 semester	25.98 d-m	30.52 c-k	28.60 d-m	24.53 f-m	24.79 e-m
3 semester	23.36 f-m	33.12 c-i	27.86 d-m	22.85 f-m	23.78 f-m
4 semester	26.06 d-m	23.69 f-m	27.64 d-m	28.16 d-m	22.87 f-m
5 semester	18.25 j-m	16.67 klm	16.57 klm	19.78 i-m	15.45 Lm
SASSARI					
	MAR1	GIO1	SAN3	PAL1	FAN3
1 semester	27.53 d-m	33.15 c-i	39.67 bcd	24.81 e-m	30.21 c-l
2 semester	25.50 d-m	20.78 h-m	28.97 d-m	19.95 h-m	24.56 f-m
3 semester	21.39 h-m	27.07 d-m	24.46 f-m	21.40 h-m	22.78 f-m
4 semester	23.01 f-m	25.98 d-m	27.20 d-m	21.72 g-m	26.16 d-m
5 semester	19.00 i-m	25.27 d-m	26.25 d-m	19.38 i-m	23.01 f-m
VILLASOR					
	MAR1	GIO1	SAN3	PAL1	FAN3
1 semester	48.45 B	36.86 b-f	31.28 c-k	23.28 f-m	29.04 d-m
2 semester	25.81 d-m	25.20 d-m	31.96 c-j	29.14 d-m	28.74 d-m
3 semester	30.96 c-k	26.00 d-m	39.42 b-e	15.08 m	32.28 c-j
4 semester	26.56 d-m	24.23 f-m	18.53 i-m	25.25 d-m	23.82 f-m
5 semester	28.80 d-m	28.97 d-m	25.00 e-m	43.18 bc	32.48 c-j

Table 12. Correlations (r) between the semester ICC and leaves fresh weight, dry weight and leaves dry matter of each clone (n.s. = not significant, * = significant for $p < 0.05$, ** = significant $p < 0.01$).

		ICC (0 °C)	ICC (5 °C)	ICC (10 °C)
Leaves fresh weight (g)	MAR1	0.419 n.s.	0.234 n.s.	0.316 n.s.
	GIO1	-0.096 n.s.	-0.201 n.s.	-0.246 n.s.
	SAN3	-0.002 n.s.	-0.176 n.s.	-0.044 n.s.
	PAL1	0.259 n.s.	-0.043 n.s.	-0.006 n.s.
	FAN3	0.337 n.s.	0.101 n.s.	0.178 n.s.
Leaves dry weight (g)	MAR1	0.352 n.s.	0.162 n.s.	0.261 n.s.
	GIO1	-0.057 n.s.	-0.174 n.s.	-0.184 n.s.
	SAN3	0.012 n.s.	-0.165 n.s.	-0.031 n.s.
	PAL1	0.267 n.s.	-0.047 n.s.	-0.018 n.s.
	FAN3	0.251 n.s.	0.045 n.s.	0.131 n.s.
Leaves dry matter (%)	MAR1	-0.271 n.s.	-0.270 n.s.	-0.162 n.s.
	GIO1	-0.208 n.s.	-0.100 n.s.	0.043 n.s.
	SAN3	-0.307 n.s.	-0.316 n.s.	-0.214 n.s.
	PAL1	-0.066 n.s.	-0.085 n.s.	-0.117 n.s.
	FAN3	-0.442 n.s.	-0.397 n.s.	-0.268 n.s.

Table 13. Correlations (r) between ICC of all test period (5 semesters) and the production of leaves of each clone (n.s. = not significant, * = significant for $p < 0.05$, ** = significant for $p < 0, 01$).

		ICC (0 °C)	ICC (5 °C)	ICC (10 °C)
Leaves fresh weight (g)	MAR1	0.417 n.s.	0.396 n.s.	0.435 n.s.
	GIO1	-0.733 n.s.	-0.747 n.s.	-0.732 n.s.
	SAN3	-0.238 n.s.	-0.256 n.s.	-0.213 n.s.
	PAL1	0.235 n.s.	0.213 n.s.	0.243 n.s.
	FAN3	0.294 n.s.	0.273 n.s.	0.316 n.s.
Leaves dry weight (g)	MAR1	0.408 n.s.	0.386 n.s.	0.426 n.s.
	GIO1	-0.679 n.s.	-0.692 n.s.	-0.679 n.s.
	SAN3	-0.265 n.s.	-0.283 n.s.	-0.240 n.s.
	PAL1	0.429 n.s.	0.411 n.s.	0.428 n.s.
	FAN3	0.109 n.s.	0.087 n.s.	0.132 n.s.
Leaves dry matter (%)	MAR1	-0.479 n.s.	-0.461 n.s.	-0.501 n.s.
	GIO1	-0.370 n.s.	-0.376 n.s.	-0.385 n.s.
	SAN3	-0.991 **	-0.99 **	-0.989 **
	PAL1	0.007 n.s.	0.016 n.s.	-0.028 n.s.
	FAN3	-0.786 n.s.	0.773 n.s.	-0.801 n.s.

4.4.2. Analysis of branches

As regards to the quantitative analysis of branches production for plant, also in this case, the fresh weight and dry weight decreased with the time. As we can see from Table 14 by the analysis of variance, the greatest fresh weight was observed in the growing plants in Tempio (16.35 g), while in Villasor there was the lowest production of branch biomass per plant (9.53 g).

The clone with the highest biomass production of the branches is the clone FAN3, which in fact appeared among the most vigorous clones and abundantly rich in woody biomass. The clone with the lowest production of branches also in this case was the clone GIO1. As we can see the differences between clones are statistically significant ($p < 0.01$), while the differences between the productions in different locations and location-clone interactions are not statistically different. Productions are different in different semesters ($p < 0.01$), the most productive semester in terms of fresh biomass was the first, the least productive was the last semester.

Tables 15 and 16 show the production of fresh and dry biomass in 4 different sites. In the site of Oristano, in the first three semesters, the clone showed the largest branch fresh weight reaching 37.14 g in the first semester. The lowest fresh weight of the branches on the same site is relative to the clone GIO1 (7.46 g). Also for the dry weight the clone MAR1 had the highest values (18.38 g). In Tempio the greatest fresh weight in about all semesters was that of the clone FAN3, that in the first semester showed values of 39.80 g fresh weight for plant, while the minimum values are those related to clone GIO1 (5.21 g to second semester). The dry weight of the branches followed the trend of the fresh weight, showing values ranging from a minimum of 2.93 g (GIO1 in the second semester) to a maximum of 25.15 g (FAN3 in the fourth semester).

In Sassari, as for the leaves, in the first 3 semesters was the clone GIO1 to present the lowest values of branch fresh weight respectively of 10.52 g, 9.25 g and 6.70 g. The same clone instead in the last 2 semesters showed fresh weight values highest than the other clones. The greatest fresh weight was reached in first semester by the clone FAN3 (45.71 g), while in the second and third semester the clone with the highest fresh weight was the clone MAR1, which recorded values respectively of 26, 84 g and 22.37 g. The trend of dry weight reflects in all semesters that of the fresh weight. The branch dry weight

ranged from a minimum of 1.10 g (MAR1 in the fourth semester) to a maximum of 27.34 g (FAN3 in the first semester).

Even in the site of Villasor the production of branches per plant was reduced over time. During the first three semesters the clone that gave the highest fresh weight of branches was the clone FAN3 showing a fresh weight per plant respectively of 23.91 g, 30.21 g and 12.98 g. At the same time it was the clone GIO1 to show the values of fresh weight lowest in the first semester and the highest values in the last two semesters. The greatest production of branches in these last two semesters was that of the clone MAR1, which showed values of 1.47 g (fourth semester) and 1.90 g (fifth semester). The dry weight of the branches followed the trend of the fresh weight.

As regards to the percentage of dry matter of the branches, in the site of Oristano, the highest percentage was that of the first two semesters in clone GIO1 (59.64% and 60.94%) (Table 17). At the same time the lowest percentage of dry matter was found in clones PAL1 and MAR1. In plants growing in the site of Tempio the percentage of dry matter ranged from a minimum of 46.12 in the first semester with the clone MAR1 to a maximum of 75.49% in the fifth semester with the clone GIO1. The percentage of dry matter in branches of the plants growing in Sassari varies from a minimum of 45.09% (MAR1 in the first semester) to a maximum of 66.52% (PAL1 in the third semester). The clone MAR1 was the clone that in all semesters, excluding the third semester, showed smallest percentages of dry matter. In the first, second and fourth semesters was the clone FAN3 to present the highest percentages of dry matter, with values of 59.14%, 58.04% and 56.14%, respectively.

In the site of Villasor, also as regards the production of branches, the highest percentages of dry matter were observed, especially in the third semester, reaching a maximum of 83.98% with the clone GIO1. The lowest percentage of dry matter was that of the clone SAN3 in the second semester (41.58%).

4.4.2.1. Influence of temperature on the production of branches

In Table 18 are reported the results of the correlation between the ICC and the production of branches. In particular, we have analyzed the relationship between the temperature sums and the fresh weight, dry weight and dry matter of the branches. The analysis showed that for most of the clones, there was no correlation between production of branches and critical temperature sums. With regard to the six-month data, only the

fresh weight ($p < 0.01$) and dry weight ($p < 0.05$) of the branches of the clone PAL1 were positively correlated ($p < 0.01$) with the threshold of 0 °C. If we look at the Table 19, relative to the entire period of the test, we note some differences. In fact, in this case, fresh and dry weight of the branches of the clone FAN3 were positively correlated ($p < 0.05$) with all the three thresholds of temperature.

Table 14. Averages and *F* values obtained from the analysis of variance of the dependent variables: fresh weight of branches (f.w. branches), dry weight of branches (d.w. branches) and % of dry matter (% d.m. branches).

	f.w. branches(g)	d.w. branches (g)	d.m. branches (%)
LOCALITY (A)			
Oristano	15.288	9.169	60.716
Tempio	16.352	9.499	58.057
Sassari	12.833	6.898	53.53
Villasor	9.534	5.459	55.636
F	7.29	7.06	36.93
P	0.019	0.021	0.001
CLONE (B)			
MAR1	15.657	8.5	54.949
GIO1	8.808	5.115	58.916
SAN3	12.688	6.947	54.192
PAL1	11.221	6.51	57.278
FAN3	19.136	11.707	59.589
F	7.28	8.25	12.27
P	0.001	0.0008	0.001
LOCALITY x CLONE (AxB)			
F	1.26	1.13	1.97
P	0.288	0.37	0.061
SEMESTER (C)			
1	19.58	10.457	53.437
2	14.187	7.674	54.252
3	13.705	8.279	61.852
4	10.838	6.711	58.075
5	9.2	5.66	57.308
F	13.57	8.9	27.99
P	0.0008	0.0009	0.001
LOCALITY x SEMESTER (AxC)			
F	3.73	5.34	26.69
P	0.001	0.001	0.001
LOCALITY x CLONE x SEMESTER (AxBxC)			
F	1.23	1.35	1.69
P	0.171	0.085	0.008

Table 15. Fresh weight (g) of the branches produced per plant in every site and in every semester. Data with letters in common are not statistically different for $p < 0.01$.

ORISTANO					
	MAR1	GIO1	SAN3	PAL1	FAN3
1 semester	37.14 a-d	7.46 e-j	20.53 b-j	10.06 e-j	20.53 b-j
2 semester	21.70 b-j	8.57 e-j	11.06 e-j	11.21 e-j	16.52 d-j
3 semester	24.88 b-i	9.36 e-j	16.66 d-j	10.66 e-j	14.51 e-j
4 semester	18.40 b-j	11.82 e-j	8.79 e-j	14.24 e-j	19.34 b-j
5 semester	10.17 e-j	13.00 e-j	17.17 d-j	12.70 e-j	15.73 d-j
TEMPIO					
	MAR1	GIO1	SAN3	PAL1	FAN3
1 semester	22.17 b-j	11.30 e-j	15.60 d-j	18.67 b-j	39.80 ab
2 semester	12.96 e-j	5.21 hij	13.06 e-j	10.97 e-j	10.76 e-j
3 semester	13.47 e-j	12.75 e-j	11.96 e-j	10.51 e-j	28.74 a-f
4 semester	15.88 d-j	9.21 e-j	15.62 d-j	17.84 c-j	39.21 abc
5 semester	15.37 d-j	6.77 f-j	11.60 e-j	17.90 c-j	21.50 b-j
SASSARI					
	MAR1	GIO1	SAN3	PAL1	FAN3
1 semester	28.54 a-g	10.52 e-j	12.52 e-j	15.38 d-j	45.71 a
2 semester	26.84 a-h	9.25 e-j	22.05 b-j	13.95 e-j	16.39 d-j
3 semester	22.37 b-j	6.70 f-j	19.33 b-j	9.09 e-j	13.37 e-j
4 semester	2.21 i-j	7.69 e-j	6.91 f-j	2.85 ij	3.45 Ij
5 semester	2.77 i-j	10.33 e-j	7.00 f-j	2.27 ij	3.33 Ij
VILLASOR					
	MAR1	GIO1	SAN3	PAL1	FAN3
1 semester	12.11 e-j	10.26 e-j	15.05 e-j	14.35 e-j	23.91 b-j
2 semester	11.67 e-j	4.74 hij	10.77 e-j	15.87 d-j	30.21 a-e
3 semester	11.13 e-j	4.96 hij	11.33 e-j	9.34 e-j	12.98 e-j
4 semester	1.47 J	10.43 e-j	3.10 ij	4.33 hij	3.97 Hij
5 semester	1.90 J	5.83 g-j	3.67 ij	2.23 ij	2.77 Ij

Table 16. Dry weight (g) of the branches produced per plant of *Atriplex halimus* in the 4 sites and 5 different semesters. Data with letters in common are not statistically different for $p < 0.01$.

ORISTANO					
	MAR1	GIO1	SAN3	PAL1	FAN3
1 semester	18.38 a-d	4.49 g-j	10.72 c-j	4.89 f-j	12.63 c-j
2 semester	10.51 d-j	5.16 f-j	5.88 d-j	5.77 d-j	10.14 d-j
3 semester	14.57 b-g	5.65 d-j	9.51 d-j	6.70 d-j	9.06 d-j
4 semester	13.08 c-j	7.82 d-j	5.51 e-j	10.01 d-j	13.79 b-i
5 semester	6.83 d-j	7.60 d-j	11.07 c-j	8.63 d-j	10.80 c-j
TEMPIO					
	MAR1	GIO1	SAN3	PAL1	FAN3
1 semester	10.13 d-j	6.12 d-j	8.06 d-j	9.81 d-j	22.61 abc
2 semester	6.18 d-j	2.93 g-j	6.94 d-j	5.91 d-j	6.37 d-j
3 semester	7.40 d-j	7.11 d-j	6.44 d-j	5.68 d-j	17.56 a-f
4 semester	10.11 d-j	5.59 e-j	8.59 d-j	11.21 c-j	25.15 ab
5 semester	9.43 d-j	5.03 f-j	8.33 d-j	10.83 c-j	13.93 b-i
SASSARI					
	MAR1	GIO1	SAN3	PAL1	FAN3
1 semester	12.70 c-j	5.87 d-j	6.92 d-j	7.54 d-j	27.34 a
2 semester	12.95 c-j	5.25 e-j	11.15 c-j	7.39 d-j	9.52 d-j
3 semester	13.20 c-j	4.22 g-j	10.04 d-j	6.13 d-j	7.63 d-j
4 semester	1.10 hij	3.82 g-j	3.58 g-j	1.46 hij	1.93 g-j
5 semester	1.30 hij	5.33 e-j	3.30 g-j	1.17 hij	1.60 hij
VILLASOR					
	MAR1	GIO1	SAN3	PAL1	FAN3
1 semester	6.60 d-j	5.39 e-j	7.63 d-j	7.35 d-j	13.97 b-h
2 semester	6.10 d-j	2.83 g-j	5.11 f-j	9.39 d-j	18.00 a-e
3 semester	7.72 d-j	4.10 g-j	6.96 d-j	7.13 d-j	8.74 d-j
4 semester	0.87 J	4.93 f-j	1.47 hij	2.17 g-j	2.03 g-j
5 semester	0.83 J	3.03 g-j	1.73 g-j	1.07 ij	1.33 hij

Table 17. Percentage of dry matter (%) in the branches of *Atriplex halimus* in different sites. Data with letters in common are not statistically different for $p < 0.01$.

ORISTANO					
	MAR1	GIO1	SAN3	PAL1	FAN3
1 semester	49.66 q-z	59.64 e-u	52.39 l-z	49.51 q-z	61.19 d-r
2 semester	48.25 r-z	60.94 d-s	53.15 k-z	51.84 m-z	60.48 e-t
3 semester	58.92 f-w	60.39 e-t	56.18 h-y	63.37 c-p	62.38 d-q
4 semester	72.25 b-e	65.54 c-l	62.26 d-q	70.13 b-g	71.09 b-f
5 semester	68.45 b-h	58.75 f-w	63.98 c-n	68.30 b-h	68.86 b-h
TEMPIO					
	MAR1	GIO1	SAN3	PAL1	FAN3
1 semester	46.12 v-z	54.27 i-z	52.18 m-z	52.54 l-z	57.40 g-y
2 semester	47.75 s-z	56.79 h-y	53.02 k-z	53.79 j-z	59.11 f-w
3 semester	54.72 i-z	56.05 h-y	54.22 i-z	54.04 i-z	59.38 e-v
4 semester	64.19 c-m	60.40 e-t	55.10 h-y	61.48 d-r	63.23 c-p
5 semester	61.21 d-r	75.49 abc	73.31 a-d	61.14 d-r	63.31 c-p
SASSARI					
	MAR1	GIO1	SAN3	PAL1	FAN3
1 semester	45.09 Xyz	55.99 h-y	54.90 i-y	50.25 p-z	59.14 f-w
2 semester	48.38 r-z	56.57 h-y	50.84 m-z	53.56 j-z	58.04 f-x
3 semester	58.43 f-w	63.75 c-o	54.75 i-z	66.52 b-j	57.65 g-x
4 semester	49.64 q-z	50.44 o-z	51.82 m-z	51.00 m-z	56.14 h-y
5 semester	46.95 u-z	50.32 p-z	47.43 t-z	51.04 m-z	49.62 q-z
VILLASOR					
	MAR1	GIO1	SAN3	PAL1	FAN3
1 semester	54.81 i-y	52.76 k-z	51.07 m-z	51.38 m-z	58.44 f-w
2 semester	52.68 l-z	59.77 e-u	41.58 z	58.59 f-w	59.92 e-u
3 semester	67.16 b-i	83.98 a	61.06 d-s	78.09 ab	65.99 b-k
4 semester	60.19 e-u	45.81 w-z	47.32 t-z	50.17 p-z	52.13 m-z
5 semester	44.13 Yz	50.65 n-z	46.12 v-z	48.81 r-z	48.29 r-z

Table 18. Correlations (r) between the semester ICC and fresh and dry weight of the branches, and dry matter of the branches of five clones (n.s. = not significant, * = significant for $p < 0.05$, ** = significant for $p < 0.01$).

		ICC (0 °C)	ICC (5 °C)	ICC (10 °C)
Branch fresh weight (g)	MAR1	0.096 n.s.	-0.037 n.s.	0.054 n.s.
	GIO1	-0.003 n.s.	0.266 n.s.	0.265 n.s.
	SAN3	0.096 n.s.	-0.008 n.s.	0.089 n.s.
	PAL1	0.556 **	0.304 n.s.	0.250 n.s.
	FAN3	0.404 n.s.	0.311 n.s.	0.309 n.s.
Branch dry weight (g)	MAR1	0.101 n.s.	-0.045 n.s.	0.036 n.s.
	GIO1	0.097 n.s.	0.310 n.s.	0.303 n.s.
	SAN3	0.188 n.s.	0.076 n.s.	0.160 n.s.
	PAL1	0.521 *	0.259 n.s.	0.196 n.s.
	FAN3	0.400 n.s.	0.300 n.s.	0.288 n.s.
Branch dry matter (%)	MAR1	0.036 n.s.	-0.031 n.s.	-0.080 n.s.
	GIO1	0.259 n.s.	0.126 n.s.	0.133 n.s.
	SAN3	0.445 n.s.	0.371 n.s.	0.351 n.s.
	PAL1	0.024 n.s.	-0.060 n.s.	-0.042 n.s.
	FAN3	0.104 n.s.	0.01 n.s.	-0.028 n.s.

Table 19. Correlations (r) between ICC of the whole test period (5 semesters) and fresh weight and dry weight of the branches, and dry matter of the branches of five clones (n.s. = not significant, * = significant for $p < 0.05$, ** = significant for $p < 0.01$).

		ICC (0 °C)	ICC (5 °C)	ICC (10 °C)
Branches fresh weight (g)	MAR1	0.034 n.s.	0.011 n.s.	0.041 n.s.
	GIO1	0.139 n.s.	0.117 n.s.	0.146 n.s.
	SAN3	0.220 n.s.	0.197 n.s.	0.232 n.s.
	PAL1	0.892 n.s.	0.887 n.s.	0.886 n.s.
	FAN3	0.984 *	0.980 *	0.985 *
Branches dry weight (g)	MAR1	0.030 n.s.	0.009 n.s.	0.033 n.s.
	GIO1	0.185 n.s.	0.165 n.s.	0.186 n.s.
	SAN3	0.283 n.s.	0.262 n.s.	0.291 n.s.
	PAL1	0.814 n.s.	0.809 n.s.	0.804 n.s.
	FAN3	0.962 *	0.956 *	0.961 *
Branches dry matter (%)	MAR1	-0.025 n.s.	-0.023 n.s.	-0.047 n.s.
	GIO1	0.437 n.s.	0.438 n.s.	0.418 n.s.
	SAN3	0.557 n.s.	0.542 n.s.	0.556 n.s.
	PAL1	-0.177 n.s.	0.178 n.s.	-0.196 n.s.
	FAN3	0.152 n.s.	0.141 n.s.	0.141 n.s.

4.4.3. Analysis of the roots

The *Atriplex halimus* is a species characterized by a root system much expanded especially when grows in arid environments, where the water content in soil is low, and the plant reacts by developing roots capable of growth in the deeper layers of the soil, and thus absorb water. In the Table 20 the analysis of variance relative to root biomass production is reported. As we can see, the highest root fresh weight per plant was recorded in the site of Tempio (18.12 g), while the lowest root weight was that of the plants located in Villasor (10.96 g). Comparing all the clones we can say that there are significant differences among these for the fresh and dry weight of root biomass, as well as for the percentage of root dry matter ($p < 0.01$).

The clone with the highest production of fresh and dried root biomass was the clone PAL1. The interaction clone and location was not significant. Differences among the productions of five semesters were instead significant. The period with the highest observed root fresh weight was the third. Probably this is due to the fact that the third semester was the most critical period for the plants. In fact, the analysis of leaf production showed that, in the majority of clones there was a contraction of the development of the aerial part of the plants that may result in a greater development of the underground part of the plants.

The percentage of root dry matter showed different trends with respect to the dry and fresh weight of roots. As we can see in the Table 15, the highest percentage of root dry matter was found to be that of the plants of Sassari. The clone GIO1 was a clone that presented the highest percentage of dry matter in the roots and in the leaves. In Table 21 are reported the values of fresh root weight for the 5 clones in the 4 sites. With regard to the production of fresh root biomass in the site of Oristano and Tempio, an increase was observed from the first to the tird semester, and then a new decrease in the fifth semester. In Oristano the greatest fresh weight of roots was reached in the third semester by the clone MAR1 (38.34 g). In the first three semesters the lowest root fresh weight was recorded in the clone GIO1 reaching a minimum value of 5.47 g in the first semester.

The dry root weight followed, with few exceptions, the performance of the fresh weight. In Tempio the lowest root fresh weight, in all semesters excluding the first was recorded in the clone GIO1. The greatest fresh weight instead was of the clone PAL1 in the fifth semester (38.03 g). The same clone showed the highest values in the third and fourth

semester. Also in this case, as in the case of shoots the dry weight of the roots follows the trend of the fresh weight (Table 22). The values of root dry weight ranged from 1.85 g in the first semester (SAN3) to 11.55 g in the fourth semester (PAL1). The clone PAL1 was the clone that in all the 5 semesters showed the highest values of dry weight.

In Sassari, as for leaves and shoots, was the clone GIO1 to show in the first 3 semesters the lowest values of root fresh weight. In general, the fresh weight ranged from 4.14 g (MAR1 in the fourth semester) to a maximum of 29.21 g (MAR1 in the third semester). The root dry weight, as the fresh weight, was highest in the first semester and then decreased in the last two semesters. In most semesters the root dry weight follows the trend of the fresh weight. The minimum values are those of the fourth semester in the clone MAR1 (1.03 g). The maximum values of root dry weight are those related to the first semester and to the clone FAN3 (11.30 g).

In Villasor, as previously observed with shoots, the clone GIO1 was the clone that in the first 3 semesters showed the lowest values of root fresh weight, while in the fourth and fifth semester was the clone MAR1 to present the lowest weight. The greatest root fresh weight was achieved by the clone FAN3 (23.3 g) in the second semester, the same clone showing the highest fresh weight also in the fifth semester (7.63 g). The lowest root dry weight, in the first 3 semesters, was reached by the clone SAN3.

With regard to the percentage of root dry matter, on the site of Oristano, the highest value was achieved in the first semester of the clone FAN3 (45.23%), the lowest by the clone MAR1 (22.9%) in the third semester (Table 23). In Tempio the highest values concerned the clone FAN3 (46.72%). In any case, in the 3 semesters subsequent to the first, the clone that showed the highest percentages was the clone GIO1. The smallest values instead were those of the second semester and in particular of the clone SAN3 (20.81%). In Sassari, the percentage of root dry matter varies from a minimum of 20.90% (PAL1 in the 5th semester) to a maximum of 37.02% (MAR1 in the first semester)

In the second, fourth and fifth semester the highest percentage of root dry matter was that of the clone FAN3, which showed values respectively 32.52%, 31.80% and 36.15%. In Villasor the percentage of dry matter in the roots ranged from a minimum of 21.94% of the clone PAL1 (fourth semester) to a maximum of 49.95% of the clone GIO1 (third semester).

4.4.3.1. Influence of temperature on root production

In Tables 24 and 25 are shown the results of the correlation between root biomass production (fresh and dry weight) and semester and total ICC. Any significant correlation was observed.

Table 20. Averages, *F* and *p* values obtained from the analysis of variance of the dependent variable fresh weight of root (f.w. root), dry weight of root (d.w. root), dry matter of root (% d.m. of root).

	f.w. root (g)	d.w. root (g)	% d.m. of root
LOCALITY (A)			
Oristano	17.622	5.386	32.724
Tempio	18.123	5.194	30.42
Sassari	12.664	4.016	34.109
Villasor	10.965	3.354	30.991
F	24.99	16.35	10.79
P	0.001	0.002	0.007
CLONE (B)			
MAR1	16.662	4.685	29.049
GIO1	10.168	3.509	36.675
SAN3	11.863	3.164	29.635
PAL1	19.971	5.579	28.883
FAN3	15.528	5.501	36.064
F	9.72	11.58	29.41
P	0.001	0.001	0.001
LOCALITY x CLONE (AxB)			
F	1.21	1.38	0.97
P	0.319	0.223	n.s.
SEMESTER (C)			
1	12.641	4.567	39.765
2	14.727	4.335	30.734
3	18.794	5.26	31.008
4	14.465	4.577	30.56
5	13.567	3.698	28.24
F	6.11	3.76	35.31
P	0.001	0.005	0.0009
LOCALITY x SEMESTER (AxC)			
F	6.75	7.56	11.52
P	0.0008	0.001	0.001
LOCALITY x CLONE x SEMESTER (AxBxC)			
F	1.36	1.42	1.39
P	0.081	0.056	0.068

Table 21. Quantitative analysis of the fresh root biomass (g) produced per plant. Data with letters in common are not statistically different for $p < 0.01$.

ORISTANO					
	MAR1	GIO1	SAN3	PAL1	FAN3
1 semester	17.69 c-k	5.47 h-k	9.78 f-k	11.37 f-k	11.56 f-k
2 semester	19.14 a-k	7.34 h-k	7.93 h-k	23.71 a-i	13.32 d-k
3 semester	38.34 A	10.86 f-k	20.75 a-k	20.50 a-k	18.33 c-k
4 semester	28.26 a-g	16.58 c-k	9.92 f-k	32.33 a-d	19.60 a-k
5 semester	6.90 h-k	16.37 c-k	24.07 a-i	31.90 a-e	18.53 c-k
TEMPIO					
	MAR1	GIO1	SAN3	PAL1	FAN3
1 semester	7.83 h-k	8.90 g-k	4.97 ijk	14.67 d-k	10.50 f-k
2 semester	20.65 a-k	8.82 g-k	15.38 d-k	19.99 a-k	9.67 f-k
3 semester	29.19 a-f	18.38 c-k	18.91 b-k	35.11 abc	22.32 a-k
4 semester	21.40 a-k	10.78 f-k	17.99 c-k	31.96 a-d	28.31 a-g
5 semester	16.43 c-k	9.40 f-k	13.90 d-k	38.03 ab	19.60 a-k
SASSARI					
	MAR1	GIO1	SAN3	PAL1	FAN3
1 semester	19.17 a-k	5.05 ijk	5.23 h-k	12.96 d-k	25.38 a-h
2 semester	19.05 b-k	13.01 d-k	19.03 a-k	18.91 b-k	15.99 c-k
3 semester	29.21 a-f	8.90 g-k	13.16 d-k	14.47 d-k	14.29 d-k
4 semester	4.14 Ijk	7.60 h-k	7.78 h-k	9.86 f-k	6.63 h-k
5 semester	6.67 h-k	12.00 e-k	9.87 f-k	11.53 f-k	6.23 h-k
VILLASOR					
	MAR1	GIO1	SAN3	PAL1	FAN3
1 semester	15.25 d-k	12.61 d-k	15.01 d-k	22.53 a-k	16.91 c-k
2 semester	11.76 f-k	6.71 h-k	6.80 h-k	14.11 d-k	23.23 a-j
3 semester	15.05 d-k	5.94 h-k	9.15 f-k	18.79 b-k	14.23 d-k
4 semester	2.90 K	11.87 f-k	3.03 jk	10.07 f-k	8.30 g-k
5 semester	4.23 Ijk	6.80 h-k	4.63 ijk	6.60 h-k	7.63 h-k

Table 22. Dry weight (g) of root biomass produced per plant in 5 semesters and 4 sites. Data with letters in common are not statistically different for $p < 0.01$.

ORISTANO					
	MAR1	GIO1	SAN3	PAL1	FAN3
1 semester	6.27 a-l	2.46 g-l	3.52 e-l	3.59 e-l	5.30 c-l
2 semester	5.28 d-l	2.92 e-l	2.37 h-l	6.23 a-l	4.93 d-l
3 semester	8.58 a-g	3.87 d-l	4.74 d-l	5.50 b-l	6.45 a-l
4 semester	11.17 abc	5.42 c-l	3.11 e-l	8.98 a-e	7.62 a-j
5 semester	2.10 h-l	5.20 d-l	6.20 a-l	8.20 a-h	4.63 d-l
TEMPIO					
	MAR1	GIO1	SAN3	PAL1	FAN3
1 semester	3.00 e-l	3.58 e-l	1.85 i-l	5.19 d-l	4.98 d-l
2 semester	4.31 d-l	2.69 f-l	3.11 e-l	4.41 d-l	2.89 e-l
3 semester	6.36 a-l	5.54 b-l	4.42 d-l	8.59 a-g	6.31 a-l
4 semester	5.92 a-l	4.02 d-l	4.12 d-l	11.55 a	9.92 a-d
5 semester	4.50 d-l	3.17 e-l	3.57 e-l	8.80 a-f	7.03 a-k
SASSARI					
	MAR1	GIO1	SAN3	PAL1	FAN3
1 semester	5.15 d-l	2.94 e-l	2.80 f-l	5.18 d-l	11.30 ab
2 semester	6.08 a-l	4.14 d-l	4.34 d-l	5.82 a-l	5.31 d-l
3 semester	7.89 a-i	3.64 e-l	3.69 e-l	4.39 d-l	5.20 d-l
4 semester	1.03 kl	2.29 h-l	2.33 h-l	2.28 h-l	2.09 h-l
5 semester	1.60 jkl	3.73 e-l	2.77 f-l	2.40 h-l	2.00 i-l
VILLASOR					
	MAR1	GIO1	SAN3	PAL1	FAN3
1 semester	4.33 d-l	3.94 d-l	3.50 e-l	6.32 a-l	6.13 a-l
2 semester	4.12 d-l	2.60 g-l	2.15 h-l	4.83 d-l	8.16 a-h
3 semester	4.09 d-l	2.86 f-l	2.60 g-l	5.60 b-l	4.88 d-l
4 semester	0.77 l	3.20 e-l	0.83 l	2.20 h-l	2.67 g-l
5 semester	1.13 kl	1.97 i-l	1.27 kl	1.50 jkl	2.20 h-l

Table 23. Percentage of dry matter (%) of the roots of *A. halimus* of 5 clones placed in 4 different sites. Data with letters in common are not statistically different for $p < 0.01$.

ORISTANO					
	MAR1	GIO1	SAN3	PAL1	FAN3
1 semester	34.43 e-p	44.23 b-h	38.56 c-l	32.15 e-p	45.23 a-g
2 semester	27.83 i-p	39.60 c-k	29.94 g-p	26.77 j-p	36.90 d-n
3 semester	22.49 m-p	36.02 d-p	23.22 l-p	29.36 h-p	34.86 d-p
4 semester	39.45 c-k	36.01 d-p	31.08 g-p	27.86 i-p	38.27 c-m
5 semester	31.25 f-p	31.98 e-p	26.56 j-p	26.17 j-p	27.92 i-p
TEMPIO					
	MAR1	GIO1	SAN3	PAL1	FAN3
1 semester	38.49 c-l	41.08 c-j	37.14 d-n	35.79 d-p	46.72 a-f
2 semester	21.03 op	30.40 g-p	20.81 p	22.46 m-p	30.32 g-p
3 semester	22.50 m-p	29.97 g-p	24.96 k-p	24.56 k-p	29.73 g-p
4 semester	22.80 i-p	39.35 c-k	22.56 m-p	37.38 d-n	35.74 d-p
5 semester	27.45 i-p	34.86 d-p	25.93 j-p	23.15 l-p	35.34 d-p
SASSARI					
	MAR1	GIO1	SAN3	PAL1	FAN3
1 semester	37.02 d-n	58.32 a	57.35 ab	46.88 a-e	52.50 abc
2 semester	31.78 e-p	32.19 e-p	23.07 l-p	30.21 g-p	32.52 e-p
3 semester	26.92 j-p	42.76 c-i	32.66 e-p	30.80 g-p	36.03 d-p
4 semester	25.78 j-p	31.45 e-p	30.00 g-p	23.22 l-p	31.80 e-p
5 semester	24.12 k-p	30.30 g-p	27.96 i-p	20.90 p	36.17 d-p
VILLASOR					
	MAR1	GIO1	SAN3	PAL1	FAN3
1 semester	29.38 h-p	31.13 g-p	24.71 k-p	28.06 i-p	36.13 d-p
2 semester	36.00 d-p	38.15 c-m	33.21 e-p	34.66 d-p	36.83 d-o
3 semester	27.13 i-p	49.95 a-d	28.37 i-p	32.42 e-p	35.45 d-p
4 semester	28.28 i-p	26.97 j-p	27.49 i-p	21.94 nop	33.78 e-p
5 semester	26.87 j-p	28.79 h-p	27.15 i-p	22.90 i-p	29.03 h-p

Table 24. Correlations (r) between the semester ICC and fresh and dry weight, and dry matter of the roots of every clone (n.s. = not significant, * = significant for $p < 0.05$, ** = significant for $p < 0.01$).

		ICC (0 °C)	ICC (5 °C)	ICC (10 °C)
Root fresh weight (g)	MAR1	-0.101 n.s.	0.001 n.s.	0.071 n.s.
	GIO1	-0.082 n.s.	0.290 n.s.	0.233 n.s.
	SAN3	-0.029 n.s.	0.121 n.s.	0.138 n.s.
	PAL1	0.349 n.s.	0.457 n.s.	0.355 n.s.
	FAN3	0.127 n.s.	0.116 n.s.	0.078 n.s.
Root dry weight (g)	MAR1	-0.077 n.s.	-0.083 n.s.	-0.045 n.s.
	GIO1	-0.024 n.s.	0.285 n.s.	0.269 n.s.
	SAN3	-0.048 n.s.	0.102 n.s.	0.154 n.s.
	PAL1	0.362 n.s.	0.327 n.s.	0.230 n.s.
	FAN3	0.191 n.s.	0.048 n.s.	0.035 n.s.
Root dry matter (%)	MAR1	0.098 n.s.	-0.054 n.s.	-0.050 n.s.
	GIO1	0.058 n.s.	-0.151 n.s.	-0.038 n.s.
	SAN3	-0.025 n.s.	-0.082 n.s.	0.021 n.s.
	PAL1	0.095 n.s.	-0.150 n.s.	-0.081 n.s.
	FAN3	0.231 n.s.	-0.019 n.s.	0.063 n.s.

Table 25. Correlations (r) between ICC of the whole test period (5 semesters) and fresh and dry weight, and dry matter of the roots of every clone (n.s. = not significant, * = significant for $p < 0.05$, ** = significant for $p < 0.01$).

		ICC (0 °C)	ICC (5 °C)	ICC (10 °C)
Roots fresh weight (g)	MAR1	0.310 n.s.	0.290 n.s.	0.313 n.s.
	GIO1	0.554 n.s.	0.540 n.s.	0.550 n.s.
	SAN3	0.496 n.s.	0.477 n.s.	0.499 n.s.
	PAL1	0.748 n.s.	0.741 n.s.	0.741 n.s.
	FAN3	0.832 n.s.	0.826 n.s.	0.824 n.s.
Roots dry weight (g)	MAR1	0.058 n.s.	0.038 n.s.	0.058 n.s.
	GIO1	0.403 n.s.	0.384 n.s.	0.404 n.s.
	SAN3	0.211 n.s.	0.189 n.s.	0.218 n.s.
	PAL1	0.776 n.s.	0.768 n.s.	0.77 n.s.
	FAN3	0.768 n.s.	0.756 n.s.	0.768 n.s.
Roots dry matter (%)	MAR1	-0.899 n.s.	-0.902 n.s.	-0.905 n.s.
	GIO1	-0.528 n.s.	-0.547 n.s.	-0.508 n.s.
	SAN3	-0.661 n.s.	-0.673 n.s.	-0.641 n.s.
	PAL1	-0.135 n.s.	-0.149 n.s.	-0.109 n.s.
	FAN3	-0.216 n.s.	-0.238 n.s.	-0.194 n.s.

4.5. Chemical analysis of the biomass

These sections contain the data of chemical analysis of samples of leaves, branches and roots of plants of *A. halimus*. As we have seen, the plant can respond in different ways at different temperatures.

4.5.1. Analysis of the leaves

Table 26 shows the summary data of the analysis of the main macronutrients in the leaves and the data of analysis of variance. Differences among locations, with respect to the content in nitrogen, were not statistically significant. Statistically different were instead interactions among clones and semesters. The clone with the highest average content of nitrogen was PAL1 (1.68%), while the lowest content has been registered in the clone FAN3 (1.25%). The interactions between locations and clones, and among locations, clones and semesters were statistically significant. Regarding phosphorus content differences among localities, clones and semesters were statistically significant, whereas no significant interactions between location and clone were recorded. The content of P ranged from a minimum of 0.84% (FAN3) to a maximum of 1.29% (GIO1).

Regarding the sodium content in the leaves, the highest result was in the site of Sassari. PAL1 was the clone with the highest sodium content (2.62%). In addition, the percentage content of sodium varied depending on the semester. In the fourth semester the levels of Na reached 3.18%. As regards the content of potassium in the leaves, the lowest value was found in the leaves from the site of Oristano (2.75% against 4.09% of Sassari). The differences among clones were statistically significant ($p < 0.01$). The clone with the highest content of potassium was the clone MAR1 (4.52%), while the content of potassium was lower in the clone SAN3 (2.66%). Clone x location interactions were not significant, while there were significant differences between semesters ($p < 0.01$). The highest content of K was found in the leaves sampled in the third semester (4.10%).

The highest content of Ca in the leaves was observed in the site of Oristano (2.49%), the lowest content was reported in the leaves sampled in Villasor. The analysis of variance has detected statistically significant differences ($p < 0.01$) among clones and locations ($p < 0.01$) for the content of Ca. The clone with the highest content of Ca in the leaves was SAN3 (2.73%). The highest Ca content was found in the leaves sampled in the 5th semester. The highest magnesium content in the leaves was recorded in Oristano

(0.84%), the lowest in Villasor (0.704%). As for the Ca, SAN3 was the clone that showed the highest content of Mg (0.9%). The highest content of Mg was that of the leaves of the first semester. As regards the analysis of microelements, in Table 27 are the data report and the analysis of variance. The copper greatest content has been reported for the leaves of the site of Tempio (15.37 ppm) and the lowest in the leaves of Villasor (6.38 ppm). The greatest copper content was of the clone GIO1 (13.7 ppm) and the lowest of the clone FAN3 (8.45). The zinc in the leaves was found to range between 85.81 ppm (Sassari) and 121.70 ppm (Villasor). The clone with the highest content of Zn was SAN3 (128.43 ppm), while the clone with the lowest content was FAN3 (82.2 ppm). For both copper and zinc differences among clones, semesters and locations were statistically significant ($p < 0.01$). Interactions location x semester were significant for the contents of all analyzed microelements ($p < 0.001$), however clone x location interactions were not significant.

The largest amount of iron was found in the leaves grown in the locality of Villasor (315.37 ppm). The clone MAR1 showed the highest Fe content (263 ppm). In addition, the Fe content varied by semester, the highest levels are related to fifth semester (316.17 ppm). The manganese was found to range between 128.76 ppm of Villasor and 264.8 ppm of Oristano.

In Table 28 to Table 37 are reported data for every element as observed for locality and semester.

In Table 28 the content of N in the leaves ranged from a minimum of 0.28% of the clone FAN3 in Tempio to a maximum of 2.57% of the clone GIO1 in Oristano. In Tempio and Oristano sites, the clone that showed the highest N content was the clone GIO1 in the fifth semester. In the sites of Sassari and Villasor was the clone PAL1 to show highest rates of N. The clones that showed the highest content of P in leaves were SAN3 (in the site of the Tempio, Sassari and Villasor) and the clone GIO1 (in the site of Oristano) (Table 29).

As regard the sodium content in the leaves this varied from a minimum of 0.22% of the clone MAR1 (Tempio) to 4.45% of the clone PAL1 (Tempio). PAL1 was the clone that in all 4 sites showed the highest content of sodium. In the site of Sassari GIO1 showed lowest values of Na (1.77%), while in Villasor the lowest content was observed for SAN3 (0.93%) and in Oristano for FAN3 (1.70%). The amount of sodium almost always increased with the time. This is because the plant, being a halophyte, absorbs and accumulates salts in the course of its life (Table 30).

In all 4 sites, the largest amount of K in the leaves was in the clone MAR1. The highest values were reached in Villasor and Tempio with 7.37% and 6.65%. The lowest content instead was recorded in the clone PAL1 at the start of test in Oristano. In the remaining sites the lowest levels of K were in the leaves of the clone SAN3 (Table 31).

Ca content in the leaves ranged from a minimum of 0.51% of the clone MAR1 (Sassari) to a maximum of 6.56% in the clone PAL1 (Oristano). In the site of Tempio and Oristano the maximum values were those of the clone PAL1, while in Sassari and Villasor maximum values were for SAN3. Also for the Ca, the maximum levels were reached in the last semesters (Table 32).

The magnesium content ranged from a minimum of 0.43% of the clone FAN3 (in Villasor) to a maximum of 1.27 of the clone FAN3 (in Oristano). The clone MAR1 showed in Oristano, Sassari and Tempio the lowest Mg content in the leaves. GIO1 showed highest values of Mg in the sites of the Tempio, Sassari and Villasor (Table 33).

As regards the analysis of microelements present in the leaves, the copper content was found to range between 0 and 27 ppm. The lowest values were generally in the last semesters and in Sassari and Villasor sites. The highest content of Cu was found in the leaves of clone GIO1 in all sites except Villasor where was the clone SAN3 to present the highest levels (Table 34).

The content of zinc was higly variable and ranged from a minimum of 44 ppm (FAN3 in Sassari) to a maximum of 212.33 ppm (SAN3 to Tempio). The clone FAN3 showed the lowest content of Zn in the leaves of the sites of Oristano, Sassari and Tempio (Table 35).

The Fe content varied from a minimum of 31.33 ppm (SAN3 in Sassari) to a maximum of 556.67 (MAR1 in Villasor) (Table 36). The lowest manganese content was in leaves of the clone FAN3 (16 ppm), which in Tempio, Sassari and Villasor showed the lowest levels of Mn as compared to the other clones (Table 37).

Table 26. Averages and analysis of variance of the content of main macroelements in the tissues of the leaf of the species *A. halimus*.

	N (%)	P (%)	Na (%)	K (%)	Ca (%)	Mg (%)
LOCALITY (A)						
Oristano	1.48	1.28	2.45	2.75	2.49	0.84
Tempio	1.42	1.06	2.08	4.06	2.15	0.79
Sassari	1.47	0.97	2.82	4.09	2.25	0.83
Villasor	1.39	0.95	2.62	4.01	1.59	0.70
F	4.47	96.75	33.83	19.05	21.36	82.53
p	0.056	0.001	0.001	0.001	0.001	0.0009
CLONE (B)						
MAR1	1.57	1.06	2.45	4.52	1.66	0.63
GIO1	1.35	1.29	2.33	4.01	1.81	0.88
SAN3	1.35	1.20	2.53	2.66	2.73	0.9
PAL1	1.68	0.95	2.62	3.83	2.66	0.8
FAN3	1.25	0.84	2.55	3.63	1.74	0.76
F	20.9	35.75	2.39	33.52	29.11	39.45
p	0.001	0.0008	0.071	0.001	0.001	0.001
LOCALITY x CLONE (AxB)						
F	8.77	1.18	0.83	0.98	1.17	3.74
p	0.001	0.340	n.s.	n.s.	0.344	0.001
SEMESTER (C)						
1	1.38	1.25	1.91	3.63	1.57	0.92
2	1.49	1.29	1.93	3.31	1.14	0.72
3	1.54	0.98	2.62	4.10	2.26	0.86
4	1.28	0.93	3.18	4.04	2.06	0.74
5	1.52	0.88	2.83	3.56	3.57	0.74
F	15.42	36.61	90.98	11.49	152.49	36.22
p	0.001	0.001	0.001	0.0008	0.0009	0.001
LOCALITY x SEMESTER (AxC)						
F	19.94	7.29	59.33	23.62	17.61	7.91
p	0.001	0.001	0.0009	0.001	0.001	0.001
LOCALITY x CLONE x SEMESTER (AxBxC)						
F	12.82	2.41	2.42	2.2	1.36	2.55
p	0.001	0.001	0.0009	0.001	0.081	0.001

Table 27. Averages and analysis of variance of the content of main microelements in the leaf tissues of the species *A. halimus*.

	Cu (ppm)	Zn (ppm)	Fe (ppm)	Mn (ppm)
LOCALITY (A)				
Oristano	10.45	111.77	213.95	264.8
Tempio	15.37	120.85	204.67	242.32
Sassari	11.32	85.81	212.08	186.00
Villasor	6.39	121.71	315.37	128.76
F	87.49	45.88	11.54	32.98
p	0.001	0.0009	0.006	0.0009
CLONE (B)				
MAR1	9.983	102.567	263	228.15
GIO1	13.700	123.883	253.033	182.6
SAN3	11.8	128.433	191.117	174.617
PAL1	10.483	113.1	250.317	308.333
FAN3	8.45	82.2	225.117	133.65
F	8.4	18.51	4.25	31.62
p	0.001	0.001	0.007	0.001
LOCALITY x CLONE (AxB)				
F	0.47	2.01	1.42	3.47
p	n.s.	0.057	0.207	0.002
SEMESTER (C)				
1	14.367	114.367	112.167	288.45
2	11.15	86.633	232.767	140.65
3	12.5	140.683	306.667	234.4
4	11.15	122.35	214.867	179.983
5	5.25	86.15	316.117	183.867
F	48.13	64.55	58.81	24.14
p	0.001	0.001	0.001	0.001
LOCALITY x SEMESTER (AxC)				
F	24.97	16.19	32.05	5.28
p	0.001	0.001	0.001	0.001
LOCALITY x CLONE x SEMESTER (AxBxC)				
F	1.89	2.26	1.43	2.27
p	0.001	0.001	0.051	0.001

Table 28. Nitrogen content (%) in leaf tissues of the various clones of *A. halimus*, located in four sites. Data with letters in common are not statistically different for $p < 0.01$.

ORISTANO					
	MAR1	GIO1	SAN3	PAL1	FAN3
1 semester	-	-	-	-	-
2 semester	2.36 ab	1.66 d-p	1.19 m-v	1.84 b-j	1.22 l-v
3 semester	1.36 f-s	1.29 i-u	1.68 c-p	1.62 d-q	1.04 q-x
4 semester	1.18 m-v	1.05 q-x	0.88 s-x	1.04 q-x	1.01 r-x
5 semester	1.94 b-f	2.57 a	1.12 o-v	1.45 f-s	1.24 k-u
TEMPIO					
	MAR1	GIO1	SAN3	PAL1	FAN3
1 semester	1.27 j-u	0.76 t-y	1.10 p-w	1.65 d-p	1.66 d-p
2 semester	1.34 g-s	0.57 wxy	1.34 g-s	1.86 b-i	1.32 h-t
3 semester	-	-	-	-	-
4 semester	1.43 f-s	1.91 b-g	1.88 b-h	1.37 f-s	0.28 y
5 semester	1.55 e-r	2.15 a-d	1.87 b-i	1.54 e-r	1.89 b-h
SASSARI					
	MAR1	GIO1	SAN3	PAL1	FAN3
1 semester	-	-	-	-	-
2 semester	1.68 c-p	0.52 xy	1.26 j-u	2.36 ab	1.59 d-r
3 semester	1.71 c-n	1.80 c-l	2.23 abc	2.16 a-d	1.74 c-m
4 semester	1.41 f-s	0.88 s-x	1.15 n-v	1.60 d-q	1.29 i-u
5 semester	1.42 f-s	1.16 m-v	0.74 u-y	1.44 f-s	1.18 m-v
VILLASOR					
	MAR1	GIO1	SAN3	PAL1	FAN3
1 semester	0.66 v-y	1.32 h-t	0.91 s-x	1.29 i-u	1.27 j-u
2 semester	1.82 b-k	1.71 c-n	1.27 j-u	1.70 c-o	1.27 j-u
3 semester	-	-	-	-	-
4 semester	1.70 c-o	1.18 m-v	1.46 f-s	1.54 e-r	1.35 g-s
5 semester	1.45 f-s	1.16 m-v	1.20 m-v	2.08 a-e	1.27 j-u

Table 29. Phosphorus content (%) in leaf tissues of the various clones of *A. halimus*, located in four sites. Data with letters in common are not statistically different for $p < 0.01$.

ORISTANO					
	MAR1	GIO1	SAN3	PAL1	FAN3
1 semester	1.48 b-h	2.18 a	1.68 a-e	1.26 d-q	1.07 e-v
2 semester	1.43 b-j	1.91 a-c	1.90 a-c	1.26 d-q	1.44 b-j
3 semester	1.44 b-j	1.56 b-g	1.07 e-v	1.33 c-n	0.62 q-v
4 semester	1.01 f-v	1.36 c-m	1.23 d-r	1.01 f-v	0.73 m-v
5 semester	0.98 f-v	0.99 f-v	1.01 f-v	0.83 i-v	1.23 d-r
TEMPIO					
	MAR1	GIO1	SAN3	PAL1	FAN3
1 semester	1.11 d-x	1.17 d-w	1.04 e-x	0.85 g-x	0.68 o-x
2 semester	1.06 d-x	1.39 b-l	0.98 f-x	1.00 f-x	0.81 j-x
3 semester	-	-	-	-	-
4 semester	1.19 d-v	1.26 c-s	1.42 b-k	1.03 f-x	0.80 j-x
5 semester	1.01 f-x	1.15 d-w	1.50 b-g	0.77 k-x	0.58 t-x
SASSARI					
	MAR1	GIO1	SAN3	PAL1	FAN3
1 semester	1.26 c-r	1.28 c-q	1.34 b-n	1.22 c-t	0.86 g-x
2 semester	1.30 b-p	0.87 g-x	1.85 abc	1.01 f-x	0.87 g-x
3 semester	0.68 o-x	1.19 d-v	0.85 g-x	0.96 f-x	0.68 o-x
4 semester	0.91 f-x	1.16 d-w	0.54 vwx	0.73 l-x	0.81 j-x
5 semester	0.67 p-x	1.19 d-v	0.83 h-x	0.46 x	0.74 l-x
VILLASOR					
	MAR1	GIO1	SAN3	PAL1	FAN3
1 semester	1.18 d-w	1.47 b-i	1.70 a-d	1.18 d-w	0.91 f-x
2 semester	1.37 b-m	1.56 b-f	1.16 d-w	1.36 b-m	1.21 d-u
3 semester	-	-	-	-	-
4 semester	0.63 q-w	0.92 f-x	0.82 i-x	0.53 wx	0.61 s-x
5 semester	0.56 uvx	1.10 d-x	0.75 l-x	0.56 u-x	0.70 n-x

Table 30. Sodium content (%) in leaf tissues of the various clones of *A. halimus* located in four sites. Data with letters in common are not statistically different for $p < 0.01$.

ORISTANO					
	MAR1	GIO1	SAN3	PAL1	FAN3
1 semester	1.80 r-y	1.75 r-y	2.37 k-w	2.10 n-y	2.33 k-w
2 semester	1.90 p-y	2.40 k-v	1.87 p-y	1.75 r-y	1.70 s-y
3 semester	2.63 i-v	1.80 r-y	2.72 h-v	2.15 n-x	2.25 m-x
4 semester	3.98 a-f	2.55 j-v	3.28 a-n	4.08 a-e	3.90 a-g
5 semester	2.80 g-u	2.10 n-y	2.30 l-x	2.35 k-w	2.45 j-v
TEMPIO					
	MAR1	GIO1	SAN3	PAL1	FAN3
1 semester	0.22 Z	0.24 z	0.27 z	0.31 z	0.26 z
2 semester	1.72 r-y	2.08 n-y	2.17 n-x	2.18 n-x	2.07 n-y
3 semester	-	- i-v	-	-	-
4 semester	2.27 m-x	2.66 i-v	2.19 n-x	1.96 p-y	2.16 n-x
5 semester	3.85 a-h	2.02 o-y	3.20 c-o	4.45 a	4.43 ab
SASSARI					
	MAR1	GIO1	SAN3	PAL1	FAN3
1 semester	2.48 j-v	2.50 j-v	2.40 k-v	2.73 g-v	3.08 d-p
2 semester	2.17 n-x	1.98 o-y	1.92 p-y	2.33 k-w	2.15 n-x
3 semester	2.32 k-w	1.77 r-y	2.60 i-v	1.92 p-y	2.33 k-w
4 semester	2.85 f-s	3.77 a-i	3.45 a-m	3.07 d-q	2.80 g-u
5 semester	3.50 a-l	3.77 a-i	4.30 abc	4.42 ab	3.98 a-f
VILLASOR					
	MAR1	GIO1	SAN3	PAL1	FAN3
1 semester	2.43 j-v	2.95 e-r	2.55 j-v	2.57 j-v	2.82 f-t
2 semester	1.58 t-y	1.53 v-y	1.62 s-y	1.82 r-y	1.68 s-y
3 semester	-	-	-	-	-
4 semester	3.52 a-k	3.27 a-n	4.12 a-e	4.17 a-d	3.62 a-j
5 semester	1.08 xyz	1.57 u-y	0.93 yz	1.85 q-y	1.17 w-z

Table 31. Potassium content (%) in leaf tissues of the various clones of *A. halimus* located in four sites. Data with letters in common are not statistically different for $p < 0.01$.

ORISTANO					
	MAR1	GIO1	SAN3	PAL1	FAN3
1 semester	0.93 W	1.13 vw	0.82 w	0.75 w	0.90 w
2 semester	3.33 f-t	3.92 c-r	2.22 q-w	2.97 i-v	3.15 g-u
3 semester	4.47 c-o	4.45 c-o	2.17 q-w	4.00 c-q	3.02 h-v
4 semester	4.55 c-o	3.35 f-t	2.63 m-w	4.45 c-o	2.60 n-w
5 semester	4.55 c-o	1.90 r-w	1.62 s-w	3.37 f-t	1.40 t-w
TEMPIO					
	MAR1	GIO1	SAN3	PAL1	FAN3
1 semester	4.47 c-o	4.37 c-p	3.58 e-s	3.80 c-r	4.30 c-p
2 semester	4.95 b-j	5.25 b-f	2.93 j-v	3.35 f-t	5.10 b-h
3 semester	-	-	-	-	-
4 semester	4.08 c-q	3.80 c-r	2.37 p-w	3.85 c-r	3.68 d-r
5 semester	6.65 Ab	3.86 c-r	3.37 f-t	5.48 b-e	4.53 c-o
SASSARI					
	MAR1	GIO1	SAN3	PAL1	FAN3
1 semester	5.85 Abc	5.05 b-i	3.37 f-t	4.72 b-m	4.43 c-p
2 semester	3.23 f-u	3.48 e-s	1.88 r-w	2.97 i-v	2.97 i-v
3 semester	5.73 a-d	4.05 c-q	3.28 f-t	4.80 b-k	4.20 c-q
4 semester	5.08 b-h	4.50 c-o	2.57 n-w	4.35 c-p	4.75 b-l
5 semester	4.48 c-o	5.52 b-e	2.48 o-w	4.48 c-o	4.10 c-q
VILLASOR					
	MAR1	GIO1	SAN3	PAL1	FAN3
1 semester	7.37 A	4.78 b-k	3.63 e-s	4.22 c-q	4.17 c-q
2 semester	3.53 e-s	3.60 e-s	1.25 uvw	3.37 f-t	2.68 l-w
3 semester	-	-	-	-	-
4 semester	5.12 b-g	4.58 c-n	4.62 c-n	4.60 c-n	5.20 b-g
5 semester	2.72 k-w	4.17 c-q	1.43 t-w	2.53 n-w	2.47 o-w

Table 32. Content of Ca (%) in leaf tissues of the various clones of *A. halimus* located in four sites. Data with letters in common are not statistically different for $p < 0.01$.

ORISTANO					
	MAR1	GIO1	SAN3	PAL1	FAN3
1 semester	1.16 p-z	1.61 k-z	2.39 f-u	1.41 n-z	0.93 r-z
2 semester	0.57	1.01 q-z	2.09 i-z	1.78 j-z	0.90 s-z
3 semester	2.74 f-o	2.17 g-z	3.17 e-k	3.72 def	3.09 e-l
4 semester	1.86 j-z	1.20 o-z	1.78 j-z	3.20 e-j	1.96 j-z
5 semester	3.02 e-m	3.71 def	5.81 ab	6.56 a	4.44 cde
TEMPIO					
	MAR1	GIO1	SAN3	PAL1	FAN3
1 semester	1.12 q-z	1.64 j-z	2.33 f-v	1.33 n-z	1.12 q-z
2 semester	0.95 q-z	0.78 v-z	2.04 j-z	1.23 n-z	0.82 u-z
3 semester	-	-	-	-	-
4 semester	1.82 j-z	1.87 j-z	2.38 f-u	2.48 f-r	2.12 i-z
5 semester	3.13 e-k	2.74 f-p	4.70 bcd	4.89 bcd	3.59 d-i
SASSARI					
	MAR1	GIO1	SAN3	PAL1	FAN3
1 semester	1.40 n-z	1.43 n-z	2.47 f-s	2.26 f-x	1.21 o-z
2 semester	0.51	1.02 q-z	1.72 j-z	1.05 q-z	0.70 xyz
3 semester	1.56 l-z	2.42 f-t	2.34 f-v	2.11 i-z	1.70 j-z
4 semester	1.86 j-z	2.35 f-u	2.79 f-n	2.75 f-o	1.33 n-z
5 semester	3.69 d-g	3.65 d-h	5.86 ab	5.56 abc	2.52 f-q
VILLASOR					
	MAR1	GIO1	SAN3	PAL1	FAN3
1 semester	1.32 n-z	1.22 n-z	1.87 j-z	2.28 f-w	0.87 t-z
2 semester	0.65 Z	0.69 yz	2.25 f-y	1.23 n-z	0.76 w-z
3 semester	-	-	-	-	-
4 semester	1.49 m-z	1.67 j-z	2.41 f-t	2.33 f-w	1.55 l-z
5 semester	1.11 q-z	1.35 n-z	1.51 m-z	2.16 h-z	1.43 n-z

Table 33. Magnesium content (%) in leaf tissues of the various clones of *A. halimus* located in four sites. Data with letters in common are not statistically different for $p < 0.01$.

ORISTANO					
	MAR1	GIO1	SAN3	PAL1	FAN3
1 semester	0.60 v-z	1.19 ab	1.05 a-j	0.74 k-z	1.27 a
2 semester	0.58 xyz	0.82 e-z	1.06 a-h	0.72 m-z	0.75 j-z
3 semester	0.92 b-t	1.03 a-k	1.18 abc	0.97 b-q	1.03 a-l
4 semester	0.52	0.69 o-z	0.77 g-z	0.66 r-z	0.55 yz
5 semester	0.58 xyz	0.68 p-z	0.97 b-q	0.91 b-u	0.77 g-z
TEMPIO					
	MAR1	GIO1	SAN3	PAL1	FAN3
1 semester	0.63 s-z	1.12 a-d	1.09 a-f	1.12 a-d	0.76 h-z
2 semester	0.58 xyz	0.82 d-z	0.85 d-y	0.70 o-z	0.68 p-z
3 semester	-	-	-	-	-
4 semester	0.53 Z	0.71 n-z	1.01 a-n	0.82 d-z	0.79 f-z
5 semester	0.57 xyz	0.73 k-z	0.89 c-w	0.84 d-z	0.74 k-z
SASSARI					
	MAR1	GIO1	SAN3	PAL1	FAN3
1 semester	0.62 s-z	1.08 a-f	0.99 a-o	1.07 a-g	1.06 a-i
2 semester	0.52	0.63 s-z	0.73 k-z	0.75 i-z	0.70 n-z
3 semester	0.73 l-z	1.12 a-e	0.90 b-v	0.87 d-x	0.79 f-z
4 semester	0.74 k-z	1.03 a-m	0.94 b-r	0.68 p-z	0.63 s-z
5 semester	0.81 f-z	0.92 b-s	0.99 a-p	0.87 d-x	0.65 r-z
VILLASOR					
	MAR1	GIO1	SAN3	PAL1	FAN3
1 semester	0.68 p-z	0.83 d-z	0.87 d-x	0.81 f-z	0.73 k-z
2 semester	0.56 Yz	0.84 d-z	0.73 k-z	0.64 r-z	0.64 r-z
3 semester	-	-	-	-	-
4 semester	0.60 u-z	1.01 a-n	0.71 n-z	0.70 o-z	0.70 o-z
5 semester	0.59 w-z	0.67 q-z	0.51	0.61 t-z	0.43

Table 34. Copper content (ppm) in leaf tissues of the various clones of *A. halimus* located in four sites. Data with letters in common are not statistically different for $p < 0.01$.

ORISTANO					
	MAR1	GIO1	SAN3	PAL1	FAN3
1 semester	11.67 d-t	15.67 b-o	15.33 b-p	10.67 f-t	8.33 k-u
2 semester	14.33 b-q	20.33 a-g	9.33 i-u	9.00 j-u	13.00 c-s
3 semester	5.00 q-u	12.33 d-s	8.00 k-u	8.00 k-u	1.67 tu
4 semester	10.33 g-t	14.00 b-r	11.00 f-t	11.00 f-t	8.33 k-u
5 semester	9.00 j-u	10.67 f-t	7.33 m-u	8.67 j-u	8.33 k-u
TEMPIO					
	MAR1	GIO1	SAN3	PAL1	FAN3
1 semester	15.33 b-p	20.33 a-g	17.00 b-m	13.33 c-s	16.33 b-n
2 semester	6.67 n-u	7.67 l-u	7.67 l-u	5.67 o-u	6.33 n-u
3 semester	-	-	-	-	-
4 semester	18.00 a-k	27.00 a	23.67 ab	22.67 abc	19.67 a-h
5 semester	8.67 j-u	9.00 j-u	10.00 h-u	11.33 e-t	7.00 m-u
SASSARI					
	MAR1	GIO1	SAN3	PAL1	FAN3
1 semester	19.33 a-i	21.67 a-d	18.00 a-k	14.33 b-q	7.67 l-u
2 semester	18.67 a-j	11.00 f-t	17.67 a-l	20.67 a-f	13.00 c-s
3 semester	8.33 k-u	22.67 abc	20.33 a-g	14.00 b-r	11.00 f-t
4 semester	9.00 j-u	6.00 o-u	3.33 stu	6.00 o-u	5.33 p-u
5 semester	0.00 u	6.00 o-u	0.00 u	4.00 q-u	5.00 q-u
VILLASOR					
	MAR1	GIO1	SAN3	PAL1	FAN3
1 semester	9.33 i-u	15.67 b-o	21.33 a-e	9.67 h-u	6.33 n-u
2 semester	8.00 k-u	17.00 b-m	3.67 r-u	8.67 j-u	4.67 q-u
3 semester	-	-	-	-	-
4 semester	5.00 q-u	5.00 q-u	9.33 i-u	4.67 q-u	3.67 r-u
5 semester	0.00 u	0.00 u	0.00 u	0.00 u	0.00 u

Table 35. Zinc content (ppm) in leaf tissues of the various clones of *A. halimus* located in four sites. Data with letters in common are not statistically different for $p < 0.01$.

ORISTANO					
	MAR1	GIO1	SAN3	PAL1	FAN3
1 semester	105.00 e-t	149.00 b-f	126.67 b-o	121.67 b-p	71.67 l-t
2 semester	102.33 e-t	101.33 e-t	104.00 e-t	115.00 d-q	45.67 st
3 semester	160.00 a-e	140.67 b-h	129.33 b-m	148.67 b-g	92.67 f-t
4 semester	116.33 d-p	98.33 f-t	106.33 e-s	131.67 b-l	81.00 h-t
5 semester	83.67 h-t	99.33 e-t	129.00 b-m	139.33 b-i	95.67 f-t
TEMPIO					
	MAR1	GIO1	SAN3	PAL1	FAN3
1 semester	78.00 i-t	180.33 ab	212.33 a	79.33 h-t	79.33 h-t
2 semester	72.00 k-t	92.33 f--t	106.33 e-s	98.00 f-t	72.00 k-t
3 semester	-	-	-	-	-
4 semester	135.33 b-j	172.33 a-d	177.00 abc	135.00 b-j	103.67 e-t
5 semester	109.33 e-r	72.00 k-t	128.33 b-o	122.00 b-p	73.00 k-t
SASSARI					
	MAR1	GIO1	SAN3	PAL1	FAN3
1 semester	99.00 f-t	95.67 f-t	128.67 b-n	135.67 b-j	78.33 i-t
2 semester	70.33 l-t	68.67 m-t	121.33 b-p	77.67 j-t	44.00 t
3 semester	103.67 e-t	147.67 b-g	98.33 f-t	133.33 b-k	64.67 p-t
4 semester	68.67 m-t	80.67 h-t	65.00 p-t	53.67 q-t	50.67 rst
5 semester	64.00 p-t	86.00 h-t	88.33 f-t	73.33 k-t	48.00 rst
VILLASOR					
	MAR1	GIO1	SAN3	PAL1	FAN3
1 semester	87.33 g-t	138.33 b-j	130.00 b-m	120.67 b-p	70.33 l-t
2 semester	67.00 o-t	88.67 f-t	118.00 c-p	106.00 e-s	62.00 p-t
3 semester	-	-	-	-	-
4 semester	174.00 a-d	211.00 a	177.67 abc	129.33 b-m	179.33 ab
5 semester	46.00 st	72.00 k-t	67.33 n-t	77.33 j-t	49.00 rst

Table 36. Iron content (ppm) in leaf tissues of the various clones of *A. halimus* located in four sites. Data with letters in common are not statistically different for $p < 0.01$.

ORISTANO										
	MAR1		GIO1		SAN3		PAL1		FAN3	
1 semester	73.67	q-v	82.67	p-v	54.00	s-v	91.67	o-v	48.33	tuv
2 semester	237.67	i-v	335.67	b-n	210.33	i-v	220.67	i-v	184.33	k-v
3 semester	266.00	e-t	289.33	d-q	215.00	i-v	209.67	i-v	200.00	j-v
4 semester	161.00	m-v	149.33	m-v	118.67	n-v	139.67	m-v	108.00	o-v
5 semester	471.33	a-g	518.00	abc	290.67	d-q	430.00	a-i	243.00	i-v
TEMPIO										
	MAR1		GIO1		SAN3		PAL1		FAN3	
1 semester	59.33	r-v	49.00	t-v	34.00	uv	63.00	r-v	47.67	tuv
2 semester	364.67	a-m	283.67	d-r	229.33	i-v	469.33	a-h	463.67	a-h
3 semester	-		-		-		-		-	
4 semester	206.00	j-v	258.67	f-u	137.67	n-v	167.33	m-v	205.33	j-v
5 semester	229.67	i-v	300.00	d-q	163.00	m-v	194.33	k-v	216.00	i-v
SASSARI										
	MAR1		GIO1		SAN3		PAL1		FAN3	
1 semester	251.33	h-v	159.00	m-v	119.00	n-v	237.33	i-v	206.33	j-v
2 semester	181.00	l-v	273.33	d-t	168.33	m-v	193.67	k-v	159.67	m-v
3 semester	304.67	c-p	317.00	c-o	236.33	i-v	397.33	a-l	273.00	d-t
4 semester	60.00	r-v	36.00	uv	31.33	v	31.67	v	36.67	uv
5 semester	291.33	d-q	317.67	c-o	256.67	g-v	482.00	a-e	281.33	d-r
VILLASOR										
	MAR1		GIO1		SAN3		PAL1		FAN3	
1 semester	212.33	i-v	140.00	m-v	74.00	q-v	157.00	m-v	83.67	p-v
2 semester	150.67	m-v	199.33	j-v	112.33	n-v	134.33	n-v	83.33	p-v
3 semester	-		-		-		-		-	
4 semester	556.67	a	406.67	a-k	533.33	ab	463.33	a-h	490.00	a-d
5 semester	420.00	a-j	280.00	d-s	167.33	m-v	293.33	d-q	476.67	a-f

Table 37. Manganese content (ppm) in leaf tissues of the various clones of *A. halimus* located in four sites. Data with letters in common are not statistically different for $p < 0.01$.

RISTANO					
	MAR1	GIO1	SAN3	PAL1	FAN3
1 semester	369.67 b-j	413.00 a-f	361.67 b-k	330.33 b-o	192.33 d-w
2 semester	173.33 f-w	134.00 j-w	161.67 h-w	426.00 a-e	71.33 p-w
3 semester	453.00 abc	314.00 b-p	314.00 d-w	425.67 a-e	252.67 b-w
4 semester	224.00 c-w	159.67 h-w	219.33 c-w	405.00 b-g	163.67 g-w
5 semester	124.67 k-w	54.00 q-w	255.00 b-w	431.33 a-d	112.33 l-w
TEMPIO					
	MAR1	GIO1	SAN3	PAL1	FAN3
1 semester	396.33 b-h	313.00 b-p	170.33 g-w	216.67 c-w	292.00 b-q
2 semester	184.33 f-w	89.67 o-w	152.33 i-w	180.33 f-w	91.33 o-w
3 semester	-	-	-	-	-
4 semester	281.00 b-s	93.67 o-w	226.67 c-w	348.00 b-m	258.67 b-w
5 semester	346.67 b-m	270.00 b-v	210.00 d-w	488.00 ab	241.00 c-w
SASSARI					
	MAR1	GIO1	SAN3	PAL1	FAN3
1 semester	431.67 a-d	161.00 h-w	271.33 b-u	381.67 b-i	156.33 h-w
2 semester	186.67 e-w	28.00 uvw	172.67 f-w	145.33 i-w	16.00 w
3 semester	223.00 c-w	352.33 b-l	152.67 i-w	286.33 b-r	95.33 o-w
4 semester	136.67 j-w	277.00 b-t	70.67 p-w	139.00 i-w	64.00 q-w
5 semester	155.67 h-w	242.00 c-w	122.33 k-w	342.67 b-n	39.67 s-w
VILLASOR					
	MAR1	GIO1	SAN3	PAL1	FAN3
1 semester	230.67 c-w	197.33 d-w	295.00 b-q	426.00 a-e	162.67 g-w
2 semester	100.33 n-w	45.67 r-w	89.33 o-w	290.33 b-r	74.33 p-w
3 semester	-	-	-	-	-
4 semester	104.00 m-w	179.00 f-w	54.67 q-w	142.33 i-w	52.67 q-w
5 semester	56.33 q-w	56.00 q-w	33.00 t-w	71.33 p-w	25.33 vw

4.5.1.1. Influence of temperature on the accumulation of macro and microelements in the leaves

In Table I and II (appendix) are reported the results of the analysis of the relationship between the content of the main macroelements in the leaves and the ICC relating to critical temperatures of 0, 5 and 10 °C.

For most of the elements significant correlation with the ICC have not been found. Whereas in the case of data grouped for semester, temperatures below 0 °C were found to correlate positively with the nitrogen content in the leaves of clone SAN3 (r = 0.488). Analyzing the data for the entire test period, there was not statistically significant correlation between ICC and the content of main macroelements analyzed in leaves, with the exception of a positive and statistically significant relationship between temperatures below 10 °C and the magnesium content of the leaves of the clone FAN3 ($p < 0.01$).

Tables III and IV shows the results of the correlation between ICC and microelements in the leaves. Statistical analysis did not reveal any influence of the ICC, for temperatures lower than 0. 5 and 10 °C, on the accumulation of Zn, Fe, Cu and Mn, both in relation to the data grouped for semester and in the data for the whole period of the test.

4.5.2. Analysis of branches

Tables 38 and 39 shows data and analysis of variance for the content of the major macro and microelements present in the *Atriplex halimus* branches.

The analysis of variance reported differences among clones statistically significant for the N content, as well as the interaction between localities and semesters. No significant differences were found among localities and semesters. The average phosphorus content in the branches in different localities ranged from 0.40% to Oristano to 0.47% in Sassari. Comparing the clones the highest content of P in the branches was for MAR1 (0.50%). The lowest content was in FAN3 (0.29%). These differences were statistically significant for $p < 0.01$. Also the differences among the five semesters were significant ($p < 0.01$). Interactions between localities and clones were not significant, while significant were those between localities and semesters ($p < 0.01$).

The content of sodium in the branches varied depending on localities from 0.25% (Tempio) to 0.32% (Sassari). The differences among clones were significant ($p < 0.01$) and the Na content varied from 0.23% to 0.30% in FAN3 in SAN3. The Na content varied among semesters from 0.33% to 0.21% in the first and fifth semesters respectively. Potassium is found to range between 0.53% (Oristano) and 1.07% (Sassari).

The clone with the highest K content in the branches was the clone MAR1 (1.022%). The differences among localities, clones and semesters were statistically significant ($p < 0.01$). There were no significant interactions between localities and clones.

The content of Ca in the branches varied from a minimum of 0.28% in Villasor plants to 0.77% in Oristano plants. Interactions between localities and clones were significant only for the content of Ca ($p < 0.01$) but not for the other macroelements.

In Tables 40-49 specific data for every macro and microelements present in the branches have been analyzed. In particular, data were shown for every clone in different semesters and in different localities.

In Table 40 the nitrogen content was almost constant for all the clones, for all sites and in all semesters. Mean separation not revealed statistically significant differences among the clones. The only difference was accounted for SAN3 in the fourth semester in Tempio, which showed highest levels of nitrogen (2.42%).

As regards the phosphorus content we can state that in the sites of Oristano, Tempio and Sassari and in all semesters the clone that showed lowest values was the FAN3, while

the highest content was of MAR1 clone (0.71%). In the Villasor site the P highest content was for SAN3 (0.82%) (Table 41).

The lowest content of Na recorded in Tempio and Villasor was in FAN3 (0.18% and 0.11%). The highest percentages were observed in MAR1, while Oristano, Tempio and Sassari showed values respectively 0.39%, 0.41% and 0.43% (Table 42).

The MAR1 was the clone in which all 4 sites showed the highest content of potassium. At Oristano percentages of K, in almost all the semesters, were recorded in clone FAN3. At Tempio the K content was found to range between 0.34% (GIO1) and 1.62% (MAR1) (Table 43).

Regarding the content of calcium in the branches the clone MAR1 showed the highest levels especially in plants of the Oristano site (2.30%), Tempio (1.89%) and Villasor (0.96%). In Sassari the highest percentages were of PAL1 (1.06%). The clones with the lowest content of Ca were the clone FAN3 and GIO1 (Table 44).

The magnesium content was not different in the 4 sites. The clone with the highest percentage of magnesium in most sites was MAR1, except in Villasor where was SAN3 to present the highest percentage of Mg (Table 45).

In the analysis of microelements, the clone MAR1 showed the highest content of Cu, in Oristano, in Tempio and Sassari. In Villasor the largest quantities of Cu were in the clone GIO1 (10.33 ppm) (Table 46). Also highest levels of zinc, in all 4 sites, were in the clone MAR1 (Table 47).

In the sites of Oristano, Sassari and Tempio the highest iron content was found in PAL1 clone, while in Villasor was in MAR1 (357 ppm) (Table 48). The manganese was found to range between 0 ppm (FAN3 in Villasor) and 64.67 ppm (SAN3 in Tempio) (Tab. 49).

Table 38. Averages and analysis of variance of the content of main macroelements in the branches of the species *A. halimus*.

	N(%)	P(%)	Na(%)	K(%)	Ca(%)	Mg(%)
LOCALITY (A)						
Tempio	0.51	0.40	0.27	0.53	0.77	0.19
Oristano	0.47	0.43	0.25	0.90	0.49	0.17
Sassari	0.48	0.48	0.32	1.07	0.48	0.17
Villasor	0.56	0.45	0.26	1.00	0.28	0.16
F	0.119	10.46	103.99	291.06	54.82	8.38
p	0.81	0.008	0.001	0.001	0.001	0.014
CLONE (B)						
MAR1	0.59	0.51	0.28	1.02	0.62	0.20
GIO1	0.44	0.50	0.27	0.81	0.44	0.17
SAN3	0.59	0.50	0.31	1.01	0.49	0.19
PAL1	0.51	0.38	0.27	0.78	0.58	0.16
FAN3	0.39	0.30	0.23	0.76	0.39	0.16
F	4.08	41.29	6.46	27.33	13.2	8.5
p	0.008	0.001	0.001	0.001	0.001	0.001
LOCALITY x CLONE (AxB)						
F	1.05	1.59	1.33	0.9	3.24	1.41
p	0.427	0.145	0.252	n.s.	0.003	0.213
SEMESTER (C)						
1	046	0.47	0.33	1.00	0.26	0.17
2	0.42	0.48	0.28	0.95	0.18	0.17
3	0.52	0.40	0.29	0.85	0.61	0.21
4	0.58	0.43	0.26	0.91	0.44	0.16
5	0.54	0.41	0.21	0.68	1.03	0.16
F	2.36	7.52	22.63	31.13	182.59	14.91
p	0.055	0.001	0.0008	0.001	0.001	0.001
LOCALITY x SEMESTER (AxC)						
F	2.67	5.65	5.91	25.39	32.22	5.52
p	0.002	0.001	0.0008	0.001	0.001	0.001
LOCALITY x CLONE x SEMESTER (AxBxC)						
F	1.30	1.08	1.43	1.91	1.73	1.33
p	0.114	0.351	0.053	0.001	0.006	0.095

Table 39. Averages and analysis of variance for the content of the main microelements in branches of the clones of the species *A. halimus*.

	Cu (ppm)	Zn (ppm)	Fe (ppm)	Mn (ppm)
LOCALITY(A)				
Oristano	4.79	33.63	149.84	30.29
Tempio	3.79	30.55	129.97	33.64
Sassari	3.64	25.77	132.52	21.73
Villasor	4	32.08	126.96	15.41
F	3.05	16.95	1.936	17.12
p	0.113	0.002	0.226	0.002
CLONE (B)				
MAR1	4.83	55.47	167.07	32.23
GIO1	4.48	23.37	124.40	17.83
SAN3	4.72	21.17	105.30	31.35
PAL1	2.97	32.23	156.62	30.48
FAN3	3.27	20.3	120.73	14.45
F	20.3	108.07	13.06	34.75
p	0.001	0.001	0.0009	0.0008
LOCALITY x CLONE (AxB)				
F	2.95	1.15	3.98	2.09
p	0.007	0.357	0.001	0.047
SEMESTER (C)				
1	7.63	28.50	56.38	22.93
2	4.3	27.10	143.15	19.10
3	4.3	29.97	181.67	32.42
4	3.17	36.83	168.52	20.66
5	0.87	30.13	124.40	31.27
F	100.52	9.19	43.48	24.01
p	0.001	0.001	0.001	0.001
LOCALITY x SEMESTER (AxC)				
F	17.53	6.61	5.31	13.81
p	0.001	0.001	0.001	0.001
LOCALITY x CLONE x SEMESTER (AxBxC)				
F	1.71	3.64	2.32	1.53
p	0.007	0.0007	0.0009	0.026

Table 40. Nitrogen content (%) in the branches of the 5 clones of *A. halimus*, located in four sites. Data with letters in common are not statistically different for $p < 0.01$.

ORISTANO					
	MAR1	GIO1	SAN3	PAL1	FAN3
1 semester					
2 semester	0.60 b	0.34 b	0.34 b	0.48 b	0.29 b
3 semester	0.60 b	0.56 b	1.01 b	1.05 b	0.49 b
4 semester	0.50 b	0.38 b	0.41 b	0.57 b	0.45 b
5 semester	0.74 b	0.55 b	0.48 b	0.49 b	0.28 b
TEMPIO					
	MAR1	GIO1	SAN3	PAL1	FAN3
1 semester	0.62 b	0.33 b	0.46 b	0.50 b	0.53 b
2 semester	0.65 b	0.38 b	0.50 b	0.54 b	0.35 b
3 semester					
4 semester	0.36 b	0.75 b	0.49 b	0.35 b	0.36 b
5 semester	0.66 b	0.37 b	0.39 b	0.45 b	0.46 b
SASSARI					
	MAR1	GIO1	SAN3	PAL1	FAN3
1 semester					
2 semester	0.49 b	0.35 b	0.51 b	0.41 b	0.27 b
3 semester	0.61 b	0.42 b	0.70 b	0.49 b	0.49 b
4 semester	0.65 b	0.39 b	0.47 b	0.53 b	0.44 b
5 semester	0.75 b	0.37 b	0.50 b	0.60 b	0.44 b
VILLASOR					
	MAR1	GIO1	SAN3	PAL1	FAN3
1 semester	0.76 b	0.43 b	0.48 b	0.51 b	0.44 b
2 semester	0.52 b	0.33 b	0.35 b	0.36 b	0.27 b
3 semester	0.39 b	0.32 b	0.29 b	0.35 b	0.32 b
4 semester	0.73 b	0.55 b	2.42 a	0.48 b	0.39 b
5 semester	0.72 b	0.52 b	0.75 b	0.77 b	0.52 b

Table 41. Phosphorus content (%) in the branches of clones of *A. halimus*, located in four sites. Data with letters in common are not statistically different for $p < 0.01$.

ORISTANO					
	MAR1	GIO1	SAN3	PAL1	FAN3
1 semester	0.56 b-l	0.49 b-q	0.53 b-o	0.54 b-n	0.27 p-u
2 semester	0.45 c-t	0.48 b-r	0.50 b-q	0.46 c-t	0.31 l-u
3 semester	0.42 d-u	0.49 b-q	0.51 b-q	0.31 l-u	0.23 r-u
4 semester	0.36 h-u	0.50 b-q	0.41 d-u	0.30 m-u	0.21 tu
5 semester	0.42 d-u	0.45 c-t	0.36 h-u	0.33 j-u	0.19 u
TEMPIO					
	MAR1	GIO1	SAN3	PAL1	FAN3
1 semester	0.72 a-b	0.48 b-r	0.54 b-n	0.32 j-u	0.31 l-u
2 semester	0.63 a-f	0.50 b-q	0.56 b-m	0.38 d-u	0.37 e-u
3 semester	0.48 b-r	0.63 a-e	0.56 b-l	0.45 c-t	0.32 j-u
4 semester	0.40 d-u	0.56 b-m	0.44 c-u	0.34 i-u	0.32 j-u
5 semester	0.37 f-u	0.45 c-t	0.33 j-u	0.28 o-u	0.23 r-u
SASSARI					
	MAR1	GIO1	SAN3	PAL1	FAN3
1 semester	0.71 ab	0.50 b-q	0.53 b-o	0.51 b-q	0.34 i-u
2 semester	0.62 a-h	0.46 b-t	0.57 b-k	0.39 d-u	0.31 k-u
3 semester	0.46 b-t	0.50 b-q	0.45 c-t	0.32 j-u	0.32 j-u
4 semester	0.63 a-g	0.56 b-m	0.44 c-u	0.41 d-u	0.34 j-u
5 semester	0.64 a-d	0.55 b-m	0.68 abc	0.37 f-u	0.31 l-u
VILLASOR					
	MAR1	GIO1	SAN3	PAL1	FAN3
1 semester	0.58 b-j	0.53 b-o	0.57 b-k	0.41 d-u	0.26 q-u
2 semester	0.52 b-p	0.52 b-p	0.82 a	0.41 d-u	0.29 n-u
3 semester	0.43 c-u	0.33 j-u	0.34 j-u	0.22 stu	0.25 q-u
4 semester	0.57 b-k	0.60 a-i	0.53 b-o	0.40 d-u	0.37 g-u
5 semester	0.48 b-r	0.55 b-m	0.47 b-s	0.36 i-u	0.31 l-u

Table 42. Sodium content (%) in the branches of five clones of *A. halimus* located in four sites. Data with letters in common are not statistically different for $p < 0.01$.

ORISTANO					
	MAR1	GIO1	SAN3	PAL1	FAN3
1 semester	0.39 b-e	0.34 b-i	0.29 b-l	0.32 b-j	0.25 b-l
2 semester	0.28 b-l	0.30 b-l	0.25 b-l	0.29 b-l	0.24 b-l
3 semester	0.25 b-l	0.21 d-l	0.32 b-j	0.26 b-l	0.20 f-l
4 semester	0.29 b-l	0.26 b-l	0.29 b-l	0.23 c-l	0.24 b-l
5 semester	0.18 h-l	0.23 c-l	0.31 b-k	0.22 c-l	0.22 c-l
TEMPIO					
	MAR1	GIO1	SAN3	PAL1	FAN3
1 semester	0.26 b-l	0.23 c-l	0.25 b-l	0.22 c-l	0.18 h-l
2 semester	0.21 d-l	0.25 b-l	0.26 b-l	0.25 b-l	0.18 h-l
3 semester	0.41 bc	0.30 b-l	0.33 b-i	0.27 b-l	0.27 b-l
4 semester	0.36 b-i	0.22 d-l	0.28 b-l	0.25 b-l	0.21 d-l
5 semester	0.27 b-l	0.18 h-l	0.21 d-l	0.24 b-l	0.20 e-l
SASSARI					
	MAR1	GIO1	SAN3	PAL1	FAN3
1 semester	0.30 b-k	0.38 b-f	0.40 bcd	0.38 b-f	0.35 b-i
2 semester	0.43 b	0.34 b-i	0.41 bcd	0.38 b-f	0.27 b-l
3 semester	0.33 b-j	0.37 b-h	0.38 b-f	0.36 b-i	0.28 b-l
4 semester	0.25 b-l	0.31 b-k	0.31 b-k	0.33 b-i	0.26 b-l
5 semester	0.28 b-l	0.23 c-l	0.17 i-l	0.30 b-l	0.20 e-l
VILLASOR					
	MAR1	GIO1	SAN3	PAL1	FAN3
1 semester	0.26 b-l	0.38 b-g	0.61 a	0.35 b-i	0.32 b-j
2 semester	0.23 c-l	0.23 c-l	0.33 b-j	0.25 b-l	0.21 d-l
3 semester	0.35 b-i	0.22 c-l	0.31 b-k	0.23 c-l	0.22 c-l
4 semester	0.20 e-l	0.27 b-l	0.22 c-l	0.21 d-l	0.18 g-l
5 semester	0.12 kl	0.21 d-l	0.21 d-l	0.13 jkl	0.11 l

Table 43. Potassium content (%) in the branches of five clones of *A. halimus* located in four sites. Data with letters in common are not statistically different for $p < 0.01$.

ORISTANO					
	MAR1	GIO1	SAN3	PAL1	FAN3
1 semester	0.29	0.20	0.21	0.20	0.13
2 semester	0.99 d-s	0.77 l-z	0.96 e-t	0.75 l-z	0.85 h-x
3 semester	0.83 i-y	0.58 q-z	0.73 l-z	0.58 q-z	0.47 v-z
4 semester	0.55 s-z	0.60 p-z	0.85 h-x	0.45 w-z	0.37 z
5 semester	0.43 xyz	0.43 xyz	0.58 q-z	0.28	0.20
TEMPIO					
	MAR1	GIO1	SAN3	PAL1	FAN3
1 semester	1.62 a	1.04 c-p	1.42 a-d	1.01 d-r	1.14 b-l
2 semester	1.14 b-l	0.70 l-z	1.33 a-g	0.80 i-z	0.95 e-u
3 semester	0.79 k-z	1.02 c-q	1.07 c-o	0.96 e-t	0.87 g-x
4 semester	0.93 f-u	0.78 k-z	0.93 f-u	0.77 l-z	0.75 l-z
5 semester	0.65 o-z	0.34	0.44 xyz	0.50 u-z	0.52 t-z
SASSARI					
	MAR1	GIO1	SAN3	PAL1	FAN3
1 semester	1.52 ab	1.15 b-l	1.40 a-e	1.40 a-e	1.04 c-p
2 semester	1.07 c-o	1.14 b-l	1.04 c-p	0.80 i-z	0.87 h-x
3 semester	1.25 a-j	0.98 d-t	1.38 a-f	0.74 l-z	1.10 b-o
4 semester	1.37 a-f	0.98 d-t	1.25 a-j	1.13 b-m	1.09 b-o
5 semester	1.05 c-p	0.78 k-z	0.67 n-z	0.84 h-y	0.73 l-z
VILLASOR					
	MAR1	GIO1	SAN3	PAL1	FAN3
1 semester	1.47 abc	1.09 b-o	1.42 a-d	1.16 b-l	1.08 b-o
2 semester	1.12 b-n	0.71 l-z	1.12 b-n	1.02 c-r	0.78 k-z
3 semester	1.07 c-o	0.67 m-z	0.89 g-w	0.39 yz	0.56 r-z
4 semester	1.25 a-i	0.96 e-t	1.29 a-h	0.91 g-v	0.93 f-u
5 semester	1.05 c-p	1.23 a-k	1.13 b-n	0.95 e-u	0.79 j-z

Table 44. Content of Ca (%) in the branches of the five clones of *A. halimus* located in four sites. Data with letters in common are not statistically different for $p < 0.01$.

ORISTANO					
	MAR1	GIO1	SAN3	PAL1	FAN3
1 semester	0.58 g-v	0.30 n-v	0.26 o-v	0.31 n-v	0.27 o-v
2 semester	0.44 j-v	0.08 uv	0.15 s-v	0.22 q-v	0.23 q-v
3 semester	0.92 d-j	0.56 h-v	0.72 f-q	0.87 d-l	0.49 i-v
4 semester	0.87 d-l	0.56 h-v	0.35 m-v	1.04 d-h	0.77 e-p
5 semester	2.30 a	1.89 ab	1.61 bc	2.07 a	1.32 cd
TEMPIO					
	MAR1	GIO1	SAN3	PAL1	FAN3
1 semester	0.28 o-v	0.23 q-v	0.23 q-v	0.15 s-v	0.15 s-v
2 semester	0.25 p-v	0.15 s-v	0.20 q-v	0.21 q-v	0.14 s-v
3 semester	0.30 n-v	0.34 m-v	0.35 m-v	0.67 f-r	0.30 n-v
4 semester	0.62 g-t	0.38 l-v	0.44 j-v	0.43 j-v	0.34 m-v
5 semester	1.89 ab	0.98 d-i	1.22 cde	1.00 d-h	0.88 d-l
SASSARI					
	MAR1	GIO1	SAN3	PAL1	FAN3
1 semester	0.38 k-v	0.26 o-v	0.49 i-v	0.25 p-v	0.23 q-v
2 semester	0.20 q-v	0.07 v	0.31 n-v	0.22 q-v	0.07 uv
3 semester	0.44 j-v	0.59 g-u	0.77 e-o	1.06 d-g	0.43 j-v
4 semester	0.36 m-v	0.28 o-v	0.35 m-v	0.50 i-v	0.31 m-v
5 semester	0.88 d-k	0.79 e-n	0.68 f-r	1.16 def	0.82 e-m
VILLASOR					
	MAR1	GIO1	SAN3	PAL1	FAN3
1 semester	0.14 s-v	0.16 r-v	0.23 q-v	0.20 q-v	0.15 s-v
2 semester	0.16 r-v	0.13 s-v	0.16 r-v	0.14 s-v	0.11 tuv
3 semester	0.96 d-i	0.64 g-s	0.72 f-q	0.50 i-v	0.43 j-v
4 semester	0.21 q-v	0.22 q-v	0.21 q-v	0.24 q-v	0.20 q-v
5 semester	0.18 r-v	0.24 q-v	0.22 q-v	0.28 n-v	0.23 q-v

Table 45. Magnesium content (%) in the branches of the five clones of *A. halimus* located in four sites. Data with letters in common are not statistically different for $p < 0.01$.

ORISTANO					
	MAR1	GIO1	SAN3	PAL1	FAN3
1 semester	0.28 ab	0.21 b-o	0.19 c-q	0.16 h-t	0.19 c-q
2 semester	0.15 i-t	0.16 h-t	0.18 d-q	0.13 m-t	0.22 a-l
3 semester	0.27 abc	0.23 a-i	0.24 a-g	0.22 a-l	0.17 g-s
4 semester	0.19 c-q	0.18 c-q	0.14 j-t	0.14 j-t	0.14 j-t
5 semester	0.26 a-d	0.21 a-n	0.22 a-l	0.22 a-l	0.16 g-t
TEMPIO					
	MAR1	GIO1	SAN3	PAL1	FAN3
1 semester	0.21 a-n	0.16 g-t	0.17 f-s	0.12 p-t	0.15 i-t
2 semester	0.21 a-n	0.15 i-t	0.19 c-q	0.17 f-s	0.20 b-p
3 semester	0.17 f-s	0.19 c-q	0.26 a-e	0.22 a-j	0.14 k-t
4 semester	0.18 c-q	0.13 n-t	0.13 l-t	0.13 m-t	0.15 i-t
5 semester	0.24 a-g	0.17 f-s	0.18 e-r	0.15 h-t	0.15 i-t
SASSARI					
	MAR1	GIO1	SAN3	PAL1	FAN3
1 semester	0.19 c-q	0.15 i-t	0.17 g-s	0.16 g-s	0.13 m-t
2 semester	0.16 h-t	0.12 o-t	0.18 d-q	0.17 f-s	0.17 f-s
3 semester	0.21 a-n	0.21 a-m	0.22 a-k	0.22 a-l	0.19 c-q
4 semester	0.21 a-n	0.17 g-s	0.18 d-q	0.20 b-p	0.16 g-t
5 semester	0.17 f-s	0.17 f-s	0.09 st	0.15 h-t	0.13 l-t
VILLASOR					
	MAR1	GIO1	SAN3	PAL1	FAN3
1 semester	0.16 g-s	0.15 i-t	0.19 c-q	0.14 j-t	0.15 h-t
2 semester	0.17 f-s	0.13 l-t	0.29 a	0.15 i-t	0.15 h-t
3 semester	0.25 a-f	0.19 c-q	0.21 a-n	0.15 i-t	0.18 e-r
4 semester	0.18 c-q	0.16 g-s	0.17 f-s	0.13 n-t	0.15 h-t
5 semester	0.09 rst	0.13 n-t	0.13 n-t	0.11 q-t	0.08 t

Table 46. Copper content (ppm) in the branches of the five clones of *A. halimus* located in four sites. Data with letters in common are not statistically different for $p < 0.01$.

ORISTANO					
	MAR1	GIO1	SAN3	PAL1	FAN3
1 semester	14.00 a	10.00 a-d	10.00 a-d	6.33 c-l	9.67 a-e
2 semester	5.00 d-o	3.67 g-o	4.67 e-o	0.67 no	1.33 l-o
3 semester	9.33 b-f	8.00 c-g	6.00 c-m	5.33 d-n	5.00 e-o
4 semester	3.33 g-o	6.67 c-k	4.67 e-o	2.67 h-o	3.33 g-o
5 semester	0.00 o	0.00 o	0.00 o	0.00 o	0.00 o
TEMPIO					
	MAR1	GIO1	SAN3	PAL1	FAN3
1 semester	7.67 c-h	6.00 c-m	6.00 c-m	6.00 c-m	6.67 c-k
2 semester	5.00 e-o	6.33 c-l	6.67 c-k	4.00 g-o	3.67 g-o
3 semester	0.00 o	0.00 o	3.00 g-o	0.00 o	3.00 g-o
4 semester	5.33 d-n	7.00 c-j	7.33 c-i	4.00 g-o	4.67 e-o
5 semester	2.33 i-o	0.00 o	0.00 o	0.00 o	0.00 o
SASSARI					
	MAR1	GIO1	SAN3	PAL1	FAN3
1 semester	12.67 ab	5.67 c-n	9.67 a-e	10.33 f-o	5.00 e-o
2 semester	6.33 c-l	5.00 d-o	6.67 c-k	4.67 e-o	0.00 o
3 semester	2.67 h-o	3.00 g-o	5.00 d-o	2.00 j-o	2.33 i-o
4 semester	1.00 mno	0.00 o	0.00 o	0.00 o	0.00 o
5 semester	2.33 i-o	2.67 h-o	1.67 k-o	4.67 e-o	3.67 g-o
VILLASOR					
	MAR1	GIO1	SAN3	PAL1	FAN3
1 semester	8.00 c-g	10.33 abc	7.00 c-j	4.33 f-o	3.33 g-o
2 semester	2.67 h-o	4.33 f-o	7.67 c-h	2.33 i-o	5.33 d-n
3 semester	5.67 c-n	8.00 c-g	6.33 c-l	5.00 d-o	6.33 c-l
4 semester	3.33 g-o	3.00 g-o	2.00 j-o	3.00 g-o	2.00 j-o
5 semester	0.00 o	0.00 o	0.00 o	0.00 o	0.00 o

Table 47. Zinc content (ppm) in the branches of the five clones of *A. halimus* located in four sites. Data with letters in common are not statistically different for $p < 0.01$.

ORISTANO					
	MAR1	GIO1	SAN3	PAL1	FAN3
1 semester	52.00 e-l	19.33 n-w	12.33 uvw	28.67 k-w	13.00 t-w
2 semester	40.67 g-r	13.33 t-w	12.33 uvw	27.67 l-w	19.00 n-w
3 semester	55.67 b-h	40.00 g-s	18.33 n-w	31.33 g-w	15.67 p-w
4 semester	79.00 a	38.67 g-t	22.00 n-w	55.00 c-i	29.00 j-w
5 semester	78.33 ab	41.67 f-p	40.00 g-s	37.00 g-u	20.67 n-w
TEMPIO					
	MAR1	GIO1	SAN3	PAL1	FAN3
1 semester	56.00 b-g	19.67 n-w	21.67 n-w	20.67 n-w	17.33 o-w
2 semester	53.67 d-k	18.33 n-w	19.67 n-w	30.67 h-w	21.67 n-w
3 semester	17.67 o-w	18.67 n-w	23.67 m-w	80.00 a	30.33 i-w
4 semester	77.67 abc	29.33 j-w	19.67 n-w	30.33 i-w	23.67 m-w
5 semester	66.67 a-e	17.00 p-w	14.00 s-w	20.33 n-w	15.33 q-w
SASSARI					
	MAR1	GIO1	SAN3	PAL1	FAN3
1 semester	49.33 e-l	27.00 l-w	19.67 n-w	23.67 m-w	20.33 n-w
2 semester	51.00 e-l	9.00 w	34.00 g-w	22.00 n-w	14.67 r-w
3 semester	50.67 e-l	16.00 p-w	15.33 q-w	22.67 m-w	16.67 p-w
4 semester	49.67 e-l	14.00 s-w	10.00 vw	29.33 j-w	15.00 q-w
5 semester	54.33 d-j	21.00 n-w	15.00 q-w	28.33 k-w	15.67 p-w
VILLASOR					
	MAR1	GIO1	SAN3	PAL1	FAN3
1 semester	44.00 e-n	35.33 g-v	16.33 p-w	32.67 g-w	41.00 g-q
2 semester	48.00 e-m	19.00 n-w	37.00 g-u	31.33 g-w	19.00 n-w
3 semester	65.33 a-f	17.67 o-w	19.00 n-w	28.00 l-w	16.67 p-w
4 semester	76.67 a-d	39.00 g-t	35.00 g-w	34.33 g-w	29.33 j-w
5 semester	43.00 e-o	13.33 t-w	18.33 n-w	30.67 h-w	12.00 uvw

Table 48. Iron content (ppm) in the branches of five clones of *A. halimus* located in four sites. Data with letters in common are not statistically different for $p < 0.01$.

ORISTANO					
	MAR1	GIO1	SAN3	PAL1	FAN3
1 semester	85.67 h-r	51.00 m-r	12.00 r	30.67 o-r	36.00 n-r
2 semester	136.67 d-r	177.33 c-p	69.67 j-r	129.00 d-r	211.67 a-l
3 semester	178.67 c-p	232.67 a-i	158.67 c-r	272.33 a-d	182.00 c-p
4 semester	247.00 a-g	192.33 b-n	104.67 f-r	264.67 a-e	232.00 a-i
5 semester	225.33 a-j	168.33 c-r	97.00 g-r	159.33 c-r	91.33 g-r
TEMPIO					
	MAR1	GIO1	SAN3	PAL1	FAN3
1 semester	27.33 pqr	18.33 qr	16.33 q-r	17.67 q-r	18.33 q-r
2 semester	177.67 c-p	127.33 d-r	102.00 g-r	332.33 ab	259.00 a-f
3 semester	122.67 d-r	146.67 d-r	173.67 c-q	300.00 abc	167.33 c-r
4 semester	225.67 a-j	181.67 c-p	118.00 d-r	116.33 e-r	101.00 g-r
5 semester	182.67 c-p	84.33 h-r	62.67 l-r	101.67 g-r	68.67 j-r
SASSARI					
	MAR1	GIO1	SAN3	PAL1	FAN3
1 semester	106.33 f-r	65.67 k-r	75.67 i-r	75.50 i-r	117.33 d-r
2 semester	132.33 d-r	68.67 j-r	152.00 c-r	203.00 b-m	130.00 d-r
3 semester	225.00 a-j	98.67 g-r	102.00 g-r	119.33 d-r	134.33 d-r
4 semester	188.33 b-o	100.33 g-r	148.67 c-r	233.67 a-h	133.67 d-r
5 semester	139.00 d-r	87.33 h-r	97.67 g-r	234.33 a-h	126.00 d-r
VILLASOR					
	MAR1	GIO1	SAN3	PAL1	FAN3
1 semester	89.33 h-r	85.33 h-r	54.00 l-r	84.33 h-r	42.67 n-r
2 semester	160.00 c-r	86.00 h-r	59.67 l-r	91.00 g-r	57.67 l-r
3 semester	357.00 a	300.67 abc	184.67 b-p	102.00 g-r	75.00 i-r
4 semester	221.33 a-k	132.67 d-r	180.67 c-p	121.33 d-r	126.33 d-r
5 semester	113.33 e-r	82.67 h-r	136.33 d-r	125.67 d-r	104.33 f-r

Table 49. Manganese content (ppm) in the branches of five clones of *A. halimus* located in four sites. Data with letters in common are not statistically different for $p < 0.01$.

ORISTANO					
	MAR1	GIO1	SAN3	PAL1	FAN3
1 semester	42.33 a-o	12.33 r-z	17.00 o-z	18.33 m-z	4.00 yz
2 semester	26.67 f-z	17.33 n-z	26.00 f-z	31.67 d-x	5.67 xyz
3 semester	38.33 b-r	48.00 a-j	47.67 a-k	56.00 a-d	19.67 m-z
4 semester	39.00 b-q	24.67 h-z	22.67 i-z	55.67 a-d	21.67 k-z
5 semester	41.33 a-p	37.33 b-t	52.67 a-e	41.67 a-o	9.67 u-z
TEMPIO					
	MAR1	GIO1	SAN3	PAL1	FAN3
1 semester	47.33 a-l	14.33 q-z	27.67 e-z	14.00 q-z	16.67 o-z
2 semester	29.00 e-z	2.33	21.67 k-z	18.00 m-z	8.67 v-z
3 semester	31.67 d-x	51.33 a-f	51.00 a-g	57.33 abc	31.67 d-x
4 semester	43.33 a-n	28.67 e-z	39.00 b-q	35.33 b-u	32.67 c-w
5 semester	60.67 ab	27.67 e-z	64.67 a	52.67 a-e	33.67 c-v
SASSARI					
	MAR1	GIO1	SAN3	PAL1	FAN3
1 semester	48.33 a-i	10.00 u-z	25.00 h-z	25.33 g-z	19.67 m-z
2 semester	32.67 c-w	4.33 yz	49.67 a-h	30.33 d-y	9.00 u-z
3 semester	26.67 f-z	11.67 s-z	23.33 i-z	21.33 l-z	9.67 u-z
4 semester	13.33 q-z	4.00 yz	11.33 t-z	16.67 o-z	3.33 z
5 semester	26.67 f-z	16.67 oz	43.67 a-m	42.33 a-o	18.33 m-z
VILLASOR					
	MAR1	GIO1	SAN3	PAL1	FAN3
1 semester	27.33 e-z	15.33 p-z	31.00 d-x	28.33 e-z	14.33 q-z
2 semester	15.00 q-z	2.00	22.00 j-z	23.00 i-z	7.00 w-z
3 semester	38.00 b-s	19.33 m-z	23.67 h-z	24.67 h-z	17.33 n-z
4 semester	6.33 w-z	2.00	7.33 v-z	5.67 xyz	0.00
5 semester	10.67 u-z	7.33 v-z	20.00 m-z	11.33 t-z	6.33 w-z

4.5.2.1. Influence of temperature on accumulation of macro-and microelements in the branches.

As observed for leaves, also for branches, the only influence of ICC on the accumulation of macronutrients regards the accumulation of nitrogen in the branches.

As we can see in Table V, temperatures below 10 °C seem to positively influence the accumulation of N in the branches of the clone FAN3 (r = 0.517).

For all the other elements statistically significant correlations between the accumulation and ICC of the semester considered have been not found. Using the ICC for the entire test period test, the only significant correlation was with the nitrogen, in particular for the clone MAR1. On the branches, there was an opposite tendency with respect to the leaves. In fact in branches temperatures below 0 and 5 °C have limited the accumulation of nitrogen in the clone MAR1 ($p < 0.05$) (Table VI) .

The correlation index was very high for 0 and 5 °C critical temperatures (r = -0.95). In Tables VII and VIII the content of microelements is presented. Only the manganese content is correlated positively and significantly with temperature, both by using the data grouped per semester and those for the entire period. Instead, the tendency of all other elements is towards a negative correlation with the temperature, in any case this relationship was not statistically significant.

Considering the semesters ICC, the manganese content in the branches was positively affected by temperatures below 0 °C ($p < 0.01$) and 10 °C ($p < 0.05$) in the clone MAR1, less 5 and 10 °C in clone GIO1 ($p < 0.05$) and less than 0, 5 and 10 °C in the clone FAN3. Taking into account the ICC for the entire period of the test, significant correlations related exclusively to temperatures below 0, 5 and 10 °C and the manganese content of the branches of the clone FAN3.

4.5.3. Analysis of roots.

In Table 50 are reported the results of the analysis of variance for content of the main macroelements in roots.

Differences in the content of the main macroelements, among localities, clones and semesters were significant. In particular, comparing the means among "localities" for nitrogen and phosphorus, the differences were significant for $p < 0.01$, and for the other macroelements were significant for $p < 0.01$.

Differences among clones, as regards to the content of the main macroelements, were statistically significant ($p < 0.01$).

The highest percentage of nitrogen in the roots was found in the site of the Tempio. The clone with the highest nitrogen content was MAR1. The clone GIO1 showed the highest percentage of P in the roots.

The lowest sodium content in the roots of the plants was in the Tempio site (0.347%), while the highest percentages were recorded in Sassari (0.503%). PAL1 showed the highest content of Na and Mg. The clone SAN3 showed in the roots the highest amounts of K and Ca. Like sodium also potassium, calcium and magnesium in the roots of the plants of Sassari have had the greatest percentages. Interactions between localities and clones were significant only for some elements such as nitrogen ($p < 0.05$) and calcium ($p < 0.01$).

For the content of some microelements in the roots in Table 51 variance analysis and averages data are reported. The highest content of Cu and Zn was in the site of Oristano, while the highest content of Fe was in the site of Sassari, and that of manganese in the site of the Tempio. In Villasor instead was recorded the lowest content of Cu, Fe and Mn. Differences among sites of all microelements were statistically significant. Instead, the differences among clones for the Cu content, were not statistically significant. The locality x clone interactions were significant only for Cu.

Tables 52-61 showed the data for every macro and microelements and the separation of the averages. In the Table 52 is reported the content of nitrogen in the roots that ranged from a minimum of 0.37% of the clone FAN3 in Oristano to a maximum of 1.18% in MAR1 in the site of Villasor. The highest percentages were in almost all sites those relating to the clone MAR1.

In the Tempio and Oristano sites the highest percentages of P were found in the clone GIO1, while in Sassari and Villasor sites the highest content of P was related to clone MAR1 (Table 53).

In the roots of Oristano plants the sodium was found to range between a minimum of 0.30% (PAL1) and a maximum of 0.69% (SAN3). In Tempio ranged from 0.33% (MAR1) and 0.50% (PAL1), in Sassari was between 0.26% (MAR1 and FAN3) and 0.85% (GIO1), and in Villasor between 0.13% (FAN3 and SAN3) and 0.72% (SAN3) (Table 54).

Regarding potassium content in Oristano site in all semesters were recorded lower content than Tempio, Villasor and Sassari. The content of K in the roots of plants growing in Oristano ranged from 0.27% (FAN3) to 1.95% (SAN3). Considering data for semesters we note that the highest content of K, in 4 semesters of 5, was recorded for GIO1 clone. In Tempio the K content varied from 0.57% (FAN3) and 2.15% (GIO1): values slightly higher than those of Oristano, with the exception of the maximum and minimum that were equivalents. In Sassari minimum values of K was in the clone MAR1 (1.09%), while the maximum value of 2.82% was recorded on the 3rd semester in SAN3 clone (Table 55).

The higher calcium content in all sites, was recorded in SAN that in the site of Oristano showed a maximum content of 2.43%, which is the highest percentage recorded in four sites (Table 56).

The content of Mg in Oristano ranged from a minimum of 0.19% (GIO1 and FAN3) to a maximum of 0.47 (PAL1), in Tempio was between 0.18% (FAN3) and 0.44% (GIO1), in Sassari between 0.24% (FAN3) and 0.47% (PAL1), and in Villasor ranged from 0.24% (FAN3) to 0.45% (MAR1) (Table 57).

Regarding the analysis of the microelements, in Tempio, Sassari and Oristano sites, the clone MAR1 showed the highest content in Zn, Fe and Mn. In Villasor was the clone SAN3 to record the highest concentrations of Zn (59.33 ppm) (Table 58-61).

The Fe content was very high in all sites and for all clones. This confirms the great capacity of the species *A. halimus* in absorption of any metals present in contaminated soils. The Fe content varied from a minimum of 210.33 ppm (FAN3 in Tempio) to a maximum of 2806.67 ppm (MAR1 in Villasor).

Table 50. Averages and analysis of variance of the content of main macroelements in the roots of the clones of *A. halimus.*

	N (%)	P (%)	Na (%)	K (%)	Ca (%)	Mg (%)
LOCALITY (A)						
Oristano	0.74	0.72	0.44	1.01	0.64	0.33
Tempio	0.75	0.64	0.35	1.46	0.37	0.29
Sassari	0.72	0.78	0.50	1.80	0.38	0.34
Villasor	0.64	0.77	0.40	1.75	0.26	0.33
F	10.44	16.06	75.92	88.84	88.31	21.16
p	0.008	0.002	0.001	0.001	0.001	0.001
CLONE (B)						
MAR1	0.81	0.73	0.41	1.47	0.39	0.34
GIO1	0.70	0.86	0.42	1.59	0.35	0.30
SAN3	0.70	0.73	0.45	1.72	0.59	0.33
PAL1	0.75	0.72	0.46	1.47	0.37	0.35
FAN3	0.61	0.60	0.37	1.26	0.35	0.29
F	25.16	27.64	8.29	14.1	27.29	8.34
p	0.001	0.0009	0.001	0.0009	0.001	0.0009
LOCALITY x CLONE (AxB)						
F	2.33	1.76	0.69	1.34	5.4	0.9
p	0.028	0.099	n.s.	0.244	0.001	n.s.
SEMESTER (C)						
1	0.70	0.91	0.51	1.41	0.19	0.33
2	0.69	0.73	0.45	1.46	0.45	0.33
3	0.71	0.68	0.50	1.72	0.41	0.38
4	0.70	0.64	0.36	1.52	0.27	0.27
5	0.78	0.68	0.30	1.40	0.73	0.30
F	7.91	42.43	53	8.63	134.8	27.66
p	0.0009	0.001	0.001	0.001	0.001	0.001
LOCALITY x SEMESTER (AxC)						
F	6.83	45.29	10.56	14.44	48.36	7.72
p	0.001	0.001	0.001	0.0008	0.0009	0.001
LOCALITY x CLONE x SEMESTER (AxBxC)						
F	3.26	1.32	2.1	1.78	4.69	1.53
p	0.001	0.101	0.001	0.004	0.0001	0.026

Table 51. Averages and analysis of variance of the content of main microelements (ppm) in the roots of the clones of the species *A. halimus*.

	Cu	Zn	Fe	Mn
LOCALITY (A)				
Oristano	8.84	39.93	1167.33	42.03
Tempio	7.57	37.01	1163.53	44.01
Sassari	6.52	33.65	1195.60	40.12
Villasor	4.947	37.36	941.87	31.21
F	43.12	5.98	6.95	10.67
p	0.001	0.031	0.022	0.008
CLONE (B)				
MAR1	7.03	48.60	1435.52	51.77
GIO1	6.40	34.30	846.12	25.70
SAN3	7.17	30.13	1041.85	38.12
PAL1	6.13	42.98	1297.92	47.08
FAN3	8.12	28.93	964.02	34.05
F	2.09	62.38	30.02	34.67
p	0.104	0.001	0.001	0.001
LOCALITY x CLONE (AxB)				
F	5.39	1.23	1.71	1.36
p	0.001	0.307	0.11	0.235
SEMESTER (C)				
1	12.47	43.18	668.92	35.95
2	6.33	33.00	1290.17	33.88
3	7.50	37.98	1611.50	51.37
4	4.517	36.43	1189.833	33.6
5	4.033	34.35	825	41.917
F	44.29	11.75	57.67	28.27
p	0.001	0.001	0.0009	0.001
LOCALITY x SEMESTER (AxC)				
F	11.75	9.28	21.26	14.6
p	0.001	0.0008	0.0009	0.001
LOCALITY x CLONE x SEMESTER (AxBxC)				
F	4.81	2.6	2.48	2.66
p	0.001	0.0008	0.001	0.0005

Table 52. Nitrogen content (%) in the roots of the 5 clones of *A. halimus*, located in four sites. Data with letters in common are not statistically different for $p < 0.01$.

ORISTANO					
	MAR1	GIO1	SAN3	PAL1	FAN3
1 semester	-	-	-	-	-
2 semester	0.81 b-m	0.66 d-s	0.66 d-s	0.78 b-o	0.52 o-t
3 semester	0.90 b-g	0.82 b-m	0.88 b-h	0.78 b-o	0.37 t
4 semester	0.85 b-j	0.76 b-p	0.80 b-o	0.92 bcd	0.7 b-r
5 semester	0.97 ab	0.95 abc	0.70 b-r	0.77 b-o	0.54 m-t
TEMPIO					
	MAR1	GIO1	SAN3	PAL1	FAN3
1 semester	0.82 b-m	0.57 j-t	0.69 d-s	0.87 b-i	0.56 k-t
2 semester	0.89 b-g	0.58 j-t	0.88 b-g	0.83 b-l	0.70 b-r
3 semester	-	-	-	-	-
4 semester	0.76 b-p	0.92 bcd	0.65 d-s	0.69 b-r	0.74 b-q
5 semester	0.91 b-e	0.66 bcd	0.81 b-n	0.74 b-q	0.79 b-o
SASSARI					
	MAR1	GIO1	SAN3	PAL1	FAN3
1 semester	-	- -	-	-	-
2 semester	0.90 b-f	0.76 b-p	0.72 b-r	0.66 c-s	0.55 l-t
3 semester	0.84 b-k	0.75 b-q	0.70 b-r	0.81 b-m	0.69 b-r
4 semester	0.79 b-o	0.62 f-t	0.55 l-t	0.78 b-o	0.76 b-p
5 semester	0.69 b-r	0.70 b-r	0.70 b-r	0.88 b-g	0.63 e-t
VILLASOR					
	MAR1	GIO1	SAN3	PAL1	FAN3
1 semester	0.76 b-p	0.59 j-t	0.78 b-o	0.70 b-r	0.65 d-t
2 semester	0.71 b-r	0.56 k-t	0.48 p-t	0.59 h-t	0.54 m-t
3 semester	0.63 e-t	0.44 rst	0.62 f-t	0.61 g-t	0.52 n-t
4 semester	0.39 st	0.66 c-s	0.59 i-t	0.58 j-t	0.46 q-t
5 semester	1.18 a	0.63 e-t	0.81 b-m	0.93 bcd	0.65 d-s

Table 53. Phosphorus content (%) in the roots of the five clones of *A. halimus*, located in four sites. Data with letters in common are not statistically different for $p < 0.01$.

ORISTANO					
	MAR1	GIO1	SAN3	PAL1	FAN3
1 semester	0.71 h-r	0.81 g-o	0.64 i-r	0.71 h-r	0.56 n-r
2 semester	0.75 h-q	1.01 c-h	0.80 g-o	0.80 g-o	0.61 j-r
3 semester	0.65 i-r	0.96 d-i	0.78 h-p	0.80 g-o	0.62 j-r
4 semester	0.56 n-r	0.71 h-r	0.65 i-r	0.56 n-r	0.46 pqr
5 semester	0.76 h-q	1.10 b-g	0.71 h-r	0.51 n-r	0.66 i-r
TEMPIO					
	MAR1	GIO1	SAN3	PAL1	FAN3
1 semester	0.79 h-p	0.70 h-r	0.72 h-r	0.52 n-r	0.54 n-r
2 semester	0.70 h-r	0.71 h-r	0.61 k-r	0.65 i-r	0.63 j-r
3 semester	0.60 l-r	0.95 e-j	0.83 g-n	0.74 h-q	0.56 n-r
4 semester	0.62 j-r	0.94 e-k	0.79 h-p	0.58 m-r	0.50 n-r
5 semester	0.71 h-r	0.93 e-l	0.71 h-r	0.75 h-q	0.51 n-r
SASSARI					
	MAR1	GIO1	SAN3	PAL1	FAN3
1 semester	1.45 a	1.33 ab	1.23 a-e	1.28 abc	1.14 b-f
2 semester	0.83 g-n	0.78 h-p	0.65 i-r	0.71 h-r	0.57 n-r
3 semester	0.57 n-r	0.73 h-r	0.58 m-r	0.74 h-q	0.58 m-r
4 semester	0.67 i-r	0.69 i-r	0.56 n-r	0.74 h-q	0.54 n-r
5 semester	0.68 i-r	0.70 h-r	0.61 l-r	0.64 i-r	0.49 o-r
VILLASOR					
	MAR1	GIO1	SAN3	PAL1	FAN3
1 semester	1.32 ab	1.47 a	1.25 a-d	1.22 a-e	0.97 d-i
2 semester	0.74 h-q	0.90 f-m	0.82 g-o	0.71 h-r	0.50 o-r
3 semester	0.58 m-r	0.59 m-r	0.75 h-q	0.53 n-r	0.51 n-r
4 semester	0.64 i-r	0.77 h-q	0.63 j-r	0.58 m-r	0.66 i-r
5 semester	0.64 i-r	0.74 h-q	0.65 i-r	0.67 i-r	0.45 q-r

Table 54. Sodium content (%) in the roots of the five clones of *A. halimus* located in four sites. Data with letters in common are not statistically different for $p < 0.01$.

ORISTANO					
	MAR1	GIO1	SAN3	PAL1	FAN3
1 semester	0.46 c-v	0.47 b-u	0.46 c-v	0.48 b-s	0.45 d-v
2 semester	0.58 b-m	0.41 f-w	0.48 b-s	0.47 b-t	0.36 j-x
3 semester	0.43 e-w	0.42 e-w	0.69 a-d	0.66 a-e	0.43 e-w
4 semester	0.38 i-x	0.40 f-w	0.42 e-w	0.42 e-w	0.36 k-x
5 semester	0.31 n-x	0.43 e-w	0.35 l-x	0.30 o-x	0.32 n-x
TEMPIO					
	MAR1	GIO1	SAN3	PAL1	FAN3
1 semester	0.33 m-x	0.28 q-x	0.31 o-x	0.29 p-x	0.24 s-x
2 semester	0.32 m-x	0.33 m-x	0.34 l-x	0.50 b-s	0.25 s-x
3 semester	0.34 l-x	0.46 c-u	0.49 b-s	0.36 j-x	0.47 b-t
4 semester	0.26 r-x	0.42 e-w	0.37 j-x	0.33 m-x	0.49 b-s
5 semester	0.30 o-x	0.31 o-x	0.30 o-x	0.39 i-w	0.18 wx
SASSARI					
	MAR1	GIO1	SAN3	PAL1	FAN3
1 semester	0.59 b-l	0.85 a	0.64 a-i	0.66 a-f	0.70 a-d
2 semester	0.64 a-h	0.47 c-u	0.63 a-i	0.57 b-n	0.42 e-w
3 semester	0.48 b-t	0.42 e-w	0.71 abc	0.61 b-k	0.54 b-p
4 semester	0.36 k-x	0.36 j-x	0.30 o-x	0.55 b-p	0.21 u-x
5 semester	0.43 e-w	0.40 g-w	0.35 k-x	0.46 c-v	0.26 s-x
VILLASOR					
	MAR1	GIO1	SAN3	PAL1	FAN3
1 semester	0.55 b-o	0.52 b-r	0.72 ab	0.65 a-g	0.48 b-s
2 semester	0.42 e-w	0.42 e-w	0.54 b-q	0.42 e-w	0.41 f-w
3 semester	0.61 a-j	0.34 l-x	0.59 b-l	0.49 b-s	0.35 l-x
4 semester	0.27 r-x	0.39 h-w	0.25 s-x	0.40 g-w	0.26 s-x
5 semester	0.20 vwx	0.22 t-x	0.13 x	0.20 vwx	0.13 x

Table 55. Potassium content (%) in the roots of the five clones of *A. halimus* located in four sites. Data with letters in common are not statistically different for $p < 0.01$.

ORISTANO					
	MAR1	GIO1	SAN3	PAL1	FAN3
1 semester	0.28 rs	0.31 qrs	0.29 rs	0.28 rs	0.27 s
2 semester	1.57 c-o	1.92 a-k	1.71 b-m	1.45 d-p	0.92 l-s
3 semester	1.06 j-s	1.47 c-p	1.95 a-k	1.42 d-p	1.04 k-s
4 semester	0.65 o-s	1.19 g-r	1.16 g-s	1.17 g-s	0.69 n-s
5 semester	0.88 m-s	1.42 d-p	0.89 m-s	0.58 p-s	0.58 p-s
TEMPIO					
	MAR1	GIO1	SAN3	PAL1	FAN3
1 semester	2.09 a-g	1.55 c-o	1.85 b-l	1.35 d-p	1.29 e-p
2 semester	1.77 b-m	1.26 f-p	1.42 d-p	1.29 e-p	1.25 f-p
3 semester	1.09 i-s	2.15 a-f	2.07 a-h	1.59 c-n	1.64 c-m
4 semester	1.46 d-p	1.88 b-k	1.54 c-o	1.30 e-p	1.75 b-m
5 semester	1.16 g-s	1.09 i-s	0.90 m-s	1.06 j-s	0.57 p-s
SASSARI					
	MAR1	GIO1	SAN3	PAL1	FAN3
1 semester	1.61 c-n	2.04 a-i	1.88 b-k	1.97 a-k	1.68 b-m
2 semester	1.62 c-n	1.20 f-q	1.28 e-p	1.21 f-q	1.13 h-s
3 semester	1.09 i-s	2.00 a-j	2.82 a	2.59 ab	1.56 c-o
4 semester	1.77 b-m	1.63 c-m	2.05 a-h	1.90 b-k	1.86 b-l
5 semester	2.40 abc	2.29 a-d	2.30 a-d	1.60 c-n	1.42 d-p
VILLASOR					
	MAR1	GIO1	SAN3	PAL1	FAN3
1 semester	2.00 a-k	1.72 b-m	1.94 a-k	2.03 a-i	1.67 b-m
2 semester	1.62 c-n	1.68 b-m	2.22 a-e	1.36 d-p	1.38 d-p
3 semester	1.98 a-k	1.88 b-k	2.57 ab	1.32 e-p	1.16 g-s
4 semester	1.55 c-o	1.44 d-p	1.68 b-m	1.94 a-k	1.74 b-m
5 semester	1.75 b-m	1.71 b-m	1.88 b-k	1.96 a-k	1.55 c-o

Table 56. Content of Ca (%) in the roots of the five clones of *A. halimus* located in four sites. Data with letters in common are not statistically different for $p < 0.01$.

ORISTANO					
	MAR1	GIO1	SAN3	PAL1	FAN3
1 semester	0.19 rst	0.17 rst	0.29 m-t	0.19 rst	0.20 q-t
2 semester	0.85 d-g	0.79 d-i	2.43 a	1.07 cde	0.84 d-h
3 semester	0.46 i-t	0.29 m-t	0.64 g-m	0.41 j-t	0.41 j-t
4 semester	0.44 j-t	0.28 m-t	0.42 j-t	0.43 j-t	0.37 k-t
5 semester	1.23 bc	0.59 g-p	1.27 bc	1.11 cd	0.64 g-n
TEMPIO					
	MAR1	GIO1	SAN3	PAL1	FAN3
1 semester	0.12 t	0.15 t	0.27 o-t	0.12 t	0.14 t
2 semester	0.18 rst	0.15 t	0.28 n-t	0.17 rst	0.18 rst
3 semester	0.44 j-t	0.36 k-t	0.25 o-t	0.23 p-t	0.26 o-t
4 semester	0.25 o-t	0.18 rst	0.31 m-t	0.22 p-t	0.20 q-t
5 semester	0.84 d-g	1.11 cd	1.44 b	0.74 e-j	0.70 f-k
SASSARI					
	MAR1	GIO1	SAN3	PAL1	FAN3
1 semester	0.20 q-t	0.13 t	0.23 p-t	0.12 t	0.16 rst
2 semester	0.14 t	0.17 rst	0.23 p-t	0.17 rst	0.19 rst
3 semester	0.48 i-t	0.42 j-t	0.61 g-o	0.38 k-t	0.40 j-t
4 semester	0.25 o-t	0.27 o-t	0.49 i-t	0.26 o-t	0.27 o-t
5 semester	0.71 f-k	0.80 d-i	1.03 c-f	0.57 g-q	0.70 f-l
VILLASOR					
	MAR1	GIO1	SAN3	PAL1	FAN3
1 semester	0.18 rst	0.17 rst	0.36 k-t	0.20 q-t	0.19 rst
2 semester	0.16 st	0.16 st	0.25 o-t	0.21 q-t	0.33 m-t
3 semester	0.34 m-t	0.34 m-t	0.54 g-r	0.53 g-s	0.49 h-t
4 semester	0.16 st	0.13 t	0.22 p-t	0.13 t	0.13 t
5 semester	0.24 p-t	0.24 p-t	0.31 m-t	0.19 rst	0.22 q-t

Table 57. Magnesium content (%) in the roots of the 5 clones of *A. halimus* located in four sites. Data with letters in common are not statistically different for $p < 0.01$.

ORISTANO					
	MAR1	GIO1	SAN3	PAL1	FAN3
1 semester	0.33 a-u	0.31 c-u	0.33 a-t	0.38 a-l	0.39 a-k
2 semester	0.35 a-r	0.31 c-u	0.34 a-r	0.35 a-r	0.32 c-u
3 semester	0.38 a-m	0.41 a-h	0.45 abc	0.47 ab	0.37 a-n
4 semester	0.22 o-u	0.19 stu	0.20 r-u	0.24 j-u	0.19 tu
5 semester	0.35 a-r	0.37 a-n	0.33 a-u	0.29 e-u	0.26 j-u
TEMPIO					
	MAR1	GIO1	SAN3	PAL1	FAN3
1 semester	0.29 e-u	0.21 q-u	0.23 n-u	0.25 j-u	0.20 r-u
2 semester	0.31 c-u	0.26 i-u	0.34 a-r	0.35 a-r	0.27 g-u
3 semester	0.29 e-u	0.44 a-e	0.37 a-n	0.39 a-j	0.42 a-g
4 semester	0.24 l-u	0.21 p-u	0.24 j-u	0.24 k-u	0.23 m-u
5 semester	0.32 c-u	0.25 j-u	0.30 d-u	0.32 b-u	0.18 u
SASSARI					
	MAR1	GIO1	SAN3	PAL1	FAN3
1 semester	0.42 a-g	0.32 c-u	0.33 a-t	0.36 a-p	0.31 c-u
2 semester	0.36 a-p	0.29 d-u	0.33 a-u	0.33 a-u	0.34 a-s
3 semester	0.32 c-u	0.29 d-u	0.38 a-l	0.44 a-d	0.36 a-q
4 semester	0.34 a-s	0.29 d-u	0.29 d-u	0.47 a	0.28 f-u
5 semester	0.37 a-o	0.36 a-o	0.34 a-r	0.34 a-s	0.24 j-u
VILLASOR					
	MAR1	GIO1	SAN3	PAL1	FAN3
1 semester	0.44 a-d	0.33 a-t	0.39 a-k	0.45 abc	0.33 a-t
2 semester	0.42 a-f	0.29 f-u	0.43 a-f	0.31 c-u	0.32 b-u
3 semester	0.45 abc	0.30 d-u	0.41 a-i	0.29 d-u	0.27 h-u
4 semester	0.31 c-u	0.29 f-u	0.27 g-u	0.34 a-s	0.26 i-u
5 semester	0.26 i-u	0.29 d-u	0.26 j-u	0.32 b-u	0.24 l-u

Table 58. Copper content (ppm) in the roots of the various clones of *A. halimus* located in four sites. Data with letters in common are not statistically different for $p < 0.01$.

ORISTANO					
	MAR1	GIO1	SAN3	PAL1	FAN3
1 semester	18.00 b	8.33 b-g	14.67 bc	5.33 c-g	57.33 a
2 semester	11.00 b-f	6.33 c-g	8.67 b-g	6.33 c-g	11.67 b-f
3 semester	7.33 c-g	9.66 b-g	11.67 b-f	7.33 c-g	6.33 c-g
4 semester	6.67 c-g	5.67 c-g	7.00 c-g	6.67 c-g	5.00 c-g
5 semester	0.00 g	0.00 g	0.00 g	0.00 g	0.00 g
TEMPIO					
	MAR1	GIO1	SAN3	PAL1	FAN3
1 semester	9.00 b-g	11.67 b-f	8.33 b-g	7.67 b-g	9.00 b-g
2 semester	6.67 c-g	5.00 c-g	5.33 c-g	7.00 c-g	4.67 c-g
3 semester	8.33 b-g	8.67 b-g	7.33 c-g	8.00 b-g	7.33 c-g
4 semester	5.67 c-g	5.67 c-g	7.33 c-g	5.00 c-g	5.33 c-g
5 semester	10.33 b-g	13.00 b-e	8.33 b-g	10.33 b-g	4.33 c-g
SASSARI					
	MAR1	GIO1	SAN3	PAL1	FAN3
1 semester	14.33 bc	6.33 c-g	13.33 b-e	14.00 bcd	9.00 b-g
2 semester	5.00 c-g	5.67 c-g	7.67 b-g	5.67 c-g	5.33 c-g
3 semester	5.67 c-g	6.33 c-g	5.67 c-g	5.00 c-g	5.67 c-g
4 semester	3.00 efg	2.00 fg	3.33 d-g	3.00 efg	2.67 efg
5 semester	7.33 c-g	5.67 c-g	7.00 c-g	8.00 b-g	6.33 c-g
VILLASOR					
	MAR1	GIO1	SAN3	PAL1	FAN3
1 semester	7.33 c-g	8.67 b-g	10.00 b-g	9.33 b-g	7.67 b-g
2 semester	5.00 c-g	5.00 c-g	6.67 c-g	3.00 efg	5.00 c-g
3 semester	8.33 b-g	8.67 b-g	9.33 b-g	6.67 c-g	6.67 c-g
4 semester	1.67 fg	5.67 c-g	1.67 fg	4.33 c-g	3.00 efg
5 semester	0.00 g	0.00 g	0.00 g	0.00 g	0.00 g

Table 59. Zinc content (ppm) in the roots of the five clones of *A. halimus* located in four sites. Data with letters in common are not statistically different for $p < 0.01$.

ORISTANO					
	MAR1	GIO1	SAN3	PAL1	FAN3
1 semester	43.67 c-u	31.00 i-x	27.67 m-x	36.67 d-x	67.00 ab
2 semester	45.33 b-s	26.67 n-x	21.33 s-x	39.67 c-x	21.00 s-x
3 semester	57.33 a-g	33.00 h-v	32.67 h-x	47.67 b-q	23.67 q-x
4 semester	60.00 a-d	47.00 b-r	37.67 d-x	53.00 b-k	31.00 i-x
5 semester	76.33 a	37.67 d-x	34.67 f-x	42.33 c-v	24.33 p-x
TEMPIO					
	MAR1	GIO1	SAN3	PAL1	FAN3
1 semester	43.67 b-u	33.33 g-x	25.67 o-x	40.00 c-x	23.67 q-x
2 semester	53.67 b-k	31.33 i-x	27.67 m-x	39.00 c-x	19.67 u-x
3 semester	28.67 l-x	30.33 j-x	42.00 c-v	54.00 b-j	49.33 b-o
4 semester	52.33 b-l	43.00 c-v	36.33 d-x	44.33 b-t	28.33 l-x
5 semester	56.33 a-h	35.33 e-x	28.33 l-x	43.33 c-u	15.67 x
SASSARI					
	MAR1	GIO1	SAN3	PAL1	FAN3
1 semester	53.67 b-k	38.67 c-x	29.67 k-x	50.67 b-n	33.67 g-x
2 semester	49.00 b-o	29.67 k-x	31.67 i-k	37.00 d-x	24.00 q-x
3 semester	48.33 b-p	30.67 j-x	25.33 o-x	40.33 c-w	21.67 s-x
4 semester	31.33 i-x	31.33 r-x	18.67 vwx	41.67 c-w	22.33 s-x
5 semester	37.33 d-x	32.67 h-x	31.67 i-x	38.00 d-x	20.67 t-x
VILLASOR					
	MAR1	GIO1	SAN3	PAL1	FAN3
1 semester	56.67 a-h	62.33 abc	59.33 a-e	51.67 b-m	55.00 a-i
2 semester	44.00 b-u	33.33 g-x	25.67 o-x	38.00 d-x	22.33 s-x
3 semester	58.33 a-f	35.67 e-x	26.67 n-x	48.33 b-p	25.67 o-x
4 semester	39.67 c-x	27.67 m-x	22.67 r-x	39.00 c-x	29.67 k-x
5 semester	36.33 d-x	23.67 q-x	17.33 wx	35.00 f-x	20.00 t-x

Table 60. Iron content (ppm) in the roots of the 5 clones of *A. halimus* located in four sites. Data with letters in common are not statistically different for $p < 0.01$.

ORISTANO										
	MAR1		GIO1		SAN3		PAL1		FAN3	
1 semester	486.67	r-z	223.33	yz	353.33	w-z	356.67	v-z	380.00	t-z
2 semester	2296.67	a-d	973.33	i-z	1023.33	h-z	2000.00	a-i	1513.33	c-r
3 semester	1526.67	c-q	1130.00	e-z	1280.00	e-x	1340.00	d-w	1186.67	e-z
4 semester	1813.33	b-m	1386.67	d-w	1803.33	b-m	1446.67	d-s	1280.00	e-x
5 semester	1510.00	c-r	960.00	j-z	976.67	i-z	1453.33	d-s	483.33	r-z
TEMPIO										
	MAR1		GIO1		SAN3		PAL1		FAN3	
1 semester	373.67	u-z	222.33	yz	267.00	xyz	361.67	u-z	210.33	z
2 semester	1176.67	e-z	526.67	q-z	463.33	s-z	1073.33	g-z	693.33	o-z
3 semester	1070.00	g-z	1600.00	c-o	1420.00	d-t	2736.67	ab	2103.33	a-f
4 semester	2320.00	a-d	1090.00	f-z	1606.67	c-o	2040.00	a-h	1906.67	a-j
5 semester	2070.00	a-g	813.33	m-z	946.67	j-z	1506.67	c-r	490.00	q-z
SASSARI										
	MAR1		GIO1		SAN3		PAL1		FAN3	
1 semester	1263.33	e-y	876.67	j-z	1203.33	e-z	1396.67	d-v	1223.33	e-z
2 semester	2150.00	a-e	1566.67	c-p	1600.00	c-o	1746.67	c-n	1836.67	b-m
3 semester	2466.67	abc	1016.67	h-z	1276.67	e-x	1873.33	a-k	1260.00	e-y
4 semester	880.00	j-z	480.00	r-z	1063.33	g-z	1366.67	d-w	663.33	o-z
5 semester	610.00	o-z	296.67	x-z	673.33	o-z	633.33	o-z	466.67	s-z
VILLASOR										
	MAR1		GIO1		SAN3		PAL1		FAN3	
1 semester	1400.00	d-u	426.67	s-z	760.00	n-z	830.00	l-z	763.33	n-z
2 semester	1520.00	c-r	713.33	o-z	966.67	j-z	1100.00	f-z	863.33	k-z
3 semester	2806.67	a	1850.00	a-l	1800.00	b-m	1780.00	b-n	706.67	o-z
4 semester	423.33	s-z	370.00	u-z	710.00	o-z	486.67	q-z	660.00	o-z
5 semester	546.67	p-z	400.00	t-z	643.33	o-z	430.00	s-z	590.00	o-z

Table 61. Manganese content (ppm) in the roots of the various clones of *A. halimus* located in four sites. Data with letters in common are not statistically different for $p < 0.01$.

ORISTANO					
	MAR1	GIO1	SAN3	PAL1	FAN3
1 semester	50.33 c-p	19.67 s-z	24.67 n-z	28.67 l-z	20.67 p-z
2 semester	45.67 d-w	20.00 r-z	29.00 l-z	41.00 g-z	28.33 l-z
3 semester	65.33 b-i	32.00 j-z	45.00 e-x	71.67 b-f	43.33 f-x
4 semester	42.00 g-z	48.00 d-u	50.00 c-q	59.67 c-k	58.67 c-k
5 semester	54.67 c-m	25.33 m-z	57.00 c-l	69.67 b-g	20.33 q-z
TEMPIO					
	MAR1	GIO1	SAN3	PAL1	FAN3
1 semester	41.33 g-z	16.00 w-z	32.67 j-z	36.00 j-z	26.00 m-z
2 semester	42.00 g-z	15.67 xyz	30.67 k-z	35.67 j-z	23.67 n-z
3 semester	58.67 c-k	52.00 c-o	41.67 g-z	73.67 b-e	74.00 bcd
4 semester	53.33 c-n	20.33 q-z	37.00 i-z	48.67 c-t	36.00 j-z
5 semester	87.00 ab	40.33 h-z	66.33 b-h	76.67 bc	35.00 j-z
SASSARI					
	MAR1	GIO1	SAN3	PAL1	FAN3
1 semester	67.00 b-h	24.33 n-z	46.67 d-v	49.00 c-t	49.67 c-r
2 semester	61.33 b-j	24.00 n-z	41.33 g-z	34.33 j-z	43.67 f-x
3 semester	110.00 a	23.00 o-z	36.00 j-z	49.33 c-s	34.33 j-z
4 semester	22.33 o-z	13.00 yz	21.33 p-z	34.33 j-z	17.00 v-z
5 semester	43.67 f-x	32.67 j-z	43.33 f-x	48.67 c-t	32.67 j-z
VILLASOR					
	MAR1	GIO1	SAN3	PAL1	FAN3
1 semester	50.00 c-q	18.67 u-z	38.33 h-z	47.67 d-u	31.67 k-z
2 semester	42.67 g-y	17.00 v-z	30.67 k-z	38.00 h-i	33.00 j-z
3 semester	49.67 c-r	42.67 g-y	40.00 h-z	54.67 c-m	30.33 k-z
4 semester	24.00 n-z	16.67 w-z	24.00 n-z	22.33 o-z	23.33 o-z
5 semester	24.33 n-z	12.67 z	26.67 m-z	22.00 p-z	16.00 w-z

4.5.3.1. Influence of temperature on the content of macro-and microelements in the roots.

Regarding the accumulation of the macroelements in the roots, it seems that, only in some cases that may be influenced by the ICC relative to the 3 critical temperatures (0, 5 and 10 °C) considered by us.

As we can see in table IX only the ICC at the temperature of 0 °C appears to be correlated significantly and negatively with the sodium content in the roots of the clone PAL1 ($p < 0.05$). Another correlation ($p < 0.01$) was found between the ICC of 0 °C and the Mg content in the roots of the clone SAN3. Considering the cold index accumulated the entire time of the test did not reveal any statistically significant correlation between temperature and accumulation of macroelements (Table X).

By analyzing the relationships between temperatures and accumulation of microelements in the roots, correlations were found positive and statistically significant between ICC for the three critical temperatures and accumulation of copper and manganese in the roots (Table XI). In particular, by using the semester data has emerged a positive correlation between the ICC concerning the temperatures of 0, 5 and 10 °C and the accumulation of copper in the roots of the clone GIO1. This correlation was also confirmed taking into account the ICC for the whole period of the test ($p < 0.01$) (Table XII). The content in manganese was positively correlated with the ICC concerning the temperatures below 5 and 10 °C.

4.6. Response of plants to pruning

Figure 21 shows the production of fresh biomass of the canopy, consisting of branches and leaves of the plants placed in the sites of Oristano and Tempio. At the time of pruning, done in the spring, it was then possible to determine the biomass produced up to that moment, and after six months, the regrowth of plants through biometric linear measure.

As the graph shows, the clone FAN 3, in the Tempio site, was the clone that showed the largest amount of fresh biomass at the pruning time, the canopy weighed on average 44 g.

Figure 22 shows the growth rate (RGR) of plants subjected to pruning. The increase in Oristano site was much greater than that of the Tempio site.

The plants of the clone clone SAN3 and GIO1, in Tempio, were the most negatively affected by the intervention of pruning. In Oristano all clones responded positively to cuts of biomass, especially the clone FAN3.

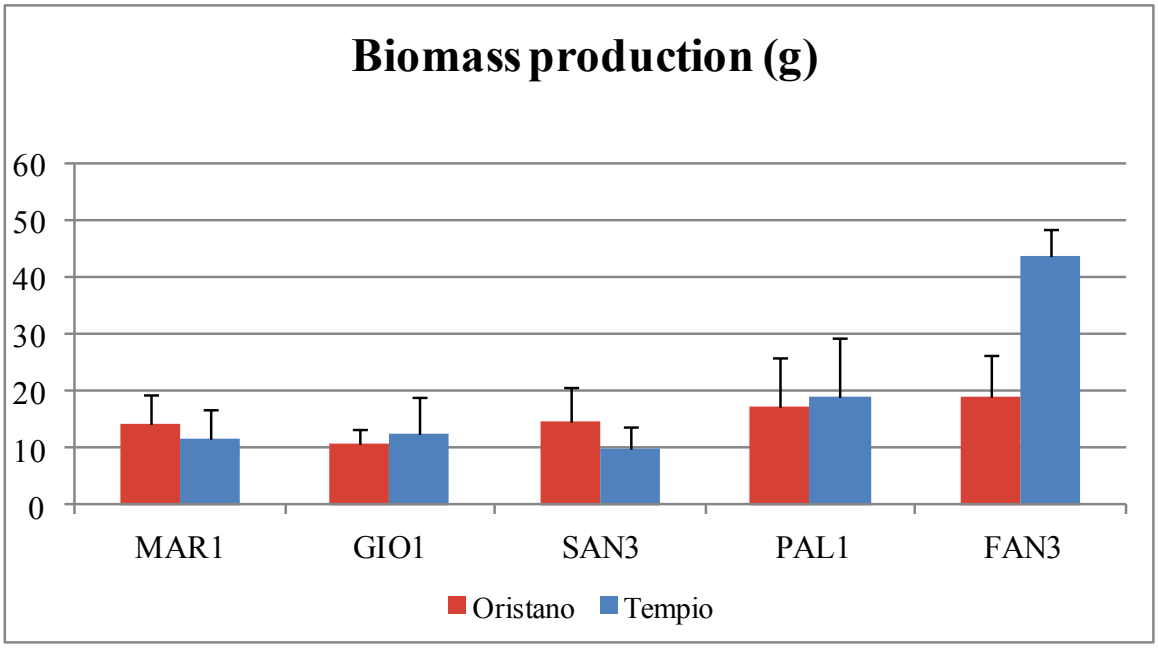


Figure 21. Biomass production per plant in the sites of Tempio and Oristano.

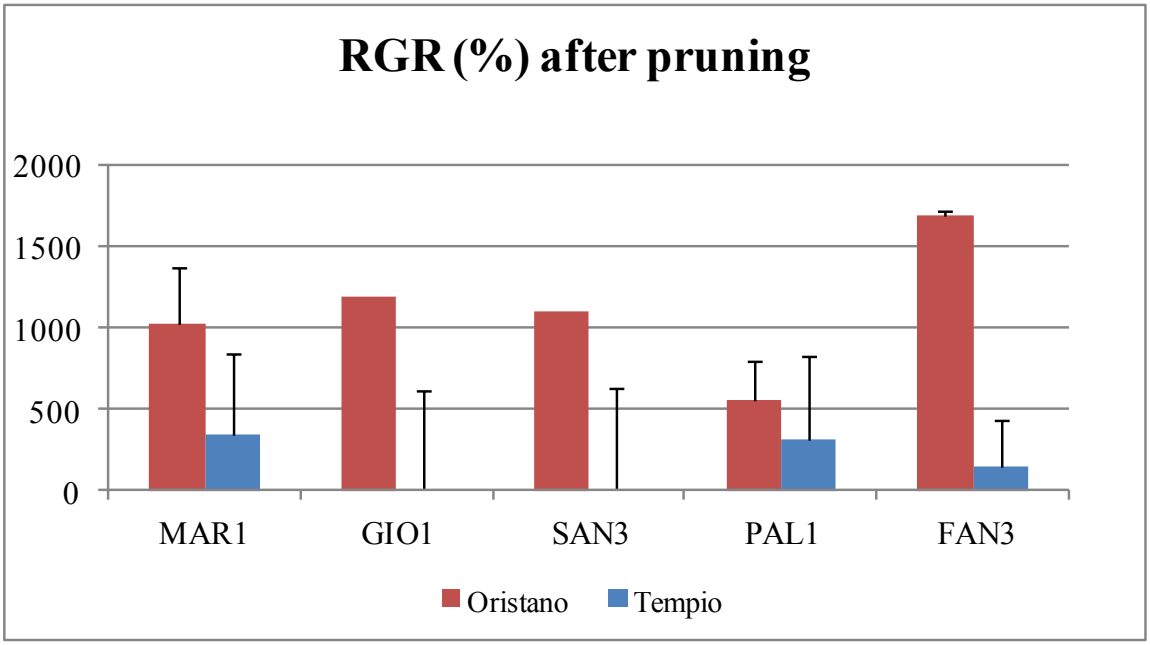


Figure 22. Growth rate 6 months after pruning.

5. DISCUSSION AND CONCLUSIONS

Climatic characterization of the experimental sites and micrometeorological trend during the three years of study

Thirty-year analysis of climate data of the four stations showed the typical characteristics of Mediterranean climate characterized by a long period of summer drought and mild winters, with occasional frosts. The combination of dry summers and rainy winters is a characteristic feature of the Mediterranean climate. In particular, the period from June to August appears to be the most arid, rainfall, in fact, are concentrated in autumn and winter. As we know *Atriplex halimus* is a perennial wild species of arid and semi-arid regions of the Mediterranean. The species is native of North Africa and is able to withstand drought and high temperatures typical of these areas (Belkheiri *et al.*, 2010).

However, in Sardinia as in other Mediterranean regions the species colonizes spontaneously only coastal areas, while in the internal regions, where winter temperatures are considerably lower, do not meet the potential for dissemination of the species. With our study we tried to evaluate the influence of different thermal regimes on growth of this species, in particular 5 clones selected from the spontaneous flora of Sardinia. Due to the high intraspecific polymorphism in this case it is essential to evaluate the response of every clone at different temperatures. To this end it was fundamental the analysis of the reference stations from the point of view thermo-pluviometric.

With reference to the stations of our research, as evidenced in the thermo graphs pluviometric of thirty years reported in Figures 5 and 6, the site characterized by highest temperatures is Villasor with an average of the maximum temperatures in July (31.56 °C). In the same month, the average of the maximum temperatures of Tempio site is lower (28 °C) than the other sites. The average minimum temperatures of thirty years is shown in the site of Tempio where the lowest value was of 5.03 °C in the month of December. A value of 8.54 °C was observed in the site of Villasor, in the month of December, as highest average of minimum temperatures. The Oristano and Sassari site have intermediate temperatures.

In the last three years the minimum temperatures, especially in February 2012, were slightly different from the seasonal average. In February 2012, the minimum temperatures have dropped under zero even in sites considered "hotter" as Oristano and Villasor. In this last site were recorded average minimum temperatures of 2.7 °C, and in Oristano, in the same month temperatures were found to be lower (1.16 °C) with respect to Tempio site (2.4 °C), which is located at higher altitude than other localities.

In order to have a clearer idea on temperature differences among sites, calculation of the Index of Cumulated Cold (ICC) was essential. From the analysis of the ICC it can be seen, in most cases, that the average minimum temperature is almost never below zero. The only site where the ICC (<0 °C) were positive result is Tempio. As we can see the ICC of the Tempio site are much higher than other sites. As regards the other two temperature thresholds (5 and 10 °C) is always Tempio site to present the highest ICC. The Villasor site instead resulted that for both critical temperatures has accumulated the lowest ICC.

Propagation of the species A. halimus

Regarding the propagation plant material, the tests performed have provided different results despite were carried both in spring, more favorable period to the rooting of the species *A. halimus*, being the plants less susceptible to attack by pathogens (Malan and Rethman, 2002). Moreover, the treatment with the hormone IBA showed a lower percentage of rooting with respect to the untreated control. These results are in agreement with those of Accardo Palumbo *et al.* (2004). In fact, even in this case the treatment with hormone both liquid and powder inhibited rooting. Instead Arya *et al.* (1993) claim that the use of the hormone in terminal cuttings of *Atriplex* ssp. stimulates rooting. The higher percentage of rooting was reached in the second test without hormone treatment, reaching a maximum value of 78% with the clone SAN3 and 66%, with the clone PAL1. The clone that showed the lowest percentage of rooting was GIO1 (19.74%). In any case, we can confirm that the *Atriplex halimus* resulted a species of easy propagation and therefore can be used without difficulty for the recovery of natural coastal or degraded areas.

Temperatures and growth rates.

In many studies the low temperatures were mentioned among the most powerful inhibitors of plants growth (Rapacz *et al*., 2001;. Ercoli *et al*., 2004; Xia *et al*., 2009). The extent of inhibition depends on the species, the stage of plant growth and nutritional status.

Species susceptible to cold damage are numerous and there are numerous studies performed to evaluate the response to this stress. Observing the data relating to the rate of plant growth of *A. halimus* in four different sites we can note that lower values of growth relate to plants located in Tempio, site characterized by the lowest average temperatures. So we can say that as regards the *A. halimus*, and for some clones in particular, the low temperatures limit the growth of plants. Even Cao *et al.* (2011), in oil palm exposed to cold temperatures recorded an increase in height lower than the control. The behavior of the different clones varies from site to site. Only the clone GIO1 has a slow growth or even a loss of biomass in all locations. Not taking into account the location factor and semester, the clone with the highest RGR was the clone MAR1, perhaps this clone having a more prostrate habit and presenting more ramifications of the other clones was able to fit better. In Tempio, clone that has adapted with more difficulty was the clone SAN3 that in almost all the semesters presented negative growth rates of biomass. The greatest growth for all clones was recorded in the second semester. Certainly the growth in the first semester was low as the plants have had to adapt to the new environment. Plants have also been transferred in the month of October, so they should immediately tackle the low winter temperatures. From the correlation between the growth rate of different clones and the interim ICC, taking into account only the average temperatures below 0, 5 and 10 °C, there were some significant results. The use of the ICC for the temperature range of 0 °C shows significant correlations only with the growth rate of the clone GIO1 ($p < 0.05$). The correlation was negative ($r = -0.50$). So we can say that in the clone GIO1, low temperatures lead to a reduction in growth and regression of vegetation. For the other clones the correlation is not statistically significant. The ICC for temperatures below the threshold of 5 and 10 °C show negative correlations only for the clone SAN3.

Temperature and biomass production

The results for the quantitative analysis of biomass produced per plant in many cases confirm the information provided from the overall development of the plant canopy (RGR). The analysis of biomass produced per plant by the clone GIO1 showed that this was the clone with the lowest production, and as we have seen previously was also the clone that showed the lowest rate of growth. Such behavior has been observed in all sites but especially in Villasor, which has been above mentioned as the site with highest average

maximum temperatures. In the winter of 2010 and 2012 the minimum temperatures fell below zero. This could be the reason because this site has registered the lowest dry weight of leaves, branches and roots. In fact, according to the hypothesis of Steffen *et al.* (1989) exposure to low temperatures inhibits photosynthesis while respiration proceeds. Even in different varieties of tomato exposure to low temperatures has reduced the production of dry matter (Hnilickova *et al*., 2002). The clones with the highest fresh leaf biomass production were clones PAL1 (Oristano and Villasor) and MAR1 (Tempio and Sassari). The analysis of correlations between leaf production and critical temperatures showed only a negative correlation, and statistically significant negative relationship were observed between dry matter of the leaves and the three SAN3 ICC for the three critical temperatures. Therefore, we can say that in this clone the three critical temperatures cause a reduction of leaf dry matter. The water leaf content is consequently higher and the plants are more susceptible to cold damage. This could indicate a lack of fit of the clone SAN3 at three different temperatures.

The water content reduction in the leaves is a common feature of cold adaptation (Strand *et al*., 1999; Kalberer *et al*., 2006; Pràsil *et al*., 2007). Even Walker *et al.* (2008), comparing the species *A. halimus* in two sites of Spain, characterized by different minimum temperatures, found a positive correlation between the cold resistance and the percentage of dry matter in the leaves. This correlation was most evident at the site characterized by lower minimum temperatures.

Observing the indices of correlation between the temperature and the amount of dry matter radical produced, we note that there is a trend toward a negative correlation. However, this correlation was not significant. Even in this case, as for the leaves, upon exposure to low temperatures the plant reduces the production of root dry matter. The clone PAL1 was among the clones with the highest fresh weight of roots, this behavior has been observed in all sites except in Sassari. In any case no correlation was found between temperature and the production of root biomass. The clone FAN3 showed the highest fresh and dry weight of the branches, this result was observed in all sites except in Oristano, where the clone MAR1 showed the highest fresh and branch dry weight. The results of the analysis of the relationship between temperature and production of branch biomass, using the data grouped for semester, only showed a significant positive correlation between fresh and dry weight of the branches of clone PAL1 and ICC for the critical temperature of 0 °C. Considering the data for the whole period of the test, positive correlations emerged

between all three ICC indexes and fresh and dry weight of the branches of clone FAN3.

The interaction between locality and clone was not significant either as regards the leaf and woody biomass but not for root biomass.

Temperature and accumulation of macro and microelements

The entity of cold damage varies with the plant species, the stage of development of the crop and with the conditions of irradiance. Moreover, it also varies with the nutritional status of mineral plant at the time of stress. The results of the chemical analyzes have given further information on the nutritional status of the various clones. The plants grown with limited supply of mineral nutrients are more susceptible to low temperatures (Starck *et al*., 2000). The knowledge of macro and microelement content in the biomass could be used to assess the ability of the species for the use as livestock feed.

Nitrogen is the element that has the greatest importance for the growth and development of plants. In a research concerning the spinach, was demonstrated that the nitrogen deficiency significantly reduced the rate of acclimation to low temperatures (Martindale and Leegood, 1997). As regards the *Atriplex halimus*, it was found that the highest nitrogen content in the leaves was related to the clone PAL1 (1.68%). This clone was that, together with the clone MAR1, showing the greatest amount of leaves per plant. Moreover, the majority of the nitrogen content in the leaves was related to the site of Sassari where, in fact, has been registered the highest growth rate. However, the differences between sites with regard to the accumulation of N were not significant. Regarding the P, at low temperatures the absorption of this decreases more than the absorption of other elements (Bravo and Uribe, 1981). The results of the relationship between the three ICC for the three critical temperatures and the phosphorus content in leaves and roots showed a trend towards a negative correlation, however, this relationship is not statistically significant. The content of P in the leaves, although slightly lower, is close to that found by van Niekerk *et al.* (2004) (1.4 to 1.92%) in the *A. halimus*. The content of P in the work reported by El-Shatnawi and Turuk (2002) is significantly lower, in fact, the maximum value reported is 0.3%, while Haddi *et al.* (2009) reported P values equal to 2.8%. The highest content of P in the leaves was related to Oristano site, while the lowest content was recorded in the Villasor site. The analysis of the content of P in the branches shows that the sites of Oristano Sassari and Tempio have smaller percentages than FAN3, while the highest content was recorded in MAR1. Regarding the content of P

in the roots the highest content was found in the clone GIO1. About the resistance to low temperatures, it has recently been demonstrated that the tolerance of the populations of *A. halimus* to freezing seems to be related to the concentrations of Na and K in the leaves (Walker *et al*., 2008). Low temperatures can cause the plant reacts accumulating Na and Ca ions or other substances that may increase the osmotic concentration and limit its dehydration.

High salinity of the soil seems improve the freezing tolerance of halophytic species such as *A. halimus* and the accumulation of Na in the vacuole could be the mechanism that contributed to the freezing tolerance of *Atriplex* plants on saline soils. For this reason, the chemical analysis of the tissues of leaves, branches and roots was essential.

The sodium content in leaves varies from a minimum of 0.22% of the MAR1 clone (Tempio) to 4.45% of PAL1 clone (Tempio). Percentages are very similar (3.5 to 4.4%) were reported by El-Shatnawi and Turuk (2002). In their studies, they found levels of Na different depending on the season, and indicate Na values of 3.5% in October, 3.3% in December and 2.9% in February. Alvarez *et al*. (2003) have found sodium content significantly higher (18.05%), as well as Abbad *et al.* (2004b), depending on the locality found percentages ranging between 9.84% and 18.41%. The clone PAL1 in all 4 sites showed the highest content of sodium. Differences among clones with regard to the sodium content in the leaves were not significant.

The clone PAL1 provided the greatest amount of fresh biomass. The sodium levels found in leaves of five clones were not exceeding the threshold of toxicity, for this reason could be used in animal feed. The lowest concentration of K in the leaves was found in Sassari site (2.77%), while higher concentrations were recorded in Oristano (4.09%). The clone with highest content of K is the result clone MAR1 (4.51%), the one with the lowest content is the clone SAN3 (2.66%). The potassium in plants exposed to low temperatures has a fundamental role in reducing cold damage. The low content of K in the clone SAN3 could be due to a lack of adaptation of it. The accumulation of Ca in plant cells may be favoured by a possible stress to which it is subjected the plant. In our case the low-temperature may influence the accumulation of this element. In fact, from the analysis, the highest content in Ca was found in clone SAN3 (2.73%) that at the same time is a clone that has been most affected by the temperature, which has determined a regression in the growth. Van Niekerk *et al.* (2004) reported values of Ca significantly higher (11.5 - 21.5%). Even the Mg content in the leaves was lower than that found by van Niekerk *et al.*

(2004) and by Abbad *et al.* (2004b). The zinc content in the leaves was very variable, in fact, ranged from a minimum of 44 ppm (FAN3 in Sassari) to a maximum of 212.33 ppm (SAN3 in Tempio). This variability within the species *A. halimus* has been already reported in the work of Van Niekerk *et al*. (2004). From the analysis of the roots we have observed the high capacity of the species *A. halimus* in the accumulation of some heavy metals such as Zn, Fe, Cu and Mn. In particular, the levels of Fe in the roots were very high ranging from 210.33 ppm for FAN3 in Tempio at 2806 ppm to MAR1 in Villasor. The accumulation of copper in the roots can cause an unbalance in the absorption and translocation of nutrients towards the upper part of the plants. The copper, as mentioned, can inhibit the absorption of K (important for the turgor maintenance and for the leaves expansion) causing a reduction in the cellular extension (Alaoui-Sossè *et al.,* 2004). Also this work showed the high ability of the species *A. halimus* accumulation of some heavy metals, such as copper and iron. A very important factor when intervention of environment restoration are located in areas contaminated or affected by mining activities in the past, as already observed in previous experiments (Mulas *et al*., 2010c).

In conclusion, the present study may be useful to give an indication on the clones to be used, if the species *A. halimus* will be used in rehabilitation projects in areas where low temperatures are a problem. Among the five clones are emerged differences as regards the influence of low temperatures on growth, on the production of biomass and accumulation of macro and microelements. Some clones were more sensitive to cold than others, and this confirms the high intraspecific variability of the *A. halimus*. In fact, between five clones tested, the GIO1 and SAN3 clones results more sensitive to low temperature. In particular, the plant growth of the clone Gio1 was found to be adversely affected by temperatures below zero, while the growth clone SAN3 was adversely affected by temperatures below 5 and 10 °C. The clones MAR1, PAL1 and FAN3 results less sensitive to low temperatures and also in the site characterized by the lowest minimum temperatures have shown greater adaptability, and therefore a positive growth on average. The resistance and tolerance to low temperatures, as we have seen, are influenced by high number of factors and from the synergy of these. The analyzes most important to signal the adaptability of various clones of *Atriplex halimus* at low temperatures were those relating to the content of some macro-elements, such as the content of sodium, potassium and calcium. Other analyzes, which could be of potential indicators of adaptability, such as the content of nitrogen, were not significant.

This work constitutes the starting point for a future wider study that can take account of other aspects of the cold resistance of the clones of *A. halimus* selected in Sardinia.

The study of adaptability to the cold of the species *A. halimus* can be deepened through more targeted analysis. As we have seen, exposure to cold can affect the metabolism of plants and enzymatic activities, therefore the analysis of metabolites that accumulate after exposure to low temperatures may provide more immediate results.

The analysis will include:

- the sugar content, in fact they increase with cold acclimation;
- the lipid composition, which then tends to acclimation to a higher degree of unsaturation;
- the soluble protein content that increase as a result of stress, in particular of glycinebetaine that increase as a result of stress from low temperatures;
- the abscisic acid content (ABA), which further develops in plants acclimated;
- the content of reactive oxygen species (ROS) and the enzyme activity involved in the antioxidants production, since the stress at low temperatures favours the production and accumulation of ROS and consequently, in plants resistant to cold, it has an increased activity of antioxidant enzymes that act on ROS.

In terms of application this research has permitted to identify the genotypes more suitable for introduction into a field trial that may better identify the capacities of the species in ecosystems characterized by lower temperatures compared to coastal areas.

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7. APPENDIX

Table I. Correlations (r) between semester ICC and the contents of the main macroelements in the leaves of clones of *A. halimus* (ns = not significant, * = significant for $p < 0.05$, ** = significant for $p < 0.01$).

		ICC (0 °C)	ICC (5 °C)	ICC (10 °C)
N (%)	MAR1	0.132 n.s.	0.099 n.s.	0.084 n.s.
	GIO1	0.468 n.s.	0.150 n.s.	0.151 n.s.
	SAN3	0.488 *	0.277 n.s.	0.310 n.s.
	PAL1	0.076 n.s.	0.189 n.s.	0.211 n.s.
	FAN3	0.251 n.s.	0.366 n.s.	0.397 n.s.
P (%)	MAR1	0.017 n.s.	-0.133 n.s.	-0.133 n.s.
	GIO1	-0.179 n.s.	-0.244 n.s.	-0.161 n.s.
	SAN3	0.104 n.s.	-0.010 n.s.	-0.051 n.s.
	PAL1	-0.177 n.s.	-0.329 n.s.	-0.254 n.s.
	FAN3	-0.357 n.s.	-0.340 n.s.	-0.360 n.s.
Na (%)	MAR1	-0.118 n.s.	-0.100 n.s.	-0.184 n.s.
	GIO1	-0.414 n.s.	-0.437 n.s.	-0.514 n.s.
	SAN3	-0.254 n.s.	-0.236 n.s.	-0.275 n.s.
	PAL1	-0.075 n.s.	-0.011 n.s.	-0.110 n.s.
	FAN3	-0.051 n.s.	-0.022 n.s.	-0.081 n.s.
K (%)	MAR1	0.251 n.s.	0.251 n.s.	0.272 n.s.
	GIO1	0.047 n.s.	0.032 n.s.	0.039 n.s.
	SAN3	0.319 n.s.	0.271 n.s.	0.262 n.s.
	PAL1	0.297 n.s.	0.282 n.s.	0.231 n.s.
	FAN3	0.294 n.s.	0.208 n.s.	0.154 n.s.
Ca (%)	MAR1	0.197 n.s.	0.319 n.s.	0.343 n.s.
	GIO1	0.148 n.s.	0.304 n.s.	0.359 n.s.
	SAN3	0.183 n.s.	0.356 n.s.	0.368 n.s.
	PAL1	0.090 n.s.	0.260 n.s.	0.264 n.s.
	FAN3	0.247 n.s.	0.355 n.s.	0.351 n.s.
Mg (%)	MAR1	-0.198 n.s.	-0.097 n.s.	-0.096 n.s.
	GIO1	-0.031 n.s.	-0.027 n.s.	0.166 n.s.
	SAN3	0.224 n.s.	0.152 n.s.	0.249 n.s.
	PAL1	0.404 n.s.	0.430 n.s.	0.589 **
	FAN3	-0.014 n.s.	-0.087 n.s.	0.086 n.s.

Table II. Correlations (r) between ICC of all test period (5 semesters) and the content of the main macroelements in the leaves of clones of *A. halimus* (ns = not significant, * = significant for $p < 0.05$, ** = significant for $p < 0.01$).

		ICC (0 °C)	ICC (5 °C)	ICC (10 °C)
N (%)	MAR1	-0.547 n.s.	-0.565 n.s.	-0.543 n.s.
	GIO1	-0.015 n.s.	-0.017 n.s.	-0.034 n.s.
	SAN3	0.915 n.s.	0.910 n.s.	0.926 n.s.
	PAL1	-0.199 n.s.	-0.200 n.s.	-0.177 n.s.
	FAN3	0.001 n.s.	0.001 n.s.	0.021 n.s.
P (%)	MAR1	0.100 n.s.	0.086 n.s.	0.093 n.s.
	GIO1	-0.233 n.s.	-0.240 n.s.	-0.248 n.s.
	SAN3	0.434 n.s.	0.424 n.s.	0.423 n.s.
	PAL1	-0.276 n.s.	-0.286 n.s.	-0.287 n.s.
	FAN3	-0.662 n.s.	-0.666 n.s.	-0.674 n.s.
Na (%)	MAR1	-0.710 n.s.	-0.726 n.s.	-0.697 n.s.
	GIO1	-0.775 n.s.	-0.777 n.s.	-0.760 n.s.
	SAN3	-0.764 n.s.	-0.775 n.s.	-0.747 n.s.
	PAL1	-0.794 n.s.	-0.796 n.s.	-0.779 n.s.
	FAN3	-0.604 n.s.	-0.618 n.s.	-0.583 n.s.
K (%)	MAR1	0.499 n.s.	0.507 n.s.	0.510 n.s.
	GIO1	0.280 n.s.	0.290 n.s.	0.292 n.s.
	SAN3	0.615 n.s.	0.625 n.s.	0.622 n.s.
	PAL1	0.422 n.s.	0.422 n.s.	0.440 n.s.
	FAN3	0.563 n.s.	0.569 n.s.	0.575 n.s.
Ca (%)	MAR1	0.212 n.s.	0.188 n.s.	0.227 n.s.
	GIO1	-0.029 n.s.	-0.052 n.s.	-0.008 n.s.
	SAN3	0.162 n.s.	0.138 n.s.	0.178 n.s.
	PAL1	-0.192 n.s.	-0.214 n.s.	-0.186 n.s.
	FAN3	0.286 n.s.	0.268 n.s.	0.284 n.s.
Mg (%)	MAR1	-0.711 n.s.	-0.723 n.s.	-0.692 n.s.
	GIO1	-0.403 n.s.	-0.419 n.s.	-0.379 n.s.
	SAN3	0.317 n.s.	0.295 n.s.	0.326 n.s.
	PAL1	0.559 n.s.	0.540 n.s.	0.577 n.s.
	FAN3	-0.068 n.s.	-0.090 n.s.	-0.062 n.s.

Table III. Correlations (r) between semester ICC and the contents of the main microelements in the leaves of clones of *A. halimus* (ns = not significant, * = significant for $p < 0.05$, ** = significant for $p < 0.01$).

		ICC (0 °C)	ICC (5 °C)	ICC (10 °C)
Cu (ppm)	MAR1	0.233 n.s.	0.021 n.s.	-0.042 n.s.
	GIO1	0.184 n.s.	-0.034 n.s.	0.005 n.s.
	SAN3	0.231 n.s.	0.017 n.s.	0.073 n.s.
	PAL1	0.306 n.s.	0.067 n.s.	0.006 n.s.
	FAN3	0.422 n.s.	0.210 n.s.	0.115 n.s.
Zn (ppm)	MAR1	0.057 n.s.	-0.112 n.s.	-0.318 n.s.
	GIO1	0.154 n.s.	0.009 n.s.	0.059 n.s.
	SAN3	0.542 n.s.	0.392 n.s.	0.346 n.s.
	PAL1	-0.053 n.s.	-0.151 n.s.	-0.072 n.s.
	FAN3	0.077 n.s.	-0.020 n.s.	-0.066 n.s.
Fe (ppm)	MAR1	-0.269 n.s.	-0.153 n.s.	-0.121 n.s.
	GIO1	-0.160 n.s.	-0.068 n.s.	-0.092 n.s.
	SAN3	-0.236 n.s.	-0.175 n.s.	-0.202 n.s.
	PAL1	-0.280 n.s.	-0.150 n.s.	-0.079 n.s.
	FAN3	-0.151 n.s.	-0.087 n.s.	-0.080 n.s.
Mn(ppm)	MAR1	0.299 n.s.	0.024 n.s.	0.100 n.s.
	GIO1	0.109 n.s.	-0.085 n.s.	0.016 n.s.
	SAN3	0.105 n.s.	-0.060 n.s.	0.002 n.s.
	PAL1	0.284 n.s.	0.023 n.s.	0.058 n.s.
	FAN3	0.495 n.s.	0.110 n.s.	0.096 n.s.

Table IV. Correlations (r) between ICC of all test period (5 semesters) and the content of the main microelements in the leaves of clones of *A. halimus* (ns = not significant, * = significant for $p < 0.05$, ** = significant for $p < 0.01$).

		ICC (0 °C)	ICC (5 °C)	ICC (10 °C)
Cu (ppm)	MAR1	0.564 n.s.	0.545 n.s.	0.581 n.s.
	GIO1	0.619 n.s.	0.600 n.s.	0.628 n.s.
	SAN3	0.852 n.s.	0.841 n.s.	0.864 n.s.
	PAL1	0.648 n.s.	0.632 n.s.	0.666 n.s.
	FAN3	0.799 n.s.	0.785 n.s.	0.810 n.s.
Zn (ppm)	MAR1	0.098 n.s.	0.093 n.s.	0.079 n.s.
	GIO1	0.506 n.s.	0.521 n.s.	0.483 n.s.
	SAN3	0.903 n.s.	0.909 n.s.	0.891 n.s.
	PAL1	-0.095 n.s.	-0.099 n.s.	-0.112 n.s.
	FAN3	0.253 n.s.	0.271 n.s.	0.228 n.s.
Fe (ppm)	MAR1	-0.443 n.s.	-0.422 n.s.	-0.461 n.s.
	GIO1	-0.525 n.s.	-0.522 n.s.	-0.545 n.s.
	SAN3	-0.677 n.s.	-0.659 n.s.	-0.692 n.s.
	PAL1	-0.506 n.s.	-0.496 n.s.	-0.495 n.s.
	FAN3	0.206 n.s.	0.230 n.s.	0.197 n.s.
Mn (ppm)	MAR1	0.616 n.s.	0.597 n.s.	0.624 n.s.
	GIO1	0.106 n.s.	0.082 n.s.	0.122 n.s.
	SAN3	0.085 n.s.	0.067 n.s.	0.083 n.s.
	PAL1	-0.018 n.s.	-0.034 n.s.	-0.025 n.s.
	FAN3	0.834 n.s.	0.827 n.s.	0.828 n.s.

Table V. Correlations (r) between semester ICC and the contents of the main macroelements in the branches of clones of *A. halimus* (ns = not significant, * = significant for $p < 0.05$, ** = significant for $p < 0.01$).

		ICC (0 °C)	ICC (5 °C)	ICC (10 °C)
N (%)	MAR1	-0.072 n.s.	0.185 n.s.	0.280 n.s.
	GIO1	-0.028 n.s.	-0.017 n.s.	-0.200 n.s.
	SAN3	-0.175 n.s.	-0.179 n.s.	-0.183 n.s.
	PAL1	-0.200 n.s.	-0.081 n.s.	0.063 n.s.
	FAN3	0.333 n.s.	0.406 n.s.	0.517 *
P (%)	MAR1	-0.031 n.s.	-0.046 n.s.	0.014 n.s.
	GIO1	-0.158 n.s.	0.121 n.s.	0.059 n.s.
	SAN3	-0.264 n.s.	-0.140 n.s.	-0.134 n.s.
	PAL1	-0.362 n.s.	-0.172 n.s.	-0.142 n.s.
	FAN3	-0.095 n.s.	-0.088 n.s.	-0.121 n.s.
Na (%)	MAR1	-0.014 n.s.	0.108 n.s.	0.118 n.s.
	GIO1	-0.422 n.s.	-0.323 n.s.	-0.209 n.s.
	SAN3	-0.304 n.s.	-0.256 n.s.	-0.146 n.s.
	PAL1	-0.256 n.s.	-0.270 n.s.	-0.190 n.s.
	FAN3	-0.286 n.s.	-0.172 n.s.	-0.085 n.s.
K (%)	MAR1	0.065 n.s.	-0.074 n.s.	-0.040 n.s.
	GIO1	-0.176 n.s.	-0.031 n.s.	-0.010 n.s.
	SAN3	-0.099 n.s.	-0.103 n.s.	-0.080 n.s.
	PAL1	-0.047 n.s.	0.017 n.s.	0.011 n.s.
	FAN3	0.066 n.s.	0.056 n.s.	0.054 n.s.
Ca (%)	MAR1	0.285 n.s.	0.244 n.s.	0.231 n.s.
	GIO1	0.121 n.s.	0.195 n.s.	0.228 n.s.
	SAN3	0.213 n.s.	0.231 n.s.	0.274 n.s.
	PAL1	-0.022 n.s.	0.141 n.s.	0.178 n.s.
	FAN3	0.120 n.s.	0.182 n.s.	0.180 n.s.
Mg (%)	MAR1	0.179 n.s.	0.052 n.s.	0.136 n.s.
	GIO1	-0.144 n.s.	0.075 n.s.	0.231 n.s.
	SAN3	-0.174 n.s.	0.097 n.s.	0.157 n.s.
	PAL1	-0.309 n.s.	0.037 n.s.	0.142 n.s.
	FAN3	-0.082 n.s.	-0.246 n.s.	-0.229 n.s.

Table VI. Correlations (r) between ICC of all test period (5 semesters) for and the content of the main macroelements in the branches of clones of *A. halimus* (ns = not significant, * = significant for $p < 0.05$, ** = significant for $p < 0.01$).

		ICC (0 °C)	ICC (5 °C)	ICC (10 °C)
N (%)	MAR1	-0.951 *	-0.950 *	-0.944 n.s.
	GIO1	0.486 n.s.	0.488 n.s.	0.466 n.s.
	SAN3	-0.564 n.s.	-0.544 n.s.	-0.579 n.s.
	PAL1	-0.534 n.s.	-0.547 n.s.	-0.540 n.s.
	FAN3	0.827 n.s.	0.825 n.s.	0.840 n.s.
P (%)	MAR1	0.001 n.s.	-0.001 n.s.	0.022 n.s.
	GIO1	0.683 n.s.	0.687 n.s.	0.695 n.s.
	SAN3	-0.270 n.s.	-0.256 n.s.	-0.264 n.s.
	PAL1	-0.700 n.s.	-0.716 n.s.	-0.685 n.s.
	FAN3	0.375 n.s.	0.379 n.s.	0.391 n.s.
Na (%)	MAR1	0.302 n.s.	0.282 n.s.	0.325 n.s.
	GIO1	-0.602 n.s.	-0.613 n.s.	-0.581 n.s.
	SAN3	-0.778 n.s.	-0.769 n.s.	-0.772 n.s.
	PAL1	-0.282 n.s.	-0.297 n.s.	-0.257 n.s.
	FAN3	-0.471 n.s.	-0.487 n.s.	-0.448 n.s.
K (%)	MAR1	0.018 n.s.	0.029 n.s.	0.029 n.s.
	GIO1	-0.093 n.s.	-0.083 n.s.	-0.080 n.s.
	SAN3	0.098 n.s.	0.112 n.s.	0.107 n.s.
	PAL1	0.087 n.s.	0.096 n.s.	0.100 n.s.
	FAN3	0.215 n.s.	0.222 n.s.	0.229 n.s.
Ca (%)	MAR1	0.116 n.s.	0.099 n.s.	0.110 n.s.
	GIO1	-0.128 n.s.	-0.146 n.s.	-0.130 n.s.
	SAN3	0.026 n.s.	0.003 n.s.	0.035 n.s.
	PAL1	-0.214 n.s.	-0.236 n.s.	-0.207 n.s.
	FAN3	-0.124 n.s.	-0.143 n.s.	-0.123 n.s.
Mg (%)	MAR1	0.067 n.s.	0.048 n.s.	0.066 n.s.
	GIO1	-0.225 n.s.	-0.242 n.s.	-0.230 n.s.
	SAN3	-0.258 n.s.	-0.241 n.s.	-0.283 n.s.
	PAL1	0.001 n.s.	-0.023 n.s.	0.020 n.s.
	FAN3	0.098 n.s.	0.080 n.s.	0.094 n.s.

Table VII. Correlations (r) between semester ICC and the content of microelements in the main branches of the clones of *A. halimus* (ns = not significant, * = significant for $p < 0.05$, ** = significant for $p < 0.01$).

		ICC (0 °C)	ICC (5 °C)	ICC (10 °C)
Cu (ppm)	MAR1	0.011 n.s.	-0.264 n.s.	-0.143 n.s.
	GIO1	-0.094 n.s.	-0.407 n.s.	-0.313 n.s.
	SAN3	-0.121 n.s.	-0.335 n.s.	-0.246 n.s.
	PAL1	-0.028 n.s.	-0.270 n.s.	-0.144 n.s.
	FAN3	0.028 n.s.	-0.074 n.s.	0.041 n.s.
Zn (ppm)	MAR1	0.248 n.s.	-0.233 n.s.	-0.323 n.s.
	GIO1	-0.129 n.s.	-0.189 n.s.	-0. 160 n.s.
	SAN3	-0.153 n.s.	-0.052 n.s.	-0.118 n.s.
	PAL1	-0.303 n.s.	0.292 n.s.	0.268 n.s.
	FAN3	-0.145 n.s.	0.023 n.s.	0.004 n.s.
Fe (ppm)	MAR1	-0.197 n.s.	-0.309 n.s.	-0.354 n.s.
	GIO1	-0.280 n.s.	-0.243 n.s.	-0.252 n.s.
	SAN3	-0.399 n.s.	-0.146 n.s.	-0.180 n.s.
	PAL1	-0.341 n.s.	-0.031 n.s.	-0.072 n.s.
	FAN3	-0.369 n.s.	-0.237 n.s.	-0.283 n.s.
Mn(ppm)	MAR1	0.590 **	0.435 n.s.	0.461 *
	GIO1	0.118 n.s.	0.448 *	0.480 *
	SAN3	0.368 n.s.	0.536 n.s.	0.508 n.s.
	PAL1	0.092 n.s.	0.340 n.s.	0.310 n.s.
	FAN3	0.506 *	0.591 **	0.550 **

Table VIII. Correlations (r) between ICC of all test period (5 semesters) and the content of the main microelements in branches of the clones of *A. halimus* (ns = not significant, * = significant for $p < 0.05$, ** = significant for $p < 0.01$).

		ICC (0 °C)	ICC (5 °C)	ICC (10 °C)
Cu (ppm)	MAR1	-0.460 n.s.	-0.478 n.s.	-0.457 n.s.
	GIO1	-0.370 n.s.	-0.365 n.s.	-0.393 n.s.
	SAN3	-0.333 n.s.	-0.345 n.s.	-0.342 n.s.
	PAL1	-0.434 n.s.	-0.444 n.s.	-0.410 n.s.
	FAN3	0.301 n.s.	0.305 n.s.	0.278 n.s.
Zn (ppm)	MAR1	-0.179 n.s.	-0.182 n.s.	-0.198 n.s.
	GIO1	-0.323 n.s.	-0.322 n.s.	-0.344 n.s.
	SAN3	-0.343 n.s.	-0.322 n.s.	-0.364 n.s.
	PAL1	0.534 n.s.	0.535 n.s.	0.515 n.s.
	FAN3	0.298 n.s.	0.318 n.s.	0.275 n.s.
Fe (ppm)	MAR1	-0.734 n.s.	-0.720 n.s.	0.751 n.s.
	GIO1	-0.247 n.s.	-0.243 n.s.	-0.269 n.s.
	SAN3	-0.436 n.s.	-0.420 n.s.	-0.432 n.s.
	PAL1	0.352 n.s.	0.329 n.s.	0.368 n.s.
	FAN3	0.049 n.s.	0.026 n.s.	0.059 n.s.
Mn (ppm)	MAR1	0.676 n.s.	0.660 n.s.	0.680 n.s.
	GIO1	0.471 n.s.	0.459 n.s.	0.462 n.s.
	SAN3	0.763 n.s.	0.747 n.s.	0.771 n.s.
	PAL1	0.344 n.s.	0.325 n.s.	0.344 n.s.
	FAN3	0.978 *	0.973 **	0.981 *

Table IX. Correlations (r) between semester ICC and the contents of the main macroelements in the roots of the clones of *A. halimus* (ns = not significant, * = significant for $p < 0.05$, ** = significant for $p < 0.01$).

		ICC (0 °C)	ICC (5 °C)	ICC (10 °C)
N (%)	MAR1	0.108 n.s.	0.216 n.s.	0.269 n.s.
	GIO1	-0.048 n.s.	-0.110 n.s.	-0.147 n.s.
	SAN3	0.143 n.s.	0.203 n.s.	0.308 n.s.
	PAL1	0.097 n.s.	0.235 n.s.	0.308 n.s.
	FAN3	0.345 n.s.	0.270 n.s.	0.169 n.s.
P (%)	MAR1	-0.049 n.s.	-0.125 n.s.	-0.036 n.s.
	GIO1	-0.073 n.s.	-0.024 n.s.	0.035 n.s.
	SAN3	-0.057 n.s.	-0.019 n.s.	0.063 n.s.
	PAL1	-0.194 n.s.	-0.154 n.s.	-0.045 n.s.
	FAN3	-0.200 n.s.	-0.206 n.s.	-0.082 n.s.
Na (%)	MAR1	-0.363 n.s.	-0.384 n.s.	-0.280 n.s.
	GIO1	-0.332 n.s.	-0.267 n.s.	-0.174 n.s.
	SAN3	-0.344 n.s.	-0.300 n.s.	-0.153 n.s.
	PAL1	-0.372 n.s.	-0.467 *	-0.342 n.s.
	FAN3	-0.323 n.s.	-0.268 n.s.	-0.149 n.s.
K (%)	MAR1	0.091 n.s.	-0.006 n.s.	-0.024 n.s.
	GIO1	-0.174 n.s.	0.056 n.s.	0.089 n.s.
	SAN3	-0.238 n.s.	-0.091 n.s.	-0.007 n.s.
	PAL1	-0.206 n.s.	-0.132 n.s.	-0.070 n.s.
	FAN3	-0.170 n.s.	-0.089 n.s.	-0.093 n.s.
Ca (%)	MAR1	0.069 n.s.	0.211 n.s.	0.199 n.s.
	GIO1	0.322 n.s.	0.386 n.s.	0.334 n.s.
	SAN3	0.125 n.s.	0.085 n.s.	0.035 n.s.
	PAL1	0.031 n.s.	0.059 n.s.	0.031 n.s.
	FAN3	0.062 n.s.	0.099 n.s.	0.071 n.s.
Mg (%)	MAR1	-0.301 n.s.	-0.293 n.s.	-0.170 n.s.
	GIO1	-0.493 n.s.	0.061 n.s.	0.201 n.s.
	SAN3	-0.403 n.s.	-0.188 n.s.	-0.041 n.s.
	PAL1	-0.397 n.s.	-0.193 n.s.	-0.056 n.s.
	FAN3	-0.566 **	-0.181 n.s.	-0.039 n.s.

Table X. Correlations (r) between ICC for all the test period (5 semesters) and the content of the main macroelements in the roots of the clones of *A. halimus* (ns = not significant, * = significant for $p < 0.05$, ** = significant for $p < 0.01$).

		ICC (0 °C)	ICC (5 °C)	ICC (10 °C)
N (%)	MAR1	0.333 n.s.	0.313 n.s.	0.338 n.s.
	GIO1	-0.092 n.s.	-0.114 n.s.	-0.085 n.s.
	SAN3	0.576 n.s.	0.564 n.s.	0.570 n.s.
	PAL1	0.205 n.s.	0.182 n.s.	0.217 n.s.
	FAN3	0.724 n.s.	0.720 n.s.	0.741 n.s.
P (%)	MAR1	-0.549 n.s.	-0.545 n.s.	-0.534 n.s.
	GIO1	-0.539 n.s.	-0.538 n.s.	-0.557 n.s.
	SAN3	-0.240 n.s.	-0.217 n.s.	-0.257 n.s.
	PAL1	-0.606 n.s.	-0.606 n.s.	-0.589 n.s.
	FAN3	-0.731 n.s.	-0.730 n.s.	-0.717 n.s.
Na (%)	MAR1	-0.871 n.s.	-0.878 n.s.	-0.857 n.s.
	GIO1	-0.614 n.s.	-0.629 n.s.	-0.594 n.s.
	SAN3	-0.887 n.s.	-0.895 n.s.	-0.875 n.s.
	PAL1	-0.714 n.s.	-0.725 n.s.	-0.695 n.s.
	FAN3	-0.466 n.s.	-0.485 n.s.	-0.445 n.s.
K (%)	MAR1	0.066 n.s.	0.081 n.s.	0.073 n.s.
	GIO1	-0.007 n.s.	0.001 n.s.	0.008 n.s.
	SAN3	-0.254 n.s.	-0.242 n.s.	-0.245 n.s.
	PAL1	-0.249 n.s.	-0.239 n.s.	-0.236 n.s.
	FAN3	0.074 n.s.	0.086 n.s.	0.084 n.s.
Ca (%)	MAR1	-0.097 n.s.	-0.116 n.s.	-0.098 n.s.
	GIO1	0.322 n.s.	0.299 n.s.	0.331 n.s.
	SAN3	-0.196 n.s.	-0.214 n.s.	-0.199 n.s.
	PAL1	-0.269 n.s.	-0.283 n.s.	-0.275 n.s.
	FAN3	-0.385 n.s.	-0.402 n.s.	-0.386 n.s.
Mg (%)	MAR1	-0.851 n.s.	-0.841 n.s.	-0.852 n.s.
	GIO1	-0.926 n.s.	-0.934 n.s.	-0.924 n.s.
	SAN3	-0.926 n.s.	-0.917 n.s.	-0.927 n.s.
	PAL1	-0.757 n.s.	-0.766 n.s.	-0.739 n.s.
	FAN3	-0.870 n.s.	-0.882 n.s.	-0.862 n.s.

Table XI. Correlations (r) between semester ICC and the contents of the main microelements in the roots of the clones of *A. halimus* (ns = not significant, * = significant for $p < 0.05$, ** = significant for $p < 0.01$).

		ICC (0 °C)	ICC(5 °C)	ICC (10 °C)
Cu (ppm)	MAR1	0.177 n.s.	0.135 n.s.	0.219 n.s.
	GIO1	0.573 **	0.512 *	0.541 **
	SAN3	0.094 n.s.	-0.006 n.s.	0.093 n.s.
	PAL1	0.277 n.s.	0.262 n.s.	0.319 n.s.
	FAN3	-0.068 n.s.	-0.090 n.s.	-0.003 n.s.
Zn (ppm)	MAR1	0.087 n.s.	-0.197 n.s.	-0.157 n.s.
	GIO1	0.056 n.s.	-0.100 n.s.	-0.076 n.s.
	SAN3	0.062 n.s.	0.106 n.s.	0.155 n.s.
	PAL1	-0.064 n.s.	0.174 n.s.	0.222 n.s.
	FAN3	-0.257 n.s.	-0.008 n.s.	0.094 n.s.
Fe (ppm)	MAR1	0.013 n.s.	-0.174 n.s.	-0.208 n.s.
	GIO1	-0.185 n.s.	0.014 n.s.	-0.007 n.s.
	SAN3	-0.230 n.s.	-0.173 n.s.	0.225 n.s.
	PAL1	-0.087 n.s.	0.157 n.s.	0.104 n.s.
	FAN3	-0.243 n.s.	-0.048 n.s.	-0.110 n.s.
Mn (ppm)	MAR1	0.218 n.s.	0.213 n.s.	0.314 n.s.
	GIO1	0.038 n.s.	0.401 n.s.	0.404 n.s.
	SAN3	0.339 n.s.	0.388 n.s.	0.385 n.s.
	PAL1	0.204 n.s.	0.434 *	0.459 *
	FAN3	-0.074 n.s.	0.301 n.s.	0.298 n.s.

Table XII. Correlations (r) between ICC for all the test period (5 semesters) and the content of the main microelements in the roots of the clones of *A. halimus* (ns = not significant, * = significant for $p < 0.05$, ** = significant for $p < 0.01$).

		ICC (0 °C)	ICC (5 °C)	ICC (10 °C)
Cu (ppm)	MAR1	0.353 n.s.	0.332 n.s.	0.361 n.s.
	GIO1	0.980 *	0.979 *	0.975 *
	SAN3	0.092 n.s.	0.069 n.s.	0.101 n.s.
	PAL1	0.676 n.s.	0.665 n.s.	0.695 n.s.
	FAN3	-0.248 n.s.	-0.262 n.s.	-0.254 n.s.
Zn (ppm)	MAR1	-0.203 n.s.	-0.211 n.s.	-0.217 n.s.
	GIO1	-0.020 n.s.	-0.001 n.s.	-0.045 n.s.
	SAN3	0.638 n.s.	0.644 n.s.	0.618 n.s.
	PAL1	0.621 n.s.	0.617 n.s.	0.607 n.s.
	FAN3	-0.276 n.s.	-0.272 n.s.	-0.272 n.s.
Fe (ppm)	MAR1	-0.272 n.s.	-0.294 n.s.	-0.262 n.s.
	GIO1	0.039 n.s.	0.017 n.s.	0.044 n.s.
	SAN3	-0.659 n.s.	-0.675 n.s.	-0.641 n.s.
	PAL1	0.618 n.s.	0.599 n.s.	0.634 n.s.
	FAN3	0.447 n.s.	0.427 n.s.	0.467 n.s.
Mn (ppm)	MAR1	0.318 n.s.	0.298 n.s.	0.341 n.s.
	GIO1	0.554 n.s.	0.540 n.s.	0.551 n.s.
	SAN3	0.529 n.s.	0.510 n.s.	0.536 n.s.
	PAL1	0.551 n.s.	0.535 n.s.	0.551 n.s.
	FAN3	0.662 n.s.	0.645 n.s.	0.677 n.s.