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Adaptation of legumes to multiple stresses in Mediterranean-type environments

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SUMMARY – Humid and mild winter and hot and dry summer characterize the areas with Mediterranean climate. Plants developing in such climates are periodically subjected to a combination of stresses including not only the lack of water and high temperature coupled to high evaporative demand and high light intensity in summer but also limitation in the content of nitrogen, phosphorus and other nutrients in the soil. Cold winter is also relevant in some areas. This situation is sometimes aggravated by salinity, fire, overgrazing, etc. The culture of forage legumes adapted to a wide range of soil types, climate conditions and management systems will contribute to increase soil fertility and to maintain soil structure in such environments. In this article, the behaviour of different legumes adapted to Mediterranean climate is discussed. Physiological aspects of the symbiosis \textit{Rhizobium}-legume-arbuscular mycorrhizae are analysed in relation to adaptive responses to relative humidity, water stress, nutrient availability and atmospheric carbon dioxide enrichment.

Key words: Alfalfa, arbuscular mycorrhizae, carbon dioxide enrichment, Mediterranean climate, \textit{Rhizobium}, water stress.

RESUME – “Adaptation des légumes aux nombreux stress dans les environnements méditerranéens”. Des hivers doux et humides ainsi que des étés chauds et secs caractérisent les régions ayant un climat méditerranéen. Les plantes qui poussent sous un tel climat sont périodiquement soumises à une série de stress incluant non seulement le manque d'eau et les hautes températures associées à une grande évaporation et une intensité lumineuse élevée en été, mais également la limitation de la teneur en azote, phosphore et autres nutriments dans le sol ainsi que de rudes hivers dans certaines régions. Cette situation est parfois aggravée par la salinité, le feu, la surexploitation des pâturages, etc. La culture des légumineuses fourragères adaptées à de nombreux types de sol contribuera à augmenter la fertilité du sol et à maintenir sa structure dans de tels environnements. Dans cet article, nous allons discuter du comportement de différentes légumineuses adaptées au climat méditerranéen. Les aspects physiologiques de la symbiose \textit{Rhizobium}-légumineuse-mycorhize arbusculaire sont analysés en fonction des réponses adaptatives à l'humidité relative, au stress hydrique, à l'abondance des nutriments et à la richesse de l'atmosphère en dioxyde de carbone.


Introduction

Aschmann (1973) defined the regions with Mediterranean climate in the world like areas in which at least 65% of the year’s precipitation occurs in winter. Annual precipitation ranges between 275 and 900 mm, the average temperature in winter months is below 15°C and the hours per year at which the temperature falls below freezing (0°C) do not exceed 3% of the total.

There are five regions in the world with this type of climate: the Mediterranean Basin, California, Chile, South Africa and Southwestern and Southern Australia. The worldwide distribution of this singular type of climate shows a pattern of notable regularity, displaying a direct relationship to the general circulation of the world’s atmosphere and its seasonal displacements. Areas of Mediterranean climate are found between latitudes 32° and 40° north and south of the equator on the west coast of continents. The summer drought is associated with the presence of the subtropical high pressure belt at these latitudes, emphasized on the west coast by stable equatorward moving air from persistent oceanic anticyclonic cells. In winter the high pressure belt and the anticyclonic cells are displaced toward the equator and mid-latitude cyclones penetrate the Mediterranean zone. The relative mildness of the winters results from the fact that polar air masses associated with cyclonic storms almost always come from the west, hence from oceanic sources.
Mediterranean climates have a definitive relation with the oceanic cold currents: the Portugal and Canary Currents, the California and Alaska Currents, the Pern (Humboldt) and Falkland Currents, and the Benguela (West Africa) and Western Australia Coast Currents. The currents are associated with the melting of the poles and the climates they produced are not more than one million years old. The Mediterranean climates, based on general meteorological considerations could never exist in the tropics. If world climate changes suppressed these currents, the Mediterranean climate would disappear as a transient period in the geological scale.

Among the most significant characteristics of these environments the following can be mentioned (Castri, 1981):

(i) They are transition zones between humid and arid ecosystems.

(ii) They have been subjected to paleoclimatic changes and, therefore, to the alternated advance and suppression of different plant associations.

(iii) With the exception of the South of Australia, they are characterized by relatively young orogenic systems with a rolling topography, with high and sharp mountains and hills rising close to the coast. Also, frequently, the territories are fragmented in several isolated small valleys.

(iv) Of all the Mediterranean-climate zones of the world, the Mediterranean Basin region suffers the most important “continental” influences. These influences are manifested by the occurrence of summer rainfall (continental trend) in some areas, by relatively greater thermal amplitudes, and by the significance of winter cold as a limiting factor.

(v) The Mediterranean environments, because of the topographic situation and the characteristics of the plant cover, and partly because of their relic nature in some cases, are very prone to degradation and desertification.

Taking into account differences in the soil nutrient content, two groups of Mediterranean land can be differentiated: Chile, California and the Mediterranean Basin on the one hand, South Africa and Australia on the other.

The Mediterranean Basin shows a high plant-species diversity as compared with temperate Europe, since it has played a role of refuge and conservation of (primarily) thermophilous and xerophilous lines during the Quaternary glaciations. It has also been a centre of origin of numerous cultivated species, such as wheat, barley, lentil, chick-pea, bean, olive, grape, almond, apricot, peach, cherry and lucerne. Undeniably, the Mediterranean Basin has influenced all the other Mediterranean-climate regions, through the introduction of typical crops (vineyards and wine production characterize all of them) and domestic animal breeds (mostly sheep and goats), and through the dispersion of Mediterranean grasses and weeds.

Another characteristic of the ecosystems with Mediterranean climate is the high frequency of fires. The decrease in productivity with the increased frequency of fires can be related to losses of nitrogen. It has been estimated that, approximately, one fourth of the total amount of nitrogen stored in the soil, can be lost through volatilization and erosion as a consequence of fire. The microclimate is modified due to the disappearance of the plant cover. The soil conditions are transformed due to the burning of organic matter; as a consequence, there are losses in carbon, nitrogen and phosphorus reserve, also transformation of several mineral elements into directly assimilable elements coupled to an increase of pH in acid soils. The disappearance of plant cover suppresses the interactions of competition, inhibition, depredation and parasitism. Also soil erosion and run-off may be frequent.

The modifications produced on the environment will favour some ecological groups; as occurs with some heliophytes with detriment to sciophytes, with neutrophilous species at the expense of acidophilous species. Nitrophilic and legume species will also be promoted as a consequence of stimulation of nitrifying and nitrogen fixing microflora by the increase of pH. It is clear that the nitrogen fixing ability which characterizes many typical species of Mediterranean shrublands is an adaptation to fire.
Adaptation to multiple stresses in Mediterranean-type environments

As has been previously mentioned, the areas with Mediterranean climate are characterized by humid and mild winters and hot and dry summers. Plant growth takes place mainly under favourable conditions in spring and autumn, the metabolic activity being limited as water stress together with high radiation and evaporative demand in the atmosphere are increased during summer. Although low temperature stress can damage cold sensitive species in winter, summer drought plays the most important role in the limitation of plant growth. Nevertheless, if we want to go deeper into the study of the physiological and ecological meaning of adaptive responses of these plants, it can not be ignored that, periodically, they are subjected to a combination of stresses. Not only the lack of water and high temperature coupled to high evaporative demand and high light intensity in summer but, also, limitation in the content of N, P and other nutrients in the soil. In some areas this situation is more important due to salinity, fire, overgrazing, etc.

All mentioned adverse conditions have shaped the characteristics of the regional vegetation. Woody and perennial herbaceous plants dominate in the more humid places while winter annuals are dominant in soils with low water retention capacity. In general, the character arido-active (avoid dehydration), rather than arido-tolerant (tolerate dehydration) is the most important adaptive trait (Sánchez-Díaz, 1989). In this context, we can separate the water limitation effects throughout the growth cycle from the severe dehydration reached during summer. In the first period, the progression of water stress can be very slow allowing perennial plants to acclimate to water stress. In this process, sensitive stomata, diminishing growth rate – especially through leaf area reduction – and changes in root/shoot ratio are important. The effects on the stomatal behaviour are mainly characterized by changes in the daily opening pattern, decreasing the stomatal conductance during (or after) noon. During the "dry period" (summer) when stomata are closed almost all day, it is known that interaction between high light intensity and high temperature with water stress may drastically affect the photosynthetic capacity. Most of these conclusions are based on studies done with natural vegetation (sclerophyl shrubs). Recently, the effect of water stress and multiple stresses has been studied on the ecophysiology of herbaceous species including forage legumes. These research works are done, both under field and controlled conditions but subjecting the plants to the typical water stress observed under natural conditions. For instance, drought must be imposed very slowly or even in a cyclical or temporary way. Recovery after rewatering must also be studied.

Stress tolerance in lucerne and annual legumes

In Europe there is currently considerable emphasis on low input, efficient agricultural systems that reduce production costs, promote environmental production policy and maintain a living countryside. The sustainability of these production systems relies on the cultivation of forage legumes. This is due to their ability to contribute to the nitrogen economy of swards though nitrogen fixation, their high feeding value and their ability to improve and maintain soil structure. Legumes have a high level of productive diversification and a flexible utilization. The same species can be usefully exploited for different purposes such as soil protection from erosion, green manure crop, living mulching, cover crop in vineyards, orchards and firebreak lines, high quality honey production, landscape enhancement, and medicinal use. Consequently, forage legumes, adapted to a wide range of soil types, climatic conditions and management systems, will become increasingly important components of sustainable agriculture production systems in Europe.

A great part of our studies on stress responses of legumes adapted to Mediterranean climate have been done in lucerne, frequently called "the queen of forage legume species". It is not surprising since in arab the term "alfalfa" means "the best forage". Almost practically all countries which surround the Mediterranean offer similar characteristics which are very appropriate for lucerne cultivation. Therefore, it is not surprising that this crop be traditional in all this region and that most of the varieties used in the rest of the world come from ecotypes grown in the Mediterranean Basin. The nitrogen fixing ability of lucerne and other forage legumes is quite superior to that of grain legumes, but in the case of lucerne the difference is still more pronounced.

Some of our studies have also been performed with other Mediterranean adapted legumes such as subclover and annual legumes. By using different species and varieties we have tried to discover different types of stress tolerance.
The role of galactomannans in Trifolieae

The seeds of legumes we are considering belong to the Trifolieae tribe and have an endosperm which contains a type of reserve polysaccharide not found in cotyledons; these are the galactomannans (Reid, 1971).

Although the cotyledons of the endospermic leguminous seeds are capable of synthesizing reserve starch, it only occurs after germination. By contrast, in non-endospermic legume seeds, the endosperm is absorbed during maturation and starch is stored in the cotyledons. Consequently, the galactomannan usually plays a storage role analogous to that of the starch in the rest of the leguminous seeds (Bewley and Black, 1978).

From a purely nutritional point of view the role of galactomannan is not qualitatively different from that of the reserves in the cotyledons. However, due to its spatial location and its hydrophilic properties, the galactomannan is the molecular basis of a mechanism whereby the endosperm imbibes a large quantity of water during seed hydration and is able to buffer the germinating embryo against desiccation during subsequent periods of drought-stress (Reid and Bewley, 1979). Therefore the galactomannan has two biological functions which it fulfills in succession. It is involved in the interaction between seed and water during germination and it serves as a substrate reserve for the developing embryo following germination.

Apparently in the Trifolieae tribe of legumes, which appears to have spread out from the comparatively dry Eastern Mediterranean region (Hegi, 1935), the galactomannan has been retained because of its high capacity to imbibe water. In this region the species are capable of surviving the summer period when available water may become very limited. Nevertheless, the small amount of galactomannan found in subclover endosperm (González Murúa et al., 1985) shows that such polysaccharides are not important for the germinative physiology of an annual plant of typical Mediterranean origin, such as subclover.

Water stress and Rhizobium-legume symbiosis

In a drying soil, the soil water potential decreases and so does the soil hydraulic conductivity. Thus it is more difficult for plants to extract water and, as a consequence, the plant water potential tends to decrease. This decrease may directly affect the physical aspects of some physiological processes.

As has been shown, a low osmotic potential or the capacity to accumulate solutes, as well as being highly elastic, and small cells, aid in maintaining positive turgor as the water content of plant tissue decreases. The presence and degree of osmotic adjustment varies with species or cultivars.

There will be other direct effects of decreased plant water potential on physiology, but the indirect effects are likely to be as important. Decreased leaf expansion and stomatal closure both restrict photosynthesis and therefore dry matter accumulation, this reduction in assimilate supply may affect many physiological processes. Shortage of assimilates at the roots may not only decrease root growth but, as a consequence of this decreased growth, the roots may be less able to utilize all the soil’s reserves of water.

The particular way water stress is imposed might be of special importance in understanding the field response of photosynthesis to drought, and also in evaluating the plant’s capacity to acclimate to stress (Kaiser, 1987; Chaves, 1991). For short-term experiments, withholding water is the most common way, but to allow for a more realistic response to drought, sustained or cyclic water stress is needed. In the first case, the stress is imposed more rapidly than when it occurs naturally.

When leaves are subjected to water stress, it is often observed that net photosynthesis rate (Pn) is decreased whereas the calculated intercellular CO₂ concentration (Ci) is more or less tolerant (Tenhunen et al., 1984; Wong et al., 1985). This response pattern has often been attributed to a combination of increased stomatal resistance (stomatal limitation) and limitation of the mesophyll photosynthetic capacity (non-stomatal limitation). In studies of the effects of temporary droughts on photosynthesis in alfalfa, Antolín and Sánchez-Díaz (1993) suggest the major implication of non-stomatal factors in the decline of photosynthesis, under cyclic drought conditions.

Microbial cells, including Rhizobium, are able to withstand lower water potentials than most higher plant cells. As a consequence, from the beginning of infection by Rhizobium until the functioning of
differentiated nodules, the most important factors which limit the fixation under water stress, will probably depend on the host plant.

Several experiments have shown the adverse effect of drought on nodule activity which, in turn, can be more pronounced due to the consequences on photosynthesis. On the other hand, drought directly affects the nodule oxygen permeability (Aguirreolea and Sánchez-Díaz, 1989). Due to the reduction in nodule permeability, nodules will have limited ability to do oxidative phosphorylation in spite of maintaining high photosynthetic rates. Work made on different lucerne cultivars suggests that those adapted to dry conditions are likely to show smaller water stress effects on nitrogen fixation than those less well adapted. For example, the dryland cultivar of Medicago sativa, ‘Tierra de Campos’, was able to grow, transpire and fix nitrogen at lower water potentials than the cultivar ‘Aragon’. Recovery from stress was also more rapid in the dryland adapted cultivar.

Although less studied, water stress also affects the degree of infection of legumes by Rhizobium. Aguirreolea et al. (1985) studying the effect of water stress on the infectious capacity of different strains of Rhizobium meliloti, have found that there is a drastic decrease in the number of nodules and delay in their appearance, which increase with the severity of the stress. On the other hand, the infectivity of one strain under optimal water conditions does not necessarily imply the same type of behaviour under water stress.

Recent studies point out the possibility of selecting drought tolerant Rhizobium strains, some of which would be very efficient as nitrogen fixers. It is likely that the continuous advances in Rhizobium genetic engineering will allow strains which will show still higher drought tolerance to be obtained.

**Water stress and Rhizobium-legume-arbuscular mycorrhiza-symbiosis**

As has already been pointed out besides N, one of the most limiting factors in Mediterranean regions, is the availability of P which, in many cases is immobilized by the presence of iron oxides. With regard to that aspect it is important to pay attention to the role of double symbiosis Rhizobium-legume-arbuscular mycorrhizal fungi (AMF). It is known that one of the benefits of these fungi is due to the improvement in phosphorus uptake into the plant.

AMF effect on plant water status has also been associated with improved host nutrition, particularly P (Nelsen and Safr, 1982; Fitter, 1988). However, several authors have reported that drought resistance of mycorrhizal plants is independent of plant P concentration (Sweatt and Davies, 1984; Augé et al., 1986; Bethlenfalvay et al., 1988; Peña et al., 1988; Sánchez-Díaz et al., 1990). Along drought, in the Medicago-Rhizobium-Glomus symbiosis, nodule activity was significantly higher in infected than in non infected plants (Peña et al., 1988). In this case, the higher activity could not be explained by improved P uptake caused by the fungus since P concentrations in mycorrhizal plants were always lower than in phosphorus fertilized ones. AMF may also increase drought resistance of plants by means of several mechanisms, including enhancing water uptake due to hyphal extraction of soil water (Ruiz-Lozano and Azcón, 1995; Duan et al., 1996), lowering leaf osmotic potential for greater turgor maintenance, by regulating stomatal conductance (Augé et al., 1986) or photosynthesis (Sánchez-Díaz et al., 1990), independently of the phosphorous content in plant tissues. More recently, Augé et al. (1994) found that mycorrhizal symbiosis can alter non hydraulic root-to-shoot signalling in drying soil.

A major problem concerning many water relations studies has been that AMF infected plants were of different size and tissue P content than non-infected ones. If plants are grown in containers of equal size, a plant size differential should lead to differential rates of soil water depletion. Also, plants with optimum P concentration should be more vigorous with higher photosynthetic rates and stomatal conductances than those with limiting P (Radin, 1984; Radin and Eidentock, 1986), and might respond differently to drought. Few arbuscular mycorrhizal water relations studies have documented leaf tissue macro-and microelement levels, but AMF could influence levels of elements other than P (Bildusas et al., 1986). To test for possible mechanisms of drought resistance in mycorrhizal plants, both mycorrhizal and non-mycorrhizal controls should be equal in size and tissue elemental concentration, especially P concentration (Davies et al., 1992).

Working with alfalfa, Goicoechea et al. (1997a) found that endogenous phytohormone levels seem to be involved in the control of photosynthetic gas exchange in plants subjected to drought. When photosynthetic gas exchange was compared in stressed symbiotic and non-symbiotic plants, it was found
that treatments with a lower ABA production in roots and a lower ABA to cytokinin ratio in leaves, as well as higher leaf cytokinin concentrations, maintained increased photosynthetic activity, leaf conductance and transpirational flux. The higher rates under water deficit were found in mycorrhizal plants, with a double-symbiosis mycorrhizal fungi-nitrogen fixing bacteria resulting in an even more beneficial effect for the host plant than that of a simple symbiosis with AM fungi. Besides, when drought-subjected mycorrhizal alfalfa plants are compared with non-mycorrhizal ones, the former maintained cytokinin levels and also delayed leaf senescence and stimulated stem production (Goicoechea et al., 1995). The same authors also found that double symbiotic plants showed significantly higher root and nodule activities during drought that could be related to smaller decreases in cytokinin content of mycorrhizal plants (Goicoechea et al., 1996). Other results (Goicoechea et al., 1997b) indicate an enhancement of nutrient content in mycorrhizal alfalfa plants during drought that affected leaf water relations during drought stress.

**Water stress and Rhizobium-legume symbiosis under atmospheric carbon dioxide enrichment**

The frequency and/or the intensity of drought is expected to increase in the Mediterranean area as a response to climate change. Thus, it was of interest to study the effects of drought and high CO$_2$ and, particularly, the interaction between them as has not been investigated before in N$_2$-fixing alfalfa plants. De Luis et al. (1999) in alfalfa cv. ‘Aragon’ found that under well-watered conditions, elevated CO$_2$ improved growth and this effect was due to a better performance of plant tissues, and not related to a better water status. When subjected to soil water deficit, elevated CO$_2$ alleviated the negative effects of drought and improved drought tolerance – meaning the ability to maintain plant productivity under given water stress – by improving carbon fixation and plant growth. As a result, plant biomass production was even higher than in well-watered plants grown under ambient CO$_2$. Plant growth stimulation in droughted plants was especially strong in below-ground production, increasing the availability of photosynthates to the nodules and nodule biomass production, which was reflected on a higher amount of total fixed nitrogen.

**Conclusions**

The growth of legumes in Mediterranean areas is seriously limited by a combination of stresses. In this respect, the stress resistance showed by nodulated legumes associated to arbuscular mycorrhizae is remarkable. The frequency and intensity of drought and temperature stresses is expected to increase in the Mediterranean area as a response to climate change. However, it has been demonstrated that the negative effect of drought is alleviated under high CO$_2$ conditions in nodulated alfalfa. Forage legumes adapted to a wide range of soil types, climatic conditions and management systems will become increasingly important components of sustainable agriculture in Europe.

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