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EDITORIAL

Special issue on "Modulating Agents of Plant Physiology and Products"

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There are large regions of the earth where conditions for plant growth are positively or negatively conditioned. The manifold favorable or unfavorable (but not necessarily immediately lethal conditions occurring either permanently or sporadically in a locality) is modulating agents of plant physiology and products. Wherever they grow, plants are subjected to a large variety of modulating agents tending to restrict or stimulate their possibilities of development or even survival. Even if the modulating agents are merely temporary, the vitality of plants might positively or negatively change and, if the limit of the plant´s ability to adjust is reached, hitherto maximum productivity or a latent damage develops (into chronic disease or irreversible injury).

The modulating agents of plant physiology and products follow the ratio of tension to strain (i.e. the elastic module) and the transition from elastic (reversible) to plastic (irreversible) change of plant physiology and biochemistry. Accordingly, organisms respond differentially to a particular physiological modulation, depending on their genetically determined "reaction norm". Moreover, also characteristic for a modulating agent of plant physiology is the occurrence of non-specific manifestations, which are primarily an expression of the degree of optimization or disturbance, following a stereotypic pattern, whatever the nature of the associated modulation factors.

At a physiological level the response to a modulating agent depends upon the background conditions, such as diurnal light rhythm, or the changes of seasons. Responses under the variable climatic conditions prevailing in natural surroundings are often much stronger than under controlled laboratory conditions. Also the way in which plant species reply to climacteric and many other modulating agents further depends of the seasonal growth rhythm.

Joined modulating agents or its sequential series may further reinforce, weaken, mask or even reinforce the response of a plant to a single agent. Moreover, enhancement of a physiological modulation in combination with others can often be observed and lead to additional stimulation or inhibitory variations.

This special issue highlights some research studies carried out by researchers working on the field of modulating agents of plant physiology and products, selected on the basis of a rigorous peer-review process. The Guest Editor wishes to express his thankfulness to the contributors and reviewers of the manuscripts and recognizes the key role of the Editorial Board of the Emirates Journal of Food and Agriculture in the publication of this issue.

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REVIEW ARTICLE

Impact of human activities on coastal vegetation – A review

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Abstract

Coastal dunes constitute complex interface ecosystems between the land and sea, providing important ecological and socio-economic services like coastal protection, recreation, regulation, food supply and biodiversity. They are characterized by high ecological diversity and also by extremely specialized and endemic species. Coastal areas, because of their position between land and sea, are attractive for many human activities. Unfortunately coastal systems have had a long history of exploitation and mismanagement.

Key words: Coastal dune systems, Iberian Peninsula, Vegetation, Human impacts, Managements

Introduction

Coastal zones constitute interface ecosystems between the land and sea (Leewis et al., 2012, Gonçalves et al., 2013; Martins et al., 2013). They exhibit complex multilevel interactions: they are under the influence of a great variety of pressures both of natural and anthropogenic origin and they display complex relationships between the physical and biological processes (Nobre and Ferreira, 2009). Besides, they are some of the most dynamic areas on Earth. In fact, a crucial feature of the coastal zones is the variability in the intensity of the multiple influences that they are subjected to. Consequently, these zones are always striving for dynamic equilibrium without almost ever achieving it (Dias, 2004).

Coastal ecosystems are among the most productive systems in the world and provide disproportionately more services relating to human well-being than most other systems, even those covering larger total areas (Millennium Ecosystem Assessment, 2005). These services include regulation (climate regulation, nutrient regulation, carbon sequestration, detoxification of polluted waters), support (shoreline stabilisation, marine life nursery functions, buffering from natural hazards), provision (supply of food, energy resources, natural products), cultural services (recreation, culture and amenity) and biodiversity (EEA, 2006, Thompson and Schlacher, 2008). As a consequence, nearly 40% of the world's population lives within 100 km of the coastline and concentrates approximately 61% of the world's total Gross Domestic product (GDP) (Millenium Ecosystem Assessment, 2005). These facts stand out the importance of coastal areas for human well-being.

However, coastal zones are extremely sensitive due to a number of reasons. One of them is the dependency of the morphology of the sea-land line and variations in its relative location on a dynamic sea-land balance (Valpreda and Simeoni, 2003). Another is the fact that coastal areas are subject to harsh environmental conditions and constitute challenging habitats to be colonized by vegetation (Maun, 2009). Plants have to manage with living in soils usually deficient in major nutrients, highly saline and generally with low water content. Besides, plants must withstand intense and almost constant winds and salt spray (Martins et al., 2013).

This study intends to provide a review of the main impacts of human activities on coastal vegetation and also of management measures that

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have been proposed to restore and/or protect these systems.

Coastal dunes

Coastal dunes systems are eolian landforms developing in coastal areas where there is a plentiful supply of appropriate sediment, a prevailing wind that carries the sand inland and vegetation capable of sand stabilization (Macedo, 2008; Maun, 2009; Acosta et al., 2013). Sand or mixed sand and rock shorelines represent around 75% of the world's ice-free coastlines (Brown, 2001 in Brown and McLachlan, 2002). As they are distributed worldwide, the climate and biomes developing on coastal dunes are very diverse, encompassing a wide range of ecological habitats (Hesp, 2002; van der Maarel 1993a in Martínez et al., 2004a). However, in spite of their wide geographical range, coastal dune ecosystems occupy a very low percentage of the terrestrial environment (Castillo and Moreno-Casasola, 1996) and, of all the coastal ecosystems sand dunes have suffered the greatest degree of human pressure (Carter, 1988).

The Spanish coast is about 8000 km in length (5000 km in the Iberian Peninsula and 3000 km on the islands), consisting of 4000 km of cliffs, 2000 km of beaches, 1400 km of coastal lowlands and 600 km of artificial coast (Dias et al., 2012). Part of the coastline faces the Atlantic Ocean and part the Mediterranean Sea. The Portuguese mainland coast is about 900 km long, with low sandy beaches and cliffs alternating irregularly (Andrade et al., 2002 in Martins et al., 2013). Beaches constitute approximately 590 km of the coastline extension (Leal, 2007 in Martins et al., 2013). From its northern end to about a third of the coastline (Figueira da Foz) the shore is essentially flat and sandy (Martins et al., 2013). To the south of this location until Cape S. Vicente cliffs alternate with lowlands with sand dunes (Dias et al., 2013). The southern coast (Algarve) shows a differentiation between the western sector, mainly composed of cliffs and the eastern sector characterized by continuous sandy beaches (Dias et al., 2013; Martins et al., 2013).

Sandy coastline ecosystems perform several important ecological functions, one of the most relevant consisting in the dissipation of incoming wave and wind energy, thus protecting and stabilizing the shoreline (Gómez-Pina et al., 2002; Gonçalves et al., 2013; Valles and Cambrollé, 2013). They also provide services very significant from the economic point of view, such as recreation, culture and amenity (Martínez et al., 2004a; Gonçalves et al., 2013). Indeed, coastal dunes are highly valuable multifunctional ecosystems that occupy a unique natural niche (Martínez et al., 2004b).

Coastal sandy environments, being situated at the interface of land and sea, are highly dynamic and plastic systems (Brown and McLachlan, 2002; Gonçalves et al., 2013; Vallés and Cambrollé, 2013). In fact, sandy shoreline ecosystems consist, in general, of three components: a surf zone, intertidal beach and dune systems (Short and Hesp, 1982), each one being colonized by different species. There is a continuous exchange of sand and biological matter between dunes, intertidal beaches and surf zones (Brown and McLachlan, 2002). The action of waves and tides mainly controls not only morphology but also species diversity, biomass and community structure (Brown and McLachlan, 2002) although species inhabiting dunes must be able to colonize an unstable substrata, to tolerate recurrent sand engulfment and sandblast (Brown and McLachlan, 2002; Fenu et al., 2012).

Biodiversity

Coastal dune ecosystems experience well marked littoral-inland gradients as regards environmental factors such as substrate coherence (which influences probability of burial by sand) and salinity, wind, salt spray and wave regime, which differ with distance from the sea and topographic sheltering (Ranwell, 1972; Guara-Requena, 1989; Acosta et al., 2009; Lortie and Cushman, 2007 in Macedo, 2008; Carranza et al., 2008; Acosta et al., 2013). These gradients act as a strong selective force, which determines the occurrence of species developing different strategies (Martínez et al., 2004b; Fenu et al., 2012). According to these gradients, structurally and floristically different vegetation types develop (Brown and McLachlan, 2002; Carranza et al., 2008), thus accounting for the coexistence of many different communities within a relatively limited area as compared with other natural ecosystems, which results in a high ecological diversity (Van der Maarel, 2003; Martínez et al., 2004b; Carranza et al., 2008, Acosta et al., 2009; Fenu et al., 2012; Acosta et al., 2013). Moreover, sandy coastal ecosystems possess extremely specialized biotic assemblages rarely shared with adjacent terrestrial ecosystems (Acosta et al., 2005; Schlacher et al., 2008; Defeo et al., 2009). Several animal groups such as arthropods, gastropods, reptiles and birds are closely associated to different habitats in the dune system (McLachlan and Brown, 2006 in Acosta et al., 2013) because of dissimilarities in food sources, protection from

predators, resting sites and vegetation structure (Kritzinger and van Aarde, 1998 in Martínez et al., 2004b). Dunes support the richest thermophilic invertebrate faunas in Britain (Kirby, 1992 in Howe et al., 2010), including many of the rarer species of bees and wasps (Howe et al., 2010). Both the high biodiversity and the high level of peculiar plants and habitats constitute a crucial factor that allows rapid adjustment to environmental change (Carter, 1988; Acosta et al., 2013). All these factors contribute to a high intrinsic value of dune ecosystems (Martínez et al., 2004a; Acosta et al., 2005).

The Portuguese coast is known for the richness of its flora, due to its special biogeographic position (Braun-Blanquet et al., 1972) and also for its importance as regards conservation value. In fact, in the total of Portuguese Natura 2000 habitats, 35% correspond to coastal habitats (Martins et al., 2013). Dune vegetation possesses a high percentage of endemic plant species as a result of speciation due to environmental isolation (Neto et al., 2007). In Portugal mainland, beaches south of Tagus River are characterized by the highest degree of endemism (Neto et al., 2007). The Mediterranean habitats are generally richer as regards floristic composition than the Atlantic ones and they also include almost all the psammophilic Portuguese endemisms (Neto et al., 2007) like for example *Linaria lamarckii*, *Thymus carnosus* and *Herniaria maritima* (Martins et al., 2013).

Beach zonation

Development of the dunes mainly depends on plant cover and height, wind velocity and quantity of sand. Secondary factors such as the rate of occurrence of swash inundation, storm wave power and wind direction can also be important in determining subsequent dune dynamics (Ranwell, 1972). As a consequence of the strong environmental gradients, sand dune ecosystems consist in a succession of shore-parallel bands. At each stage of the succession the plant community alters the soil and microclimate, allowing the establishment of another group of species.

At the upper edge of the beach where only high tides and storm waves arrive and debris accumulates in spring, the first vegetation strip develops. Very few and highly specialized vascular plant species can survive here, with specific morphological and physiological traits (García-Mora et al., 1999; Acosta et al., 2009). These pioneer species must possess a high salt tolerance and features that allow the survival to sand instability and burial. Additionally they have fast reproductive strategies, which allow them to exploit the spring-summer quasi-stability of the beach. Litter brought by storms and tides is essential for the survival of these plants as it provides nutrients, moisture and shelter (Carter, 1988; Costa et al., 1994). The vegetation is ephemeral, composed mostly of annual species which regenerate and re-establish new populations in spring/summer (Acosta et al., 2009). This first plant community is characterized by low vegetation cover and reduced floristic diversity. In Continental Portugal *Salsola kali* and *Cakile maritima* are characteristic species (martins et al., 2013). These communities constitute the habitat 1210 of the European Ecological Network "Natura 2000": "Annual vegetation of drift lines" (Martins et al., 2013). In all the dune system the upper beach habitat contains a significantly small number of species, but it shows the highest rarity values and amount of endangered species (Acosta et al., 2009). In the Portuguese coast these communities have suffered a decrease over the last 20 years due to coastline retreat and intensive recreational use during summer (Martins et al., 2013). They are characterized by a variable conservation status, average or good in the best preserved areas (Martins et al., 2013).

At the very top of the beach (high beach) there is only a sporadic penetration of the waves during storms and winter (Martins et al., 2013). Perennial plant species with rural strategies are capable of colonizing this area (Carter, 1988). The aerial parts of the plants obstruct the wind and absorb the energy of windblown sand grains. Wind velocity near plants is thus reduced, and the sand is deposited around the stems (Carter, 1988; Maun, 2009). Gradually, a small dune develops around the plant. Sand trapping by these species is, however, not sufficient for big dune formation (Carter, 1988) and as a consequence, they constitute low mounds of sand, known as incipient or embryonic dunes. These dunes form habitat 2110: Mobile embryonic dunes. These are inherently species-poor and sparsely vegetated communities (Martins et al., 2013), being dominated, in continental Portugal, by two subspecies of the grass *Elytrigia juncea*: *Elytrigia juncea* subsp. *boreoatlantica* and *Elytrigia* subsp. *juncea* (Costa et al., 1994; Honrado et al., 2002; Lomba et al., 2008; Martins et al., 2013). The high beach is an extremely selective habitat for plants (Ranwell, 1972) and, as a consequence, it possesses a low diversity. However, this habitat type is of exceptional importance as an indicator of the general structural and functional 'health' of a dune system. In fact, embryonic

shifting dunes are particularly vulnerable to trampling by beach users and to mechanical cleaning of beaches. In Portugal the high beach vegetation has suffered a decrease in its area, with different conservation status along the coast (Martins et al., 2013). Good conservation is observed where human pressure is low (Martins et al., 2013), although most areas are not well preserved.

Inland, perennial species with broader and denser stems constitute a major barrier to the wind and as a consequence a greater quantity of sand is deposited around their stems. As sand accumulates, dunes grow, becoming much higher than embryonic dunes. The most important characteristic of these perennial plants is their ability not only to survive but also to grow horizontally and vertically in response to sand burial (Maun, 2009). Thus, dune starts to grow in area and height over time. Although their growth is stimulated by sand deposition, there is, however, a limit for burial survival. Unless the plant is able to grow more rapidly than the rate of sand deposition, growth ceases (Carter, 1988). These communities correspond to habitat 2120: Shifting dunes along the shoreline with *Ammophila arenaria* ("white dunes"). Vegetation cover is incomplete and loose sand is subject to wind-blow. The white dunes are colonized by herbaceous vegetation dominated by *Ammophila arenaria* (Honrado et al., 2002; Lomba et al., 2008; Martins et al., 2013) and are characterized by low to medium diversity levels (Martins et al., 2013). These plant communities perform important ecological services such as the prevention of storms, since they constitute the first natural barrier against the sea (Martins et al., 2013). In continental Portuguese dunes endemic species like *Linaria lamarckii* and *Thymus carnosus* are frequent (Martins et al., 2013).

Both the embryonic and the shifting dunes with *A. arenaria* possess alow/medium diversity as regards vegetation. However, despite the low number of plant species, these habitats are characterized by high rarity and conservation values, because of the high proportion of endangered and exclusive plant taxa (Van der Maarel, 2003; Acosta et al., 2009, Martins et al., 2013). In a comprehensive study of invertebrates in UK and Wales, Howe et al. (2010) found that the most specialized dune invertebrates tend to occur in the earlier stages of dune succession, the number of specialists declining as the proportion of bare sand decreases.

Further inland the substrate becomes more and more stabilized and there is lower influence of salt spray as the result of a progressively increasing distance from the sea (Lomba et al., 2008). These conditions allow sand colonization by more species, especially shrubs that form communities of high cover value, thus the name of grey dunes (Carter, 1988; Martins et al., 2013). Grey dunes constitute a priority habitat in "Natura 2000": *2130 Fixed dunes with herbaceous vegetation. These communities are characterized by a high diversity and are very important in sand fixation (Martins et al., 2013). Most vegetation communities in grey dunes of the northwest Iberian Peninsula are characterized by endemic taxa (Lomba et al., 2008). *Jasione lusitanica* and *Coincya monensis* subsp. *cheiranthos* var. *johnstonii* are two Portuguese endemisms (Martins et al., 2013).

The different communities of the sequence described show an increase in species richness, cover, stature and biomass (Lubke, 2004; Martins et al., 2013). On the first communities vegetation is constrained by extreme abiotic conditions while on the stabilized dunes, plant communities are structured mostly due to biotic interactions (Doing, 1985).

Alterations of the zonation

Modification of this general scheme may occur because of natural factors, due to the ever-changing nature of the environment. Aeolian coastal dune systems morphology and plant species composition are the product of complex histories of alternating events of shoreline advance or retreat (González-Villanueva et al., 2013), driven by climate variables, sediment quantity and characteristics, vegetation cover, storm frequency and severity and ocean-level fluctuations (Klijn, 1990; Pye, 1993 in González-Villanueva et al., 2013). However, nowadays alteration of the vegetation zonation scheme occurs mostly due to human activities. A large extension of the Portuguese coastal sandy systems is being subjected to intense erosion processes (Martins et al., 2013) and, as a consequence, beach species tend to migrate to the mobile dunes, or even mobile dunes advance inland, overlapping stabilized dunes, thus changing the normal sequence of communities. Transgressive dune systems in the north of Portugal display a lower number of plant species in comparison to those of stable coastal systems (Macedo, 2008). Disturbances related to trangressive coastal dynamics induce dramatic changes in the composition, structure and function of plant communities by the selection of species holding particular traits (Schlacher et al., 2008). Macedo (2008) identified an increase of ruderal species and

a decrease in the importance of stress-tolerant specialist species in over-disturbed foredune communities in the north of Portugal.

One of the most important aspects to realize with the coastal environment is that it is dynamic, like the forces that shape it, so it is endlessly changing. In fact, at a world level 70% of beaches are receding, 20% are stable, and only 10% are advancing (O'Riordan, 1995 in Valpreda and Simeoni, 2003). The causes of shoreline shift can be of local importance, such as a reduction of sediment input or of global importance, such as a worldwide sea level rise (Taveira Pinto, 2004; Feagin et al., 2005) and they can act at short, medium and long-term timescales (Veloso-Gomes et al., 2004; Valpreda and Simeoni, 2003).

Human activities and their impacts

The influence of humans on the coastal environment is not a modern phenomenon. In fact, it has occurred since prehistoric times (Heslenfeld et al., 2004; Nordstrom, 2000 in Schlacher et al., 2008). Iberian populations have influenced coastal regions for centuries (Dias et al., 2013). Romans caused severe environmental impacts to the vegetation cover of the Peninsula due to agriculture, deforestation and mining activities. The Muslims brought new impacts as a result of new agricultural processes and salt exploitation in estuaries and lagoons (Dias et al., 2013). After the conquest of the Peninsula by the Christians there was an increase in demographic growth, which caused growing demand for resources, especially open land for agriculture. Thus deforestation increased significantly, causing widespread erosion and reinforcing littoral drift (Dias et al., 2013). In the Aveiro region in the northwest of Portugal, in response to a huge increase in the littoral drift, the original open bay area was transformed gradually into a closed lagoon (Dias et al., 2013). The Portuguese maritime expansion required renewed shipyards and a rapid manufacture of vessels to assure the maritime trade between the mainland and recent discovered territories, which increased forest cutting in the mainland and contributed to the import of large volumes of timber from different sources (Reboredo and Pais, 2012, 2013). The same authors state that the deforestation in the mainland was mainly due to shipbuilding, since the fluctuations in the demography from the foundation of the nationality until the beginning of the XVII century were narrow, and it is well know that fuelwood consumption by inhabitants was the main energy source at that epoch.

Conversely, from the XVII onwards a demographic boom occurred in parallel with a strong decline of maritime trade, indicating that the almost total depletion of the forest cover in the mainland in the beginning of the XVIII was mainly related with human needs (fuelwood) and not vessel construction (Reboredo and Pais, 2012, 2013).

Since the XXth century the construction of dams has caused a negative sediment budget to the Portuguese coast (Martins et al., 2013), as beach particles originate mainly from inland erosion, being transported to sea via rivers (Pereira, 1996; Brown and McMachlan, 2002). These facts have resulted in erosional processes and thus inland displacement of the shoreline (Martins et al., 2013).

As coastal ecosystems provide a variety of goods and services, they have long been used for many different activities such as settlement, fishing, agriculture, afforestation, coastal defence, urban development, industrial expansion, mining and recreation (Carter, 1991 in Martínez et al., 2004a; Heslenfeld et al., 2004). However, many of these activities have accelerated remarkably in the last two centuries. All these activities generate economic profits to the human populations; yet, they result in more or less severe impacts on the coastal ecosystems (Martínez et al., 2004a). Population growth and demographic flows towards the coastline are nowadays putting pressures on these ecosystems at unprecedented scales (Brown and McLachlan, 2002; Schlacher et al., 2006, 2007a in Schlacher et al., 2008). The increase of human occupation is, in many cases, incompatible with the natural dynamics of the coastal areas (Veloso-Gomes et al., 2004) and, as a result, worldwide many coastal dune systems are in advanced stages of degradation, irreversibly altered or even lost (Martínez et al., 2004b). For example, the sandy coasts in the Mediterranean Basin are considered among the most endangered environments in Europe (van der Meulen et al., 2004; Carboni et al., 2009).

The Portuguese coastline presents a significant variety of dynamic situations (Ferreira et al., 2008 in Macedo, 2008). Some beaches are subjected to erosion processes, showing an average retreat rate of almost 4 m/year (Ferreira and Dias, 1993 in Ferreira et al., 1995). For the region around the city of Porto, in the north, shoreline retreat is estimated as a result of a reduction of sand areas and increased storminess (Ferreira et al., 2008 in Macedo, 2008). Some beaches are stable and show little evidence of erosion or accretion (Ferreira and Dias, 1993 in Ferreira et al., 1995). Nowadays, as a

whole, high erosion rates seriously affect over 30% of the Portuguese coastline (Freire et al., 2009) as a consequence of several factors like the reduction of the sediment supply of the main rivers and construction too close to the shoreline (Silva et al., 2007).

Urban pressure and tourism

In Portugal the coastal area possesses a high landscape and conservation value and it is therefore no surprise that it is subjected to intense pressures (Silva et al., 2007). In fact, coastal areas are the most populated areas in the country - over 75% of the population lives on the coast. Urban, industrial and touristic areas near the coast are responsible for 85% of the GDP (CNADS, 2001). In Portugal the two largest metropolitan areas are located near the coast, concentrating 40% of the country´s total population (CNADS, 2001). As the result of bad planning and management, which allowed chaotic construction in hazard prone areas (Silva et al., 2007), coastal areas suffer from many problems. Indeed, almost every winter erosion problems occur on the western Portuguese coast due to the construction of tourist resorts close to the shoreline without suitable planning or management (Ferreira et al., 1995). In a study by the EEA (2006), among 17 European countries, mainland Portugal experienced, during 1990-2000, the largest increase (34%) in artificial areas within a 10-km strip from the coast. This rate is much higher than the increase in resident population in the same period (Freire et al., 2009).

The establishment of populations in coastal areas was, in many cases, only possible with modification of the coastal front and the building of heavy man-made structures. These infrastructures and coastal defenses, although offering more favorable conditions for economic growth, have caused magnification of coastal erosion, because important physical processes have not been taken into account (Taveira-Pinto, 2004). In fact, structures such as harbours, breakwaters, jetties and groins, which disrupt sand transport, may lead to severe erosion because they withdraw down-drift beaches of sand (Brown and McMachlan, 2002). As a result, all over the world coastal inhabitants and man-made infrastructures are facing a high-risk condition due to coastal erosion (Veloso-Gomes et al., 2004). In our present-day society, which emphasizes socio-economic criteria, this phenomenon is seen as unacceptable (Valpreda and Simeoni, 2003). Thus, management of coastal areas has traditionally focused almost exclusively on maintaining and restoring physical and geomorphological features important for coastal defence (James 2000b; Micallef and Williams 2002 in Sclacher et al., 2008).

In the 19th Century health benefits of oceanic baths were recognized (Corbin, 1989 in Dias et al., 2013; Dias, 2005; Freitas, 2010), a fact that triggered a new behaviour as regards sandy coasts, which in turn was responsible for a new and profitable economic activity: tourism. Nowadays many populations living in coastal areas depend largely on the income derived from the recreational usage of beaches and associated activities. The massification of tourism originated a boom in coastal areas demand during the 1960s and 1970s, which was responsible for the rapid and many times uncontrolled urban expansion, an example being the extensive southern coastal areas of Spain and Portugal (Dias et al., 2013). Benidorm, in Spain, increased from 5000 inhabitants in 1950, to 70,000 in 2008 and can additionally offer more than 38,000 beds (Giussani et al., 2010 in Dias et al., 2013). The resident population in Albufeira, in the South of Portugal, was 31 500 inhabitants in 2001, but peak summer population reached around 300,000 people (CNADS, 2001). In these regions many natural areas were transformed, irreversibly, into artificial coasts (Dias et al., 2013).

As a consequence of the increase of human population in coastal areas, which has often resulted in chaotic demographic expansion, some dunes systems have been completely destroyed, in the process of providing living space for buildings, residential development, tourist resorts, and recreation areas (Martínez et al., 2004b; Martins et al., 2013). Massive tourism demand has led to verticalization of the coastline (tall buildings) such as occurs in Benidorm in Spain or in Albufeira, in Portugal (Dias et al., 2013). Too often, buildings have been constructed too close to the shoreline, therefore being threatened by erosion processes (Freire et al., 2009). In Spain, the large-scale urban development carried out on the foredunes during the tourist boom of the sixties and seventies caused the destruction of many Spanish dune systems (Gómez-Pina et al., 2002). As a result of such massive dune occupation, most of the Spanish coastline started to show signs of erosive patterns, particularly in most of the tourist spots (Gómes-Pina et al., 2002). In many shorelines mere fragments of dune now remain between the high density residential and coastal amenity structures with a consequent reduction in their coastal protection role and amenity value (Curr et al., 2000).

Even when the coastal systems were spared, dunes are squeezed between sea water on the marine side and human settlements on the landward side (Feagin et al., 2005; Schlacher et al., 2008), which results in a delicate condition. In situations of rising sea level, beach erosion causes an inland movement of dune habitats but, owing to the existence of man-made barriers, species are no longer able to disperse and grow (Martinez and Psuty, 2004 in Feagin et al., 2005; Martins et al., 2013). And in fact coastal erosion actually impacts about 70% of the world´s beaches (Bird, 1985 in Feagin et al., 2005).

The Mediterranean region plays host to ca. 33% of the world's tourism industry. This high quantity of visitors, as expected, exerts a huge impact on its natural coastal resources (Curr et al., 2000). In fact, Salman observed that in 1994, approximately 75 % of European Mediterranean sand dunes had already been destroyed (Salman 1994 in Martins et al., 2013). Unfortunately destruction has continued although we are not able to quantify the remaining destruction degree of sand dunes.

In Portugal the construction on top of the dunes and the land use change in the more interior stages of the dune system have damaged the ecological gradients (Martins et al., 2013) reducing the very few good spots of natural communities in the more interior dunes.

Recreation

Recreation activities severely impact dune systems and are often responsible for the damage and even destruction of habitats (Acosta et al., 2013). Sandy beaches are the most popular areas of the seashore. In fact, more people use sandy beaches than any other type of marine or coastal habitat. As a consequence, recreation activities are tremendously concentrated (Defeo et al., 2009), which implies a high density of occupation. These activities include sunbathing, swimming and other activities such as walking, windsurfing and kite surfing (Leewis et al., 2012). Recreation activities produce various impacts on the natural environment with extreme negative effects, one of the most important being trampling by animals and humans and crushing by vehicles (Liddle and Greig-Smith, 1975; Defeo et al., 2009).

Dune vegetation is adapted to the stressful natural conditions and highly dynamic environment of sandy seashores; however, it is very vulnerable to man-made disturbances such as those connected to trampling (Liddle and Greig-Smith, 1975; Defeo et al., 2009; Acosta et al., 2013). The most obvious direct impacts of trampling on vegetation originate from the crushing and shearing of plants which decreases the fitness of certain species (Gallet and Rozé, 2001 in Acosta et al., 2013) and thus reduces biodiversity (Bonte and Hoffman, 2005 in Acosta et al., 2013; Hesp et al., 2010). On the other hand a permanent loss of vegetation cover may occur in cases of high intensity of trampling. That is, trampling intensity has a profound influence on the establishment of bare ground (Hesp et al., 2010). The destruction of plants that trap and hold sand particles exposes the underlying sand to the onshore wind, promoting the development of breaches, called blowouts. After a blowout is initiated, its margins continue to erode which results in extensive areas of open sand within what was previously a stabilized zone. Moreover, trampling destroys the complex spatial structure of the dune ecosystem that results from the strong environmental gradients (Acosta et al., 2013). As trampling is the result of heavy but usually seasonal demand at medium-high latitudes, there may be some opportunity of recovery during periods of low use. Sousa (2010) refers that during periods of low use vegetation develops in footpaths but is later destroyed by holidaymakers during high use season. Acosta et al. (2013) mention that dune vegetation, if protected from trampling, recovers fast as regards biodiversity, cover and spatial structure. The capacity for rapid recovery is a distinctive characteristic of dune plant species which are indeed adapted to high levels of disturbance. However, if certain thresholds are reached, irreversible damage can occur and then recovery is unlikely to happen (Alveirinho-Dias et al., 1994 in Curr et al., 2000).

Gheskiere et al. (2005) demonstrated, both for a Mediterranean as well as for a Baltic coastal sandy beach, that tourism related activities affect the meio-nematofauna and contribute to higher stress levels, lower species diversity of the nematode assemblages compared to nearby untouched beaches.

Off-road vehicles (motorcycles, 4 x 4 vehicles and vehicles of the buggy type) are usually used on beaches and dunes all over the world for recreation (Sclacher et al., 2008). Due to weight and type of motion, the damage caused by these vehicles is more widespread than that caused by human trampling (Westhoff 1967 in Kindermann and Gormally, 2010). The negative impacts they cause include damage of the physical properties and stability of the substrate, destruction of vegetation and disturbing, injuring or killing fauna (Brown and McLachlan, 2002; Defeo et al., 2009). Direct

mortality affects not only vertebrates like turtles and birds (destruction of eggs and young) but also invertebrates such as isopods, amphipods, crabs and certain echinoderms (Brown and McLachlan, 2002; Martin et al., 2006 in Defeo et al., 2009). The passage of motorised vehicles causes severe damage, the dune system takingyears to recover.

Exotic species

The introduction of exotic vegetation on coastal dunes was, in a not distant past, a common practice along much of the world's coastlines for the purpose of stabilizing the sand substrate as is the case of *Casuarina equisetifolia* L. in the Indian Ocean and Caribbean Sea regions, *Tamarix gallica* L. in the Gulf of Mexico and *Acacia* spp. in the Mediterranean (Cronk and Fuller, 2001 in Feagin et al., 2010). Thus, nowadays, coastal sand dunes stand as some of the most threatened ecosystems due to the invasion of alien plant species (Gallego-Fernández et al., 2006). Almost always short-term stabilization of the sand was reached at the expense of long-term ecological sustainability (Feagin et al., 2010). In fact, the presence of invasive species not only alters community composition leading to a decrease in biodiversity, but also affects ecosystem functioning and structure (Gallego-Fernández et al., 2006). *Acacia* species, nitrogen-fixing plants, fill a niche that is vacant on dunes, characterized by nitrogen-poor soil (Parker et al., 1999). Nonetheless, these alien species alter soil characteristics and processes, namely soil C and N pools, which disrupt colonization by native plants (Marchante et al., 2008).

In Portugal, as happened in other parts of the world, management of coastal areas focused, for a long time, on sand fixation. In fact, in coastal areas denuded of vegetation sand was set freely in movement by the wind and was carried inland, causing serious threat to farmlands and infrastructures. Several *Acacia* tree species (*Leguminosae*) were introduced to stabilize the sand (Marchante et al., 2009). Successful colonization resulted in the growth of woodlands dominated by these exotic species, which had a consequent loss of native species diversity. On the other hand, acacia trees, covering almost the entire soil surface, brought over-stabilisation of the dunes, which resulted in the loss of bare sand patches (Marchante et al., 2009). That is, these invasive species changed the environment of the dune, which is naturally dynamic. Currently, it is documented that over-stabilised dune systems are disadvantageous (Pye et al., 2007) because they are unable to change as a consequence of fluctuations of natural forcing factors. For instance, they are incapable of supplying sand to beaches in periods of need (Lubke, 2004). The introduction of exotic plants can also be harmful to the native fauna. In India, sandy coastline in many areas has been flattened to make way for plantations with exotic species, thus destroying sea turtle nesting habitat and altering dune topography which can have serious negative impacts on coastal protection ability (Feagin et al., 2012).

Well-preserved coastal dune systems provide effective defence against the sea and at a lower cost than massive and rigid engineering interventions, although dune stabilization throughout the use of exotic species does not seem to us the proper form of management of coastal areas.

Global climate change

In addition to direct anthropogenic impacts, global climate change is expected to have intense, extensive and long-lasting consequences for the coastal systems, particularly when coastlines are retreating in response to rising sea levels (Feagin et al. 2005; Harley et al., 2006 in Schacher et al., 2008). The expected rise in sea level, if coupled with an increase in the frequency and/or intensity of storms, as predicted for some regions, is likely to lead to escalating erosion and consequent loss of habitat (Brown and McLachlan, 2002).

Pollution

Pollution is defined by the Directive 2000/60/EC of the European Parliament and of the Council of 23th October 2000 as "the direct or indirect introduction, as a result of human activity, of substances or heat into the air, water or land which may be harmful to human health or the quality of aquatic ecosystems or terrestrial ecosystems directly depending on aquatic ecosystems, which result in damage to material property, or which impair or interfere with amenities and other legitimate uses of the environment" (OJEC, 2000).

Coastal zones, particularly those with large population densities received large inputs of pollutants from anthropogenic origin (domestic and industrial sewages) despite airborne particulates from distant industrial sources. For example, trace metals were likely transported from the industrial sites to the area of their deposition as sulphur-bearing coatings on small anthropogenic particles. After deposition, these sulphur-bearing compounds reacted with organic matter within the sediment (Mastalerz et al., 2001). In that sense, several studies have been undertaken in different coastal world areas, in order to evaluate the PCBs, PAHs

and heavy metal contamination degree of fauna, flora and substrata. For example, in Portugal mainland, estuarine sediments of Tejo River (Reboredo, 1981; Reboreda and Caçador, 2007), Sado River (Reboredo and Ribeiro, 1984; Reboredo, 1988) Ria Formosa Lagoon (Padinha et al., 2000) Ria de Aveiro Lagoon (Ramalhosa et al., 2005) and Lima River (Almeida et al., 2011), were monitored in terms of their metal content.

Also, some representative halophytic species colonizing the substrata such as *Halimione portulacoides* and *Spartina maritima* (Reboredo, 1983, 1985, 1993; Padinha et al., 2000; Reboreda and Caçador, 2007), or the former species plus *Sarcocornia fruticosa* and *Sarcocornia perennis* (Duarte et al. 2010) or even *Juncus maritimus*, *Spartina patens*, *Phragmites australis* and *Triglochin striata* (Almeida et al., 2011) were evaluated in terms of their metal content.

Although these halophytes were not representative of the dune vegetation, in some cases may live in close contact with the dune formation, which is the case of *Halimione portulacoides* colonizing a sandy environment in particular areas of Sado river estuary (Reboredo, 1988, 1992).

Thus, dunes, as interface ecosystems between land and sea, receive and suffer from a wide range of pollutants and debris, from land and/or sea origin. Figure 1 describes the most common pollutants identified in dune systems, and its main sources. Defeo et al. (2009) summarize, by a schematic diagram the variety of spatial and temporal scales of pollutants acting on sandy beach macrofaunal living communities. This conceptual model eventually can be applied to dunes systems because, according to Defeo et al. (2009), a variety of anthropogenic materials, ranging in size from molecules to large debris, can be found in dune sands impairing the physiology, survival, reproduction and behaviour of dune species.

Metals, coming from wastewater and industry tends to accumulate on fine-grained sands (reference in Defeo et al., 2009) or are carried to underwater streams by run-off, although the binding capacity of sand was weak compared with substrata with high organic and silt-clay contents (Reboredo and Pais, 1984) Fine-grained beaches usually support relatively high diversity and biomass (Defeo et al., 2009), and metal pollution may cause significant reductions both in biodiversity and in the population density of economically valuable species (references in Defeo et al., 2009), by high levels inputs or by cumulative processes.

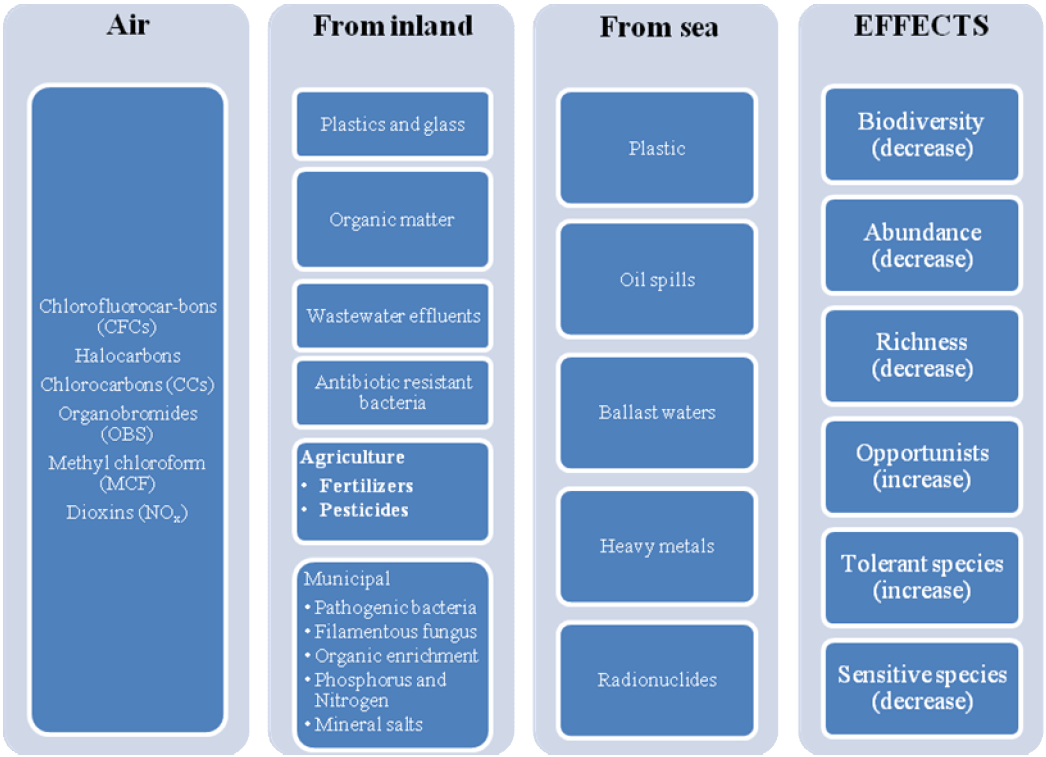

Figure 1. Most common pollutants found in dunes and their main effects in dune community systems.

Debris

Coastal areas, on one hand, receive litter coming from the sea, brought by currents and waves, and, on the other hand, litter left by beach visitants. These are known as anthropogenic marine debris (AMD).

A study from Santos et al. (2005) concerning litter composition found on beaches and dunes show that small fragments of plastics, cigarette butts, organic matter and wood compose the most abundant residues. This study showed that residues of fruits, corncob and corn leaves were the main organic matter components, whilst matches and ice cream sticks were the most representative residues of wood. A study of Madzena and Lasiak (1997) found Styrofoam as the second most important item after plastic. Cigarette butts are usually found mixed with sand and, according to Derraik (2002) referred by Santos et al. (2005), are mainly related to tourism activities.

According to Bravo et al. (2009), AMD is a ubiquitous problem, which has motivated public participation in activities such as beach surveys and clean-up campaigns. A study carried out by Bravo et al. (2009) along the Chilean coast showed that public participation in surveys and cleaning activities raised awareness and thereby contributed to an improvement of the situation. However, Bravo et al. (2009) refer that the quantities and types of AMD in beaches and coastline systems remain largely unknown.

Plastic and glass

Plastics are synthetic organic polymers, their existence being relatively recent (Gorman, 1993 in Derraik, 2002). Being lightweight, strong, durable and cheap (reference in Derraik, 2002) and with large versatility, led to a great increase in their use into all aspects of everyday life (references in Derraik, 2002) over the past decades. Plastic debris may be dispersed over long distances, because they are buoyant (Derraik, 2002; Aliani et al., 2003). They may reach the dunes by wave action, or being part of the litter deposited by beach visitants or even by wastewater effluents. Drifting plastic debris was reported by Masó et al. (2003) to be a potential vector for dispersing Harmful Algal Blooms (HAB) species.

Plastic objects dominate the visible litter on sandy beaches worldwide (references in Defeo et al., 2009; Bravo et al., 2009), constituting a danger since they may persist for centuries (references in Derraik, 2002). Plastics also constitute a potential smothering agent when swallowed by animals, nor only by swimmers like turtles and sperm whales (Stephanis et al., 2013) but also by land animals, found in dune systems.

Glass bottles have been recorded to cause wounds in humans (Santos et al., 2005) and animals crossing dune systems. Madzena and Lasiak (1997), in a study on the South African coastline, found that glass was the third most important item of the composition of the abandoned litter, both in terms of number and weight.

Organic matter

People using beaches frequently leave organic matter such as food leftovers. Dunes are often used as deposits of dog (Santos et al., 2005) and human excrements, even when there are sanitary infrastructures or waste bins in the vicinity. This organic matter causes contamination of sand with pathogenic bacteria and filamentous fungus, gives bad smell and causes aesthetic disturbances. It also constitutes a source of attraction to numerous unwanted insects like wasps and flies. The organic matter constitutes a source of enhanced nutrient inputs, especially nitrogen, which causes a change in vegetation communities, with the appearance of nitrophilous species.

Antibiotic resistant bacteria

The uncontrolled and extensive use of pharmaceutical substances mainly antibiotics in human and veterinary medicine, animal husbandry, agriculture and aquaculture has caused the increased introduction of those antimicrobial agents in the aquatic environment (references in Mudryk et al., 2010). The study of Mudryk et al. (2010) conducted at the Southern Baltic Sea coast, showed that the majority of bacteria inhabiting the sand of the studied beach were resistant to only one antibiotic out of 18 tested antibiotics in the study. This study also showed that the bacteria inhabiting the middle part of the beach and the dune were more antibiotic resistant than bacteria isolated from the seawater and the shoreline-seawater contact zone. These results also show a no significance difference in antibiotic resistance between bacteria isolated from the surface and the subsurface sand layers. The authors conclude that: a). the antibiotics are a significant selection factor and probably play an important role in regulating the composition of bacterial communities of the marine beach; b). human activities might contribute to the level of antibiotic resistant bacteria inhabiting such sites; c). the occurrence of resistant bacteria in the sand of marine beaches can possibly be treated as an alternative bioindicator of marine sand

contamination influenced by anthropogenic activities and of the returning rate of resistance genes to the human population through the seawater and the marine sand beach usage.

Wastewater and sewage systems and freshwater effluents

Pathogenic bacteria and filamentous fungus can be found in beaches (Defeo et al., 2009) and sand dunes from wastewater and sewage systems by illegal discharge despite the existence of the wastewater treatment stations, as European legislation demands. This is a serious public health especially when health standards limits are exceeded which may cause beach closures (Defeo et al., 2009).

Freshwater effluents mostly charged with organic matter and mineral salts may cause a broader deterioration in the quality of the surrounding habitat (reference in Defeo et al., 2009). These effluents, arising from human activities, cause negative impacts at the survival, growth and fecundity rates (references in Defeo et al., 2009) of individuals and populations of dune species, changes in diversity and structure of the communities (reference according to Defeo et al., 2009).

Radionuclides

Radionuclides become a part of the soil, or as part of the Earth's original crust, or produced and deposited by cosmic ray interactions, or through man-made releases (EPA, 2006). Copplestone et al. (2001) examined the spatial, temporal and depth distributions of radionuclide (134Cs, 137Cs, 238Pu, 239+240Pu and 241Am) activity concentrations in the soil and in two dune plant species (*Festuca rubra* – red fescue and *Ammophila arenaria* – marram grass), in the vicinity of the British Nuclear Fuels, receiving a continuous input of entrained radionuclides, principally via sea-to-land transfer. From this study, Copplestone et al. (2001) found the greater activity concentrations in deeper regions of the soil profile, due to the leaching of radionuclides in percolating drainage water accentuated by the coarse texture, low organic matter and clay mineral content of coastal sands. The results also showed, in soil, a similar spatial distribution for all radionuclides studied, despite differences in the vegetation cover and a little evidence of any temporal changes over the study period. Copplestone et al. (2001) study found that the dominant influence of external contamination was by adherence to the plant foliage and not exclusively by root uptake. So, according to Copplestone et al. (2001) work, the transfer of radionuclides through higher trophic levels of grassland ecosystems occurs mainly by external adherence to plant foliage.

Oil pollution

Oil pollution causes a great public attention because it has a visible impact on shores, especially on bathing beaches and on aquaculture farming, and causes serious ecological and socio-economical negative impacts on coastlines reached by oil spills. They result from accidents like vessel collision, run aground ships, pipeline ruptures, petroleum shafts/platforms accidents, or during armed conflicts (i.e. Great Gulf War) (Wieczorek et al., 2007).

The oil spill reaching the coast spreads over the surface of the sediment and may go deeper in the sediment during days or weeks following the spill event (reference in Fernández-Fernández et al., 2011). Regarding the subsurface contamination two mechanisms have been described (Fernández-Fernández et al., 2011): oil penetration and oil burial. Oil penetration is driven by the percolation of oil within the sediment (Fernández-Fernández et al., 2011). This type of contamination depends on sediment texture, mean grain size and oil physical properties (viscosity), wave energy, temperature and other factors, including fungal degradation (references in Defeo et al., 2009; Fernández-Fernández et al., 2011), generating low level oiling, up to sixty centimetre in depth (Fernández-Fernández et al., 2011). In contrast, oil burial generates strong oil contamination, up to several meters in depth (Fernández-Fernández et al., 2011). The results of this study also revealed from fieldworks that the oil was still present on beaches, seven years after an oil spill.

According to Sinderman (1996) (referred by Defeo et al., 2009), sheltered beaches are usually more sensitive to pollution than exposed ones, even when sediments are fine and oil does not penetrate deeply, since they are less well flushed by wave action. Dunes in its ocean-exposed side, can suffer oil spill impact and vegetation may be used to protect coastlines against petroleum spreading to the inner regions.

The oil spill in the Gulf of Mexico was the largest accidental marine release in the petroleum era (reference in Brame et al., 2013), as a result of the explosion at the *Deepwater Horizon* drilling rig and subsequent oil spill in April of 2010 (references in Brame et al., 2013) off the coast of Louisiana (Harlow et al., 2011). The ensuing oil spill caused

substantial economic and environmental damage to states on the U.S. Golf Coast (Harlow et al., 2011).

Prestige oil tanker sank off on the 19th of November 2002 off the NW coast of Spain, spilling approximately 64000 tons of heavy fuel oil (Fernández-Fernández et al., 2011), about twice the size of the Exxon Valdez spill off the Alaska in 1989, and is already the worst shipping disaster off Spain since the tanker *Aegean Sea* ran aground near La Coruña in 1992 (Junoy et al., 2005). The oil spill affected approximately 1000 km of coast belonging to Portugal, France and Spain and more than 600 sandy beaches were polluted along the Atlantic coast of Spain (Fernández-Fernández et al., 2011) having serious socio-economical impacts on shellfish farming of Rias Galegas. As a result of the Prestige oil-spill and the clean-up activities, beach populations were reduced, with *Eurydice* and *S. squamata* as the most affected taxa of the macroinfauna of the Galician sandy beaches affected by the Prestige oil-spill (Junoy et al., 2005).

Management

The coastal zone is strategically important from environmental, economic and societal points-of-view (Veloso-Gomes et al., 2008). Therefore, solving or mitigating some of its problems is of vital consideration when shaping policy for sustainable development, and needs integrated and co-ordinated management policies (Veloso-Gomes et al., 2008).

Dunes, because of their very position at the border between land and sea, are attractive for many human activities (Van der Meulen et al., 2004). Unfortunately coastal systems have had a long history of exploitation and mismanagement (Martínez et al., 2004b). The three primary ecological services coastal areas provide are defence, nature conservation and recreation. However, human activities on these systems introduce conflicting uses that hamper some of the services. Human populations and their associated activities on the coast are expected to increase in the coming decades (Brown and McLachlan, 2002; Martínez et al., 2004a), placing bigger pressure on the coastline. This increased pressure may well be mitigated by improved legislation and management practices resulting from a better understanding of coastal dune ecosystems. In the past coastal regions have often been inappropriately managed mainly due to an absence of adequate planning (Carboni et al., 2009). Even nowadays, in general, ecological aspects as well as suitable policies towards sustainability are still missing (Veloso-Gomes et al., 2004). How should coastal systems be managed so that their geomorphological and ecological characteristics are preserved and are thus able to provide the maximum of goods and services for future generations? (Feagin et al., 2010).

Management of coastal areas is undoubtedly a complex issue (Silva et al., 2007). It should be conducted in an integrated, sustainable and flexible way, so as to be able to answer to the challenges brought up by such a dynamic but sensible environment. Recently there has been a change in approach as regards dune ecosystems management: it should take into account the various aspects (physical as well as the biological, social and economic aspects) (Weinstein et al., 2007 in Macedo, 2008), the dynamic nature of the system, and operate within a long-term strategic framework at regional and national level. This strategy requires an understanding of the morphological and sedimentological features of the dune system and the knowledge of the main processes acting upon the ecosystem and prediction of future changes. Poorly planned development can increase the exposure of coastal communities to extreme events, particularly where such development is encouraged or unregulated (Feagin et al., 2010). Management should aim at maintaining the diversity and the natural dynamics of these ecosystems (Avis, 1992 in Martínez et al., 2004b).

It is known that tourism is currently a major contributor to the occupation of the coast. In Spain beach tourism is responsible for 74% of foreign tourism (Yepes, 1998 in Silva et al., 2007). Tourism is an important economic activity in Portugal, representing about 4.2% of national GDP and employing close to 5% of the workforce (Veloso-Gomes and Taveira-Pinto, 1997). The economic importance beaches have nowadays requires that recreation is included as an important component in management. Tourism was for a long time considered as a clean activity with almost no negative effects on the environment (Gheskiere et al., 2005). However, it has major impacts on coastal dune systems, which must be evaluated and monitored.

If one ecological service is over-valuated at the expense of the other services, as happens with coastal protection, it may be eventually chosen to replace dune ecosystems by heavy man-made structures (Koch et al., 2009) or in other areas to plant exotic tree species for sand stabilization. These measures may no doubt be short-sighted because they reduce or even prevent adaptive capacity of the coastal systems. Thus, over the long-term they may prove ineffective not only for

the purpose they intended to tackle but also they may preclude other ecological services (Acosta et al., 2013).

In face of the numerous threats endangering coastal habitats the need for monitoring and active management of these environments has emerged (Carboni et al., 2009). Vegetation plays an essential role in determining the size, shape and stability of dune systems (Kim, 2005). In fact, vegetation quality is essential to maintain the integrity of dunes, since plants trap and stabilize sand particles against water and wind erosion. It is thus fundamental to preserve the natural vegetation cover so that coastal dune ecosystems are able to provide the full range of services (Martínez et al., 2004a). Wherever possible, the management of coastal areas should consider the succession of plant communities that occur in the dune system, from pioneer stages near the sea to the mature stages inland. These communities need to be managed as a whole, as there is a close interdependence of all the dune succession stages. Only then can the dune system adjust to erosion processes, whether natural or man-induced. Mobile dunes are part of the coastal system, with unique flora and fauna, and should be allowed to function normally which means that natural sand movements ought to be permitted (Richie, 2001 in Howe et al., 2010).

An essential aspect of management of coastal areas is thus the monitoring of vegetation changes, as vegetation composition and cover manifest the sedimentary dynamics of the system (Levin et al, 2007). To accomplish this goal reliable indicators of the condition of vegetation in coastal ecosystems must be developed (Espejel et al., 2004). The study of key species represents a suitable and an importantapproach (Gonçalves et al., 2013). Macedo (2008) suggests, based on a study performed in the northwest coast of Portugal, that, in the framework of monitoring protocols, shifts in the floristic composition of plant dune communities as regards functional traits can be used as consistent indicators of the state of coastal systems and consequently of their dynamics. Compositional shifts in phytosociological or functional spectra may be useful for the establishment of indicators of change of coastal dynamics (García-Mora et al., 1999; Lomba et al., 2008).

Conclusions

Coastal zones constitute complex interface ecosystems between the land and sea and are among the most productive systems in the world. As they provide important services and goods, they have long been used for many different human activities. However, many of these activities result in more or less severe impacts on the coastal ecosystems. Pollution, urbanization, disruption of sand transport, tourism and climate change seriously affects sandy shores. Coastal systems should be managed so that their geomorphological and ecological characteristics are preserved for future generations. This implies the existence of well-preserved coastal dune systems, since plants trap sand and stabilize the dunes. Vegetation monitoring is thus essential, which can be accomplished using consistent indicators of the state of coastal systems and consequently of their dynamics.

References

Acosta, A., M. L. Carranza and C. F. Izzi. 2005. Combining Land cover mapping for coastal dune with vegetation analysis. Appl. Veg. Sci. 8:133–138.

Acosta, A., M. L. Carranza and C. F. Izzi. 2009. Are there habitats that contribute best to plant species diversity in coastal dunes? Biodivers. Conserv. 18:1087–1098.

Acosta, A., T. Jucker, I. Prisco and R. Santoro. 2013. Passive Recovery of Mediterranean Coastal Dunes Following Limitations to Human Trampling. In: M. L. Martínez, J. B. Gallego-Fernández and P. A. Hesp (Eds.). Restoration of Coastal Dunes. Springer-Verlag. ISBN: 978-3-642-33444-3 (Print) 978-3-642-33445-0 (Online).

Aliani, S., A. Griffa and A. Molcard. 2003. Floating debris in the Ligurian Sea, North-western Mediterranean. Mar. Pollut. Bull. 46:1142-1149.

Almeida, C. M. R., A. P. Mucha and M. T. Vasconcelos. 2011. Role of different salt marsh plants on metal retention in an urban estuary (Lima estuary, NW Portugal). Estuar. Coast. Shelf Sci. 91:243–249.

Brame, J. A., S. W. Hong, J. Lee, S-H. Lee and P. J. J. Alvarez. 2013. Photocatalytic pre-treatment with food-grade TiO_2 increases the bioavailability and bioremediation potential of weathered oil from the *Deepwater Horizon* oil spill in the Gulf of Mexico. Chemosphere 90:2315-2319.

Braun-Blanquet, J., G. Braun-Blanquet, A. Rozeira and A. R. Pinto da Silva. 1972. Résultats de Trois Excursions Géobotaniques à travers le

Portugal Septentrional et Moyen. IV. Esquisse sur la végétation dunale. Agron. Lusit. 33:217-234.

Bravo, M., M. A. Gallardo, G. Luna-Jorquera, P. Núñez, N. Vásquez and M. Thiel. 2009. Anthropogenic debris on beaches in the SE Pacific (Chile): Results from a national survey supported by volunteers. Mar. Pollut. Bull. 58:1718-1726.

Brown, A. C. and A. McLachlan. 2002. Sandy shore ecosystems and the threats facing them: some predictions for the year 2025. Environ. Conserv. 29:62-77.

Carboni, M., M. L. Carranza and A. Acosta. 2009. Assessing conservation status on coastal dunes: A multiscale approach. Landscape Urban Plan. 91:17–25.

Carranza, M. L., A. T. R. Acosta, A. Stanisci, G. Pirone and G. Ciaschetti. 2008. Ecosystem classification for EU habitat distribution assessment in sandy coastal environments: An application in central Italy. Environ. Monit. Assess. 140:99-107.

Carter, R. W. G. 1988. Coastal Environments - An Introduction to the Physical, Ecological and Cultural Systems of Coastlines. Academic Press Limited, London.

Castillo, S. A. and P. Moreno-Casasola. 1996. Coastal sand dune vegetation: an extreme case of species invasion. J. Coast. Conserv. 2:13-22.

Ciccarelli, D., G. Bacaro and A. Chiarucci. 2012. Coastline dune vegetation dynamics: evidence of no stability. Folia Geobot. DOI 10.1007/s12224-011-9118-5.

CNADS (Conselho Nacional do Ambiente e do Desenvolvimento Sustentável). 2001. Reflexão sobre o Desenvolvimento Sustentável da Zona Costeira. Lisboa, CNADS: 57.

Copplestone, D., M. S. Johnson and S. R. Jones. 2001. Behaviour and transport of radionuclides in soil and vegetation of a sand dune ecosystem. J. Environ. Radioactiv. 55:93-108.

Costa, J. C., D. Espírito Santo and M. Lousã. 1994. The vegetation of dunes of Southwest Portugal. Silva Lusitana 2:51-68.

Costa, J. C., J. H. Capelo, M. F. Lousã and C. Aguiar. 1993. Communautés de *Juniperus* au Portugal. Colloq. Phytososiol. 22:499-526.

Curr, R. H. F., A. Koh, E. Edwards, A. T. Williams and P. Davies. 2000. Assessing anthropogenic impact on Mediterranean sand dunes from aerial digital photography. J. Coast. Conserv. 6:15-22.

Defeo, O., A. McLachlan, D. S. Schoeman, Th. A. Schlacher, J. Dugan, A. Jones, M. Lastra and F. Scapini. 2009. Threats to sandy beach ecosystems: a review. Estuar. Coast. Shelf Sci. 81:1-12

Derraik, J. G. B. 2002. The pollution of the marine environment by plastic debris: a review. Mar. Pollut. Bull. 44:842-852.

Dias, J. M. A. 2004. A história da evolução do litoral português nos últimos vinte milénios. In: A. A. Tavares, M. J. F. Tavares and J. L. Cardoso (Org.). pp. 157-170. Evolução Geohistórica do Litoral Português e Fenómenos Correlativos: Geologia, História, Arqueologia e Climatologia. Lisboa.

Dias, J. A. 2005. Evolução da zona costeira portuguesa: forçamentos antrópicos e naturais. Rev. Encontr. Cientif. 1:7-27.

Dias, J. A., A. Cearreta, F. I. Isla and M. M. de Mahiques. 2013. Anthropogenic impacts on Iberoamerican coastal areas: historical processes, present challenges, and consequences for coastal zone management. Ocean Coastal Manage. 77:80–88.

Doing, H. 1985. Coastal fore-dune zonation and succession in various parts of the world. Vegetatio 61:65-75.

Duarte, B., M. Caetano, P. R. Almeida, C. Vale and I. Caçador. 2010. Accumulation and biological cycling of heavy metal in four salt marsh species, from Tagus estuary (Portugal). 158:1661–1668.

Edgar, G. J., L. Kerrison, S. A. Shepherd and M. V. Toral-Granda. 2003. Impacts of the *Jessica* oil spill on intertidal and shallow subtidal plants and animals. Mar. Pollut. Bull. 47:276-283.

EEA (European Environmental Agency). 2006. The changing faces of Europe's coastal areas. EEA Report No 6/2006. EEA-European Environment Agency, Copenhagen.

Espejel, I., B. Ahumada, Y. Cruz and A. Heredia. 2004. Coastal Vegetation as Indicators for Conservation. In: Martínez, M. L. and N. P. Psuty (Eds.). pp. 297-318. Coastal Dunes. Ecology and Conservation. Springer-Verlag. Berlin. Heidelberg. New York.

EPA (Environmental Protection Agency). 2006. Radionuclides in soil. Office of Radiation and Indoor Air (6608J). EPA 402-F-06-051. [www.epa.gov/radtown/soil.html].

Feagin, R. A., D. J. Sherman and W. E. Grant. 2005. Coastal erosion, global sea-level rise, and the loss of sand dune plant habitats. Front. Ecol. Environ. 3(7):359–364.

Feagin, R. A., N. Mukherjee, K. Shanker, A. H. Baird, J. Cinner, A. M. Kerr, N. Koedam, A. Sridhar, R. Arthur, L. P. Jayatissa, D. L. Seen, M. Menon, S. Rodriguez, M. Shamsuddoha and F. Dahdouh-Guebas. 2010. Shelter from the storm? Use and misuse of coastal vegetation bioshields for managing natural disasters. Conserv. Lett. 3:1–11.

Fenu, G., M. Carboni, A. T. R. Acosta and G. Bacchetta. 2012. Environmental factors influencing coastal vegetation pattern: new insights from the Mediterranean Basin. Folia Geobot. DOI 10.1007/s12224-012-9141-1.

Fernández-Fernández, S., A. M. Bernabeu, F. Bouchette, D. Rey and F. Vilas. 2011. Beach morphodynamic influence on long-term oil pollution: the *Prestige* oil spill. J. Coastal Res. 64:890-893.

Ferreira, Ó., J. Dias, C. Cama and R. Taborda. 1995. Quantification of beach erosion caused by storms on the Portuguese coast. Directions in European Coastal Management. Healy and Doody (Eds.), Samara Publishing Limited, Cardigan. ISBN 1 873692064.

Freire, S., T. Santos, T. and J. A. Tenedório. 2009. Recent urbanization and land use/land cover change in Portugal – the coastline and coastal urban centers. The J. Coastal Res. Sp. Issue 56:1499-1503.

Freitas, J. I. R. G. 2010. O litoral português na época contemporânea: representações, práticas e consequências. Os casos de Espinho e do Algarve (c. 1851 a c. de 1990). Doutoramento em História. Faculdade de Letras da Universidade de Lisboa.

Gallego-Fernández, J. B., S. Muñoz Vallés and C. Dellafiore. 2006. Introduction of exotic plants caused by beach and dune management. pp. 335-364.

García-Mora, M. R., J. B. Gallego-Fernández and F. García-Novo. 1999. Plant functional types in coastal foredunes in relation to environmental stress and disturbance. J. Veg. Sci. 10:27-34.

Gheskiere, T., M. Vincx, J. M. Weslawski, F. Scapini and S. Degraer. 2005. Meiofauna as descriptor of tourism-induced changes at sandy beaches. Mar. Env. Res. 60:245–265.

Gómez-Pina, G., J. J. Muñoz-Pérez, J. L. Ramírez and C. Ley. 2002. Sand dune management problems and techniques, Spain. J. Coastal Res. Sp. Issue 36:325-332.

Gonçalves, S. C., P. M. Anastácio and J. C. Marques. 2013. Talitrid and Tylid crustaceans bioecology as a tool to monitor and assess sandybeaches' ecological quality condition. Ecol. Indic. 20:549–557.

González-Villanueva, R., S. Costas, M. Pérez-Arlucea, S. Jerez, S. And R. M. Trigo. 2013. Impact of atmospheric circulation patterns on coastal dune dynamics, NW Spain. Geomorphology 185:96–109.

Guara-Requena, M. 1989. La influencia de la distancia al mar en la distribucion de la flora de las dunas del Cabo de Gata. Acta Bot. Malacitana 14:151 – 159.

Harlow, W. F., B. C. Brantley and R. M. Harlow. 2011. BP initial image repair strategies after the *Deepwater Horizon* spill. Public Relat. Rev. 37:80-83.

Heslenfeld, P., P. D. Jungerius and J. A. Klijn. 2004. European coastal dunes: ecological values, threats, opportunities and policy development. In: M. L. Martínez, N. P. Psuty (Eds.). pp. 335-351. Ecological Studies, Vol. 171. Coastal Dunes, Ecology and Conservation. Springer-Verlag. Berlin. Heidelberg.

Hesp, P. 2002. Foredunes and blowouts: initiation, geomorphology and dynamics. Geomorph. 48:245–268.

Hesp, P., P. Schmutz, M. L. Martinez, L. Driskell, R. Orgera, K. Renken, N. A. R. Revelo and O. A. J. Orocio. 2010. The effect on coastal

vegetation of trampling on a parabolic dune. Aeol. Res. 2:105–111.

Honrado, J., R. Pereira, R. Araújo, G. Santos, J. Matos, P. Alves, H. N. Alves, I. S. Pinto and F. Barreto Caldas. 2002. Classification and mapping of terrestrial and intertidal vegetation in the Atlantic Coast of Northern Portugal. Littoral 2002, The Changing Coast. EUROCOAST / EUCC, Porto – Portugal. Ed. EUROCOAST – Portugal, ISBN 972-8558-09-0.

Howe, M. A., G. T. Knight, G. T. and C. Clee. 2010. The importance of coastal sand dunes for terrestrial invertebrates in Wales and the UK, with particular reference to aculeate Hymenoptera (bees, wasps & ants). J. Coast. Conserv. 14:91–102.

Junoy, J., C. Castellanos, J. M. Viéitez, M. R. de la Huz and M. Lastra. 2005. The macroinfauna of the Galician sandy beaches (NW Spain) affected by the *Prestige* oil-spill. Mar. Pollut. Bull. 50:526-536.

Kim, K. D. 2005. Invasive plants on disturbed Korean sand dunes. Estuar. Coast. Shelf S. 62:353–364.

Kindermann, G. and M. J. Gormally. 2010. Vehicle damage caused by recreational use of coastal dune systems in a Special Area of Conservation (SAC) on the west coast of Ireland. Coast Conserv. 14:173–188.

Koch, E. W., E. B. Barbier, B. R. Silliman, D. J. Reed, G. M. E. Perillo, S. D. Hacker, E. F. Granek, J. H. Primavera, N. Muthiga, S. Polasky, B. S. Halpern, C. J. Kennedy, C. V. Kappel and E. Wolanski. 2009. Non-linearity in ecosystem services: temporal and spatial variability in coastal protection. Front. Ecol. Environ. 7:29–37.

Leewis, L., P. M. van Bodegom, J. Rozema and G. M. Janssen. 2012. Does beach nourishment have long-term effects on intertidal macroinvertebrate species abundance? Estuar. Coast. Shelf S. 113:172-181.

Levin, N., G. J. Kidron and E. Ben-Dor. 2007. Surface properties of stabilizing coastal dunes: combining spectral and field analyses. Sedimentology 54:771–788.

Liddle, M. J. and P. Greig-Smith. 1975. A survey of tracks and paths in a sand dune ecosystem, I. Soils, II. Vegetation. J. Appl. Ecol. 12:893-930.

Liddle, M. J. and K. G. Moore. 1974. The microclimate of sand dune tracks: the relative contribution of vegetation removal and soil compression. J. Appl. Ecol. 11:1057–68.

Lomba, A., P. Alves and J. Honrado. 2008. Endemic Sand Dune Vegetation of the Northwest Iberian Peninsula: Diversity, Dynamics, and Significance for Bioindication and Monitoring of Coastal Landscapes. J. Coastal Res. 24-2B:113–121.

Lubke, R. A. 2004. Vegetation dynamics and succession on sand dunes of the eastern coasts of Africa. In: M. L. Martínez and N. Psuty (Eds). pp. 67-84. Coastal dunes: ecology and conservation. Springer-Verlag. Heidelberg.

MA (Millenium Ecosystem Assessment). 2005. Global Assessment Reports. Volume 1: Current State and trends. Chapter 19: Coastal systems. Island Press, Washington, DC.

Macedo, J. A. M. 2008. A functional approach to modelling vegetation environment relations in coastal sand dunes. Dissertação apresentada à Faculdade de Ciências da Universidade do Porto para a obtenção do grau de mestre em Ecologia Aplicada. Porto.

Madzena, A. and T. Lasiak. 1997. Spatial and Temporal variations in beach litter on the Transkei Coast of South Africa. Mar. Pollut. Bull. 34(11):900-907.

Marchante, E., A. Kjøller, S. Struwe and H. Freitas. 2008. Short- and long-term impacts of *Acacia longifolia* invasion on the belowground processes of a Mediterranean coastal dune ecosystem. Appl. Soil Ecol. 40:210-217.

Marchante, E., A. Kjøller, S. Struwe and H. Freitas. 2009. Soil recovery after removal of the N2-fixing invasive *Acacia longifolia*: consequences for ecosystem restoration. Biol. Invasions 11:813–823.

Martínez, M. L., N. P. Psuty, and R. A. Lubk. 2004a. A Perspective on Coastal Dunes. In: M. L. Martínez and N. P. Psuty (Eds). pp. 3-10. Coastal Dunes, Ecology and Conservation. Springer-Verlag. Berlin Heidelberg. Ecological Studies, Vol. 171.

Martínez, M. L., M. A. Maun and N. P. Psuty. 2004b. The Fragility and Conservation of the World's Coastal Dunes: Geomorphological,

Ecological, and Socioeconomic Perspectives. In: M. L. Martínez and N. P. Psuty (Eds). pp. 355-369. Coastal Dunes, Ecology and Conservation. Springer-Verlag Berlin Heidelberg. Ecological Studies, Vol. 171.

Martins, M. C., C. Neto, C. and J. C. Costa. 2013. The meaning of mainland Portugal beaches and dunes' psammophilic plant communities: a contribution to tourism management and nature conservation. Journal of Coastal Conservation, Published online 18 January. DOI 10.1007/s11852-013-0232-9.

Masó, M., E. Garcés, F. Pagès and J. Camp. 2003. Drifting plastic debris as a potential vector for dispersing Harmful Algal Blooms (HAB) species. Sci. Mar. 67(1):107-111.

Mastalerz, M., C. Souch, G. M. Filipelli, N. L. Dollar and S. M. Perkins. 2001. Anthropogenic organic matter in the Great Marsh of the Indiana Dunes National Lakeshore and its implications. Int. J. Coal Geol. 46:157-177.

Maun, M. A. 2009. The biology of coastal sand dunes. Oxford University Press, Oxford.

Moffett, M. D., A. McLachlan, P. E. D. Winter and A. M. C. De Ruyck. 1998. Impact of trampling on sandy beach macrofauna. J. Coast. Conserv. 4:87-90.

Mudryk, Z., P. Perliński and P. Skórczewski. 2010. Detection of antibiotic resistant bacteria inhabiting the sand of non-recreational marine beach. Mar. Pollut. Bull. 60:207-214.

Neto, C., J. C. Costa, J. Honrado and J. Capelo. 2007. Phytosociological associations and Natura 2000 habitats of Portuguese coastal dunes. Fitosociologia 44(2) suppl. 1:29-35.

Nobre, A. M. and J. G. Ferreira. 2009. Integration of ecosystem-based tools to support coastal zone management. J. Coastal Res. SI 56:1676-1680.

OJEC. 2000. Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 establishing a framework for Community action in the field of water policy. Official J. Eur. Commun. 327:1-72.

Padinha, C., R. Santos and M. T. Brown. 2000. Evaluating environmental contamination in Ria Formosa (Portugal) using stress indexes of *Spartina maritima*. Mar. Env. Res. 49:67–78

Parker, I. M., D. Simberloff, W. M. Lonsdale, K. Goodell, M. Wonham, P. M. Kareiva, M. H. Williamson, B. Von Holle, P. B. Moyle, J. E. Byers and L. Goldwasser. 1999. Impact: toward a framework for understanding the ecological effects of invaders. Biol. Invasions 1:3–19.

Pereira, A. R. 1996. The Alentejo and Algarve coasts in the portuguese framework. In: A. B. Ferreira and G. T. Vieira (Eds.). pp. 199-205. Fifth European Intensive Course on Applied Geomorphology – Mediterranean and Urban Areas, Departamento de Geografia, Universidade de Lisboa.

Pye K, S. E. Saye and S. J. Blott. 2007. Sand Dune Processes and Management for Flood and Coastal Defence. Part 4. Techniques for Sand Dune Management. R&D Technical Report FD1302/TR/4, Department for Environment, Food and Rural Affairs, London.

Ramalhosa, E., E. Pereira, C. Vale, M. Válega and A. C. Duarte. 2005. Distribution of mercury in the upper sediments from a polluted area (Ria de Aveiro, Portugal). Mar. Pollut. Bull. 50:682-697.

Ranwell, D. S. 1972. Ecology of salt marshes and sand dunes. Chapman and Hall, London.

Reboreda, R. and I. Caçador. 2007. Copper, zinc and lead speciation in salt marsh sediments colonized by *Halimione portulacoides* and *Spartina maritima*. Chemosphere 69:1655-1661.

Reboredo, F. 1981. Determinação de metais pesados em sedimentos do estuário do rio Tejo, por espectrofotometria de absorção atómica, por processo de chama e sem chama. Relatório Final de Estágio da Licenciatura em Biologia (Ramo Científico), Faculdade de Ciências da Universidade de Lisboa, pp. 54.

Reboredo, F. 1983. The importance of *Spartina maritima* in the recycling of metals from a polluted area of river Sado estuary. In: Biomass Utilization, W.A. Coté edit., Plenum publishers (New York), pp. 241-248.

Reboredo, F. and C. Ribeiro. 1984. Vertical distribution of Al, Cu, Fe and Zn in soil salt marshes of the Sado estuary, Portugal. Int. J. Env. Studies 23:249-253.

Reboredo, F. and J. Pais. 1984. Trace metal content of sediments of the salt marsh of Sado estuary

- Portugal. The importance of the organic matter in the binding of metal ions. Proc. 3rd Int. Symp. on the Interactions between Sediments and Water, Geneva (Switzerland), 28-31 August, pp. 276-279.

Reboredo F. 1985. Heavy metal analysis of sediments and live tissues of *Spartina maritima* from salt marshes of Sado estuary - Portugal. Proc. 5th Int. Conf. of Heavy Metals in the Environment, Athens (Greece), 10-13 September, pp. 330-333.

Reboredo F. 1988. Alguns aspectos sobre a acumulação de Fe, Cu e Zn por *Halimione portulacoides* (L.) Aellen. Dissertação de Doutoramento (Ph. D. Thesis), Faculdade de Ciências e Tecnologia/ Universidade Nova de Lisboa, pp. 165.

Reboredo F. 1992. Cadmium accumulation by *Halimione portulacoides* (L.) Aellen. A seasonal study. Mar. Environ. Res. 33:17-29.

Reboredo F. 1993. How differences in the field influence Cu, Fe and Zn uptake by *Halimione portulacoides* and *Spartina maritima*. Sci. Total Environ. 133 :111-132.

Reboredo F. and J. Pais. 2012. A construção naval e a destruição do coberto florestal em Portugal – do século XII ao século XX. Ecologia (Revista online da Sociedade Portuguesa de Ecologia) 4:31-42.

Reboredo F**.** and J. Pais. 2013. Evolution of forest cover in Portugal – from the XII to the end of the XX centuries. J. Forestry Res. *(in press)*

Santos, I. R., A. C. Friedrich, M. Wallner-Kersanach and G. Fillmann. 2005. Influence of socio-economic characteristics of beach users on litter generation. Ocean Coast. Manage. 48:742-752.

Schlacher, T. A., D. S. Schoeman, J. Dugan, M. Lastra, A. Jones, F. Scapini and A. McLachlan. 2008. Sandy beach ecosystems: key features, sampling issues, management challenges and climate change impacts. Mar. Ecol. 29:70–90.

Silva, C. P., F. L. Alves and R. Rocha. 2007. The Management of Beach Carrying Capacity: The case of northern Portugal. J. Coastal Res. Sp. Issue 50:135-139.

Short, A. D. and P. A. Hesp. 1982. Wave, beach and dune interactions in southeastern Australia. Mar. Geol. 48:259-284.

Sousa, C. V. P. 2010. Vulnerabilidade dos Sistemas Dunares da Praia do Meco. Dissertação para obtenção do grau de Mestre em Engenharia do Ambiente, Faculdade de Ciências e Tecnologia da Universidade Nova de Lisboa, Lisboa.

Stephanis, R., J. Giménez, E. Carpinelli, C-Gutierrez-Exposito and A. Cañadas. 2013. As main meal for sperm whales: Plastics debris. Mar. Pollut. Bull. 69(1–2):206–214.

Taveira-Pinto, F. 2004. The practice of coastal zone management in Portugal. J. Coast. Conserv. 10:147–158.

Thompson, L. M. C. and T. A. Schlacher. 2008. Physical damage to coastal dunes and ecological impacts caused by vehicle tracks associated with beach camping on sandy shores: a case study from Fraser Island, Australia. J. Coast. Conserv. 12:67–82.

Vallés, S. M. and J. Cambrollé. 2013. Coastal Dune Hazards. In: Ch. W. Finkl (Ed). pp. 491-510. Coastal Hazards. Springer.

Valpreda, E. and U. Simeoni. 2003. Assessment of coastal erosion susceptibility at the national scale: The Italian case. J. Coast. Conserv. 9:43-48.

Van Der Maarel, E. 2003. Some remarks on the functions of European coastal ecosystems. Phytocoenologia 33(2–3):187–202.

Van der Maarel, E. and M. van der Maarel-Versluys. 1996. Distribution and conservation status of littoral vascular plant species along the European coasts. J. Coast. Conserv. 2:73-92.

Van der Meulen, F., T. W. M. Bakker and J. A. Houston. 2004. The costs of our coasts: examples of dynamic dune management from western Europe. In: M. L. Martínez and N. P. Psuty (Eds.). pp. 297-318. Coastal Dunes. Ecology and Conservation. Springer-Verlag. Berlin Heidelberg, N. Y.

Veloso-Gomes, F., F. Taveira-Pinto, L. das Neves, J. Pais Barbosa and C. Coelho. 2004. Erosion risk levels at the NW Portuguese coast: The Douro mouth - Cape Mondego stretch. J. Coast. Conserv. 10:43-52.

Veloso-Gomes, F., A. Barroco, A. R. Pereira, C. S. Reis, H. Calado, J. G. Ferreira, M. C. Freitas and M. Biscoito. 2008. Basis for a national strategy for integrated coastal zone management in Portugal. J. Coast. Conserv. 12:3–9.

Weslawski, J. M., A. Stanek, A. Siewert and N. Beer. 2000. The sandhopper (Talitrus saltator, Montagu 1808) on the Polish Baltic coast. Is it a victim of increased tourism? Oceanol. St. 29:77-87.

Wieczorek, A., D. Dias-Brito and J. C. C. Milanelli. 2007. Mapping oil spill environmental sensitivity in Cardoso Island State Park and surrounding areas, São Paulo, Brazil. Ocean Coast. Manage. 50:872-886.

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REVIEW ARTICLE

Quality assessment of Arabica and Robusta green and roasted coffees – A review

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Abstract

This review is a synopsis on coffee quality assessment of green and roasted coffee beans of *Coffea arabica* and *Coffea canephora*. The particle size, medium sieve, most frequent sieve, share split, cumulative calibration, trade homogeneity, mass of 1000 beans, apparent density, strange bodies and defects, mass losses on drying, olfactory and visual parameters, chromatic parameters, soluble solids, pH and chemical characterization (chlorogenic acids, caffeine and trigonelline) is described and evaluated, considering the most important factor associated to the coffee trade, according to a technological perspective.

Keywords: Arabica coffee, Green coffee, Quality, Roasted coffee, Robusta coffee

Abbreviation list: 3-CQA – 3-O-caffeoylquinic acid; 3-FQA – 3-O-feruloylquinic acid; 3,4-diCQA – 3,4-O-dicaffeoylquinic acid; 3,5-diCQA – 3,5-O-dicaffeoylquinic acid; 4-CQA – 4-O-caffeoylquinic acid; 4,5-diCQA – 4,5-O-dicaffeoylquinic acid; 5-CQA – 5-O-caffeoylquinic acid; 5-FQA – 5-O-feruloylquinic acid; C* – Chroma; CGA – Chlorogenic acid; CQA – Caffeoylquinic acid; CQAtotal – Total caffeoylquinic acids; diCQA – Dicaffeoylquinic acid; diCQAtotal – Total dicaffeoylquinic acids; FQA – Feruloylquinic acid; FQAtotal – Total feruloylquinic acids; Hº – Hue angle; L* – Lightness

Introduction

Coffee is currently grown in about 80 countries of four continents. Brazil is the world's largest producer, followed by Vietnam and Colombia. Many African countries, including Uganda, Burundi, Rwanda and Ethiopia have coffee as their main source of foreign exchange. In addition, the vast majority of coffee plantations worldwide belongs to smallholders, which makes the activity highly important in maintaining rural lifestyles, providing an important source of income and wealth distribution, by promoting employment and local development in the producing and processing regions (DaMatta and Ramalho, 2006; Partelli et al., 2010).

Coffea arabica grows in 85% of coffee producing countries, predominantly in the American Continent, accounting for approximately 69-74% of the world coffee production. *Coffea canephora* is predominantly grown in Asia and Africa (this last continent with about 80% of total plantation), being responsible for producing circa 25-30% of coffee worldwide (Ramalho, 2002).

The world green coffee production has been growing since the sixties, with total production varying between 4.2 and 7-8 million tonnes, between 1960 and the last decade (ICO, 2009). Moreover, world consumption of green coffee, though rising since the nineties, stabilized close to 7 million tonnes per year, being only 25% consumed in producing countries.

Coffee beans quality depends on moisture content, defects, bean size, some chemical compounds and preparation of a sample to perform cup tasting (Leroy et al., 2006). Physical analysis to characterize technological quality of green coffee is also used due to its promptness and easiness, as well as to its lower costs. In this context, colour has been used for quality assessment of green coffee beans (Nelson, 2005; Bicho et al., 2005, 2008) and there have also been studies using image processing for grain quality inspection (Soedibyo et al., 2010). To perform physical quality analysis standard

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methods are being used to characterize technological quality of green coffee, namely particle size, medium sieve, most frequent sieve, share split, cumulative calibration, trade homogeneity, mass of 1000 coffee beans, apparent densities, strange bodies and defects, mass losses on drying and olfactory and visual parameters. Quality assessment of coffee genotypes at a chemical level can also be used, namely for identification of chemical clusters discriminators of the roast degree in Arabica and Robusta coffee beans, identification of nutritional descriptors of roasting intensity in beverages of Arabica and Robusta coffee beans, identification of chemical clusters discriminators of Arabica and Robusta green coffee and impact of roasting time on the sensory profile of Arabica and Robusta coffee (Bicho et al., 2010, 2011a,b, 2012, 2013a,b). In this work a synopsis of the most useful parameters to assess coffee quality is presented following a technological perspective.

Physical parameters

In Arabica and Robusta coffee beans the particle size that prevails, according to the fractional calibration, is the sieve number 17 and 19, respectively (Bicho, 2005; Esteves and Oliveira, 1970), with the medium sieve remaining between 17.4 and 19.2. The trade homogeneity usually is lower in Arabica coffee (94.3), relatively to Robusta coffee (97.0). The cumulative percentage of green coffee Arabica and Robusta accumulated until the sieve 17 might reach about 100%. This commercial characteristic resulting of undergoing calibration stages during the benefit prior to marketing determines the required homogeneity of the coffee beans for roasting, to avoid burning of smaller grains (Cortez, 2001). Moreover, depending of the roasting degree, the volume of coffee beans might vary between 40- 69% in Arabica, and 48-57% in Robusta (Bicho et al., 2012).

The mass of coffee beans of green Robusta coffee is typically higher relatively to Arabica (Coste, 1992; Fazuoli, 1986), with unitary values of about 0.21 g and 0.15 g, respectively. Moreover, apparent densities of Arabica and Robusta green coffee do not vary significantly, showing values around 0.6-0.7 g cm^{-3} (Bicho, 2005; Coste, 1992; Esteves and Oliveira, 1970). Moreover, apparent density of coffee beans can decrease to 0.4-0.3 g cm^{-3}, with increase of roasting time (Bicho, 2005; Bicho et al., 2012).

The moisture content of green coffee beans usually ranges from 9.1% to 9.3%, for Arabica and Robusta respectively, being therefore within the established range of the international market, below 12.5%, according to the technical regulation of identity and quality for classification of the benefited raw green coffee bean (Instrução Normativa Nº 8, 2003). Mass losses of coffee beans might increase 10.5-19.4% in Arabica, and 10.1-16.7% in Robusta, with increase of roasting time (Bicho et al., 2012).

The colour of Arabica and Robusta green coffee beans has a uniform yellowish. Moreover, one factor that positively contributes to further improve the appearance uniformity and colour of the beans is related to the minimization of the silverskin adherence to the bean by polishing during the benefit. Otherwise silverskin may confer to the seed a strong green tone, when carotenoids and chlorophylls are present in it or red if only carotenoids prevail (Cardoso, 1994; Clifford, 1987; Coste, 1992). In this context, the residual silverskin of the surveyed samples might have a reddish-brown colour, being almost absent in the furrow of the green coffee beans, which is an indicator of a dry processing.

The determination of the colour of green coffee bean, made with the illuminants D_{65} and C allow the collection of coordinates systems chromatic CIE L*a*b* and CIE L*C*Hº. In this context the parameters a* and b* (contribution of the green/red and blue/yellow, respectively) typically present positive values, pointing a greater contribution of the red and yellow components. The combined value of these two chromatic parameters justifies the yellowish colour visually detected for both coffee beans. Although the lightness (L*) might not be significantly different, the opposite can occur with chroma (C*) and hue angle (Hº). Jointly these three coordinates contribute to the colour of the green coffee beans. In fact, Arabica green coffee might have a greenish yellow, while Robusta green coffee can have a brownish yellow colour (most probably with the coordinates a* and b* being significantly higher). Accordingly, the red and yellow colours prevail in this coffee. Moreover, the wet processed coffee can reveal negative a* values, pointing to a major green contribution. Therefore, the greenish colour develops in this type of processing, in contrast to the yellowish colour of dry processed beans (that has a positive value for the chromatic coordinate a*). Accordingly, the coffee industry can use this difference in colour (green or yellow) as a quick way to identify the type of post-harvest processing of green coffee beans (Bicho, 2005). Nevertheless, it also must be pointed that the age of the grain can also be affected by the value of a* (Mendes et al., 2001).

Indeed, L*, a* and b* values of arabica samples can be similar to those obtained by Nelson (2005) for an aged arabica coffee from Jamaica.

In grinded green coffee, the coordinates CIE L*a*b* and CIE L*C*Hº, using illuminant D_{65}, usually indicates that coordinates a*, b*, C* and Hº did not significantly differ between the two coffee types, unlike the parameter L*, but when illuminant C is used, only b* and C* did not differ significantly (Bicho 2005). These data point to the existence of a significant correlation between coordinates a* and Hº, and coordinates b* and C*. The Hº value of grinded green coffee can be more influenced by the contribution of a* (that presents a higher variation) than of coordinate b*, thus suggesting that a* would determine the colour of green coffee. For C* calculation the b* coordinate assumes a higher importance than a* due to its higher value. Thus, the absence of b* variation might justify the absence of significant differences among C* values.

The analysis of the chromatic parameters, using the illuminants D_{65} and C, might show that lightness (L*) as well as parameters a*, b*, C* and H° are not significantly different between genotypes when comparing the same roasting degree. Moreover, within each type of roasted coffee beans, the pattern of lightness (L*) can reveal an antagonist interaction with increased roasting intensity. The parameter a* can increase significantly in roasting of Arabica and Robusta coffee due to the yellowish intensification in the initial phase of the burning process, but a decrease can be found thereafter. The coordinate b* also show an antagonist pattern with increased roasting intensity, due to an increasing browning of the beans. Accordingly, parameters C* and H° can decrease significantly along the roasting process showing an increased reduction from green coffee to a sharp roasting intensity (Bicho et al., 2012).

After milling, significant differences can be found between green Arabica and Robusta coffees in several colour parameters when D_{65} and C are used (Bicho et al., 2012). The parameter L* might decrease significantly with roasting, whereas a* (green/red contribution) can increase sharply in Arabica and Robusta in a lower roasting degree with the opposite developing thereafter with higher roasting (contributing therefore to the red colour of the roasted coffee powder), similarly to what can happen before milling the beans. Yet, after milling a* values in higher roasting degrees can be still much higher than in green coffee, contrary to what might happen for the whole bean. On the same way, parameter b* (yellow/blue contribution) can increase with a lower roasting intensity, contrary to what can be observed with whole beans, and might decrease significantly thereafter with higher roasting intensity. The parameter C* varies similarly to the chromatic coordinates a* and b* with a substantial increase with low roasting intensities and subsequent decreases thereafter for higher roasting intensities, although maintaining higher values than those of the whole bean. The parameter H° can further decrease sharply with increasing roasting intensity, following a similar pattern found in the whole bean. The brightness and tone of the coffee powder samples typically show similar variations, exhibiting antagonist patterns with increasing roast intensity (Bicho et al., 2012).

Chemical parameters

In Arabica and Robusta green coffees the contents of soluble solids corresponds to about 33-34% (Esteves and Oliveira, 1970; Mendonça et al., 2005). After roasting, depending of its intensity, soluble solids of coffee beans typically decreases to 30-26% in Arabica and to 31-27% in Robusta (Bicho et al., 2011a). The pH of Arabica and Robusta green coffee corresponds to 5.6 and 5.7, respectively (Leroy et al., 2006), whereas depending of roasting intensity, these values can vary between 5.45- 5.12 and 5.49-5.32 (Bicho et al., 2011a).

Caffeine contents, for Arabica and Robusta green coffees, can reach about 1.45% and 2.38%, respectively (Silvarolla et al., 2000; Viani, 1993), while with increasing roasting time, caffeine contents decreases to ca. 1.376-1.299% and to 2.163-2.327% (Bicho et al., 2011a).

The values found for trigonelline contents remains in ca. 1.39% and 1.01%, for Arabica and Robusta green coffees respectively (Ky et al., 2001). With increasing roasting time, trigonelline contents might decrease to 1.293-0.553% in Arabica and to 0.902-0.571% in Robusta (Bicho et al., 2011a).

CQA, diCQA, and FQA represent about 98% of total chlorogenic acids in coffee (Clifford and Staniforth, 1977). The 5-CQA is the most representative both in Arabica and Robusta green coffees representing ca. 77% of total CQA (Fortunato et al., 2010). Also, the levels of all caffeoylquinic acid isomers (3-CQA, 4-CQA and 5-CQA), as well as CQA_{total}, typically are significantly higher in the Robusta green coffee (Clifford, 1985; Correia, 1990; Ky et al., 2001; Viani, 1993). The content of each isomer of diCQA is usually significantly higher in the Robusta green

coffee. Quite similar amounts of 3,4-diCQA, 3,5-diCQA and 4,5-diCQA might be found in Robusta green coffee, while the isomer 3,5-diCQA predominates in green Arabica coffee (Clifford, 1985, 1997; Ky et al., 2001). The 5-FQA isomer predominates (ca. 90% of total FQA), being the contents of the others (3-FQA and 4-FQA) much less relevant (Clifford, 1985; Correia, 1990; Ky et al., 2001; Viani, 1993). Robusta green coffee usually has higher levels of 3-FQA and 5-FQA, as well as a significantly higher total amount of FQA. The total content of each group of chlorogenic acids (CQA_{total}, $diCQA_{total}$, and FQA_{total}), as well as CGA_{total}, tends to be higher in Robusta green coffees, prevailing 5-CQA in green coffee. In fact, 5-CQA represented more than half (65 and 55%) of total CGAs in Arabica and Robusta coffees. That led to the higher weight of CQAs, representing 84% and 72% of total CGAs in Arabica and Robusta green coffees, respectively. diCQA acids prevails as the second most abundant group in the $diCQA_{total}$, corresponding to ca. 12% and 20% of total CGAs, while the FQAs represented ca. 4% and 8% of the CGA_{total} value in Arabica and Robusta green coffees, respectively. The content of total CGA in Robusta green coffee remains ca. 25% higher than in the Arabica green coffee (Bicho et al., 2013a).

Roasting promotes severe decreases in CGA contents. In this context, following the increase in roasting intensity, 3-CQA, 4-CQA, and 5-CQA decreases gradually to 17-20% in Arabica and to 27-37% in Robusta beans, thereby determining the sharp decrease in CQA_{total}. 5-CQA contents are similar for Arabica, but lower for Robusta than those obtained by others (Alves et al., 2006). Concerning 3,4-diCQA, 3,5-diCQA, and 4,5-diCQA, a even higher susceptibility to degradation can be observed. In fact, if a high roasting intensity is applied, the values can reach 5-8% in Arabica and 10-14% in Robusta, and also remains significantly higher in Robusta beans. Although less affected than CQAs and diCQAs, the FQA values usually are significantly reduced under higher roasting conditions, with 5-FQA being more reduced than 3-FQA, while 4-FQA can be considered irrelevant (Clifford, 1985; Ky et al., 2001; Viani, 1986, 1993). As for the CQAs and diCQAs, the FQA_{total} of Robusta is less affected and a significantly higher value persists when compared with Arabica beans. Among CGA compounds, the diCQAs are the most sensitive, being severely degraded with more intense roasting, while FQAs, although strongly affected, suffers a lower reduction, thus increasing its relative weight. Furthermore, in the overall CGAs of roasted coffees, the CQA fraction maintained the highest relative weight, prevailing 5-CQA (Bicho et al., 2011a).

Conclusions

The chromatic parameters, which allow a fast, reliable, low cost and non-destructive analysis that integrates the result of several processes, from plant production until storage, can partially differentiate the technological quality of Arabica and Robusta green coffee. Yet, as coordinate a* strongly affects Hº value of the green coffee in the colour analysis, the illuminant type used in the measurement must be defined and/or combined. In fact, the variation of spectral composition of incident light can lead to a different colour perception. Accordingly, through the application of colour parameters the technological quality of green coffee can be assessed. In roasted coffee beans, the parameters L∗, C*, H°, and coordinate b* displays an antagonist interaction due to an increase in the roasting intensity, whereas after milling, only L* and H° might decrease progressively. Considering that the parameters L* and H° followed similar patterns using both illuminants, D65 and C, it can be concluded that they are appropriate to evaluate coffee colour changes during roasting, enabling a relationship with coffee quality.

In general, Robusta green coffee shows higher values for pH, soluble solids, caffeine, total caffeoylquinic acids, total dicaffeoylquinic acids, and total feruloylquinic acids, but the content of soluble solids might not be significantly different in both species of green coffee. The content of caffeine does not vary significantly, but trigonelline decrease with burning up intensity. The levels of chlorogenic acids also decrease with increasing roasting time. The 5-O-caffeoylquinic acid prevailed in Arabica and Robusta beverages, but the isomers of dicaffeoylquinic and feruloylquinic acids remains higher in Robusta.

References

Alves, S. T., R. C. E. Dias, M. T. Benassi and M. B. S. Scholz. 2006. Metodologia para análise simultânea de ácido nicotínico, trigonelina, ácido clorogênico e cafeína em café torrado por cromatografia líquida de alta eficiência. Química Nova, 29:1164-1168. [in Portuguese].

Bicho, N. C. C. 2005. Qualidade e segurança alimentar no café verde e aclimatação do género Coffea em condições de stress ambiental. Lisboa, Dissertation (Food

Technology Master) Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, Portugal, pp. 389. [in Portuguese].

Bicho, N. C. C., F. J. C. Lidon, J. D. C. Ramalho, A. E. Leitão and J. F. S. Oliveira. 2005. Caracterização física do café verde numa perspectiva da qualidade alimentar e do valor comercial. Biol. Veg. Agro-industrial 2:269-292. [in Portuguese].

Bicho, N. C., F. C. Lidon, J. C. Ramalho, J. F. Santos Oliveira, M. J. Silva and A. E. Leitão. 2008. Colour and quality of green coffee. Proceedings of 22nd International Conference on Coffee Science (ASIC 2008), Campinas, Brazil, 14-19 September 2008, p:588-592.

Bicho, N. C., F. C. Lidon, J. C. Ramalho and A. E. Leitão. 2010. Quality assessment of coffee genotypes from Brazil. ASIC 2010, The 23rd International Conference on Coffee Science, Bali, Indonesia, 3-8 October 2010, p:732-737.

Bicho, N. C., A. E. Leitão, J. C. Ramalho and F.C. Lidon. 2011a. Identification of chemical clusters discriminators of the roast degree in Arabica and Robusta coffee beans. Eur. Food Res. Tech. 233:303-311.

Bicho, N. C., A. E. Leitão, J. C. Ramalho, N. B. Alvarenga and F. C. Lidon. 2011b. Identification of nutritional descriptors of roasting intensity in beverages of Arabica and Robusta coffee beans. Internat. J. Food Sci. Nutr. 62:865–871.

Bicho, N. C., A. E. Leitão, J. C. Ramalho and F. C. Lidon 2012. Use of colour parameters for roasted coffee assessment. Ciência e Tecnologia de Alimentos, 32:436-442.

Bicho, N. C., A. E. Leitão, J. C. Ramalho, N. B. Alvarenga and F. C. Lidon. 2013a. Identification of chemical clusters discriminators of Arabica and Robusta green coffee. Internat. J. Food Prop. 16:895–904.

Bicho, N. C., A. E. Leitão, J. C. Ramalho, N. B. Alvarenga and F. C. Lidon. 2013b. Impact of roasting time on the sensory profile of Arabica and Robusta coffee. Ecol. Food Nutr. 52:163-177.

Cardoso, A. P. S. 1994. Café-Cultura e tecnologia primária. Instituto de Investigação Científica Tropical, Lisboa, pp.169 [in Portuguese].

Clifford, M. N. and P. S. Staniforth. 1977. A critical comparison of six spectrophotometric methods for measuring chlorogenic acids in green coffee beans. In: Huitième Colloque Scientifique International sur le Café (Abidjan). ASIC, Paris, pp. 109–114.

Clifford, M. N. 1985. Chlorogenic acids. In: R. J. Clarke and R. Macrae (Ed.) pp. 153-202. Coffee, v. I: Chemistry. Elsevier Applied Science Publishers, London.

Clifford, M. N. 1987. Chemical and physical aspects of green coffee products. In: M. N. Clifford and K. C. Wilson (Ed.) pp. 305-374. Coffee, botany, biochemistry and production of beans and beverage. Croom Helm e Methuen, New York.

Clifford, M. N. 1997. The nature of chlorogenic acids. Are they advantageous compounds in coffee? In: 17eme Colloque Scientifique International sur le Café (Nairobi). ASIC, Paris, pp. 79-91.

Correia, A. M. N. G. 1990. Influência da torra na evolução dos ácidos clorogénicos do café. PhD Thesis. Instituto Superior de Agronomia, Universidade Técnica de Lisboa. pp. 281 [in Portuguese].

Cortez, J. G. 2001. Proposal for a global system to improve coffee. Comments and suggestions. Document WP-Quality nº 3/01, 31/10/2001. International Coffee Organization, p.8.

Coste, R. 1992. Coffee: the plant and the product. MacMillan Press, London, pp. 328.

Damatta, F. M. and J. C. Ramalho. 2006. Impacts of drought and temperature stress on coffee physiology and production: A review. Braz. J. Plant Physiol. 18:55-81.

Esteves, A. B. and J. S. Oliveira. 1970. Contribuição para o estudo das características dos cafés de Angola. Junta de Investigações do Ultramar, Estudos Ensaios e Documentos, 126, pp. 177 [in Portuguese].

Fazuoli, L. C. 1986. Genética e melhoramento do cafeeiro. In: Cultura do Cafeeiro - Factores que afectam a produtividade, Associação Brasileira para Pesquisa da Potassa e do Fosfato, São Paulo, pp. 89-113. [in Portuguese].

Fortunato, A. S., F. C. Lidon, P. Batista-Santos, A. E. Leitão, I. P. Pais, A. I. Ribeiro and J. C. Ramalho. 2010. Biochemical and molecular characterization of the antioxidative system of *Coffea* sp. under cold conditions in genotypes

with contrasting tolerance. J. Plant Physiol. 167:333-342.

ICO. 2009. International Coffee Organization. Trade statistics. Available via dialog: http://www.ico.org/coffee_prices.asp. Accessed: 5th May 2013.

Instrução Normativa nº 8. 2003. Regulamento técnico de identidade e de qualidade para a classificação do café beneficiado grão cru. Ministério de Estado da Agricultura, Pecuária e Abastecimento, Brasil, 12 pp. [in Portuguese].

Ky, L. C., J. Louarn, S. Dussert, B. Guyot, S. Hamon and M. Noirot. 2001. Caffeine, trigonelline, chlorogenic acids and sucrose diversity in wild *Coffea arabica* L. and *C. canephora* P. accessions. Food Chem. 75:223–230.

Leroy, T., F. Ribeyre, B. Bertrand, P. Charmetant, M. Dufour, C. Montagnon, P. Marraccini and D. Pot. 2006. Genetics of coffee quality. Braz. J. Plant Physiol. 18:229-242.

Mendes, L. C., A. C. Menezes, M. Aparecida and A. P. Silva. 2001. Optimization of the roasting of robusta coffee (*C. canephora* conillon) using acceptability tests and RSM. Food Qual. Pref. 12:153-162.

Mendonça, L. M. V. L., R. G. F. A. Pereira and A. N. G. Mendes. 2005. Parâmetros bromatológicos de grãos crus e torrados de cultivares de café (*Coffea arabica* L.). Ciências Tecnologia Alimentar 25:239-243. [in Portuguese].

Nelson, G. 2005. Using colorimetry as analytical technique for quality assessment of green coffee beans. XXI Latin American Coffee Industry Symposium, San Salvador, El Salvador, 14-15 July 2005. Available at: http://www.docstoc.com/docs/108528086/USING-COLORIMETRY-AS-ANALYTICAL-TECHNIQUE-FOR-QUALITY-ASSESSMENT. Accessed: 5th May 2013.

Partelli, F. L., H. D. Vieira, A. P. D. Rodrigues, I., Pais, E. Campostrini, M. M. Chaves and J. C. Ramalho. 2010. Cold induced changes on sugar contents and respiratory enzyme activities in coffee genotypes. Ciência Rural 40:781-786.

Ramalho, J. C. 2002. O cafeeiro. Aspectos gerais da sua biologia e cultura. Comunicações, nº 21, Instituto de Investigação Científica Tropical, Lisboa, p. 44 [in Portuguese].

Silvarolla, M. B., P. Mazzafera and M. M. A. Lima. 2000. Caffeine content of Ethiopian *Coffea arabica* beans. Gen. Mol. Biol. 23:213–215.

Soedibyo, D. W., U. Ahmad, K. B. Seminal and I. D. M. Subrata. 2010. The development of automatic coffee sorting system based on image processing and artificial neural network. Proceedings of AFITA 2010 International Conference - The Quality Information for Competitive Agricultural Based Production System and Commerce, Bogor Agricultural University, West Java-Indonesia, p: 272-275. Available at: http://repository.ipb.ac.id/handle/123456789/41672. Accessed at: 5th May 2013.

Viani, R. 1986. Coffee. In: Ullmann's Encyclopedia of industrial chemistry, vol A7. VCH Verlagsgesellschaft, Weinheim, pp. 315-339.

Viani, R. 1993. The composition of coffee. In: S. Garattini (Ed.) pp. 17-41. Caffeine, Coffee, and Health. Raven Press. New York.

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REGULAR ARTICLE

Effect of sowing date and seeding rate on bread wheat yield and test weight under Mediterranean conditions

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Abstract

Yield and test weight are attributes of particular economic importance in wheat production systems and are strongly affected by environmental conditions. This study was conducted to determine the effects of sowing date and seeding rate on grain yield and test weight of fifteen bread wheat varieties and five advanced lines from Portuguese Wheat Breeding Program (Plant Breeding Station, Elvas, Portugal) under irrigated Mediterranean systems. Field experiments were conducted at two locations of Southeast Portugal, during 2011/2012 growing season. Two seeding rates were compared (200 and 350 seeds.m^{-2}) in two different sowing dates in each location. Results showed that sowing date and seeding rate affects yield and test weight under irrigation field conditions, for Mediterranean region of Southeast Portugal, but only sowing date had significant effects among the varieties. At Elvas, higher yield was obtained with the 2nd sowing date (21 December) for most of the varieties studied. In opposite, in Beja trials, the highest values for yield were found when varieties were sown earlier (1st sowing date - 26 October). Test weight had a similar performance in the two sites, though top values for this trait were found with the 1st sowing date. Comparing the results obtained in the two studied locations, Beja showed, for the majority of the varieties, 3t/ha higher average yield than Elvas.

Key words: Bread wheat, Sowing date, Seeding rate, Yield, Test weight, Environmental constraints

Introduction

Wheat (*Triticum aestivum* L.) is a major cereal crop in many parts of the world and it is commonly known as the king of cereals. It belongs to *Poaceae* family and globally, after maize and rice, is the most cultivated cereal (FAOSTAT, 2013). Researchers can manage wheat cultivars, fertilizer levels, irrigation regime and agricultural practices to maximize wheat crop yield under the current conditions, but environmental constraints still be the main factors affecting wheat productivity in many regions of the world (El-Maaboud et al., 2004). In the US, for instance, unfavorable environments account for over 94% of the difference between average and record yield, and less than 6% of the disparity is attributable to diseases, insects and weeds (Boyer, 1982). In Mediterranean regions rain falls mostly during autumn and winter, and the water deficit rises in spring, coinciding with the anthesis and grain-filling period. Thus, drought and heat stress usually reduce yield potential during the period of grain formation (Simane at al., 1993; Lloveras et al., 2004). The challenge to increase wheat yield is even more difficult by projected climate changes, particularly higher temperatures and changes on rainfall distribution and amount (Parry and Hawkesford, 2010; Lobell et al., 2011). Even at a single location, in addition to variation due to agronomic and genetic factors, there is often considerable year to year variation reflecting different weather patterns. It is important to recognize that for farmers, maximizing yield is not their sole objective; profitability and managing risk are the most important criteria. Test weight is especially important for several food grain crops, particularly for those on which this trait is compulsory measured. The test weight is the first measurable/weighable qualitative trait of grain cereals mentioned in history, from the 19th century. Since then a great attention has been paid to it. Although it was introduced into regulations during the 20th century, it is hardly mentioned in the seed

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legislation. Protic et al. (2007) defines seed test weight using its dependence on seed density, shape and size, highlighting the fact that test weight is an important quality trait and that it can be used to estimate the amount of grain in a warehouse. They also state that test weight increased in time as a contribution of plant breeding. It is used by breeders to evaluate variety adaptation to the specific local conditions. The main goal of this study was to evaluate the effect of sowing date and seeding rate on grain yield and test weight of bread wheat under irrigation, in farmers field conditions.

Materials and Methods

Trials location

Trials were conducted during the 2011/2012 cropping season at farmers field in two different environments: Elvas (Alto Alentejo region) and Beja (Baixo Alentejo region), representing the most important provinces in Portugal for bread wheat crop. Table 1 shows some important data about the field trial sites.

Wheat germplasm

Cultivars choice was based on its growth cycle and origin. Among the studied germplasm, fifteen bread wheat varieties are from different origins and the remaining five are advanced breeding lines from Portuguese Wheat Breeding Program of National Institute for Agrarian and Veterinarian Research (Elvas, Portugal). Table 2 presents origin and growth habit of the germplasm used in the experiments.

Table 1. Geographic location, soil type, sowing and harvesting dates of the trials.

Location	Geographic Location	Soil	Sowing date	Harvesting Date
Elvas	38°53'N 7°8'W	Shallow silt loam	1st: 05-12-2011	28-06-2012
			2nd: 21-12-2011	29-06-2012
Beja	37º58`N 7º45'W	Deep clay	1st: 26-10-2011	06-07-2012
			2nd: 29-11-2011	06-07-2012

Table 2. Origin and growth habit of bread wheat varieties and advanced lines.

Germplasm	Origin	Growth habit
Roxo	Portugal	Very early maturity; Spring
Ardila	Portugal	Very early maturity; Spring
Eufrates	Portugal	Late maturity; Facultative
Nabão	Portugal	Very early maturity; Spring
Badiel	Spain	Early maturity; Spring
Siena	Spain	Very early maturity; Facultative
Bologna	France	Very late maturity; Facultative
Ingenio	France	Very late maturity; Facultative
Pata-Negra	Spain	Very early maturity; Spring
Alabanza	Spain	Early maturity; Facultative
Inoui	France	Early maturity; Facultative
Aguila	France	Late maturity; Facultative
Linha 3	England	Late maturity; Winter
Nogal	France	Early maturity; Facultative
Mané-Nick	Spain	Very early maturity; Spring
TE 0205	Portugal	Very early maturity; Spring
TE 0206	Portugal	Very early maturity; Spring
Flycatcher"s" x …	Portugal	Early maturity; Facultative
Linha 1	England	Late maturity; Winter
Linha 2	England	Very late maturity; Winter

Table 3. Location, sowing date and sowing rate of field experiments.

Location	Sowing date	Seeding rate
Elvas	5 December 2011	200 seed m^{-2}
		350 seed m^{-2}
	21 December 2011	200 seed m^{-2}
		350 seed m^{-2}
Beja	26 October 2011	200 seed m^{-2}
		350 seed m^{-2}
	29 November 2011	200 seed m^{-2}
		350 seed m^{-2}

Field experiments

Wheat germplasm was evaluated in four experiments with two different sowing dates and two seeding densities, in two locations (Elvas and Beja), under irrigation conditions (Table 3).

All treatments were conducted with: nitrogen fertilization at sowing time (150 kg ha^{-1} as 18-46-0) and three top-dressed fertilizations (150 kg ha^{-1} as Urea 46%; 937 kg ha^{-1} as 32N Solution and 150 kg ha^{-1} as ammonium nitrate 27%); two weed control (at pre-emergence and post-emergence) and three antifungal treatments (tillering, jointing and heading stages). The experimental design was a randomized complete block design with four replications using a split plot treatment arrangement. Each plot size area was 6 m^2 (5 m long and and six rows, 20 cm apart). Details of the meteorological conditions and irrigation supply, in both environments, are presented in Figure 1.

Statistical analysis

Statistical analysis was performed on SPSS software (IBM, version 17.0). Means were compared using Tukey Student's test (significance level $P < 0.05$). Analyses of variance were done across sowing date and seed rate for each location.

Results and discussion

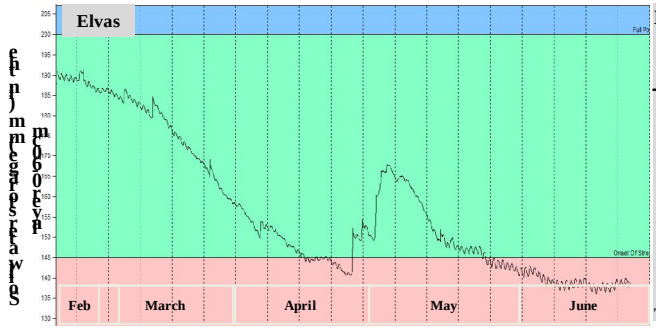
Evolution of soil water availability and climatic conditions

The evolution of water stored in the soil differed between the two studied locations. At Elvas, a shallow silt loam soil with about 50 cm of deep and with infiltration problems, applied irrigations were of low allocation to avoid losses by flooding. The irrigation had an effect only on the first 10 cm of soil deeper. With raising temperatures in March, it was observed an intensification of water consumption by plants and, consequently, a reduction of its availability on the soil (Figure 1). This situation was slightly reversed due to rainfall occurred during April and May. At Beja, with a deep and clay soil, irrigation had a visible effect in the 20-30 cm of soil deep, allowing a better "hydric comfort" to plants during the growth cycle. Only in March (when the maximum temperature increased), water consumption augmented due to crop evapotranspiration and water evaporation from the soil surface, resulting in a decrease on the amount of available water to the crop. This situation was quickly reversed by the occurrence of precipitation at the end of March (Figure 1). The soil differences of the two sites were of great importance in the wheat development. The higher water holding capacity of the soil at Beja allowed genotypes to grow under hydric comfort during grain filling period, as it can be seen in general on yield and test weight values.

Elvas experiments

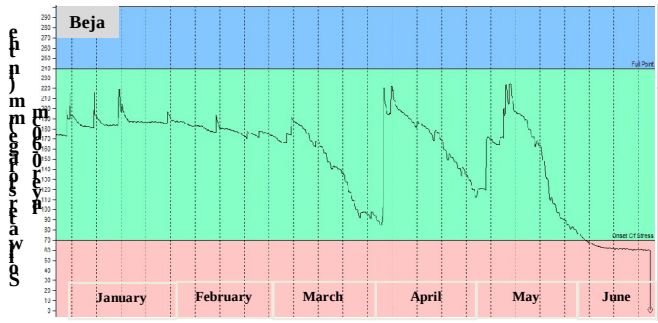
Variety, sowing date and seeding rate affected yield significantly (Table 4). A significant variety x sowing date interaction for grain yield was found result of the cultivars different growth habits (winter/facultative/spring).

Significant differences were found for grain yield among varieties, when sowed with different seeding rates and at different sowing times. This experiment also showed an overall yield advantage for the late sowing time (Table 5). Ingenio and Flycatcher"s" obtained the highest values for yield in the 2nd sowing date differing significantly from other varieties. Linha 2 obtained the smallest yield value for both sowing times and seeding rates with a significant difference from others. The greatest increase on yield between the 1st and the 2nd sowing date was observed in Ingenio, Inoui and Aguilla. These results are in disagreed with other authors (Cutforth et al., 1990, Ozturk et al., 2006) who reported that delaying sowing date leads to a decrease in wheat yield. Our results revealed that facultative and winter wheat varieties showed an advantage for grain yield when sown later as, in this specific crop season (2011/2012), temperatures during the grain filling period (April and May) where moderate as shown in Figure 1. Thus, it was possible for the varieties to elongate the cycle and increase the individual thousand grain weight with a positive response on yield. Nevertheless, spring wheat varieties (Ardila and Badiel) showed a decrease in grain yield for 2nd sowing date, data that are in accordance with several authors (Cutforth et al., 1990; Lloveras et al., 2004; Ozturk et al., 2006).



Elvas

Months	Minimum temperature (ºC)	Maximum temperature (ºC)	Rainfall (mm)	Irrigation (mm)
Sept 11	14,9	31,0	23,0	-
Octo11	12,3	27,9	48,0	-
Nov 11	8,7	17,6	100,0	-
Dec 11	4,0	14,2	9,0	-
Jan 12	1,7	14,8	6,0	23,0
Feb 12	0,1	16,5	0,0	32,0
Mar 12	6,3	21,4	2,0	25,0
Apr 12	7,8	18,8	54,0	26,0
May 12	12,0	27,6	18,0	13,0
June 12	15,5	31,5	2,0	0,0
TOTAL	-	-	**262,0**	**119,0**



Beja

Months	Minimum temperature (ºC)	Maximum temperature (ºC)	Rainfall (mm)	Irrigation (mm)
Sept 11	15,6	29,9	57,7	-
Octo11	12,8	28,0	66,5	-
Nov 11	9,2	18,1	110,5	-
Dec 11	4,9	15,3	10,4	-
Jan 12	4,1	16,2	14,0	24,0
Feb 12	1,2	15,9	1,0	24,0
Mar 12	6,2	21,1	40,0	48,0
Apr 12	7,2	17,8	41,0	38,0
May 12	11,6	26,8	40,0	36,0
June 12	13,7	29,9	2,0	0,0
TOTAL	-	-	**383,1**	**170,0**

Figure 1. Evolution of water stored in the soil at Elvas and Beja.

Table 4. Analysis of Variance for yield of 20 bread wheat sown at Elvas (Alto Alentejo, Portugal).

Variation Source	Yield			
	df	Mean Square	F value	Sig. (p)
Variety	19	2061981,027	12,714	***
Sowing date	1	1,025E^{7}	63,183	***
Seeding rate (seed m^{-2})	1	3245180,582	20,009	***
Variety x Sowing date	19	611466,257	3,770	***
Variety x Seeding rate	19	51106,681	0,315	ns
Sowing date x Seeding rate	1	127884,471	0,788	ns
Variety x Sowing date x Seeding rate	19	156759,565	0,967	ns
Error	240	162187,048		

***, **, *: Significant at P<0.001; P<0.01 and P<0.05, respectively; ns: no significant

Grain yield increased with an increase in seeding rate (Table 5). Similarly, Ozturk et al. (2006) found that an increasing seeding rate up to 525 seeds m^{-2}, increased spikes per square meter at harvest, resulting in increased grain yield. Seeding rate effect was less important than sowing date in maximizing grain yield in Mediterranean environments.

Table 6 shows great differences between minimum and maximum yield values obtained at Elvas. The minimum yield value was obtained with Badiel with the lower seeding rate and at the 2nd sowing data. In opposite, the maximum was obtained with Ingenio with the higher seeding rate and also at the 2nd sowing data. An high coefficient of variation confirm this finding.

Table 5. Yield differences between two sowing dates with two seeding densities for 20 varieties/advanced lines of bread wheat in Elvas trials.

Variety/advanced line	Yield (kg/ha) 1st sowing date		Yield (kg/ha) 2nd sowing date	
	Seed rate 200 seed.m^{-2}	Seed rate 350 seed.m^{-2}	Seed rate 200 seed.m^{-2}	Seed rate 350 seed.m^{-2}
Flycatcher "s"	2783^{abc}	3275^{d}	3460^{f}	3382^{b-f}
Ingenio	2844^{abc}	3165^{d}	3712^{f}	4054^{f}
Nabão	2619^{abc}	3066^{cd}	2811^{a-f}	3060^{a-f}
Alabanza	2435^{abc}	3025^{bcd}	3204^{def}	2726^{a-e}
Badiel	2949^{bc}	3012^{bcd}	1904^{a}	2477^{a-d}
Pata-negra	3039^{c}	2974^{a-d}	3176^{c-f}	3450^{b-f}
Inoui	2327^{abc}	2970^{a-d}	3529^{f}	3575^{def}
Eufrates	2781^{abc}	2898^{a-d}	2934^{b-f}	3116^{a-f}
Mané-Nick	2375^{abc}	2794^{a-d}	2908^{a-f}	2966^{a-f}
Ardila	2315^{abc}	2706^{a-d}	2178^{abc}	2406^{abc}
Aguilla	2568^{abc}	2690^{a-d}	3130^{b-f}	3697^{ef}
TE0206	2681^{abc}	2678^{a-d}	2757^{a-f}	3017^{a-f}
Linha1	2343^{abc}	2640^{a-d}	2887^{a-f}	3149^{a-f}
Bologna	2492^{abc}	2601^{a-d}	3233^{ef}	3493^{c-f}
Nogal	2274^{abc}	2531^{a-d}	3055^{b-f}	2943^{a-f}
TE0205	2342^{abc}	2528^{a-d}	2930^{b-f}	2748^{a-e}
Siena	2590^{abc}	2491^{a-d}	2869^{a-f}	3129^{a-f}
Linha3	1891^{ab}	2119^{abc}	2377^{a-e}	2677^{a-e}
Roxo	1945^{ab}	2070^{ab}	2197^{a-d}	2212^{a}
Linha2	1817^{a}	2005^{a}	2118^{ab}	2323^{ab}
Range	1817-3039	2005-3275	1904-3712	2212-4054
Mean	2471	2712	2868	3030
SE	45,59	42,29	43,51	48,47

Varieties are listed in yield decreasing order of 1st date and usual seed rate (350 seed.m-2). Different letters in the same column indicate significant difference (P<0.05).

Table 6. Descriptive statistics values of yield in 20 bread wheat at Elvas.

	n	Mean ± SE	Minimum	Maximum	CV (%)
Yield (kg/ha)	320	2770±32,42	812	5430	20,94

Table 7. Analysis of Variance for test weight of 20 bread wheat sown at Elvas (Alto Alentejo, Portugal).

Variation Source	Test weight			
	df	Mean Square	F value	Sig. (p)
Variety	19	335,961	426,584	***
Sowing date	1	281,4	357,306	***
Seeding rate (seed.m^{-2})	1	22,113	28,078	***
Variety x Sowing date	19	12,924	16,410	***
Variety x Seeding rate	19	2,148	2,727	***
Sowing date x Seeding rate	1	0,367	0,466	ns
Variety x Sowing date x Seeding rate	19	1,184	1,504	ns
Error	240	0,788		

***, **, *: Significant at P<0.001; P<0.01 and P<0.05, respectively; ns: no significant.

Table 8. Test weight differences between two sowing dates with two seeding densities for 20 varieties/advanced lines of bread wheat in Elvas trials.

Variety	Test weight (kg/hl) 1st sowing date		Test weight (kg/hl) 2nd sowing date	
	Seed rate 200 seed.m^{-2}	Seed rate 350 seed.m^{-2}	Seed rate 200 seed.m^{-2}	Seed rate 350 seed.m^{-2}
Nabão	82,67^{j}	83,31^{j}	81,66^{i}	82,30^{h}
TE0205	82,74^{j}	82,88^{ij}	81,46^{hi}	82,09^{gh}
Siena	81,90^{hij}	82,81^{hij}	80,86^{ghi}	82,46^{h}
Pata-negra	82,56^{ij}	82,73^{hij}	81,06^{hi}	81,52^{fgh}
Ardila	80,47^{ghi}	82,38^{hij}	78,73^{efg}	80,16^{e-h}
Bologna	81,53^{g-j}	82,10^{g-j}	79,26^{e-h}	80,02^{e-h}
Roxo	82,38^{hij}	82,05^{g-j}	80,36^{ghi}	80,69^{fgh}
Eufrates	81,99^{hij}	81,99^{g-j}	80,70^{ghi}	80,94^{fgh}
Badiel	80,38^{gh}	81,21^{f-i}	77,08^{de}	80,18^{e-h}
Alabanza	80,92^{g-j}	80,86^{fgh}	80,33^{ghi}	80,56^{fgh}
TE0206	80,46^{ghi}	80,35^{efg}	79,53^{f-i}	79,39^{efg}
Mané-Nick	79,57^{fg}	79,80^{def}	80,48^{ghi}	80,50^{e-h}
Nogal	78,16^{f}	78,64^{cde}	77,39^{def}	78,69^{def}
Inoui	77,95^{ef}	78,24^{cd}	75,34^{cd}	75,36^{c}
Flycatcher "s"	75,61^{cd}	77,07^{c}	75,59^{cd}	77,65^{cde}
Ingenio	75,95^{de}	76,95^{c}	74,59^{c}	76,39^{cd}
Aguilla	74,81^{cd}	74,36^{b}	69,19^{b}	70,06^{b}
Linha1	73,68^{bc}	74,01^{b}	68,69^{b}	67,70^{b}
Linha3	71,92^{b}	72,03^{a}	67,60^{a}	67,31^{b}
Linha2	69,16^{a}	70,22^{a}	66,06^{a}	63,86^{a}
Range	69,16-82,74	70,22-83,31	66,06-81,66	63,86-82,46
Mean	78,74	79,20	76,80	77,39
SE	0,092	0,083	0,095	0,123

Varieties are listed in test weight decreasing order of 1st date and usual seed rate (350 seed.m-2). Different letters in the same column indicate significant difference (P<0.05).

Test weight was significantly affected by variety, sowing date and seeding rate. Furthermore, interactions between variety and sowing date and variety and seeding rate were found to be statistically significant for test weight (Table 7).

Test weight depends on grain size, shape and density and indicates the adaptability of a variety to environment. Nabão, TE0205, Roxo and Pata-Negra, showed the highest value for test weight and reveal remarkable stability across the two sowing times (Table 8).

A test weight advantage with the 1st sowing date was observed when compared with the 2nd date (Table 8). These results are in accordance with Protic et al. (2007) who concluded that test weight of winter wheat decreased with later sowing, as a consequence of compensatory effects among yield components (Borghi et al., 1995). Portuguese variety Nabão and advanced line TE0205, both developed at Wheat Breeding Program (INIAV-Elvas), had the highest test weight values. Linha2, Linha3 and Linha1 had the lowest values for this trait (Table 8), with non-significant differences between seeding rates neither sowing dates. Genetics has an important role in regulating test weight but it can be affected also by climatic and edaphic factors.

Results of the whole data set showed that, test weight ranged from 61,84 to 83,62 kg.hl^{-1} at Elvas with a coefficient of variation showing low data dispersion (Table 9).

Beja experiments

Variety, seeding rate and sowing date showed a significant effect on yield. Interaction between sowing date and variety was also statistically significant for grain yield (Table 10).

Average yield of the top 5 varieties/ advanced lines in the 1st sowing date was almost 2 t/ha higher than the 2nd sowing date. Table 11 shows that in Beja trials, the higher yielding varieties increased grain yield when sown earlier. Results showed that

sowing with a higher seeding rate did not outcome higher yield. Grain yield obtained with higher seeding rate was slightly superior (Table 11). Moreover, under favorable edafoclimatic conditions (irrigation, soil, etc.) a higher seed density results in high-biomass production, high number of spikes per square meter though with smaller spikes and consequently with no increase in grain yield. Peltonen-Sainio (1991) showed that a higher seed rate usually produces high-biomass and the genotypes often mature late, which is usually undesirable.

For the 1st sowing date, the higher values for yield were obtained with Nogal, Inoui, Bologna, Flycatcher"s", Eufrates, Aguilla and Linha1 (Table 11), cultivars with facultative growth cycle (excepting Linha1) for which heading time occurred after April's 10 (data no shown). In Mediterranean conditions of Portugal the optimum heading time must occurred ± 10 days around April 1st. For this facultative or winter wheat, the earlier sowing date (26 October) promoted a higher expression of grain yield potential. These results are according with Malcolm et al. (2013) who reported that in many countries where only spring wheat is cultivated, the highest wheat yield of over 15 t/ha have been achieved for winter wheat grown with a long growing season at higher latitudes. Woodruff et al. (1983) also reported that the large differences on the yield of genotypes having different development cycles, within a group and from a given sowing date, were primarily due to the interactions between growth duration, water use and evaporative demand conditions around anthesis. For 1st sowing date, the lowest yield varieties were Pata-Negra, Alabanza, Mané-NicK, Ardila, Siena and Linha2 (Table 11). Except for Linha2 (winter variety), other varieties have short growth cycles, with earlier heading time, before April´s 1st. Consequently, the late sowing date, on November 29th, showed to be an advantage for grain yield of these spring wheat varieties. Ingenio, Roxo, Nabão, TE0206, Badiel and TE0205 revealed remarkable yield stability concerning sowing date with an optimum heading time around April 1st, as referred. However, yield of these varieties was always below the trial average mean.

Table 12 shows a large gap between the minimum and maximum yield values. This is in accordance with the big coefficient of variation found. The minimum yield value was obtained with Linha2 with the higher seeding rate and for the 2nd sowing date. This performance points out the importance of duration of growth cycle, indicating that this variety is not adapted, once is a very late variety (data not shown). The maximum value was obtained with Nogal with the higher seeding rate and for the 1st sowing date (Table 11).

Wheat variety, sowing date and seeding rate affected significantly test weight. Interaction between variety and sowing date was also found to have a significant effect on this trait (Table 13).

Table 9. Descriptive statistics values of test weight in 20 bread wheat on Elvas.

	n	Mean ± SE	Minimum	Maximum	CV (%)
Test weight (kg/hl)	320	78,03±0,27	61,84	83,62	6,08

Table 10. Analysis of Variance for yield of 20 bread wheat sown at Beja (Baixo Alentejo region, Portugal).

Variation Source	Yield			
	df	Mean Square	F value	Sig. (p)
Variety	19	1,297E7	35,148	***
Sowing date	1	1,552E7	42,082	***
Seeding rate (seed.m-2)	1	2271623,395	6,158	**
Variety x Sowing date	19	8064707,629	21,862	***
Variety x Seeding rate	19	542237,996	1,470	ns
Sowing time x Seeding rate	1	542238,187	1,470	ns
Variety x Sowing date x Seeding rate	19	924877,6	2,507	***
Error	240			

***, **, *: Significant at P<0.001; P<0.01 and P<0.05, respectively; ns: no significant

Table 11. Yield differences between two sowing dates with two different seeding densities for 20 varieties/advanced lines of bread wheat in Beja trials.

Variety	Yield (kg/ha) 1st sowing date		Yield (kg/ha) 2nd sowing date	
	Seed rate 200 seed m^{-2}	Seed rate 350 seed m^{-2}	Seed rate 200 seed m^{-2}	Seed rate 350 seed m^{-2}
Nogal	8585^{g}	9099^{t}	6830^{fg}	6787^{gh}
Inoui	7392^{fg}	7638^{ef}	5381^{b-f}	5758^{c-g}
Bologna	7022^{c-g}	7601^{ef}	5917^{d-g}	6348^{e-h}
Linha1	7191^{d-g}	7379^{de}	4276^{abc}	4938^{bcd}
Flycatcher "s"	5600^{a-e}	7071^{cde}	6049^{d-g}	5978^{d-g}
Ingenio	5949^{b-f}	6968^{cde}	5647^{c-f}	5535^{c-f}
Eufrates	7349^{efg}	6801^{cde}	5421^{b-f}	5179^{cde}
Aguilla	7431^{fg}	6681^{b-e}	4870^{bcd}	5626^{c-g}
Linha3	5111^{ab}	6195^{a-e}	3826^{ab}	3821^{ab}
Roxo	6242^{b-f}	6177^{a-e}	6222^{d-g}	6153^{efg}
Nabão	6914^{c-g}	6154^{a-e}	5759^{c-g}	6123^{d-g}
TE0206	5941^{b-f}	5672^{a-d}	5211^{b-e}	6416^{fgh}
Badiel	6074^{b-f}	5447^{abc}	6219^{d-g}	7445^{h}
TE0205	5453^{a-d}	5040^{ab}	5795^{c-g}	5866^{d-g}
Pata-negra	4648^{ab}	5024^{ab}	6763^{efg}	6116^{d-g}
Alabanza	5378^{abc}	4964^{a}	6621^{efg}	6838^{gh}
Mané-Nick	5692^{a-f}	4813^{a}	7319^{g}	6825^{gh}
Linha2	4164^{a}	4762^{a}	2851^{a}	2619^{a}
Ardila	4481^{ab}	4507^{a}	5416^{b-f}	6355^{e-h}
Siena	4143^{a}	4492^{a}	3911^{ab}	4597^{bc}
Range	4143-8585	4492-9099	2851-7319	2619-7445
Mean	6038	6124	5515	5766
SE	75,79	72,96	68,51	51,80

Varieties are listed in yield decreasing order of 1st date and usual seed rate (350 seed.m-2). Different letters in the same column indicate significant difference (P<0.05)

Table 12. Descriptive statistic values for test yield in 20 bread wheat varieties at Beja.

	n	Mean ± SE	Minimum	Maximum	CV (%)
Yield (kg/ha)	320	5861±72,35	1873	9492	22,08

Table 13. Analysis of variance for test weight of 20 bread wheat sown at Beja (Baixo Alentejo region, Portugal).

Variation Source	Test weight			
	df	Mean Square	F value	Sig. (p)
Variety	19	401,032	115,78	***
Sowing date	1	48,875	14,110	***
Seeding rate (seed m^{-2})	1	6,962	2,010	***
Variety x Sowing date	19	36,970	10,673	***
Variety x Seeding rate	19	2,939	0,848	ns
Sowing date x Seeding rate	1	2,938	0,848	ns
Variety x Sowing date x Seeding rate	19	4,310	1,244	ns
Error	240			

***, **, *: Significant at P<0.001; P<0.01 and P<0.05, respectively; ns: no significant

Table 14. Test weight differences between two sowing date with two seeding densities for 20 varieties of bread wheat in Beja trials.

Variety	Test weight (kg/hl) 1st sowing date		Test weight (kg/hl) 2nd sowing date	
	Seed rate 200 seed m^{-2}	Seed rate 350 seed m^{-2}	Seed rate 200 seed m^{-2}	Seed rate 350 seed m^{-2}
Roxo	83,01^{g}	82,70^{i}	82,20^{i}	81,83^{h}
Bologna	81,40^{fg}	82,23^{hi}	78,59^{f-i}	78,64^{gh}
Nabão	82,35^{g}	81,27^{hi}	79,99^{f-i}	81,19^{h}
Eufrates	80,73^{efg}	80,91^{ghi}	80,30^{ghi}	79,01^{gh}
Nogal	79,83^{efg}	80,46^{ghi}	75,87^{e-h}	76,52^{fgh}
TE0205	80,03^{efg}	79,82^{f-i}	81,14^{i}	80,89^{h}
TE0206	77,52^{def}	77,90^{e-h}	76,09^{e-h}	78,72^{gh}
Pata-negra	77,41^{def}	77,87^{e-h}	80,57^{hi}	81,09^{h}
Alabanza	76,42^{de}	76,60^{efg}	79,33^{f-i}	79,16^{gh}
Badiel	73,20^{cd}	75,62^{ef}	75,20^{ef}	77,56^{gh}
Ingenio	74,67^{cd}	75,43^{def}	70,12^{bcd}	69,53^{b-e}
Flycatcher “s”	74,56^{cd}	74,63^{de}	72,54^{cde}	74,57^{efg}
Ardila	74,15^{cd}	74,51^{cde}	75,41^{efg}	73,98^{d-g}
Inoui	74,30^{cd}	74,46^{cde}	70,03^{bcd}	70,03^{b-e}
Linha1	71,00^{bc}	73,39^{b-e}	67,85^{abc}	68,80^{bcd}
Linha3	67,43^{ab}	70,89^{a-d}	68,05^{abc}	66,12^{ab}
Mané-Nick	71,71^{bc}	69,90^{abc}	76,10^{e-h}	75,51^{fg}
Aguilla	71,16^{bc}	69,58^{ab}	65,85^{ab}	67,73^{bc}
Siena	67,82^{ab}	68,93^{ab}	73,58^{de}	71,84^{c-f}
Linha2	65,79^{a}	67,13^{a}	63,88^{a}	62,04^{a}
Range	65,79-83,01	67,13-82,70	63,88-82,20	62,04-81,83
Mean	75,22	75,71	74,63	74,74
SE	0,189	0,199	0,213	0,229

Varieties are listed in test weight decreasing order of 1st date and usual seed rate (350 seed.m-2). Different letters in the same column indicate significant difference (P<0.05)

Table 15. Descriptive statistic values for test weight in 20 bread wheat varieties at Beja.

	N	Mean ± SE	Minimum	Maximum	CV (%)
Test Weight (kg/hl)	320	75,08±0,30	60,14	83,74	7,21

Data showed that the higher and steady test weight values (including two sowing dates) were obtained with Ardila, Roxo, Nabão, Eufrates, TE0205 and TE0206, with an increase when sowed later (Table 14). Varieties with longer growth cycle presented a significant reduction on test weight in 2nd sowing date. Spaner et al. (2000) and Ozturk et al. (2006) reported that a delayed in sowing tends to decrease test weight in facultative wheat.

Maximum test weight was recorded Roxo, Bologna, Nabão, Eufrates, Nogal, TE0205 and TE0206, in the 1st sowing date (October 26th) with a significant decrease in the 2nd sowing date (November 29th) as shown in table 14. This behaviour is similar to the observed with the grain yield at Beja. This performance reflects an important adaptation of these varieties to Mediterranean conditions predominant in Portugal. In opposite, varieties developed in different environmental conditions (longer growth cycles) showed worse adaptation resulting on lower test weights.

Results showed that test weight ranged from 60,14 to 83,74 $kg.hl^{-1}$ in Beja with a small coefficient of variation indicating low dispersion of the data.

Conclusions

Results clearly confirmed that intrinsic genetic yield potential is not enough to obtain high wheat yield. Overall, in Beja, with the same varieties, the average wheat yield was around 3t/ha higher than in Elvas (Tables 6 and 12). This fact indicates that

several limitations to the expression of yield potential exist, related with agronomy (i.e., depth and physical structure of the soil and crop management practices) and climate such as frost during flowering and high temperatures during grain filling, which could cause irreversible damage to wheat crop yield. In this context, the fastest and most practical ways to increase yield are to improve agronomy in conjunction with continuing genetic improvement (Costa et al. 2012). At Beja, the highest value for grain yield was obtained by Nogal with 7,8 t/ha and heading time at April 1st. On the other hand, Linha2 showed the lowest performance with 3,6 t/ha and heading time out of adequate window (May 1st). At Elvas, Flycatcher"s", an advanced breeding line, was the best genotype with 3,2 t/ha and Linha2 revealed the end of the varieties ranking with 2 t/ha. The highest test weight was obtained with the 1st sowing date, in both locations. Increasing sowing rate did not significantly influenced test weight at Elvas and Beja, for the majority of cultivars. These results showed that both sowing date and seeding rate influence grain yield and test weight in the majority of cultivars, but the effect of sowing date was greater than that of seeding rate. Results also indicated that according with the climatic conditions occurred during 2011/2012 season in both places (Elvas and Beja), where a strong Mediterranean pattern drives wheat development, the germplasm evaluation and selection is paramount to better determine and characterize the ideotype wheat plant that breeders should strive to develop. New advanced breeding lines like Flycatcher"s", TE0205, TE0206, obtained by Cereal Breeding Program in Plant Breeding Station (Elvas, Portugal) are excellent examples resulting from this kind of approach.

References

Abd El Maaboud, M. S., A. F. Abou-Hadid, M. Edriss and M. A. Medany. 2004. Climate change and productivity of some wheat cultivars under rainfed and supplementary irrigation conditions. In: C. Cantero-Martínez and D. Gabiña (Eds.), pp. 139-146. Mediterranean rainfed agriculture: Strategies for sustainability. Zaragoza: CIHEAM. (Options Méditerranéennes: Série A. Séminaires Méditerranéens; n. 60).

Borghi, B., M. Corbellini, M. Ciaffi, E. Lafiandra, E. Stefanis, D. Sgrulletta, G. Boggini and N. Fonzo. 1995. Effect of heat shock during grain filling on grain quality of bread and durum wheats. Aust. J. Agric. Res. 46: 1365-1380.

Boyer, J. S. 1982. Plant productivity and environment. Science 218:443-448.

Costa, R., N. Pinheiro, A. S. Almeida and B. Maçãs. 2012. Influence of enhanced UV-B radiation on wheat production in relation with abiotic, biotic and socioeconomics constraints. Emir. J. Food Agric. 24(6):565-575.

Cutforth, H. W., C. A. Campbell, S. A. Brandt, J. Hunter, D. Judiesch, R. M. DePauw and M. Clarke. 1990. Development and yield of Canadian western red spring and Canada prairie spring wheats as affected by delayed seeding in the brown and dark brown soil zones of Saskatchewan. Can. J. Plant Sci. 70:639-660.

FAOSTAT 2013. Available in http://www.faostat.fao.org

Hawkesford, M., J. L. Araus, R. Park, D. Calderini, D. Miralles, T. Shen, J. Zhang and M. Parry. 2013. Prospects of doubling global wheat yields. Food Ener. Sec. 2(1):34–48.

Lloveras, J., J. Manent, J. Viudas, A. López and P. Santiveri. 2004. Seeding rate influence on yield and yield components of irrigated winter wheat in a mediterranean climate. Agron. J. 96:1258-1265.

Lobell, D. B., W. Schlenker and J. Costa-Roberts. 2011. Climate trends and global crop production since 1980. Science 333:616-620.

Ozturk, A., O. Caglar and S. Bulut. 2006. Growth and yield response of facultative wheat to winter sowing, freezing sowing and spring sowing at different seeding rates. J. Agron. Crop Sci. 192:10-16.

Parry, M. A. J. and M. J. Hawkesford. 2010. Genetic approaches to reduce greenhouse gas emissions: increasing carbon capture and decreasing environmental impact. In: M. P. Reynolds (Ed.) pp. 139-150. Climate change and crop production. CABI International, Wallingford, U.K.

Peltonen-Sainio. 1991. Productive oat ideotype for northern growing conditions. Euphytica 54:27-32.

Protic, R., M. Miric, N. Protic, Ž. Jovanovic and P. Jovin. 2007. The test weight of several winter wheat genotypes under various sowing dates and nitrogen fertilizer rates. Rom. Agric. Res. 24:43-36.

Simane, B., J. M. Peacock and P. C. Struik. 1993. Differences in development plasticity and growth rate among drought-resistant and susceptible cultivars of durum wheat (*Triticum turgidum* L. var. *durum*). Plant Soil. 157:155-166.

Spaner, D., A. G. Todd and D. B. McKenzie. 2000. The effect of seeding date, seeding rate and N fertilization on winter wheat yield and yield components in eastern Newfoundland. Can. J. Plant Sci. 80:703-711.

Woodruff, D. R. and Tonks, J. 1983. Relationship between time of anthesis and grain yield of wheat genotypes with differing developmental patterns. Aust. J. Agric. Res. 34:1-11.

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REGULAR ARTICLE

Durum wheat breeding in Mediterranean environments - influence of climatic variables on quality traits

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Abstract

Two durum wheat trials were carried out in Mediterranean conditions during 2010/2011 and 2011/2012 growing seasons at Plant Breeding Station-Elvas (Portugal). The experiments were conducted under rainfed conditions however, in 2012, due to extreme drought it was necessary to use artificial irrigation between booting and mid grain filling stage. Thirty durum wheat genotypes were studied and six quality parameters were evaluated: thousand kernel weight (TKW), test weight, vitreousness, protein content, SDS test and pigment content through Minolta CR 300 Colorimeter (L*a*b*) analysis. ANOVA showed that all sources of variation for four quality traits were highly significant ($P < 0.001$) for both years, except for SDS volume and index b* that were not significant during the two years of trials. Environmental effects showed that total water input during grain filling, appears to affect negatively grain quality by reducing test weight, TKW and semolina yield. Maximum temperatures during the same period reduced test weight, TKW, semolina yield and pigment content (L*), but increased protein content. A negative correlation was found between protein content and test weight and a positive correlation between test weight and semolina yield. Technological trait associated with pasta quality pigment index (b*) was significant different among the genotypes.

Key words: Durum wheat, Advanced breeding lines, Climatic variables, Quality traits

Introduction

Durum wheat is considered a minor cereal crop, representing only the 8 to 10% of cultivated wheat around the world (Mohammadi et al., 2011), being an important crop in the Mediterranean basin (Pedro et al., 2011). However, durum wheat cultivation have gradually decreased in some countries in the Mediterranean region such as Portugal, Spain and others due to world policies as well as the fact that high yielding durum wheat cultivars cannot compete with the best bread wheat varieties. Nevertheless, durum wheat is an economically important crop because of its unique features related to grain end use products. It is generally considered the hardiest of all wheats. Durum kernels are large, golden amber and translucent. Durum wheat quality is highly dependent of the genotype, agricultural production technology packages and fluctuations in biotic and abiotic environmental factors (Autran et al., 1983; Nachit et al., 1993). In addition, soil fertility, fertilization and water availability are the main factors affecting the quality stability (di Fonzo et al., 2000). Environmental conditions are known to have a significant influence on end-use quality characteristics, but the relative magnitude of environment, genetic and genotype x environment (GxE) effects on quality is unclear (Peterson et al., 1992). The G x E interaction effects on durum wheat pasta quality have been studied by several groups of researchers (Rharrabti et al., 2003; Kilic et al., 2005; Mohammadi et al., 2011), who found that environment and year, significantly affect protein content, sedimentation volume, gluten index and yellow pigment content (Sakin et al., 2011). Moreover, test weight, kernel size and vitreousness are also important, as they are strongly related to semolina yield and brightness appearance of semolina (Dziki and Laskowski, 2005). For breeders, stability of quality attributes is a goal to pursue for the importance in terms of genotypes changing ranks across environments and affects selection efficiency. In the last century, substantial genetic progress has been made and achieved on

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what concern to quality characteristics of durum wheat, mainly, from the last decades of XX century up to now. For grain end-users as millers, consistency in quality of cultivars is of great importance, regardless the regularity along the years (Rharrabti et al., 2003; Letta et al., 2008). However, as mentioned by Grausgruber et al. (2000) the quality of a genotype usually reacts like other quantitative characters to favorable or unfavorable environmental conditions. Improvement of durum wheat quality is one of the main goals of the Durum Breeding Program carried out by the Portuguese Plant Breeding Station (EMP, Elvas) of the National Institute of Agrarian and Veterinary Research (INIAV). The majority of the work is focusing the main quality parameters to obtain varieties with high quality for pasta production. The purposes of the study discussed on the present paper were: (i) to evaluate a group of advanced durum wheat lines obtained in EMP, considering technological performance; (ii) to study the quality parameters correlations and to estimate environmental effect in some of these parameters concerning pasta quality.

Materials and Methods

Plant materials and growing conditions

Two field trials were conducted during 2010/2011 and 2011/2012 seasons with 27 advanced durum wheat lines and three varieties Celta, Hélvio and Marialva (Table 1), developed at EMP Cereal Breeding Program, belonging to National Institute of Agrarian and Veterinary Research (INIAV). Genotypes were sown in a randomised complete block design with four replications. Seed rate was adjusted for a density of 350 viable seeds m^{-2} and plot size area was 9.6 m^2 (8 m long and six rows, 20 cm apart).

In spring 2012 it was decided to use artificial irrigation between booting and grain formation initiation with a total of 40 mm of water in order to assure the normal grain development of the plants.

In order to evaluate the germplasm utilization value the following parameters were studied: thousand-kernel weight (TKW), test weight, vitreousness, protein content and SDS sedimentation test. Semolina yield and its color were evaluated using a Minolta CR300 Colorimeter (Minolta Corp., Ramsey, NJ). Colorimeter L* values represent 'lightness', with score of 100 as white and 0 as black (Morris et al., 2000). Colorimeter a* values reflect red-green colors with '+' values indicating 'redness', and '–' values as 'greenness'. Colorimeter b* values measure yellow to blue colors, with '+' values indicating 'yellowness' and '–' values indicating 'blueness'.

Table 1. Advanced experimental lines, commercial varieties used in this experiment.

INIAV1	2003/2004	F8
INIAV2	2003/2004	F8
INIAV3	2003/2004	F8
INIAV4	2003/2004	F8
Celta		
INIAV5	2003/2004	F8
INIAV6	2003/2004	F8
INIAV7	2003/2004	F8
INIAV8	2003/2004	F8
INIAV9	2003/2004	F8
INIAV10	2003/2004	F8
INIAV11	2003/2004	F8
INIAV12	2003/2004	F8
INIAV13	2003/2004	F8
Hélvio		
INIAV14	2003/2004	F8
INIAV15	2003/2004	F8
INIAV16	2003/2004	F8
INIAV17	2003/2004	F8
INIAV18	2003/2004	F8
INIAV19	2003/2004	F8
INIAV20	2003/2004	F8
INIAV21	2003/2004	F8
INIAV22	2003/2004	F8
Marialva		
INIAV23	2003/2004	F8
INIAV24	2003/2004	F8
INIAV25	2003/2004	F8
INIAV26	2003/2004	F8
INIAV27	2003/2004	F8

Climatic variables

In each trial, total amount of water input was calculated adding natural rainfall plus irrigation (when needed), supplied during growth cycle from sowing to physiological maturity, sowing to anthesis and beginning of grain filling to physiological maturity (end of grain filling). Maximum temperature was also recorded from anthesis to end of maturity.

Statistical analysis

Statistical analysis was performed on SPSS programme (IBM, version 17.0). Means were compared using Tukey Student's test (significance level $P < 0.05$). Climatic variables were plotted against each quality parameter and relevant associations are presented graphically in the results section. Correlation coefficients are also presented for the same parameters.

Table 2. Agro-ecological characteristics of the evaluation sites of trials

Cropping Seasons	Geographic Location	Agro-ecological Zone	Soil	Precipitation (mm) Sowing – Physiological Maturity	Sowing – Anthesis	Grain Filling Period
2010/2011	38o53`N 7o08`W	Rainfed	Clayey – pH 8.0	279.5	161.6	117.9
2011/2012	38o53`N 7o08`W	Rainfed	Clayey – pH 8.0	89.3 + 40.0a	41.0 + 16.0a	48.3 + 24.0a

a Supplemental irrigation

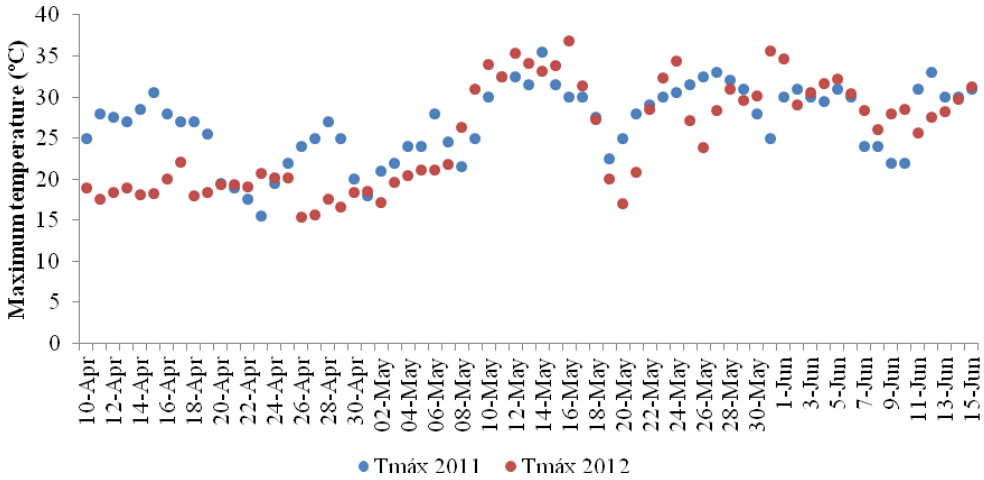

Figure 1. Maximum temperatures during grain filling in 2010/2011 and 2011/2012 seasons.

Results

Climatic variables

Agro-ecological characteristics and climatic data observed during for 2010/2011 and 2011/2012 seasons are shown in Table 2 and Figure 1, which illustrate the kind of climatic variability usual in the Mediterranean region. It can be assumed that the most important water stress constraints for wheat, in Mediterranean environments, occurred from stem elongation to booting and from anthesis to grain filling. Grain filling and grain ripening are the most important development stages that determine the final grain quality. During 2011/2012 season, artificial irrigation started in the beginning of tillering in order to promote a normal development of plant biomass and to assure enough soil humidity during anthesis. Sprinkler irrigation was applied since the beginning of grain formation up to mid grain filling in a total of 24 mm of water.

Trials were sowed on 25-01-2011 for 2010/2011 season and on 07-12-2011 for 2011/2012 season. Harvesting took place on 01-07-2011 for 2010/2011 season and on 06-07-2012 for 2011/2012 season.

Figure 2 shows the temperature between the 10th April and the 15th of June for the two trials seasons. The maximum temperatures in the first 3 weeks after beginning of grain filling in 2011 were 10ºC higher than those recorded for the same period in 2012. From May until the end of grain filling, daily temperatures were similar in both years.

Genotype and environmental conditions effects on durum wheat quality

The traits related with quality, such as protein content, test weight, thousand-kernel weight, SDS sedimentation test, semolina yield and pigment content of 30 durum wheat genotypes were evaluated. The ANOVA results (Table 3) indicated a strong influence of the year on quality traits. Only SDS and the yellowness (b*) showed no influence of environmental conditions during trials seasons. Genotypic effects were mainly observed for pigment contents a* and b* ($P < 0.001$).

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Cropping Seasons	Geographic Location	Agro-ecological Zone	Soil	Precipitation (mm) Sowing – Physiological Maturity	Sowing – Anthesis	Grain Filling Period
2010/2011	38°53`N 7°08`W	Rainfed	Clayey – pH 8.0	279.5	161.6	117.9
2011/2012	38°53`N 7°08`W	Rainfed	Clayey – pH 8.0	89.3 + 40.0[a]	41.0 + 16.0[a]	48.3 + 24.0[a]

[a] Supplemental irrigation

Table 3. Analysis of variance table for quality traits of 30 durum wheat genotype grown in two seasons with four replications.

Source	df	Test weight		df	TKW		df	Semolina yield		df
		Mean square	F value		Mean square	F value		Mean square	F value	
Year (Y)	1	2743.61	176.11***	1	986.18	59.23***	1	350.90	10.69**	1
Genotype (G)	29	7.72	0.92n.s	29	17.46	1.27n.s	29	4.37	1.53*	29
Y x G	29	9.44	1.12n.s	29	8.91	0.65n.s	29	2.71	0.54n.s	29
Error	174	8.41		174	13.73		174	2.85		174

Source	df	Vitreousness (%)		df	SDS volume		df	Protein content	
		Mean square	F value		Mean square	F value		Mean square	F value
Year (Y)	1	53.20	5.11*	1	222.34	2.84n.s	1	846.00	874.19***
Genotype (G)	29	10.67	1.03n.s	29	98.41	1.26n.s	29	0.88	1.12n.s
Y x G	29	13.07	1.26n.s	29	21.86	0.28n.s	29	0.69	0.88n.s
Error	174	10.41		174	78.40		174	0.79	

Source	df	L*		df	a*		df	b*	
		Mean square	F value		Mean square	F value		Mean square	F value
Year (Y)	1	109.76	52.15***	1	2.18	20.61***	1	0.31	0.13n.s
Genotype (G)	29	1.22	0.58n.s	29	0.66	6.20***	29	11.06	4.54***
Y x G	25	0.78	0.37n.s	25	0.10	0.98n.s	25	4.67	1.92n.s
Error	53	2.11		53	0.11		53	2.44	

***, **, * Significant at P<0.001, P<0.01 and P<0.05 respectively

n.s - no significant

The mean quality traits for each growing season are presented in Table 4. The means of TKW, test weight, vitreousness, semolina yield and L*a*b* increased in the second year (2011/2012). The mean values for SDS associated with protein content decreased in the second year also. It may be due to a decrease in production once, during 2010/2011, grain yields were significantly lowers than in 2011/2012 (data not shown). Other aspect to highlight, which was related to weather conditions during 2011/2012, were the values for TKW that were significantly higher than in 2010/11 season. It must be mentioned that the color of pasta products is mainly influenced by yellow pigment, which is largely controlled by the genotype since the average for the two year trials showed no significant differences in the values of L* and b*.

Influence of climatic variables

The effect of maximum temperature and water (precipitation and supplemental irrigation when used) during grain filling period on germplasm performance are presented in Figures 2 and 3. Test weight, semolina yield, TKW and L* index were negatively associated with maximum temperature during grain filling. Protein content showed a positive association with that climatic variable (Figure 2). L* index and TKW were positively associated with grain filling duration (Figure 3). Concerning water during grain filling, this climatic

variable was associated negatively associated with test weight (Figure 3). TKW was positively affected by grain filling duration and on the opposite, was negatively affected by maximum temperature and precipitation during the same period (Table 5). Test weight was negatively influenced by maximum temperature and precipitation but showed a positive and significant correlation with the duration of grain filling. It was found no effect of the environment in grain vitreousness (Table 5), showing all genotypes high values in both years (Table 4). Protein showed a high positive correlation with maximum temperature and rainfall, and showed a negative correlation with the duration of grain filling period. Semolina yield directly related with test weight, showed a negative correlation with maximum temperature and water availability during grain filling. Concerning the colorimeter parameters, which reflect the pigment content in the grain, brightness (L*) and a* values (redness) were negatively affected by rainfall and maximum temperature. L* was negatively correlated to protein, positively correlated with test weight and TKW (Table 5).

Redness (a* values) was negatively affected by maximum temperature during grain filling and yellowness (b*), which indicates the brightness of pasta, showed no correlation with the climatic variables (Table 5).

Table 4. Mean quality traits of 30 durum wheat genotypes in 2010/2011 and 2011/2012 seasons.

Zone	TKW (g)		Test weight (kg/hl)		Vitreousness (%)	
INIAV-Elvas	2011	2012	2011	2012	2011	2012
Mean	41.07	45.12	74.83	81.59	97	98
Min – Max	34.70 - 47.00	33.30 – 55.60	61.84 -81.58	77.01 – 84.02	72 - 100	80 - 100
SE	0.27	0.40	0.36	0.11	0.34	0.25
SD	2.93	4.34	3.96	1.25	3.72	2.77
Overall mean	43.10		78.21		97.35	

Zone	Protein content (%)		SDS (ppm)		Semolina yield (%)	
INIAV-Elvas	2011	2012	2011	2012	2011	2012
Mean	17.31	13.55	40.72	38.57	52.54	54.95
Min – Max	14.60 – 19.40	11.30 – 15.50	21 - 74	20 - 58	47.60 – 57.30	49.04 – 60.50
SE	0.79	0.84	0.86	0.75	0.16	0.19
SD	0.87	0.92	9.47	8.20	1.79	2.09
Overall mean	15.43		39.64		53.74	

Zone	L*		Pigment content a*		b*	
INIAV-Elvas	2011	2012	2011	2012	2011	2012
Mean	82.88	84.02	-1.48	-1.21	22.71	22.76
Min – Max	79.34 – 85.81	83.58 – 86.14	-2.93 – 0.14	-2.05 – 0.16	16.24 – 28.36	15.26 – 26.24
SE	0.21	0.08	0.74	0.05	0.39	0.30
SD	1.61	0.62	0.56	0.42	2.55	2.29
Overall mean	83.45		-1.34		22.74	

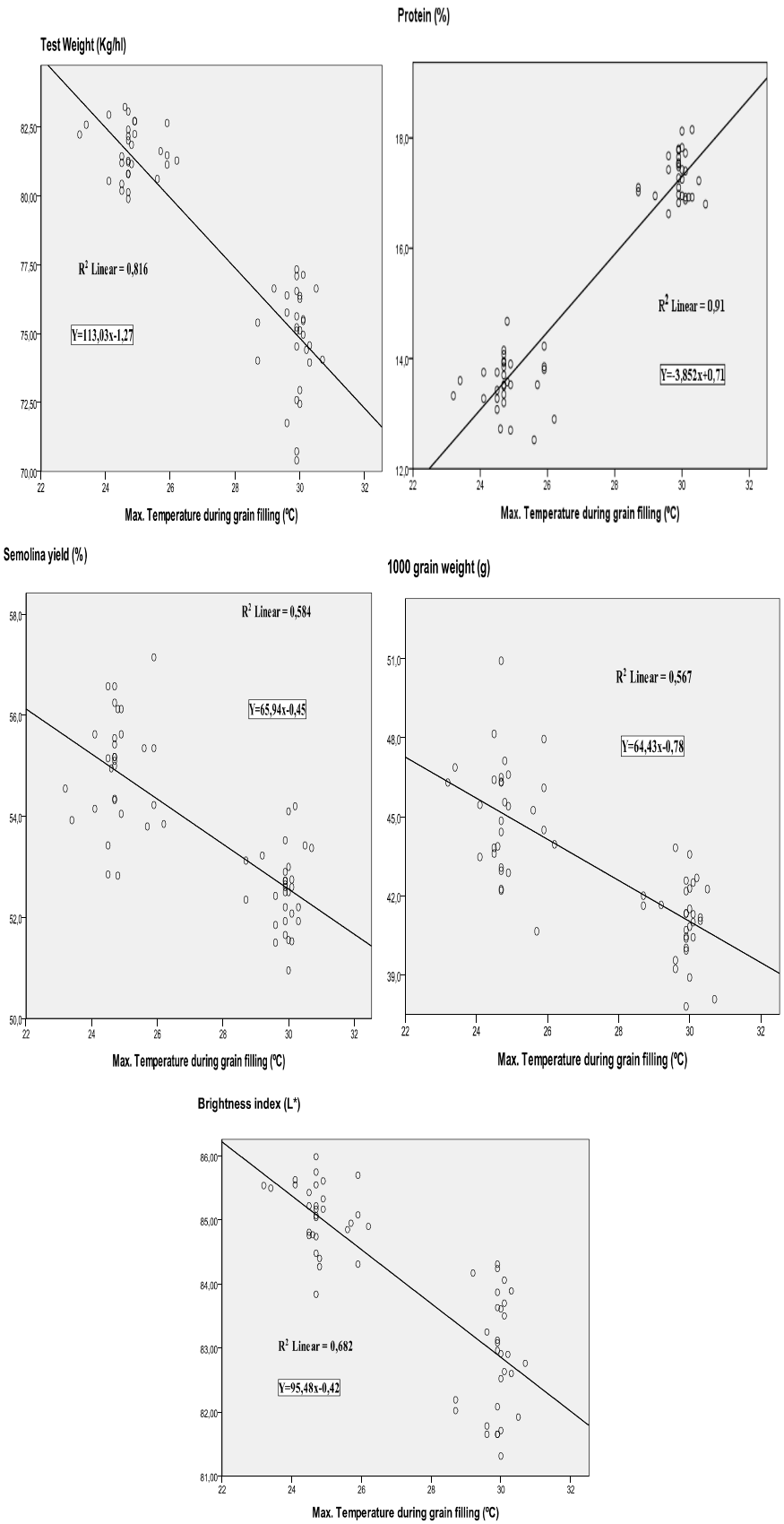

Figure 2. Regression of quality traits with maximum temperature during grain filling.

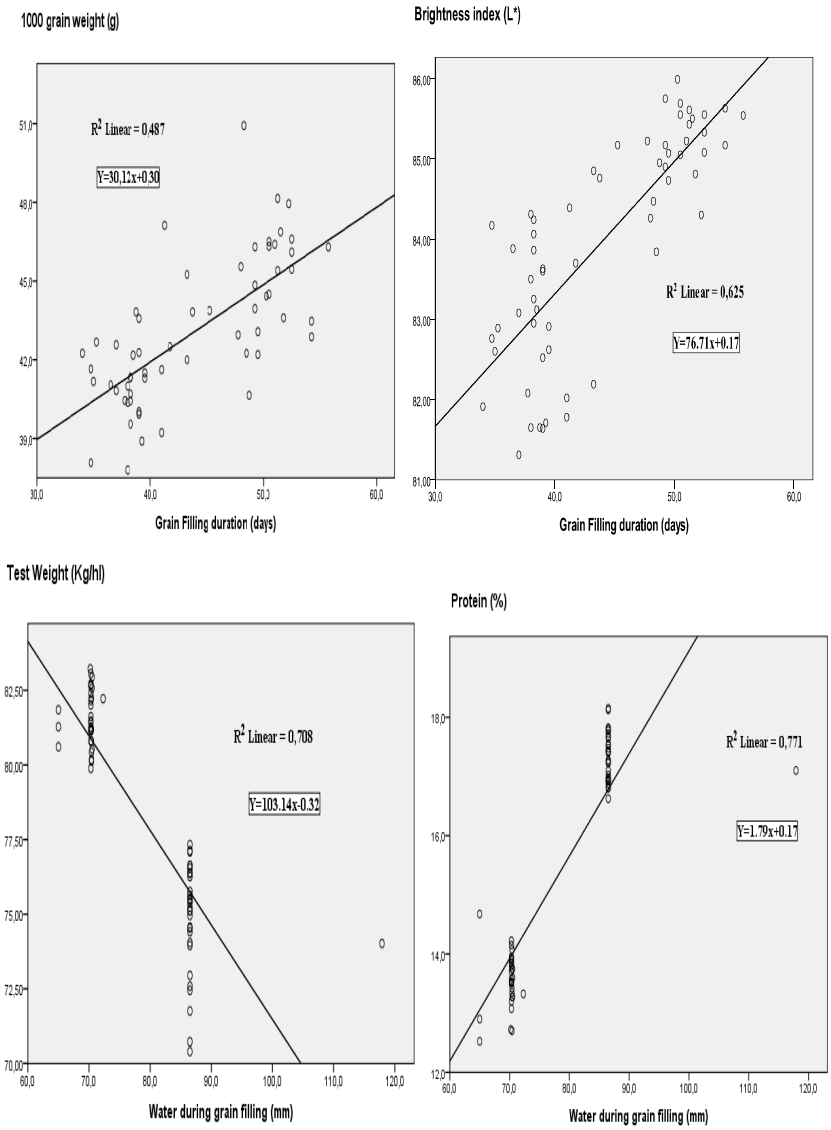

Figure 3. Regression of quality traits according to climatic variables.

Table 5. Correlation coefficients among quality parameters and climatic variables obtained from genotype and climatic variable mean value (n=60).

	Precipitation Grain Filling (mm)	Max. Temp. Grain Filling (°C)	Grain Filling (days)
Test Weight (kg/hl)	-0.841**	-0.903**	0.830**
TKW (g)	-0.662**	-0.753**	-0.698**
Vitreuosness (%)	-0.248ns	-0.233ns	-0.254ns
Protein content (%)	0.878**	0.954**	-0.878**
SDS volume (ppm)	0.267*	0.245ns	-0.311*
Semolina yield (%)	-0.730**	-0.764**	0.668**
L*	-0.778**	-0.820**	0.791**
a*	-0.270ns	-0.283*	0.206ns
b*	-0.018ns	-0.025ns	0.043ns

** correlation is significant at 0.01 level; * correlation is significant at 0.05 level; n.s. correlation is not significant

Associations between quality traits

Quality traits were, in general, significantly correlated and some interesting associations can be highlighted (Table 6). TKW was positively correlated to test weight, semolina yield and brightness, but negatively correlated with protein content. Test weight showed a positive association with vitreousness, semolina yield and brightness, although interacted negatively with protein content. Higher values of grain protein showed a negative correlation with semolina yield and brown hue (L*).

Quality traits associated with pasta technological quality

Durum wheat protein quantity and gluten quality are widely responsible for the pasta cooking characteristics, whereas yellow pigments are effective on pasta products colour (Borrelli et al., 1999; Sakin et al., 2011).

In addition, vitreousness is an important trait to durum wheat quality, once there is a strong relation with semolina yield and brightness appearance of semolina. Quality traits mean values associated with pasta quality of 30 durum wheat genotypes are present in Table 7. Protein contents of the 30 genotypes studied ranged from 14.6% to 15.9%, and there were not significant differences between genotypes (Table 7). Vitreousness is traditionally an important quality trait for pasta industry, as it is associated to commercial value; this trait is responsible for high semolina yield, good granulation and purity. The endosperm vitreousness varied between 93.5% and 98.7%. No statistically significant differences were found between genotypes. Gluten quality of durum wheat is commonly evaluated by sodium dodecyl sulfate (SDS) and gluten index (GI) tests. In this study only SDS was determined. The mean values varied from 30.8ppm and 46.8ppm although no significant differences were found between genotypes (Table 7). Semolina and pasta yellow colour is a traditional rather than functional characteristic of quality. Brightness (L*) ranged between 82.96 and 84.88 and yellowness (b*) varied from 16.01 to 25.10. For this last trait, data showed that INIAV 18 is significantly higher than commercial varieties, Hélvio and Marialva and INIAV 11 is significantly higher than Marialva (Table 7).

Table 6. Pearson´s correlation coefficients among quality parameters obtained from genotype mean values (n=60).

		TKW	Test weight	Vitreousness	Protein content	SDS volume	Semolina yield	Pigment content	
								L*	a*
Test weight		0.485**							
Vitreousness		0.130*	0.373**						
Protein content		-0.432**	0.732**	-0.052ns					
SDS volume		0.042ns	-0.148*	0.014ns	0.096ns				
Semolina yield		0.328**	0.447**	0.212**	-0.484**	-0.138*			
Pigment content	L*	0.343**	0.536**	0.267**	-0.605**	-0.049ns	0.423**		
	a*	0.116ns	0.186*	0.118ns	-0.196*	-0.118ns	0.166ns	-0.012 ns	
	b*	-0.063ns	0.031ns	-0.071ns	-0.034ns	0.129ns	0.081ns	-0.070 ns	-0.569**

** Correlation is significant at 0.01 probability level

* Correlation is significant at 0.05 probability level

ns Correlation is not significant

Table 7. Mean quality characteristics associated with pasta quality of 30 durum wheat genotypes grown during two years.

Genotype	Protein (%)	Vitreousness (%)	SDS (ppm)	semolina yield (%)	Pigment content	
					L*	b*
INIAV1	15.50^{a}	96.2^{a}	41.2^{a}	54.31^{a}	84.23^{a}	23.91^{c-g}
INIAV2	14.66^{a}	97.1^{a}	42.8^{a}	54.36^{a}	83.80^{a}	23.01^{a-g}
INIAV3	15.15^{a}	98.7^{a}	39.5^{a}	53.30^{a}	84.30^{a}	23.32^{a-g}
INIAV4	15.71^{a}	97.5^{a}	40.5^{a}	52.67^{a}	84.02^{a}	21.46^{a-f}
Celta	15.96^{a}	98.3^{a}	32.1^{a}	54.72^{a}	84.34^{a}	23.05^{a-g}
INIAV5	15.15^{a}	98.3^{a}	40.7^{a}	53.52^{a}	84.33^{a}	24.66^{efg}
INIAV6	15.51^{a}	97.8^{a}	43.0^{a}	52.88^{a}	84.22^{a}	23.61^{b-g}
INIAV7	15.07^{a}	98.0^{a}	40.6^{a}	53.11^{a}	84.57^{a}	23.16^{a-g}
INIAV8	15.51^{a}	97.7^{a}	42.5^{a}	54.11^{a}	84.60^{a}	23.99^{c-g}

Table 7. Contd..

Genotype	Protein (%)	Vitreousness (%)	SDS (ppm)	semolina yield (%)	Pigment content L*	b*
INIAV9	15.18[a]	97.1[a]	39.3[a]	53.72[a]	83.79[a]	23.38[a-g]
INIAV10	15.47[a]	98.2[a]	41.6[a]	54.75[a]	83.25[a]	23.27[a-g]
INIAV11	15.42[a]	98.1[a]	42.1[a]	53.35[a]	83.66[a]	24.48[d-g]
INIAV12	15.56[a]	97.6[a]	44.7[a]	54.65[a]	84.30[a]	25.10[fg]
INIAV13	14.81[a]	95.6[a]	46.8[a]	53.12[a]	84.75[a]	23.38[a-g]
Hélvio	15.73[a]	95.8[a]	39.3[a]	52.18[a]	83.92[a]	19.90[a-d]
INIAV14	15.75[a]	97.6[a]	36.7[a]	53.62[a]	84.81[a]	23.38[a-g]
INIAV15	15.06[a]	98.0[a]	42.3[a]	53.85[a]	84.64[a]	23.65[b-g]
INIAV16	15.52[a]	97.7[a]	41.3[a]	53.26[a]	84.59[a]	21.32[a-f]
INIAV17	15.68[a]	96.7[a]	40.8[a]	54.01[a]	83.78[a]	23.90[c-g]
INIAV18	15.76[a]	98.2[a]	36.7[a]	53.83[a]	83.44[a]	26.21[g]
INIAV19	15.71[a]	97.6[a]	41.1[a]	54.02[a]	83.49[a]	24.23[d-g]
INIAV20	15.48[a]	98.2[a]	35.0[a]	53.41[a]	82.96[a]	21.73[a-g]
INIAV21	15.26[a]	97.5[a]	42.0[a]	51.88[a]	83.28[a]	22.38[a-g]
INIAV22	14.91[a]	96.7[a]	37.6[a]	53.80[a]	84.88[a]	22.30[a-g]
Marialva	15.95[a]	98.1[a]	41.6[a]	54.77[a]	83.16[a]	18.95[a]
INIAV23	15.37[a]	97.6[a]	36.2[a]	54.50[a]	84.01[a]	20.11[a-e]
INIAV24	15.85[a]	93.5[a]	35.6[a]	54.22[a]	83.36[a]	22.69[a-g]
INIAV25	15.07[a]	94.7[a]	30.8[a]	53.56[a]	83.48[a]	22.05[a-g]
INIAV26	15.57[a]	97.5[a]	34.2[a]	54.53[a]	84.35[a]	19.08[ab]
INIAV27	15.66[a]	98.0[a]	39.5[a]	54.21[a]	84.30[a]	19.49[abc]
Range	14.66-15.95	93.5-98.7	30.8-46.8	51.88 – 54.77	82.96-84.88	18.95-26.21
Mean	15.43	97.4	39.5	53.74	84.02	22.75
MS error	0.795	10.41	78.40	3.85	1.964	2.437

Different letters in the same column indicate significant differences ($P<0.05$)

Discussion

In this paper were studied the relative contributions of genotype, environment and GxE on grain quality variation in 30 durum wheat genotypes tested across two seasons. During grain filling period, genotypes were subjected to temperature and moisture conditions that differed in the two seasons. Although the qualitative composition of the wheat grain is genetically determined, the quantitative composition could be significantly modified by growing conditions (Mpofu et al., 2006). Understanding semolina quality variations due to different environments would be useful for improving pasta quality. Several studies carried out in Italy have also reported the high influence of environment and genotype x environment interaction in determining durum wheat quality (Mariani et al., 1995; Nachit et al., 1995; Boggini et al., 1997; Novaro et al., 1997). Other results (Miezan et al., 1977; Zhu and Khan, 2001) provide the evidence that interannual and multilocal variation on thousand- kernel weight and protein content are much more influenced by environmental conditions than by genotype. As presented in our findings, the protein content was positively associated with moderately high temperatures during grain filling (Figure 2) what was in accordance with results of Rao et al. (1993) and Uhlen et al. (1998). The maximum temperature, from 25ºC to 30ºC, that occurred in the first days of grain filling period during 2011, contribute to lower TKW and test weight reducing grain yield with implications on protein contents in accordance with Williams et al. (1986), which reported that durum wheat protein content is inversely correlated with grain yield. Previous studies correlations by Matsuo and Dexter (1980) have illustrated that low test weight is an indication of shrivelled kernels and higher protein content, indicating a possible cause of high levels of protein content found in 2010/2011 season.

The study of climatic variability effect on grain quality revealed that though the Mediterranean climate irregularity causes great fluctuation on grain yield (data not shown) that irregularity may also comprise an opportunity for good expression of durum wheat quality traits, in accordance with Borghi et al. (1997). Associations between quality traits revealed a positive correlation between test weight and vitreousness, TKW, semolina yield and

pigment content (L*). Similar associations were also found by Novaro et al. (1997) and Rharrabti et al. (2003). Regarding the main technological parameters associated with pasta quality, it was found no significant differences in protein, vitreousness, SDS and brightness L* within the germplasm studied. Durum protein content ranges from 6% to 20%, depending on variety, environmental conditions and cultural practices during growth (CWC, 2005). For pasta products quality, the protein content level should be between 12% and 16%. The modern pasta manufacturing requires durum semolina to contain over 14% protein, which corresponds to 15% grain protein content (Landi and Guarneri, 1992). The values obtained in the present study for grain protein content were within the range for production of high-quality pasta products according the previously mentioned authors. Vitreousness values obtained for all genotypes were in accordance with Dexter and Matsuo (1981), Dexter et al. (1988, 1989) and Matsuo and Dexter (1980) which stand that the acceptable minimum value of kernel vitreousness is 80%. The end-product utilization of the durum wheat crop focuses on the semolina market; hence there exists the need to investigate the quality attributes required to supply this market. It is recognized that high extraction rates for durum wheat semolina (rather than the smaller particle sized flour) is of importance to the miller (Troccoli et al., 2000), and that semolina yield is related to kernel hardness. Nevertheless researchers have linked traits such as test weight and thousand kernel weight to semolina yield and therefore indirectly to grain hardness (Marshall et al., 1986). In this research all the genotypes had high grain weight, high vitreousness and moderate values of test weight. This aspect may have contributed to the high values of semolina yield. For pigment index (L*) genotypes showed values that matches the exigency for the most important pasta industry in Portugal, which defines values for this index between 82 to 83 for high quality durum wheat (personal information). Other pigment index (b*) values obtained also range in the parameters defined by Portuguese industry, which define values for this index greater than 23. The vast majority of genotypes reached this value, with exception of varieties Marialva (18.95) and Hélvio (19.90) with the lowest values and advanced lines INIAV 26 and INIAV 27 which also denote low values of yellow pigments.

Conclusion

Although environmental factors, such as maximum temperatures and water available during grain filling period, have important effects on wheat grain protein accumulation and quality for pasta technology, durum wheat quality is a genotype-dependent trait. In general, moderately high temperature, proper soil moisture (resulting from rainfall and irrigation) and adequate solar radiation may improve durum wheat quality. Some ecological factors, including soil physiological and chemical properties and geographic latitude, can also affect durum wheat quality. Durum wheat quality may be improved by breeding elite varieties, better management practices and exploiting the synergism between genotype and environment (Costa et al. 2012). A large amount of information is available on the relative importance of genotype, environment, and GXE interaction effects on the durum wheat quality traits grown in the Mediterranean region. Studies including a sizeable number of genotypes and water regimes may provide useful information not only on quality performance and stability of germplasm, but also on the specific characteristics of the tested environments. This kind of information can support decisions regarding the definition of target zones favorable for good expression of particular quality traits. In conclusion, the improvement achieved in durum wheat breeding Program developed by EMP in Portugal has contributed to identify genotypes and to obtain durum wheat varieties, able to express high yield potential and good technological quality.

References

Autran, J. C., J. Abecassis and P. Feillet. 1983. Statistical evaluation of different technological and biochemical tests for quality assessment in durum wheats. Cereal Chem. 63:390-394.

Boggini, G., M. A. Doust, P. Annicchiaro and L. Pecetti. 1997. Yielding ability, yield stability and quality of exotic durum wheat germplasm in Sicily. Plant breed. 116:541-545.

Borrelli, G. M., A. Troccoli, N. Di Fanzo and C. Fares. 1999. Durum wheat lipoxygenase activity and other quality parameters that affect pasta color. Cereal Chem. 76:335–340.

Borghi, B., M. Corbellini, C. Minoia, M. Palumbo, N. di Fonzo and M. Perenzin. 1997. Effects of mediterranean climate on wheat bread-making quality. Eur. J. Agron. 6:145-154.

Costa, R., N. Pinheiro, A. S. Almeida and B. Maçãs. 2012. Influence of enhanced UV-B

radiation on wheat production in relation with abiotic, biotic and socioeconomics constraints. Emir. J. Food Agric. 24(6):565-575.

CWC, 2005. Description of durum wheat semolina quality factors. http://www.California wheat. org.

Dexter, J. E. and R. R. Matsuo. 1981. Effect of starchy kernels, immaturity, and shrunken kernels on durum wheat quality. Cereal Chem. 58:395-400.

Dexter, J. E., B. A. Marchylo, A. W. MacGregor and R. Tkachuk. 1989. The structure and protein composition of vitreous, piebald and starchy durum wheat kernels. J. Cereal Sci. 10:19-32.

Dexter, J. E., P. C. Williams, N. M. Edwards and D. G. Martin. 1988. The relationship between durum wheat vitreousness, kernel hardness and processing quality. J. Cereal Sci. 7:169-181.

di Fonzo, N., P. De Vita, A. Gallo, C. Fares, O. Padalino and A. Troccoli. 2000. Crop management efficiency as a tool to improve durum wheat quality in Mediterranean areas. In: J. Abecassis, P. Braun, P. Feuillet, P. Joudrier, B. Pascal and P. Roumet (Eds.), pp. 44–59. Proceedings of the International Workshop on Durum Wheat, Semolina and Pasta Quality: Recent Achievements and New Trends, INRA, Montpellier, France, November 27, 2000.

Dziki, D. and J. Laskowski. 2005. Wheat kernel physical properties and milling process. Acta Agrophys. 6:59-71.

Grausgruber, H., Berforester, M., Werteker, M., Ruckenbauer, P. and Vollmann, J. 2000. Stability of quality traits in Austrian-grown winter wheats, Field Crops Res. 66: 257-267.

Kilic, H., Erdemci, I., Karahan, T., Aktas, H., Karahan, H. and Kendal, E. 2005. Determination of adaptation capability of some durum wheat cultivars in the southeastern Anatolian conditions. GAP IV, Agricultural Congress, Sep 21-23, Sanliurfa, Turkey. 768-773.

Landi, A. and R. Guarneri. 1992. Durum wheat and pasta industries: Twenty years of achievement in science and technology. In: Cereal Chemistry and Technology: A long past and a bright future. Proceeding of 9th Int. Cereal and Breed Congress, 1-5 June Paris 1992, pp. 139-142.

Letta, T., M. G. D`Egidio and M. Abinasa. 2008. Stability Analysis for Quality Traits in Durum Wheat (*Triticum durum* Desf.) Varieties under south Eastern Ethiopian conditions, World J. Agri. Sci. 4(1):53-57.

Marshall, D. R., D. J. Mares, H. J. Moss and Elliason, F.W. 1986. Effect of grain shape and size on milling yields in wheat. Experimental studies. Austr. J. Agric. Res. 37:331–342.

Mariani, B. M., M. G. D'Egidio and P. Novaro. 1995. Durum wheat quality evaluation: influence of genotype and environment. Cereal Chem. 72(2):194.

Matsuo, R. R. and J. E. Dexter. 1980. Relantionship between some durum wheat physical characteristics and semolina milling properties. Can J. Plant Sci. 60:49-53.

Miezan, K., E. G. Heyne and K. F. Finney. 1977. Genetic and environmental effects on the grain protein content in wheat. Crop Sci. 14:175-180.

Mohammadi, M., R. Karimizadeh, M. K. Shefazadeh and B. Sadeghzadeh. 2011. Statistical analysis of durum wheat yield under semi-warm dryland condition. Austr. J. Crop Sci. 5(10):1292-1297.

Morris, C. F., H. C. Jeffers and D. A. Engle. 2000. Effect of processing, formula and measurement variables on alkaline noodle color – toward an optimized laboratory system. Cer. Chem. 77:77–85.

Mpofu, A., D. Harry, H. D. Sapirstein and T. Beta. 2006. Genotype and environmental variation in phenolic content, phenolic acid composition, and antioxidant activity of hard spring wheat. J. Agric. Food Chem. 54:1265-1270.

Nachit, M. M., M. Baum, A. Impiglia and H. Ketata. 1993. Studies on some grain quality traits in durum wheat grown in Mediterranean environments. In: N. Di Fonzo, F. Kaan and M. Nachit (Eds.) pp. 17-19. Durum Wheat Quality in the Mediterranean Region, CIHEAM/ICARDA/CIMMYT, Zaragoza, Spain.

Nachit, M. M., M. Baum, A. Impiglia and H. Ketata. 1995. Studies on some grain quality traits in durum wheat grown in Mediterranean

environments. In: N. Di Fonzo, F. Kaan and M. Nachit (Eds.) pp. 181-187. Durum Wheat Quality in the Mediterranean Region, CIHEAM/ICARDA/CIMMYT, Zaragoza, Spain.

Novaro P., M. G. D'Egidio, L. Bacci and B. M. Mariani. 1997. Genotype and environment: their effect on some durum wheat quality characteristic. J. Gen. Breed. 51:247-252.

Pedro, A., R. Savin, Z. D. Habash and G. A. Slafer. 2011. Physiological attributes associated with yield and stability in selected lines of a durum wheat population, Euphytica 180:195-208.

Peterson, C. J., P. S. Graybosh, P. S. Baenziger and A. W. Grombacher. 1992. Genotype and environment effects on quality characteristics of hard red winter wheat. Agron. J. 85:1023-1028.

Rao, A. C. S., J. L. Smith, V. K. Jandhyala, R. I. Papendick and J. F. Parr. 1993. Cultivar and climatic effects on protein content of soft white winter wheat. Agron. J. 85:1023-1028.

Rharrabti, Y., D. Villegas, C. Royo, V. Martos-Núñez and L. F. Garcia del Moral. 2003. Durum wheat quality in Mediterranean environments II. Influence of climatic variables and relationships between quality parameters. F. Crops Res. 80:133-140.

Sakin, M. A., A. Sayaslan, O. Duzdemir and F. Yuksel. 2011. Quality characteristics of registered cultivars and advanced lines of durum wheats grown in different ecological regions of Turkey. Can. J. Plant Sci. 91:261-271.

Troccoli, A., G. M. Borrelli, P. De Vita, C. Fares and N. di Fonzo. 2000. Durum wheat quality: a multidisciplinar concept. J. Cereal Sci. 32:99-113.

Uhlen, K. A., R. Hafskjold, A. H. Kalhovd, S. Sahlström, Longva and E. M. Åmagnus. 1998. Effects of cultivars and temperature during grain filling on wheat protein content, composition, and dough mixing properties. Cereal Chem. 75:460-465.

Williams, P. C., M. Nachit, A. Shehadeh, A. Sategh and M. Michael. 1986. Comparative quality of Sebou with Gezira. Sham I, Rachis 5(2):55.

Zhu, J. and K. Khan. 2001. Effects of genotype and environment on glutenin polymers and bread-making quality. Cereal Chem. 78:125-130.

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SHORT COMMUNICATION

Yield and nutritional quality of greenhouse lettuce as affected by shading and cultivation season

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Abstract

Glasshouse experiments were conducted in winter and spring growing seasons in order to evaluate the effects of shading on production and nutritional quality of lettuce (*Lactuca sativa* L. cv. Parris Island), under Mediterranean climate conditions. In both seasons, plants were cultivated hydroponically under four different levels of photosynthetically active radiation intensities (26, 47, 73 and 100 % of incident light intensity). The results showed that stomatal conductance and photosynthetic rate significantly decreased in shaded plants. This strong negative correlation of leaf physiological parameters with light deficiency resulted in lower biomass yield production in both growing seasons. Moreover, the nutritional value (ascorbic acid concentration) was also significantly decreased in relation to incident light intensity decrease. In contrary, a strong positive correlation of leaf total chlorophyll content and nitrate content with light deficiency was detected. However, nitrate concentration in all treatments remained within the European Union's permissible levels being significantly lower in plants produced in spring compared to winter.

Key words: *Lactuca sativa*, Light intensity, Nitrate content

Introduction

Lettuce consists one of the most important cultivated vegetable in Greece, contributing significantly to national economy. Lettuce leaves are usually consumed raw and without any restriction to daily intake. However, lettuce is characterized by its great ability to accumulate nitrate in leaves which can be harmful to human health (Cometti et al., 2011). Thus, nitrate concentration in lettuce is considered one of the more important quality parameters.

Nitrate accumulation in plants is quite complex since it is influenced by both genetic and environmental factors (Novo et al., 2008). Among environmental factors light intensity is reported to strongly affect nitrate accumulation in plants (Novo et al., 2008). Taking into consideration the recent results which conclude that plants subjected to lower light intensities tend to accumulate more nitrates, nitrate levels are expected to be lower in plants grown in the Mediterranean zone which is characterized by relatively higher radiation intensities. However, the fact that nitrate accumulation in plants does not solely depend on irradiation intensity make it difficult the extrapolation of the results obtained in areas characterized by different environmental conditions. Furthermore, little information is available concerning the interaction of light intensity and growing season on the nitrate concentration. Thus, a more comprehensive study concerning the effects of different light intensities in each production area is needed.

Besides nitrates, ascorbic acid concentration is also considered as an important quality indicator in lettuces which is also influenced by both abiotic and biotic parameters (Cometti et al., 2011). However, the effects of different light intensities in ascorbic acid concentration in lettuces remained more or less obscure.

The aim of the present study was to examine the effects of different light intensities in nitrate concentration and nutritional value in lettuce plants

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grown in hydroponic system in two different growing seasons.

Materials and methods

Two experiments in different seasons (winter–spring) were conducted in a heated greenhouse of Technological Institute of Mesolonghi (lat. $38^0$21'N, long. $20^0$56'E), using a hydroponic system. The first experiment, which conducted during the winter period, started on November 10th, 2007 while the second experiment (spring season) started on March 8th, 2008. In both experiments, lettuce seedlings (*Lactuca sativa* L. cv. Parris Island) of three true leaves were obtained from a nursery and transplanted in 4 liters pots filled with perlite. Immediately after transplanting the seedlings were randomly divided into four groups (each one consisting of 80 plants). All plants were uniformly irrigated with a nutrient solution containing: 8 mM KNO_3, 4 mM $Ca(NO_3)_2$, 2mM $MgSO_4$, 1 mM KH_2PO_4, 4 mM $MgCl_2$, 42 μM NaFe-EDTA, 45 μM H_3BO_3, 9 μM $MnCl_2$, 0.7 μM $ZnSO_4$, 0.3 μM $CuSO_4$, 0.12 μM Na_2MoO_4. During the experimental period the nutrient solution's pH ranged between 5.6 and 6.0.

Four different light intensity treatments were applied using shade nets: (1) 100% of full ambient light (FAL) (control); (2) 73% of FAL; (3) 47% of FAL and (4) 26% of FAL. Light interception by different type nets, expressed as percentage of incident photosynthetic active radiation (PAR), was calculated at midday by taking 10 readings of PAR in rapid succession above the each type nets and 10 below the nets using a 60-cm Sunfleck Ceptometer (Decagon Devices, Pullman, WA, USA). The maximum daily PAR values measured in a sunny day in the control treatments ranged between 1290 μmol $m^{-2}s^{-1}$ and 1000 μmol $m^{-2}s^{-1}$ in spring and winter season respectively.

Gas exchange measurements

Gas exchange measurements were conducted on twenty fully expanded, mature leaves per treatment in both experimental periods. All measurements were performed one day before harvest, at the same daytime (10.00–11.30 a.m.), using a portable gas exchange system (LCpro+, ADC BioScientific Ltd). Fresh weight was measured at harvest on twenty plants per treatment.

Chlorophyll content

Chlorophyll concentration was determined on twenty leaf discs obtained from twenty randomly selected leaves per treatment. Leaf samples were collected at harvest, wrapped in plastic bags and transferred immediately in the laboratory for chlorophyll content estimation. The concentration of chlorophyll a (Chl a) was measured at 665nm and chlorophyll b (Chl b) 648nm, using a spectrophotometer (Shimadzu UV-1601 VIS) (Shinano et al., 1996). The content of the chlorophylls was calculated using the following equations (Lichtenthaler and Wellburn, 1983):

$Chla = 14{,}85xA_{665} - 5{,}14xA_{648}$ (mg Chl a/ml)
$Chlb = 25{,}4xA_{648} - 7{,}36xA_{665}$ (mg Chl b/ml)

Ascorbic acid

Ascorbic acid content was determined in the same leaves that were previously used for chlorophyll determinations according to the method described by Bajaj and Kaur (1981), using a Spectrophotometer (Shimadzu UV-1601 VIS).

Nitrates content

Nitrate concentration was measured at harvest using twenty randomly selected leaves obtained from twenty plants per treatment. Each leaf sample was homogenized in a blender and the resulting slurry was filtered through two paper filters. The filtrate was diluted (1: 10-1:200) in the eluent used and filtered through a 0.2-μm membrane filter. This solution was injected into the chromatograph according to Pentchuk et al. (1986).

Statistical data analysis

Mean values of all treatments were compared by two-way ANOVA test and significant differences were determined using Duncan's test ($p<0.05$). All data were analyzed using SPSS 15.0 program for Windows.

Results and Discussion

In both seasons (winter and spring) the accumulation of nitrates in the leaf tissues was significantly higher in lower irradiance intensity treatments compared to the control (Table 1). Furthermore, nitrate concentration was significantly higher in plants grew during the winter period compared to those in spring (Table 1) irrespective the light intensity applied, indicating that different growing seasons affect nitrate content. These higher values of nitrate concentration during the winter season could be attributed to lower PAR intensity exhibited during that period and/or to differences in other microclimatic parameters. However, the fact that there was no significant interaction between growing season and PAR intensity (Table 1) provide evidences that the above mentioned differences in nitrate concentration could be mainly attributed to changes in PAR intensity. These results clearly suggest that light intensity should be considered as the main factor influencing of nitrate

concentrations in lettuces. Novo et al. (2008) growing lettuce in hydroponic system reported that only a 18% shading resulted in an significant increase in nitrate concentration in plants. In our results the maximum concentration of nitrates found (even at lowest levels of PAR intensity in winter) does not exceed the European union permissible levels for winter season suggesting that under the studied area conditions the nitrate levels do not represent any risk for human consumption of hydroponics lettuces. Similar results concerning the significant role of light intensity in nitrate concentration have also been reported for a variety of vegetables (Blom-Zandstra and Lampe, 1985; Drews et al., 1997; Proietti et al., 2004).

Except nitrates, ascorbic acid concentration is considered as one of the most important nutritional quality factors in many vegetable crops (Lee and Kader, 2000). In our results, the higher levels of shading seemed to negatively affect ascorbic acid concentration (Table 1). Furthermore, the ascorbic acid content was significantly lower in lettuces grown during winter compared to those grown during spring (Table 1). The statistical analysis of all data indicates that ascorbic acid concentration in lettuce seemed to be light dependent. Several reports concerning the mechanism of light induced ascorbic acid synthesis tend to conclude an indirect effect of light intensity in ascorbic acid concentration rather a direct one. In particular, a close relationship between photosynthetically produced sugars and ascorbic acid concentration is suggested (Lee and Kader, 2000). If this is true, then it would be expected that photosynthetic rate would be significantly higher in those treatments exhibiting the higher ascorbic acid content. Indeed, in our results the treatments characterised by higher accumulation in ascorbic acid i.e the control treatments also exhibited the higher photosynthetic rates (Figure 1). Thus, our results tend to conclude that the amount and intensity of light during the growing season might have a considerable effect on the concentration of ascorbic acid probably via its influence on the plants photosynthetic performance. Similar results were also observed in other leafy greens where ascorbic acid concentration was also increased in relation to light exposure (Gruda, 2005; Oyama et al., 1999; Weerakkody, 2003).

Table 1. Effect of different light intensities (expressed as percentage of the control) and growing seasons on nitrates, chlorophyll and ascorbic acid content as well as on fresh weight in lettuces plants*.

Season	PAR (% Control)	Nitrates mg/ Kg f.w	Chlorophyll a+b mg /g f.w	Ascorbic acid mg /100g f.w	Fresh weight (f.w) g.plant^{-1}
Winter	100	2571.3±97.83^{a}	1.51±0.01^{a}	67.77±0.91^{d}	290.41±5.77^{d}
	73	2931.3±50.21^{b}	1.67±0.03^{a}	61.37±0.73^{c}	240.67±5.81^{c}
	47	3203.9±49.65^{c}	1.91±0.06^{b}	55.47±0.52^{b}	208.33±9.28^{b}
	26	3616.7±61.51^{d}	2.13±0.09^{c}	48.20±0.26^{a}	167.67±6.49^{a}
Spring	100	1991.1±61.26^{a}	1.37±0.03^{a}	73.57±0.88^{d}	326.67±12.02^{d}
	73	2357.1±49.8^{b}	1.57±0.03^{b}	65.47±0.58^{c}	270.33±2.91^{c}
	47	2749.7±73.76^{c}	1.77±0.03^{c}	58.43±0.56^{b}	242.67±4.33^{b}
	26	3028.3±52.74^{d}	1.97±0.02^{d}	52.47±0.61^{a}	201.67±4.41^{a}
Two-way ANOVA (p-values)					
Season (S)		<0.0001	<0.001	<0.0001	<0.0001
PAR (P)		<0.001	<0.001	<0.0001	<0.0001
S X P		0.692	0.910	0.241	0.966

*Values are means ± S.E. from 20 replicates. Means followed by different letters are significantly different ($p < 0.05$) according to the Duncan's test. Probability (p-value) of two way analysis for season and PAR is shown.

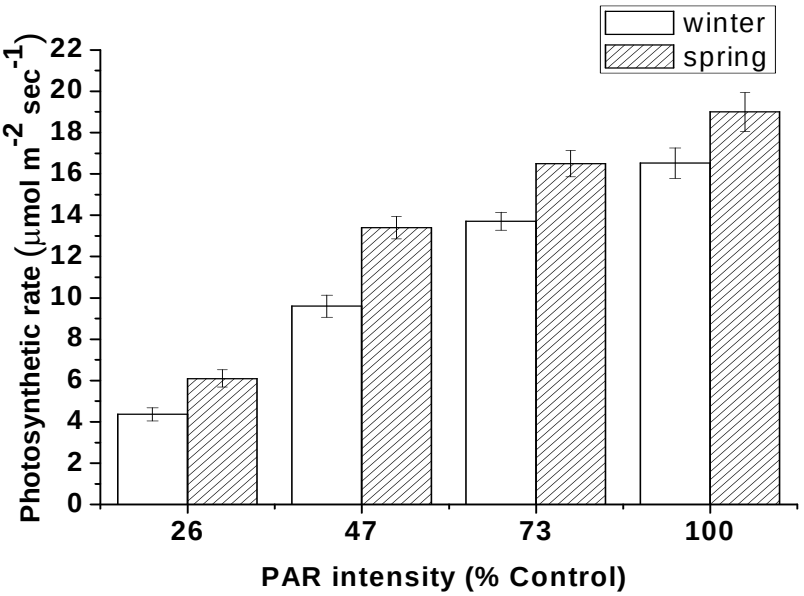

Figure 1. Effect of different light intensities (expressed as percentage of the control) and growing seasons in plants photosynthetic rate.
Data are means ± S.E of twenty replicates.

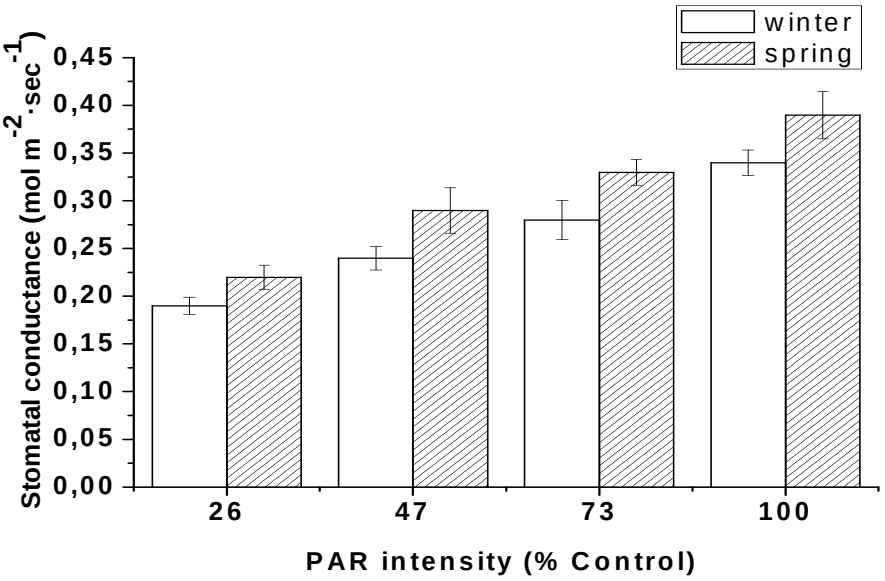

Figure 2. Effect of different light intensities (expressed as percentage of the control) and growing seasons in plants stomatal conductance.
Data are means ± S.E of twenty replicates.

As it was probably expected the increase in photosynthetic rate in plants grown under the higher light intensity was also positively related to plants fresh weight (Table 1). Similar results were also observed by Caruso et al. (2004), who reported that shading caused a reduction in the fresh weight and the content of the main taste-determining compounds (sugar, acids, mineral elements except

nitrates) in strawberries, resulting in a poor taste sometimes associated with autumn-grown vegetables. Higher fresh weight was due to largest leaves per plant rather to different leaf number per plant (data not shown). Craker and Seibert (1983) have suggested a light-dependent photo-regulatory mechanism within the plants that controls the development of leaf size. Our data with lettuce seem to support these interpretations. Higher photosynthetic rate under higher irradiance could be attributed to higher stomatal conductance since the latter exhibit a linear relationship in response changes in photosynthetic rate (Figure 2). In contrary, changes in total chlorophyll concentration in relation to photosynthetic rate followed an opposite pattern exhibiting higher values under reduced radiation intensities (Table 1). This is consistent with the results already reported for various species which indicated higher chlorophyll content in the plants grown under shaded condition (Vandana and Bhatt, 1999). This increased pigment content in shaded leaves has been attributed to the increase in number and size of chloroplast, the amount of chlorophyll per chloroplast and/or better granna development and is considered to be an efficient plants adaptive acclimation process to low light intensities.

Conclusions

Our study indicated that the reduction of light intensity represented the main environmental factor affecting lettuce quality indicators. However, although nitrate increase due to low light intensity was significant, nitrate accumulation in leaves remained within European Union's permissible levels. Furthermore, a direct light-intensity effect on fresh weight and an indirect one on nutritional value (ascorbic acid) of lettuce were also evident.

References

Bejaj, K. L. and G. Kaur. 1981. Spectrophotometric determination of L-ascorbic acid in vegetables and fruits. Analyst. 106:117-120.

Blom-Zandstra, M. and J. E. M. Lampe. 1985. The role of nitrate in the osmoregulation of lettuce *(Lactuca sativa* L.) grown at different light intensities. J. Exp. Bot. 36:1043–1052.

Caruso, G., A. Villari and G. Villari. 2004. Quality characteristics of "*Fragaria vesca* L." fruits influenced by NFT solution EC and shading. Acta Hort. 648:167–174.

Cometti, N. N., M. Martins, C. Bremenkamp and J. A. Nunes. 2011. Nitrate concentration in lettuce leaves depending on photosynthetic photon flux and nitrate concentration in the nutrient solution. Hortic. Brasil. 29:548-553.

Craker, L. E. and M. Seibert. 1983. Light and the development of Grand Rapids lettuce. Can. J. Plant Sci. 63:277-281.

Drews, M., I. Schonhof and A. Krumbein. 1997. Gehalt an inhaltstoffen in eissalat (*Lactuca sativa* L. var. capitata L.) in abhangigkeit von sorte und stadium der kopfentwicklung beim anbau im gewachshaus. Gartenbauwiss. 62(2):65–72.

Gruda, N. 2005. Impact of environmental factors on product quality of greenhouse vegetables for fresh consumption. Crit. Rev. Plant Sci. 24(3):227-247.

Lee, S. and A. A. Kader. 2000. Preharvest and postharvest factors influencing vitamin C content of horticultural crops. Postharvest Biol. Tec. 20:207-220.

Lichtenthaler, H. K. and A. R. Wellburn. 1983. Determinations of total carotenoids and chlorophylls a and b of leaf extracts in different solvents. Biochem. Soc. Trans. 11:591-59.

Novo, A. C., J. F. Medeiros, C. H. E Souza, P. R. G. Pereira, H. E. P. Martinez, P. C. R Fontes and N. N. Cometti. 2008. Influência do sombreamento so bre o crescimento e teores de nitrato em hortaliças folhosas em hidroponia. Revista Universo Acadêmico, Nova Venécia, 13.

Oyama, H., Y. Shinohara and T. Ito. 1999. Effects of air temperature and light intensity on β-carotene concentration in spinach and lettuce. J. Japan. Soc. Hort. Sci. 68(2):414-420.

Pentchuk, J., Ü. Haldna and K. Ilmoja. 1986. Determination of nitrate and chloride ions in food by single-column ion chromatography. J. Chromatogr. A 364:189-192.

Proietti, S., S. Moscatello, A. Leccese, G. Colla and A. Battistelli. 2004. The effect of growing spinach (*Spinacia oleracea* L.) at two light intensities on the amounts of oxalate, ascorbate and nitrate in their leaves. J. Hort. Sci. Biotech. 79(4):606–609.

Shinano, T., T. T. Lei, T. Kawamukai, M. T. Inoue, T. Koike and T. Tadano. 1996. Dimethylsulfoxide method for the extraction of chlorophylls a and b from the leaves of

wheat, field bean, dwarf bamboo, and oak. Photosynthetica 32(3):409-415.

Vandana, V. and R. K. Bhatt. 1999. Physiological changes in Sesbania species to reducing light intensities. J. Agron. Crop Sci. 182:43-47.

Weerakkody, W. A. P. 2003. Nutritional value of fresh leafy vegetables as affected by pre-harvest factors. Acta Hort. 604:511–515.

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REGULAR ARTICLE

The use of natural zeolites impregnated with zinc and copper ammoniates as carriers of micronutrients in growing vegetables

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Abstract

The aim of our study was to obtain prolonged micronutrients for use in organic agriculture and production of row materials for baby food. For this purpose, we proposed to use impregnated with ammoniates of zinc and copper zeolite. Field experiments were conducted during 2011 – 2012 on the territory of Kyiv region. Results of two-year experiments revealed that during the first year of impregnated zeolite implementation statistically reliable (confidence level 0.95) increase of zinc and copper in carrot and beetroot. On the second year after zeolite implementation, their influence on zinc and copper concentration in spring oats and spring barley grains was observed. Concentration of these micronutrients in oats and barley grains was significantly larger than in the control sample. Implementation of zeolites, impregnated with zinc and copper ammoniates, can be recommended for improving the soil quality and micronutrients level in grain and vegetable production.

Key words: Micronutrients, Soil, Zeolite, Baby food, Organic agriculture

Introduction

Ukraine has vast areas of fertile lands. The most fertile soils in the world – humus soils – occupy almost 27.8 million ha, which is 67.7% of total arable Ukrainian lands and 8.7% of all world humus (Pozniak, 2008). Availability of large areas of fertile lands enables the development of organic agriculture production. According to the IFOAM data, in 2011 in Ukraine there were 118 farms registered and certified by international accreditation bodies as farms with organic farming. Total area of certified for organic farming agricultural lands is of 270226 ha. Nowadays, Ukraine is ranked first in the east-European region by the area of lands certified for organic farming. These farms specialize mostly on production of grain, bean and oil cultures. The area of certified lands is almost 0.7% of all arable lands of Ukraine (Data collection, 2013).

Besides, production of baby food in Ukraine can only take place in a special raw area. The status of such zone is given after the detailed analysis of quality of soils and their pollution with radionuclides, heavy metals and persistent organic pollutants (Law of on Baby Food, 2006). The main criterion for organic farms and special raw areas is the concentration of radionuclides in their soils. This is important since almost one third of Ukraine, or 15 thousand of square kilometers, is polluted with radioactive wastes (National Report of Ukraine, 2011). This area is inhabited by almost 2.4 million people. According to the Ukrainian "Law on Baby Food", the status of special raw area can be granted to the farm with concentration of 10 kBk/m^2 for ^{137}Cs and 0.75 kBk/m^2 for ^{90}Sr. Pollution of agricultural lands with heavy metals and persistent organic pollutants also limits number of farms that can receive the special raw area status.

The important criterion for organic farms and farms with special raw area status is sufficient concentration of micronutrients in soil. Agricultural

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activities on such lands require introduction of required microelements. Important requirement for organic farming is the reduction of solubility of introduced micronutrients. That is why our research is focused on microfertilizers with prolonged action for usage in organic farming and baby food production.

Materials and Methods

Zeolite-Sokyrnit (clinoptilolite) was chosen for the prolongation of microelements effect. Usage of zeolites is allowed in organic farming without any restrictions. The necessity of application of microelements (e.g., boron, copper, iron, manganese, molybdenum, zinc) has to be recognized by the certification body or authority (Codex Alimentarius, 2007). Zeolite-Sokyrnit is the mineral of volcano origin with deposit in Zakarpattya region of Ukraine. Low cost and simplicity of application are the main reasons of economical effectiveness of zeolite technologies. Zeolite-Sokyrnit consists of: $(Na, K)_4\ CaAl_6Si_{30}O_{72}\cdot 24H_2O$ (Tarasevich Yu et al., 1979). Natural zeolite is widely used in agriculture since due to its unique properties it improves the soil quality, capable of retaining the moisture and reducing the mobility of pollutants.

We have used zeolite with the grain size of 3-10mm. Crystal structure of zeolite is formed by tetrahedral groups of $SiO_2/4$ and $AlO_2/4$, connected by common vertexes in the 3d-frame pierced with cavities and canals. Quantities of the microelements used for the impregnation were evaluated basing on adsorption capabilities of zeolite (using the data from adsorption isotherm).

To obtain zeolites impregnated with zinc and copper ammoniates they were held in the water solution of these substances for 14 hours. Quantities of zinc and copper salts were calculated according to the plants need in microelements. Quantity of zeolites was calculated provided that water capability of zeolites to be of 20%. To introduce the test plots in soil of total area of 0.4 ha, we processed 35.2 kg of 3-10mm zeolite grains with 7 liters of ammoniates solution containing 79.0 g of Zn^{2+} and 15.4 g of Cu^{2+}.

Field experiments were conducted over 2011 – 2012 on the territory of Kyiv region according to the Technique of field experiment (Dospehov, 1985). Soil of the region of experiment is the low-humus chromosomes soil (humus concentration of about 2.7– 4%). The experiment was conducted to study the effect of zeolites impregnated with zinc and copper ammoniates ($Zn(NH_3)_4SO_4$ and $Cu(NH_3)_4SO_4$) on the concentration of micronutrients in carrot and beet.

Experiment scheme for carrot

1. Control – without introduction of zeolite and microelements;
2. Non-impregnated Zeolite introduction;
3. Impregnated Zeolite introduction - 0.275 kg/m^2 (Zn – 200mg/m^2; Cu – 40 mg/m^2).

Experiment scheme for beetroot

1. Control – without introduction of zeolite and microelements;
2. Non-impregnated Zeolite introduction;
3. Impregnated Zeolite introduction - 0.275 kg/m^2; (Zn – 200mg/m^2; Cu – 40 mg/m^2)

To study the prolonged effect of zeolites impregnated with zinc and copper complexes on the content of micronutrients in agricultural raw materials, in 2012 we conducted additional experiments. We planted grains – spring oats and spring barley - at the same location without adding new portions of zeolites. Barley was planted instead of carrot and oats was planted instead of beetroot.

The experiment was repeated 3 times. The area of each test site was 0.02 ha. Plots have random positions. Root-crop samples were selected using the envelope method – in 5 points on each site 10 carrots, 5 beetroots and grain were sampled. Grain yield were collected from each plot separately.

Determination of metal concentrations in products of mineralization of samples was conducted using the method of atomic adsorption in the propane-air flame (atomic-adsorption spectrometer AAS-3, Germany). The sources of radiation were hollow cathode lamps, working wavelengths – 324.8 nm for copper and 213.9 nm for zinc.

Total metal concentration in the soil was determined using the method of dissolution of soil in hydrofluoric and perchloric acids according to ISO 14869-1:2001 followed by atomic-absorption determination according to ISO 11466:1995. Determination of metal concentrations in root-crop was repeated 3 times according to GOST 30178-96 (closest analogs - ISO 7952:1994; ISO 6636-2:1981; ISO 6869:2000).

Determination of metal concentrations in samples was conducted using atomic-absorption method in the propane-air flame (spectrometer AAS-3, Carl Zeiss, Germany). Statistical data processing was made using the StatSoft Statistica 7 software.

Measurements

Pore structure of the Zeolite-Sokyrnit (clinoptiolite) was studied using low-temperature (77.4 K) adsorption/desorption of nitrogen on the high-speed gas sorption analyzer "Autosorb-6" ("Quantachrome", USA). Before the footage samples were degassed for 20hrs under the temperature of 473K and vacuum $6.58 \cdot 10^{-5}$ Torr. Processing of the obtained results of the nitrogen adsorption/desorption was made automatically using the software Quantachrome Instruments, version 3.0. We measured specific surface area (S), specific volume (V) and average radius (R) of zeolite (clinoptiolite) pores. We also studied dependence of pore volume distribution on the radius of pores using Density Functional Theory (DFT) (Webb et al., 1997).

Mean specific surface area is 18.5 m^2/g. Mean specific pore volume (V) is 0.1 cm^3/g. Mean pore radius (R) is 5.3 nm. Differential curve obtained contains the series of peaks that correspond to the radii of 2.8, 12 nm, and the wide range of values from 12 to 22 nm, which reflects a multimodal distribution of pores in the sample. These pores are primary for the impregnation of zeolite (clinoptiolite) with zinc and copper ammoniates.

Measurements of metals concentrations in root-crop and grain-crop were made three times. Beetroot and carrot samples from each site were washed, peeled and chopped. After thorough mixing, three samples of 15-20g were selected from each variant. (Samples of root-crop and grain were 3-5 times larger compared to the minimum required sizes to obtain more representative results). Mineralization of samples was made using the method of dry ashing. Every sample was put in the heat-resisting bulb. After drying samples in a dessicator, the samples were flied ash in a muffle furnace at the temperature of 400°C. Ash of each sample was preliminary processed with nitric acid (1:1) with a few drops of hydrogen peroxide. After evaporation to the state of moist salts samples were dissolved in 1H nitric acid. The solution was then filtered and diluted to obtain the volume of 25 cm^3. As for grain, we sampled 3 samples of 10 grams each from each variant. Product of grain mineralization we obtained using the same way as described above, except drying.

Results

Concentration of microelements in vegetables in each sample is presented in Figure 1, in grain – in Figure 2. Reliability of data on metal concentrations in different samples was tested using t-test for independent samples (Table 1, 2). Averaged metal concentrations for each sample are also presented.

Concentration of zinc and copper in root-crop of carrot in control sample (Var 1) and in sample where non-impregnated zeolites were used (Var 2) is statistically insignificant (see in Table 1). At the same time, in the root-crop of beetroot there is a reliable increase in the concentration of copper when using non-impregnated zeolites as well as impregnated ones. Implementation of impregnated zeolites (Var 3) causes an increase in both metals concentrations comparing to both control (Var 1) and non-impregnated zeolite variants (Var 2). Besides, the crop increase is observed in both zeolite variants - (Var 2) and (Var 3) - comparing to the control (Var 1).

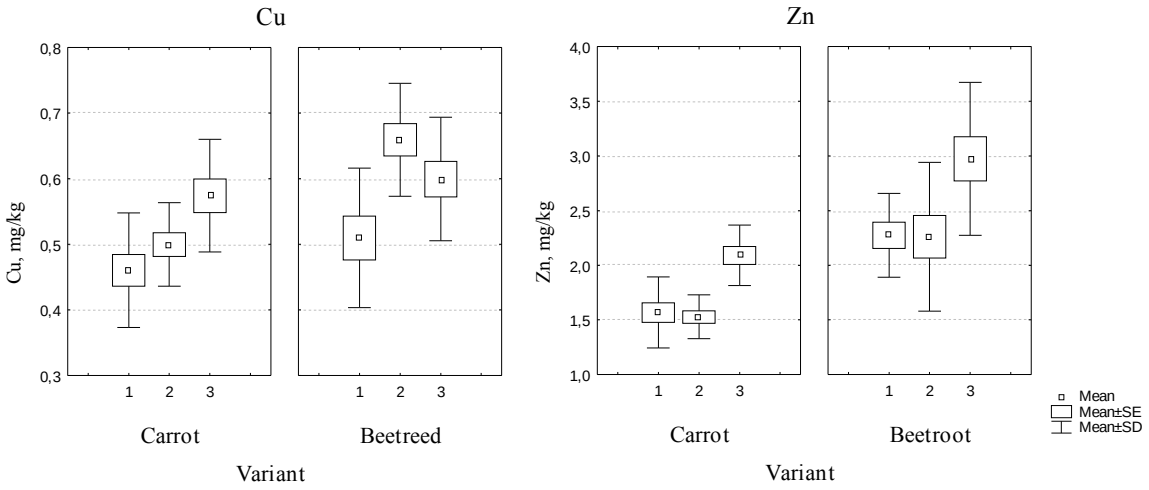

Figure 1. Concentration of Copper and Zinc in carrot and beetroot roots.

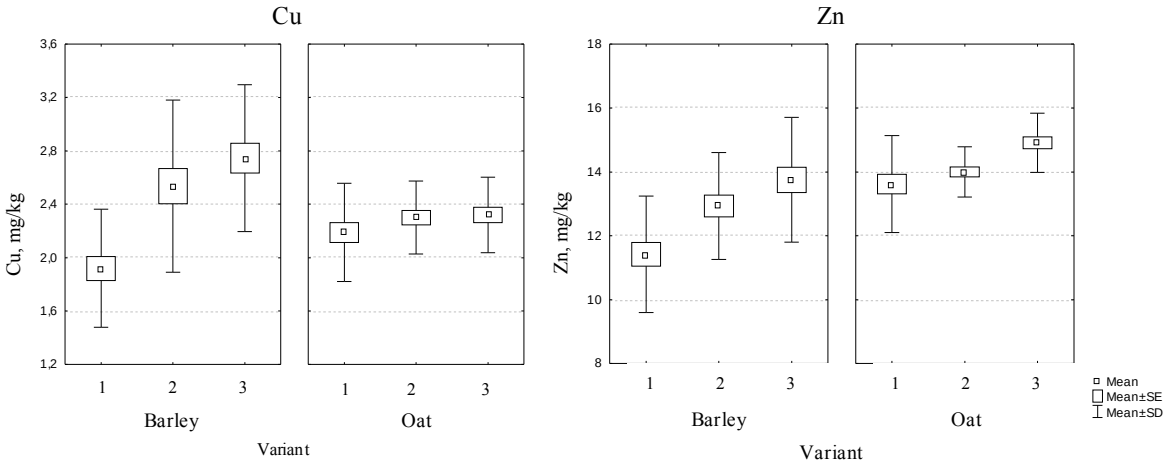

Figure 2. Concentration of Copper and Zinc in barley and outs grains.

Table 1. Averaged values and statistical significance level of the hypothesis about coincidence of group means for root-crop.

Parameters	Carrot Mean		Statistical significance	Beetroot Mean		Statistical significance
	Var 1	Var 2		Var 1	Var 2	
Cu, mg/kg	0.46	0.50	0.19	0.51	0.66	0.002
Zn, mg/kg	1.52	1.53	0.92	2.27	2.26	0.96
Yild, t/ha	21.8	22.3	0.055	17.0	19.9	<0.0001
	Var 1	Var 3		Var 1	Var 3	
Cu, mg/kg	0.46	0.57	0.00416	0.51	0.60	0.049
Zn, mg/kg	1.52	2.09	<0.0001	2.27	2.97	0.010
Yild, t/ha	21.8	22.9	<0.0001	17.0	20.6	<0.0001
	Var 2	Var 3		Var 2	Var 3	
Cu, mg/kg	0.50	0.57	0.027	0.66	0.60	0.118
Zn, mg/kg	1.53	2.09	<0.0001	2.26	2.97	0.019
Yild, t/ha	22.3	22.9	0.0004	19.9	20.6	0.016

Table 2. Averaged values and level of statistical significance of hypothesis about samples coincidence for grain crop.

Spring barley				Spring oat			
	Mean		statistical significance		Mean		statistical significance
	Var. 1	Var. 2			Var. 1	Var. 2	
Cu	1.9	2.5	<0.001	Cu	2.2	2.3	0.24
Zn	11.4	12.9	0.004	Zn	13.6	14.0	0.28
	Var. 1	Var.3			Var. 1	Var.3	
Cu	1.9	2.7	<0.001	Cu	2.2	2.3	0.17
Zn	11.4	13.8	<0.001	Zn	13.6	14.9	<0.001
	Var.2	Var.3			Var.2	Var.3	
Cu	2.5	2.7	0.23	Cu	2.3	2.3	0.79
Zn	12.9	13.8	0.125	Zn	14.0	14.9	<0.001

On the next year after the implementation of zeolites intake of microelements into agricultural crops was changed (Table 2). For grain crops concentrations of both metals increased after zeolite implementation, both impregnated and non-impregnated (beside copper in Var.2 - Var.3). Besides, for spring barley statistically significant difference in data ($p = 0.95$) is observed when

compared to control. This difference is much less significant when comparing non-impregnated and impregnated zeolites. For oats, an increase in zinc concentration in the variant with impregnated zeolite compared to other two variants is statistically reliable only.

Discussion

Zeolites are porous crystalline aluminum silicates. They consist of combined oxides of silicon, aluminum and alkali or alkaline earth metals. They can also include different chemical elements and crystal water. Properties of zeolites are defined by nano-channels and cavities that can contain extraneous ions and neutral molecules. It is known that Natural zeolite is widely used in agriculture. Thanks to its unique properties and chemical composition it improves soil quality, can retain moisture and decrease pollutants mobility. (Salavati-Niasari et al., 2011).

In our research we used the ability of zeolites to adsorb metal-ammonium complexes. Using electron paramagnetic resonance EPR (Clark et al., 1984) the mechanism of interaction of copper ammoniate complexes with aluminum silicates Allophane and Imogolite (zeolite (clinoptiolite) analogs) was studied. It was shown that during sorption of ammoniate complexes several types of complexes are formed on the surface of aluminum silicates. Aluminum silicate groups of the carrier (≡Si-OH и =Al-OH) take part in the formation of these complexes:

$$\equiv Si\text{-}OCu(H_2O)_4^{+} + 4NH_3 \leftrightarrow \equiv Si\text{-}OCu(NH_3)_4^{+} + 4H_2O \quad (1)$$

$$\equiv Si\text{-}OH + NH_3 + Cu(NH_3)_4(H_2O)_2^{2+} \leftrightarrow \equiv Si\text{-}OCu(NH_3)_4^{+} + NH_4^{+} + 2H_2O \quad (2)$$

$$=Al\text{-}OCu(NH_3)_2OH + NH_3(aq) \leftrightarrow =Al\text{-}OCu(NH_3)_3^{+} + OH(aq)^{-} \quad (3)$$

We must admit that chemical composition of a copper ammoniate complex with aluminum silicate surface and its hydrolytic stability depends on $Cu/NH_3(aq)$ proportion. If NH_3 is insufficient, the balance in reaction (1) shifts left. If the proportion is equimolar or ammonia is prevailing, the balance in reaction (1) shifts right. Besides, when ammonia is prevailing, reactions (2) and (3) become possible. Ammonium ions, which results from these reactions, are adsorbed by aluminum silicates. We can assume that cation $[Cu(NH3)_4]^{2+}$ is adsorbed by the narrow mesopores following the ion-exchange mechanisms (reactions 1-3), and the $Cu(NH3)_4SO_4 \cdot H_2O$ molecules are adsorbed by wide mesopores.

We proposed that implementation the soil of micronutrients in the form of complexes with natural zeolites of Sokirnya deposit can help to increase the zinc and copper concentration in agricultural production and prolong micronutrients presence in soil by reducing their mobility.

In field experiment in 2012 we studied the influence of non-impregnated and impregnated with copper and zinc ammoniates zeolites on the concentration of zinc and copper micronutrients in carrot and beetroot (see Figure 1).

Concentration of zinc and copper in carrot of control sample (Var. 1) non-impregnated zeolites sample (Var. 2) is statistically insignificant (Table 1). At the same time, in root-crop of beetroot a reliable increase in copper levels is observed when using both impregnated and non-impregnated zeolites. Implementation of impregnated zeolites (Var. 3) leads to an increase in concentrations of both metals comparing both to the control (Var. 1) and to non-impregnated zeolite variant (Var 2). Crop increase is observed when using both non-impregnated (Var 2) and impregnated zeolites variants (Var 3) compared to the control. These results confirmed field experiment conducted under the same soil conditions in 2011.

We also studied a prolonged effect of implementation of impregnated zeolites on the crop production (Figure 2). The next year after implementation of zeolites we observed new data, which are presented in Table 2. For grain-crop an increase in concentrations of both metals is observed when using any combination of impregnated and non-impregnated zeolites (beside copper in Var.2 - Var.3). Particularly, for barley we observe statistically reliable (confidence level 0.95) differences of implementation of impregnated zeolites (Var. 3) compared to the control; but, compared to the non-impregnated zeolites (Var. 2) there is less difference between these two. For oats, only the difference in zinc levels is statistically reliable when comparing impregnated zeolite (Var. 3) both to control (Var. 1) and non-impregnated zeolite variant (Var. 2).

Conclusions and recommendations

Environmentally safe method for improving the quality of vegetables and grain is proposed. Based on field experiments conducted in 2011 and 2012 it was shown that implementation of zinc and copper ammoniates in their complex with natural zeolites from Sokirnya deposit (Ukraine) helps to increase the concentration of zinc and copper in agricultural production.

Results of two-year experiments revealed that during the first year of impregnated zeolite implementation a statistically reliable (confidence level 0.95) increase in zinc and copper content in carrot and beetroot is observed.

On the second year after zeolite implementation, their influence on zinc and copper concentration in spring oats and spring barley was observed. Concentration of these microelements in oats and barley grains was significantly larger than in the control sample. For oats, an increase in zinc concentration compared to control variant and non-impregnated zeolite variant is statistically reliable. For barley, difference between control and impregnated zeolite samples is statistically reliable (confidence level 0.95), but difference between the impregnated zeolite variant and non-impregnated zeolite variant is not statistically significant.

Implementation of zeolites, impregnated with zinc and copper ammoniates, can be recommended for improving the soil quality and micronutrients level in grain and vegetable production.

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References

Clark, C. J. and M. B. McBride. 1984. Chemisorptions of Cu(II) and Co(II) on Allophane and Imogolite. Clays and Clay Minerals. 32(4):300-310.

Codex Alimentarius. 2007. Organically Produced Foods. Third Edition. WHO, FAO. Rome. p. 33.

Data collection on organic agriculture world-wide. 2013. The World of Organic Agriculture. 14th ed. Nuremberg, Germany.

Dospehov, B. A. 1985. Technique of field experiments (with the fundamentals of statistical processing of research results). 5th Ed., M.: Agropromizdat.

GOST 30178-96:1996. Raw material and food-stuffs. Atomic absorption method for determination of toxic elements.

ISO 11466:1995. Soil quality. Extraction of trace elements soluble in aqua regia.

ISO 14869-1:2001. Soil quality. Dissolution for the determination of total element content. Part 1: Dissolution with hydrofluoric and perchloric acids.

ISO 7952:1994. Fruits, vegetables and derived products. Determination of copper content Method using flame atomic absorption spectrometry.

Law of Ukraine. On Baby Food. 2006. Vidomosti of Verkhovnoyi Rady of Ukraine. 44: 4-33.

Pozniak, S. P. 2008. Chornozem. Environmental Encyclopedia. V. 3. pp. 368 – 369. Kyiv.

Salavati-Niasari, M. and F. Mohandes. 2011. From zeolite to host-guest nanocomposite materials, In: B. Reddy (Ed.) pp. 341–380. Diverse Industrial Applications of Nanocomposites. Intechweb.org

Tarasevich Yu, I., G. G. Rudenko, V. E. Kravchenko and V. E. Polyakov. 1979. Physical and Chemical Properties of the Transcarpathian zeolite and its application as a filter material in water treatment. Chem. Tech. Water 1(1):66-69.

Twenty-five years of the Chernobyl disaster. Safety of future. 2011. National Report of Ukraine. Kyiv, KIM.

Webb, P. A. and C. Orr. 1997. Analytical Methods in Fine Particle Technology. Micromeritics Instrument Corporation, Norcross, GA. USA.

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REGULAR ARTICLE

The uptake of macronutrients by an active silicon accumulator plant growing in two different substrata

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Abstract

Pennisetum clandestinum (*Graminae/Poaceae*) an active Si-accumulator, was cultivated in two different substrata, both with reduced Si solubility. Plants growing in organic-rich soils contained much less Ca, K, Na and Si, than species growing in sandy soils. Although the highest macronutrient concentrations were associated to the highest Si levels in the organs of *P. clandestinum*, the R correlation values indicate that Si does not influence the internal balance and the uptake of these elements. In *ca* 65% of the cases roots have the highest average values regardless of the type of culture, while the contents of Mg in the shoots and roots of *P. clandestinum* were generally not significantly different (P>0.05). A significant decline of the macronutrient levels associated to the shoots and roots of *P. clandestinum* was observed from the 4th to the 6th month assay, especially for Ca in both organs, while for Mg and Na the decline is focused mainly in the shoots; K and Si decline is generally below 10%. When average values of Si in shoots and roots of plants collected from organic-rich and sandy soils were plotted against the average concentrations of Ca, K, Mg and Na in the same organs, weak but positive R correlation values were obtained - the highest R values were observed for Na and K and the lowest for Ca and Mg, regardless of the culture. Exception for the high R value observed for K, although the influence of Si on the K status in the whole plant is time-depending - R values, diminished from the 4th to the 6th month, as it happens in the majority of the cases. In conclusion, *P. clandestinum* can grow well and healthily in substrata with acid pH values and high carbonate content and low solubility of Si suggesting that the definition of the essentiality of Si, even in a Si-accumulator plant is still a matter of great controversy.

Key words: Macronutrients, Organic-rich soil cultures, *Pennisetum clandestinum*, Sand cultures, Silicon

Introduction

Pennisetum clandestinum Hochst. ex Chiov (Kikuyu grass, Graminae/Poaceae) is an active Si accumulator, growing in altitude in the natural habitat in East and Central Africa, and a plant used in pastures all over the world (Fulkerson et al., 1998; Williams and Baruch, 2000). Although Si is not essential for higher plants, it improves fitness in nature significantly and it increases agricultural productivity (Raven, 2003). Its presence and availability influences many aspects of the biology of plants, particularly the mineral nutrition balance. Miyake and Takahashi (1985) observed that soybean plants grown without Si, accumulated unusually high concentrations of P, while the addition of Si in the medium increased K concentration in shoots and roots but reduced Na in salt-stressed barley (Liang, 1999), although Gunes et al. (2007) observed that different Si applications on barley growing in sodic-boron toxic soil does not influence their Na contents.

Moreover, Si is known to effectively mitigate various abiotic stresses such as aluminum, arsenic, cadmium, chromium or manganese toxicities, or salinity, drought, chilling and freezing stresses. The mechanisms of Si-mediated alleviation of metal toxicities in higher plants may include the stimulation of antioxidant systems, the complexation or co-precipitation of toxic metal ions with Si or the interference with the uptake processes (Liang et al., 2007). The Si-mediated alleviation of metals toxicity suggests that Si could be a candidate for Cd (Rizwan et al., 2012), Cr (Ali et al., 2013) or As (Tripathi et al., 2013)

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detoxification in crops under Cd, Cr or As-contaminated soils.

Different soil types may also influence the content of Si within the same plant, without promoting an imbalance in mineral content in general (Henriet et al., 2008). The uptake of Si by perennial ryegrass is influenced by the soil type, source of Si and rate of Si applications (Nanayakkara et al., 2008) and the Si content of the plants increased with increasing rates of applications.

Nevertheless, we agree with Epstein (1994) who claimed that Si in higher plants is beneficial but not essential, which is consistent with the findings of Ma et al. (2002) who observed a rice mutant defective in Si uptake, which is neither phenotypically different from the wild type nor different in terms of nutrient uptake especially P and K. Otherwise, the beneficial effects of Si are mainly associated with its high deposition in plant tissues enhancing their strength and rigidity and its capacity to overcome stresses, leading some authors to purpose transgenic crops with the genes controlling Si uptake (Richmond and Sussman, 2003; Ma and Yamaji, 2006).

Sandy soils are low in plant-available Si (Datnoff et al., 1997) as well as those with high silt and organic matter contents. Since the solubility of monosilicic acid is much reduced in the presence of Al, Ca and Fe and low pH values (Birchall, 1978), an experiment where several factors (quartz sand, high organic matter and carbonate contents and low pH) might probably affect the uptake of Si by *P. clandestinum* was undertaken, in order to evaluate the effects on growth, mineral balance and assess the extension of the beneficial effect of Si, if really exists.

Materials and Methods

Pennisetum clandestinum (Hochst. ex Chiov) plants used in soil and sand cultures were cultivated according to the previously described methods (Reboredo, 1988, 1991; Reboredo et al., 2006). Small cuttings had been in water for three-four days and were transported a posteriori to sand and soil cultures in pots with ca. 1700 cm3 volume under daylight.

Each pot contained a single cutting; 120 individuals were tested - half of them growing in sand (82.2% sand, 15.6% carbonate content, 1.0% organic matter and 1.2% silt-clay fraction; pH 6.9; water content 16%) and the other half in an organic-rich soil (38.2% coarse fraction, 12.6% carbonate content, 43.3% organic matter and 5.9% silt-clay fraction; pH 5.6; water content 48%).

Sand and organic-rich soil characterization was undertaken according to Rivière (1977). The elemental composition of both substrata is described in a previous work (Reboredo et al. 2006).

Cuttings were irrigated twice a week with 50 ml of a nutrient solution (Baumeister and Schmidt, 1962) at a final pH of 5.6. From the 2nd month until the end of the experiment – summer period, plants were supplied with a nutrient solution three times a week in order to avoid an excessive dryness of the substrata.

Five *P. clandestinum* plants from each different substrata (each pot contained a single plant) were collected 4 and 6 months after the beginning of the treatment, washed with bidistilled water and separated into shoots and roots and dried at 100ºC to constant weight. Vegetal material was digested by HNO3 according Vandecasteele and Block (1993), Ca, K, Mg, Mn, Na, and Si being determined by atomic absorption spectrometry.

N and P were also monitored in triplicate in plant samples. Nitrogen was determined according to the Kjeldahl method (Watts and Halliwell, 1996) and phosphorus, according to the method described by Watanabe and Olsen (1965). Fibre determination was achieved through the Weende method (Adrian et al., 2000).

An analysis of variance (ANOVA) was performed and average values were compared by the Tukey's test (Sokal and Rohlf, 1994). The adjustment of data through a linear regression analysis was performed as well as the R value.

Results

In what concerns Si, Ca, Mg, Na and K, roots of *Pennisetum clandestinum* have, in ca 65% of the cases, the highest average values regardless of the type of culture considered, except for K and Mg. In the latter case, however, shoots in sand cultures contained an average value of 1.0% Mg, against 0.9% Mg observed in roots ($P>0.05$) while shoots growing in organic-rich soils exhibited almost twice the average Mg value of roots (Table 1).

Table 1. *Average values of Si, Ca, Mg, Na, K and total N and P in *Pennisetum clandestinum* shoots and roots.

	Sandy soils				Organic-rich soils			
	Shoot		Root		Shoot		Root	
§Si*	1697b	± 260	2164c	± 226	788a	± 168	870a	± 243
†Si	1415b	± 134	1954c	± 173	732a	± 92.4	791a	± 143
decline (%)	*16.6*		*9.7*		*7.1*		*9.1*	
§Ca**	3.5c	± 0.54	5.3d	± 0.7	0.61a	± 0.10	1.5b	± 0.1
†Ca	2.3c	± 0.32	4.4d	± 0.69	0.48a	± 0.07	1.1b	± 0.16
decline (%)	*34.3*		*17.0*		*21.3*		*26.7*	
§Mg**	1.0bc	± 0.19	0.91b	± 0.13	1.2c	± 0.12	0.64a	± 0.07
†Mg	0.73b	± 0.11	0.78b	± 0.10	0.83b	± 0.06	0.59a	± 0.06
decline (%)	*27.0*		*14.3*		*30.8*		*7.8*	
§Na	2655b	± 364	3936d	± 606	1970a	± 320	3479c	± 450
†Na	2084b	± 304	3459c	± 474	1459a	± 291	3139c	± 259
decline (%)	*21.5*		*12.1*		*25.9*		*10.0*	
§K**	6.9c	± 1.3	4.6b	± 0.54	6.4c	± 1.0	3.4a	± 0.28
†K	6.5c	± 0.9	4.2ab	± 0.5	5.3bc	± 0.5	3.1a	± 0.6
decline (%)	*5.8*		*8.7*		*17.2*		*8.8*	
§Fibre**	25.5				25.8			
†Fibre	17				21			
decline (%)	*33.3*				*18.6*			
§Total N**	2.4b	± 0.07	1.4a	± 0.12	3.7c	± 0.3	1.1a	± 0.17
†Total N	2.0b	± 0.15	1.1a	± 0.13	3.1c	± 0.3	0.9a	± 0.15
decline (%)	*16.7*		*21.4*		*16.2*		*18.2*	
§Total P**	0.11b	± 0.03	0.07ab	± 0.01	0.34c	± 0.07	0.11b	± 0.03
†Total P	0.10b	± 0.03	0.06ab	± 0.01	0.27c	± 0.05	0.09b	± 0.03
decline (%)	*9.1*		*14.3*		*20.6*		*18.2*	
§N/P ratio	21.8				10.9			
†N/P ratio	20.0				11.5			

Mean values, in the same row, not followed by a common letter are significantly different (P<0.05);

(*) Values referred in Reboredo et al. 2006

Mean values are expressed as mg.kg-1 dry weight ± S deviation or as % dry weight (**)

Samples collected in the 4th (§) and 6th month assay (†)

The levels of Ca, K, Na and Si of *P. clandestinum* plants growing in sand were clearly higher than those observed in plants growing in organic-rich soils, with an exception for the Mg levels which were similar, regardless of the type of culture (Table 1). This fact was also confirmed at the end of the experiment – the Mg levels of plant shoots from different substrata and the levels of the roots collected from the sandy soils were not significantly different (P>0.05).

Despite the differences of both matrices, the average levels of Si in shoots and roots of plants cultivated in sand were, at the end of the experiment (6^{th} month), 1.9 and 2.5 times higher than the average values found in the same organs, in plants growing in organic-rich soils (Table 1). These ratios were similar in the 4^{th} month assay - 2.2 and 2.5 for shoots and roots, respectively.

When total mean values of Si in shoots and roots of plants collected from organic-rich soils and sandy soils were plotted against the average concentrations of Ca, K, Mg and Na in the same organs, R positive values were observed (Tables 2, 3, 4 and 5). Several conclusions can be enhanced such as: in general the highest R values were observed for Na and K and the lowest R values for Ca and Mg, regardless of the culture; R values decreased from the 4th month assay to the end of the experiment, in the great majority of the cases and finally the strong correlation (P<0.001) between Si and K in shoots collected from organic soils at the 4th month assay (R - 0.980).

Table 2. *Pennisetum clandestinum* R values from plants growing in organic-rich soils

	Shoots organic-rich soils 4 months					Roots organic-rich soils 4 months				
	Ca	Mg	Na	K	Si	Ca	Mg	Na	K	Si
Ca	-									
Mg	0.151	-				0.019	-			
Na	0.879**	0.103	-			0.090	0.253	-		
K	0.328	0.277	0.562	-		0.002	0.690	0.669	-	
Si	0.233	0.210	0.457	0.980***	-	0.195	0.407	0.708	0.607	-

***P<0.001; ** P < 0.01

Table 3. *Pennisetum clandestinum* R values from plants growing in organic-rich soils

	Shoots organic-rich soils 6 months					Roots organic-rich soils 6 months				
	Ca	Mg	Na	K	Si	Ca	Mg	Na	K	Si
Ca	-									
Mg	0.209	-				0.000	-			
Na	0.451	0.673	-			0.062	0.808*	-		
K	0.100	0.857**	0.307	-		0.185	0.206	0.289	-	
Si	0.057	0.560	0.176	0.568	-	0.011	0.631	0.638	0.614	-

* P < 0.05; ** P < 0.01

Table 4. *Pennisetum clandestinum* R values from plants growing in sandy soils

	Shoots sandy soils 4 months					Roots sandy soils 4 months				
	Ca	Mg	Na	K	Si	Ca	Mg	Na	K	Si
Ca	-									
Mg	0.334	-				0.112	-			
Na	0.236	0.329	-			0.794*	0.066	-		
K	0.039	0.019	0.312	-		0.195	0.065	0.338	-	
Si	0.105	0.301	0.652	0.591	-	0.107	0.040	0.438	0.730	-

* P < 0.05

Table 5. *Pennisetum clandestinum* R values from plants growing in sandy soils

	Shoots sandy soils 6 months					Roots sandy soils 6 months				
	Ca	Mg	Na	K	Si	Ca	Mg	Na	K	Si
Ca	-									
Mg	0.254	-				0.249	-			
Na	0.285	0.150	-			0.017	0.187	-		
K	0.805*	0.069	0.587	-		0.052	0.899**	0.345	-	
Si	0.489	0.207	0.468	0.514	-	0.056	0.388	0.337	0.530	-

* P < 0.05; ** P < 0.01

When average concentrations of each studied element from shoots and roots of plants collected from both substrata were plotted against the average concentrations of the remaining elements in the same organs, weak R positive values were observed, generally, although in a few cases strong correlations were noted (Tables 2, 3, 4 and 5). Nevertheless, no pattern can be established from the results here presented.

When the K, Na and Si levels at the 4th month were compared with those measured two months later, a strong decline was detected, especially for shoot Na contents – 25.9% and 21.5% for shoots growing in organic-rich soils and in sandy soils, respectively. The Si decline was high in shoots collected from sandy soils (16.6%) and low in shoots collected from organic-rich soils (7.1%), occurring the inverse for K (Table 1). The decline of K and Si at the bellow-ground organs is less 10% (Table 1).

The strong decline of Ca and Mg, from the 4th to the 6th month, was evident in shoots and roots of *Pennisetum clandestinum* collected from sandy and organic-rich soils, although the Mg decline for the roots was considerably lower, varying between 14.3% and 7.8% (Table 1).

In terms of shoot and root declines, regardless of the substrata used, we observed the following

trend: Shoot decline – Ca/Mg>Na>K/Si; Root decline – Ca>Mg/Na>K/Si

Total N is high in shoots growing in organic-rich soils with percentages varying between 3.7 and 3.1% at the 4th and 6th month assay, respectively, far above the average values found in shoots growing in sandy soils. The average root values of N in the same periods, and regardless the type of cultures, were not significantly different (P>0.05) - Table 1. Plants growing in sandy soils exhibited very low amounts of total P, while those growing in organic-rich soils were not deficient in this particular element.

The fibre contents of the shoots of plants cultivated in sandy and organic rich soils were similar at the 4th month assay, while at the 6th month shoots collected from the same substrata exhibited a decline of 33.3% and 18.6%, respectively (Table 1).

Discussion

The Si levels of *Pennisetum clandestinum* were very low, especially in plants growing in organic rich soils, which is probably related with the nature of the matrices mainly quartz, high carbonate and organic matter contents, besides Al and Fe levels of the silt-clay fraction which could lower the concentration of monosilicic acid in soil solution.

Although the pH of the nutrient solution favors the uptake of micronutrients instead of macronutrients and Si, Miles (1998) observed that *P. clandestinum* is tolerant to high soil acidity without significant effects on yield, while Sidari et al. (2004) concluded that *P. clandestinum* is a grass tolerant to pH values ranging between 4.0 and 6.0, without adverse effects on biomass production and nutritive properties (amino acid content, crude protein) although the mineral balance had not been studied.

Depending on the type of soils the average leaf Si concentration ranges from 2.7 to 3.9 g kg^{-1} for bananas cropped in Eastern slopes in Guadeloupe and from 7.7 to 9.6 g kg^{-1} for those cropped in Western slopes (Henriet et al., 2008). These differences in the Si content do not interfere with the mineral content in the leaves which were very similar, in general. For example Mg ranges from 2.9 to 3.2 g kg^{-1}, K from 28.3 to 33.3 g kg^{-1}, Ca from 4.1 to 6.6 g kg^{-1} and P from 1.8 to 2.2 g kg^{-1}. The largest variation was observed for Mn – 0.37 to 1.5 g kg^{-1}.

Nanayakkara et al. (2008) also observed that the uptake of several macro and micronutrients by perennial ryegrass (*Lolium perenne* L.) was not influenced by increasing amounts of Si applied to the soil, nor the type of soil. This non-dependence of Si, as it happens in our case, raises the question why accumulate so much Si especially in above-ground organs, if the same plants can live healthily with much less amounts without decreasing yields and the resistence to diseases.

The Si accumulation by the long-lived leaves of *Sasa veitchii* was rapid during spring and summer and slow during winter (Motomura et al., 2002). The Si decrease in *P. clandestinum*, generally less than 10%, runs in parallel with the summer season and the beginning of autumn, when ends the experiment and a decline in the mineral content of kikuyu occurred (Table 1).

The Ca levels in the shoots of *Pennisetum clandestinum* growing in sandy soils were clearly higher (3.5%) than those observed by Huett and Menary (1980) in the same plant organs and disagrees with several statements that kikuyu is deficient in Na and Ca (Fulkerson, 2007) and presented several imbalances of Ca, P, K, Mg and Na deficiency (Marais, 2001).

Sodium shoot levels of kikuyu grass range between 0.2 and 0.15% for plants growing in organic soils and 0.26 and 0.21% for those growing in sand, above the 0.14% value referred by Fulkerson (2007) for summer. Our Ca levels also indicate that instead of provide supplements to dairy cows grazing kikuyu pastures, soils can be amended with calcium compounds, besides the fertilization with N.

The application of calcium carbonate linearly increased the Ca concentration of kikuyu grass Whittet cultivar, but linearly decreased the Si concentration, as well as Mg and Mn levels (Mathews et al., 2009). Thus, it seems that the choice of the type of soil and adequate management are critical while the response of kikuyu grass is enough flexible to fit the purpose of those who search a better nutritive value.

An antagonistic effect of K on Si concentrations of the grass *Paspalum vaginatum* due to the soil treatment with potassium silicate was observed by Trenholm et al. (2001). The increased K tissue concentration reduced Si concentration, indicating preferential uptake of K and exclusion of Si.

In our case, Si does not seem to influence the internal balance and the uptake of K - an exception was noted in the 4th month assay, the strong correlation (P<0.001) between Si and K (R - 0.9801) in the shoots of *P. clandestinum* growing in organic soils. Also, the ionic balance between K and Na did not seem to be affected in *P.*

clandestinum plants - no significant correlations were observed between these elements in both substrata, regardless of the month of vegetal sample collection (Tables 2, 3, 4 and 5).

Broadley et al. (2004) observed that K is the dominant cation in shoot tissues of Poales under both experimental and natural conditions with an average value of 5.44% (n =18), which agrees with our results, while Mg ranges within Poales between 0.12% and 0.30%, clearly below our lower average value – 0.73%, indicating that Mg uptake by *P. clandestinum* is unaffected by the differences in the matrices.

Weak positive correlations between Mg and Ca levels of *P. clandestinum* shoots were observed, but not inverse correlations, as noted by Reboredo and Silvares (2007) studying the mineral balance of normal and abnormal stems (fasciated forms) of *Spartium junceum* - the elements were strongly inverse correlated.

The more silicon is present in the tissues the less phosphorus is detected. When the levels of P are high, as it happens for shoots growing in organic rich soils (approximately three times more than the levels observed in shoots growing in sand), the respective Si content is low, which agrees with Miyake and Takahashi (1985), who observed that soybean plants grown without Si, accumulated unusually high P concentrations. However, several authors confirmed that Si appplications can increase the phosphorus content in rice plants (Islam and Saha, 1969) while others showed that the addition of phosphate had not significant effect on Si absorption by wheat (Rains et al., 2006).

Comparatively with the levels of P measured in the 4th month assay, declines of 9.1% and 20.6% were observed at the end of the experiment, in the shoots of *P. clandestinum* growing in sand and organic-rich soils, respectively. These declines are parallel to the end of the summer season and beginning of autumn, which agrees with Fulkerson et al. (1998), who verified that in *P. clandestinum* the P levels changed significantly with season falling and the gradual increase of the dormancy.

From the nine tropical pasture grasses studied, Andrew and Robin (1971), observed that *P. clandestinum* was the least responsive and *Melinus minutiflora* the most responsive, in terms of growth and chemical composition, after phosphate addition to phosphate-deficient soils. The phosphate application also decreased the concentration of K in plants and increased the Mg contents in the majority of the species, without significant effects on Ca contents.

It seems that the percentage of the total N is independent from the Si content of the organs, whatever type of culture there is. Comparatively with the levels of N measured in the 4th month assay declines of 16.7% and 16.2% were observed at the end of the experiment, in the shoots of plants growing in sand and organic-rich soils, respectively.

The analysis of the N/P shoot concentration ratio partially agrees with the indication of a factor 10 (Tessier and Raynal, 2003), but only for shoots growing in organic-rich soils. The ratios of shoots growing in sandy soils reflect a P limitation, probably due to the Si content.

The average crude fibre contents at the 4th month assay is in agreement with the values observed by Muscolo et al. (2003) and Fulkerson (2007) although two months later these values had declined 33.3% and 18.6%, for shoots collected in sandy and organic rich soils, respectively.

Conclusions

Pennisetum clandestinum an active Si accumulator can grow well and healthily in the substrata where Si is mainly insoluble suggesting that the lack or poverty of this element is not a limiting factor. Only P seems to suffer the influence of Si in the case of plants growing in sandy soils. Even K, which seems to be strong correlated to the respective Si contents of shoots growing in organic soils (not in sandy soils), reveals, as the experiment develops, a gradually weaker correlation as assessed by the R values, enhancing in this particular case, that Si could be beneficial only at a certain stage of growth.

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References

Adrian, J., J. Potus, A. Poiffait and P. Dauvillier. 2000. Análisis nutricional de los alimentos. Editorial Acribia S.A., Saragoça (Espanha), pp. 292.

Ali, S., M. A. Farooq, T. Yasmeen, S. Hussain, M. S. Arif, F. Abbas, S. A. Bharwana and G. Zhang. 2013. The influence of silicon on barley growth, photosynthesis and ultra-structure under chromium stress. Ecotox. Environ. Safe. 89:66–72.

Andrew, C. S. and M. F. Robins. 1971. The effect of phosphorus on the growth, chemical composition and critical phosphorus

percentages of some tropical pasture grasses. Aust. J. Agr. Res. 22:693-706.

Baumeister, W. and L. Schmidt. 1962. Uber die rolle des natriums im pflanzlichen stoffwechsel. Flora 152:24-56.

Birchall, J. D. 1978. Silicon in the biosphere. New Trends in Bio-inorganic Chemistry. In: R. J. P. Williams and J. J. R. Frausto da Silva (Eds.), p. 489. Academic Press, London.

Broadley, M. R., H. C. Bowen, H. L. Cotterill, J. P. Hammond, M. C. Meacham, A. Mead and P. J. White. 2004. Phylogenetic variation in the shoot mineral concentration of angiosperms. J. Exp. Bot. 55:321-336.

Datnoff, L. E., C. W. Deren and G. H. Snyder. 1997. Silicon fertilization for disease management of rice in Florida. Crop Prot. 16:525-531.

Epstein, E. 1994. The anomaly of silicon in plant biology. Proc. Natl. Acad. Sci. USA, 91:11-17.

Gunes, A., A. Inal, E. G. Bagci and S. Coban. 2007. Silicon-mediated changes on some physiological and enzymatic parameters symptomatic of oxidative stress in barley grown in sodic-B toxic soil. J. Plant Physiol. 164:807-811.

Fulkerson, W. J., K. Slack, D. W. Hennessy and G. M. Hough. 1998. Nutrients in ryegrass (*Lolium* spp.), white clover (*Trifolium repens*) and kikuyu (*Pennisetum clandestinum*) pastures in relation to season and stage of regrowth in a subtropical environment. Aust. J. Agr. Res. 38:227-240.

Fulkerson, B. 2007. Kikuyu grass (*Pennisetum clandestinum*). FutureDairy, Tech. Note, 7 pp.

Henriet, C., L. Bodarwé, M. Dorel, X. Draye and B. Delvaux. 2008. Leaf silicon content in banana (*Musa* spp.) reveals the weathering stage of volcanic ash soils in Guadeloupe. Plant Soil 313:71-82.

Huett, D. O. and R. C. Menary. 1980. Effect of aluminium on growth and nutrient uptake of cabbage, lettuce and kikuyu grass in nutrient solution. Aust. J. Agr. Res. 31:749-761.

Islam, A. and R. C. Saha. 1969. Effects of silicon on the chemical composition of rice plants. Plant Soil 30:446-458.

Liang, Y. 1999. Effects of silicon on enzyme activity and sodium, potassium and calcium concentration in barley under salt stress. Plant Soil 209:217-224.

Liang, T., W. Sun, Y. -G. Zhu and P. Christie. 2007. Mechanisms of silicon-mediated alleviation of abiotic stresses in higher plants: A review. Environ. Pollut. 147:422-428.

Ma, J. F., K. Tamai, M. Ichii and G. F. Wu. 2002. A rice mutant defective in Si uptake. Plant Physiol. 130:2111-2117.

Ma, J. F. and N. Yamaji. 2006. Silicon uptake and accumulation in higher plants. Trends Plant Sci. 11:392-397.

Marais, J. P. 2001. Factors affecting the nutritive value of kikuyu grass (*Pennisetum clandestinum*) – a review. Trop. Grassl. 35:65-84.

Mathews, B. W., J. R. Carpenter and L. E. Sollenberger. 2009. In vitro digestibility and chemical composition of kikuyugrass as influenced by soil silicon, liming and genotype. Comm. Soil Sci. Plant Anal. 40:2855-2873.

Miles, N. 1998. Fertilization and liming of kikuyu. Proceedings of a kikuyu technology day. KwaZulu-Natal Department of Agriculture, Pietermaritzburg, South Africa, pp. 8-11

Miyake, Y. and E. Takahashi. 1985. Effects of silicon on the growth of soybean plants in a solution culture. Soil Sci. Plant Nutr. 31:625-636.

Motomura, H., N. Mita and M. Suzuki. 2002. Silica accumulation in long-lived leaves of *Sasa veitchii* (Carrière) Rehder (Poaceae-Bambusoideae). Ann. Bot. 90:149-152.

Muscolo, A., M. R. Panuccio and M. Sidari. 2003. Effects of salinity on growth, carbohydrate metabolism and nutritive properties ok kikuyu grass (*Pennisetum clandestinum* Hochst). Plant Sci. 164: 1103-1110.

Nanayakkara, U. N., W. Uddin and L. E. Datnoff. 2008. Application of silicon sources increases silicon accumulation in perennial ryegrass turf on two soil types. Plant Soil 303:83-94.

Rains, D. W., E. Epstein, R. J. Zasoski and M. Aslam. 2006. Active silicon uptake by wheat. Plant Soil 280:223-228.

Raven, J. A. 2003. Cycling silicon – the role of accumulation in plants. New Phytol. 158:419-421.

Reboredo, F. 1988. Alguns aspectos sobre a acumulação de Fe, Cu e Zn por *Halimione portulacoides* (L.) Aellen. Dissertação de Doutoramento (Ph.D. Thesis), Faculdade de Ciências e Tecnologia/ Universidade Nova de Lisboa, 165 pp. (In Portuguese).

Reboredo, F. 1991. Cu and Zn uptake by *Halimione portulacoides* (L.) Aellen. A long-term accumulation experiment. Bull. Environ. Contam. Toxicol. 146:442-449.

Reboredo, F., R. Bragadesto and L. Sousa. 2006. Do silicon levels in the matrix influence the mineral nutrition of *Pennisetum clandestinum* (Hochst. ex Chiov)? Arch. Agron. Soil Sci. 52:353-358.

Reboredo, F. and C. Silvares. 2007. Fasciation phenomena and mineral balance in *Spartium junceum* L. Phyton 47:123-132.

Richmond, K. E. and M. Sussman. 2003. Got silicon? The non-essential beneficial plant nutrient. Curr. Opinion Plant Biol. 6:268-272.

Rivière, A. 1977. Méthodes granulométriques. Techniques et Interprétations. Masson edit. (Paris), 170 pp.

Rizwan, M., J. -D. Meunier, H. Miche and C. Keller. 2012. Effect of silicon on reducing cadmium toxicity in durum wheat. J. Hazard. Mater. 209-210:326– 334.

Sidari, M., M. R. Panuccio and A. Muscolo. 2004. Influence of acidity on growth and biochemistry of *Pennisetum clandestinum*. Biol. Plant. 48:133-136.

Sokal, R. R. and J. Rohlf. 1994. Biometry. 3rd edit., W. H. Freeman Company (New York), pp. 896.

Tessier, J. T. and D. J. Raynal. 2003. Use of nitrogen to phosphorus ratios in plant tissue as an indicator of nutrient limitation and nitrogen saturation. J. Appl. Ecol. 40:523-534.

Tripathi, P., R. D. Tripathi, R. P. Singh, S. Dwivedi, D. Goutam, M. Shri, P. K. Trivedi and D. Chakrabarty. 2013. Silicon mediates arsenic tolerance in rice (*Oryza sativa* L.) through lowering of arsenic uptake and improved antioxidant defence system. Ecol. Eng. 52:96–103.

Trenholm, L. E., R. R. Duncan, R. N. Carrow and G. H. Snyder. 2001. Influence of silica on growth, quality, and wear tolerance of seashore *Paspalum*. J. Plant Nutr. 24:245-259.

Vandecasteele, C. and C. B. Block. 1993. Modern Methods for Trace Element Determination, John Wiley & Sons, Chichester, pp. 330.

Watts, S. and L. Halliwell. 1996. Detailed field and chemical methods for soil - Appendix 3. In: S. Watts and L. Halliwell (Eds.), pp. 475-505. Essential Environmental Science, Methods & Techniques, Routledge (London).

Watanabe, F. S. and S. R. Olsen. 1965. Test of an ascorbic acid method for determining phosphorus in water and $NaHCO_3$ extracts from the soil. Soil Sci. Soc. Am. Proc. 29:677-78.

Williams, D. G. and Z. Baruch. 2000. African grass invasion in the Americas: ecosystem consequences and the role of ecophysiology. Biol. Invasions 2:123-140.

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REGULAR ARTICLE

Fluorescence quenching as an indicator of the conformational change of humic acids

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Abstract

Humic acids as a water soluble fraction of humus play important role in the plant growth. Even though structure of humic acids is not clear, their spectroscopic properties can reveal us useful information. To investigate influence of humic acids reorganization, HPSEC fractionation at mild fractionation conditions has been carried out. Reconstruction of secondary chromatograms of separated fractions showed molecular size increase and increased response on fluorescence detector, although neither molecular size change nor significant absorbance increase was observed on UV-VIS detector. However, bigger aggregates, that didn't fluoresce in the unfractionated sample started to fluoresce. The reason for this behavior is not some change of spectral properties of a specific fraction, but a general fluorescence increase indicating humic acid reorganization. Therefore spectroscopic changes can be used as a tool for monitoring their reorganization, which might play an important role in nutrients soil plant mobility and should be studied in more details from that perspective.

Key words: Conformational change, Fluorescence, Fluorescence quenching, Humic acid, HPSEC

Introduction

In soil science humus refers to any organic matter that has reached a point of stability, where it will break down no further. Stable (or passive) humus consists of humic acids and humins (Di-Giovanni et al., 1998).

Humic acids (HA) as a water soluble fraction of humus play important role in the plant growth (Lee and Bartlett, 1976; Nardi et al., 2002). The beneficial effects of HA on plant growth may be related to their indirect (increase of fertilizer efficiency or reducing soil compaction), or direct (improvement of the overall plant biomass) effects. They play important role in ion absorption (increase the availability of micronutrients from sparingly soluble hydroxides (Stevenson, 1994)). Apart from that, HA are absorbed by plants, influencing intermediary metabolism, or plant growth and development directly, by acting as hormone-like substances (Piccolo, 1996).

Even though structure of HA is not clear, their spectroscopic properties can reveal us useful information. HA exhibit rather unusual absorption and emission characteristics: absorption decreases with increasing wavelength in an approximately exponential fashion, but extends well into visible and in some cases the near infra-red. HA spectra does not arise from a superposition of many independent chromophores (Del Vecchio and Blough, 2004), but from a continuum of coupled states formed through charge transfer interactions of a few distinct chromophores. Emission maxima shift continuously to red with increasing excitation wavelength, while fluorescence quantum yields monotonically decrease (Boyle et al., 2009).

According to available data, the fluorescence efficiency of HA is generally low. This means that a great part of absorbed energy is dissipated leading neither to fluorescence emission nor to triplet excited state production. Therefore a non-irradiative relaxation must play an important role, meaning that many aggregates do not fluoresce due to aggregation (Coelho et al., 2010). It is already reported (Engebretson and Von Wandruszka, 1999; Specht et al., 2000; Wu et al., 2003; Conte et al., 2007) that the bigger aggregates do not fluoresce. And authors generally agree that this is due to

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fluorescence quenching and consequently increased non radiative relaxation). Fluorescence quenching is strongly related to the local environment of chromophores, such as solvent or mobile phase in case of chromatographic separation or surrounding aggregates influencing the distances between fluorescence donor and fluorescence acceptor.

But with the new evidence that HA are aggregates composed mainly of fairly small subunits weakly held together by predominantly hydrophobic forces (Piccolo et al., 1996a; Sutton and Sposito, 2005; Schaumann, 2006) unusual absorption and emission characteristics make more sense: by influencing distances between donors and acceptors, aggregation of subunits does not influence only the size, but also the spectroscopic characteristics of HA.

To study the influence of the aggregation on spectroscopic characteristics of HA, mild fractionation procedure, hardly changing their natural properties is needed (Burba et al., 1998) and their characterization by physical or chemical methods should be performed under ‘in situ' or at least nature-like conditions (Buffle and Leppard, 1995). Therefore high pressure size exclusion chromatography (HPSEC) in low ionic strength mobile phase is necessary.

But even in mild, nature like condition, the fractionation changes the local environment of chromophores and therefore changes the distances between fluorescence donor and fluorescence acceptor. This should be pronounced as change in fluorescence quenching efficiency, therefore different secondary chromatograms of fractions would be expected if recorded by fluorescence or absorbance detectors. If we could confirm this hypothesis, we could use HPSEC with fluorescence detection as a tool for better understanding of aggregation behavior of HA.

Materials and Methods

Humic acid sodium salts (Aldrich) were used for the experiments, as an representative of terrestrial HA (Valencia et al., 2013). They were dissolved in ultrapure water (Barnstead NANOpure system) at a concentration of 1 g/L, to keep ionic strength as low as possible to imitate natural conditions, left on ultrasound bath for 15 minutes and then centrifuged for 30 minutes at 4000 rpm. The supernatant was filtered through a 0.45 μm PTFE filter (Chromafil).

Analyzes of HA samples were performed by aqueous high pressure size exclusion chromatography (HPSEC) using a high pressure liquid chromatograph Agilent HP 1100 Series with G1322A Degasser, G1311A QuatPump quaternary pump, G1315A DAD diode array detector, and G1321A FLD fluorescence detector.

0.001 M $NaNO_3$ (Riedel-de Haën) was used as a mobile phase at a flow rate 0.5 mL/min. The void volume was 5.5 ml.

For HPSEC 50 μL of samples were injected onto a MN Nucleogel aqua-OH 50-8 column (3000 mm x 7.7 mm, 8 μm particle size) with a MN Nucleogel aqua-OH 8P pre-column (50 mm x 7.7 mm) (Macherey-Nagel). The temperature of chromatographic column was kept constant at 25°C. The detection was performed with a diode array detector (DAD) at 280 and 230 nm and a fluorescence detector (FLD)(EX = 230 nm, EM = 440 nm). The dead volume of capillary connecting the two detectors was 0.1 mL. Data acquisition and processing were accomplished by the HP ChemStation A.07.01 software.

For the fractionation experiment, 250 μL fractions of HA, separated with HPSEC, were collected behind the second detector (FLD). 100 μL aliquots of each fraction were immediately analyzed with HPSEC again (three replicates).

Results and Discussion

From the chromatogram of fractions (Figure 1) it is obvious, that the biggest aggregates (retention times shorter than 13 minutes) do not fluoresce even after fractionation.

But before discussing the reconstructed chromatograms, we have to consider the fact that only 100 μL aliquots of collected fractions (250 μL) were analyzed with HPSEC again. Furthermore the initial sample is diluted. It has been observed in our experiments, as also reported in the literature (Swift and Posner, 1971), that decreasing concentrations of HA produced shifts of peaks from low to high molecular size ranges (shorter retention times).

Of course, the peak area decreased as well, as a result of smaller amount of HA (100 μL aliquots) and consequently smaller absorbance. On the basis of 6 replicates at 3 different concentrations we have calculated the expected retention time and peak area, as if the whole collected fraction would be injected ($R^2 > 0,90$ for retention time and $R2 > 0,99$ for peak area).

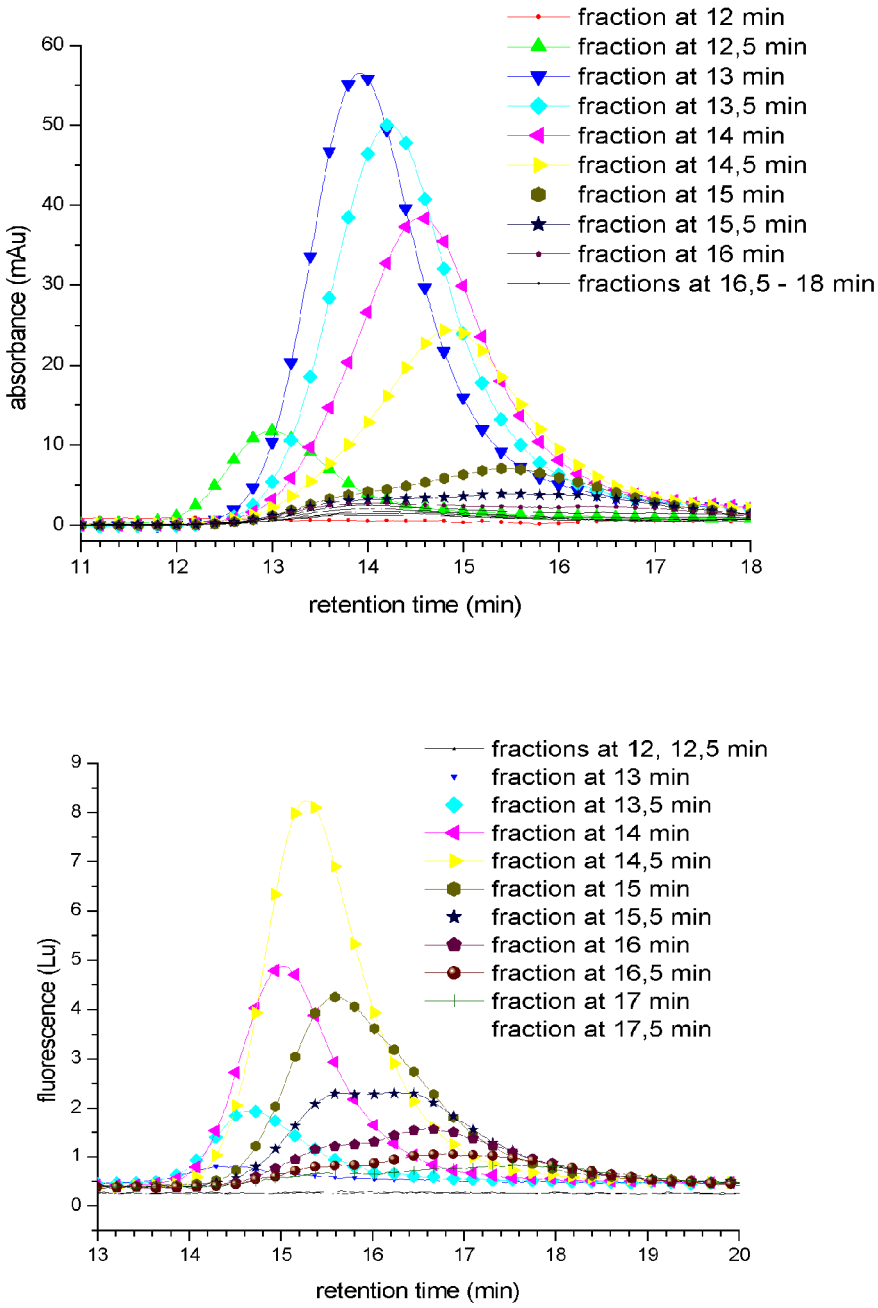

Figure 1. Chromatograms of fractions.

Therefore, when reconstructing the primary chromatogram from chromatograms of fractions (sum of signal intensities from chromatograms of fractions), 2.5 times smaller peak area and a shift of the retention time by up to 0.2 min toward shorter retention times on both detectors, compared to unfractionated sample, would be expected as a consequence of dillution (100 µL of 250 µL injected).

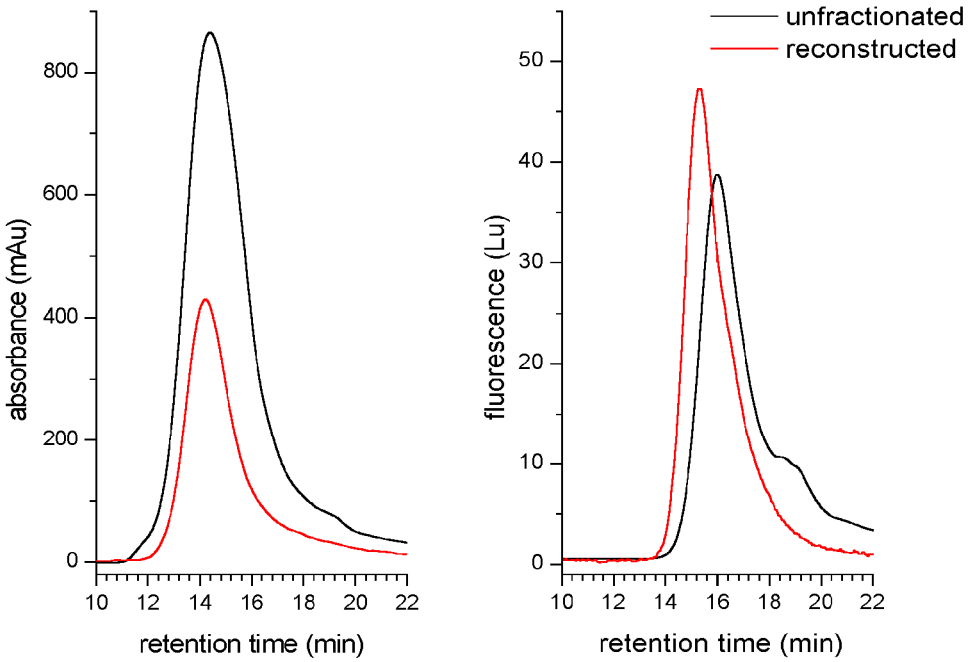

Figure 2. Reconstructed chromatogram of fractions.

However, the reconstructed DAD chromatogram exhibits a 5 times smaller peak area and 0.2 min shorter retention time as compared to the unfractionated sample. The difference in the retention time between reconstructed and original (unfractionated) chromatograms corresponds approximately to the expected retention time decrease due to dilution (0.2 min). DAD peak area of the reconstructed chromatogram, corrected by the dilution factor 2.5 is however significantly smaller (50 %) than the peak area of the unfractionated sample (Figure 2).

The decrease of absorbance in our experiment cannot be attributed to the dilution effects and neglection of fractions with retention times above 18 min, since they were estimated to represent less than 8% of the total amount of injected HA. Also material loss due to adsorption on stationary phase is not likely (a low ionic strength mobile phase) because of electrostatic repulsion between stationary phase and HA (De Nobili and Chen, 1999; Perminova, 1999). We realize that the choice of a low ionic strength mobile phase has also its drawbacks: it is not enough to eliminate all ionic interactions (Perminova, 1999), but this would be important only if we would like to interpret results with absolute values of molecular size. Which we don't.

Actually, the decrease of absorbance has already been observed in different fractionation processes (Burba et al., 1995; Aster et al., 1996; Schimpf and Petteys, 1997; Lin et al., 1999, 2000; Kitis et al., 2002; Hoque et al., 2003; Hur and Schlautman, 2003; Alberts and Takács, 2004; Zanardi-Lamardo et al., 2004; Richard et al., 2004) or changed in their aggregation through acidic treatment (Piccolo et al., 1999; Cozzolino et al., 2001; Baigorri et al., 2007) but mostly left without explanation. Piccolo explained that absorbance decrease is a consequence of the disruption of weak intermolecular bonds; holding aggregates together (Piccolo et al., 1996b). We find it as the only reasonable explanation also in our experiment. Of course fractionation under mild conditions is much less radical than organic acids treatment, therefore only partial disruption, influencing mostly spectroscopic properties has been observed.

Conformational changes of HA are even more pronounced on the fluorescence detector: the retention time at the fluorescence maximum is 0.8 min shorter than for the unfractionated sample (Figure 2). This retention time shift cannot be explained only with the dilution effect (0.2 min), and therefore indicates that bigger aggregates, which did not fluoresce in the unfractionated sample start exhibiting higher fluorescence or that smaller aggregates do not fluoresce anymore.

Table 1. Fluorescence of unfractionated sample, fraction, collected at specified retention time and sum of fractions at the retention time of maximal absorbance of each fraction (tmax). Fluorescence of fraction and sum of fractions has been corrected by the dilution factor 2.5.

Fraction, collected at (min)	13.5	14	14.5	15	15.5	16	16.5	17	17.5
Maximal fluorescence of fraction	2.5	5	12.5	20	10	5	5	2.5	2.5
Fluorescence of all fractions	10	22.5	42.5	47.5	42.5	25	20	17.5	10
Response in original chromatogram	2	5	13	20	31	35	29	25	15

An increased overall fluorescence of the fractions shown in the reconstructed chromatogram indicate that retention time shift is due to increased fluorescence of bigger aggregates: cumulative FLD peak area of individual fractions, multiplied by the dilution factor 2.5, is bigger than the peak area of unfractionated sample, regardless much smaller absorbance and therefore weaker excitation (Figure 2).

Similar observation (increase of fluorescence even if the UV absorption decreases) has been reported in the literature as a consequence of degradation: the fluorescence of the NOM samples investigated was increased upon hydrolysis (Kumke et al., 2001), after chlorination (Korshin et al., 1999), ozonation and UV irradiation of HA (Win et al., 2000).

The fact that fractionation experiment lead to the same result as photo or chemical degradation can only be explained with the decreased quenching effect, caused by change in the local environment of HA aggregates. Changes of the local environment influence the distance between fluorescent donor and acceptor and consequently the rate of energy transfer (Gilbert et al., 1991) without bond breaking.

The quenching is directly related also to rigidity, because more rigid structures decrease the possibility of fluorescence quenching (Specht et al., 2000) So the fluorescence increase could be caused by increased rigidity, such as formation of hydrogen bonds (Conte et al., 2006). But we would expect, if this is the reason, that fluorescence increase would be pronounced only on specific fractions and pronounced more like fluorescence concentration (Alberts and Takács, 2004) than general fluorescence increase.

Closer inspection of the chromatogram of fractions (Figure 1, Table 1) reveals that fluorescence increase after the process of fractionation is not characteristic for a specific fraction. Actually, the fractions collected at 14, 14.5 and 15 minutes (highest absorbance) show as high fluorescence as unfractionated samples. Lower fluorescence of fractions collected after 15.5 min (highest fluorescence of unfractionated samples) might not be a consequence of actual fluorescence decrease, but more likely a consequence of the shift of fluorescence excitation/ emission maxima (Richard et al., 2004). The shift of ex/em maxima should be taken into account also in biggest aggregates, but it is obviously less pronounced than quenching effect decrease.

Similar behavior, indicating different spectroscopic properties of fractions, collected before 15th minute and after could be observed also by comparing UV-Vis absorption ratios: absorbance at 300 nm to absorbance at 400 nm (E300/E400) of unfractionated sample and fractions.

Fractions, collected at 13 and 14 minutes, that represent major contribution to the absorbance of the unfractionated samples and practically no fluorescence, show lower E300/E400 absorbance rate compared to the unfractionated sample, indicating shift of the absorbance maxima toward shorter wavelength (blue shift). This shift can be explained with changed spectroscopic properties due to increased polarity of environment (Skoog et al., 2007) in the process of fractionation: HA aggregates, which were surrounded with bigger or smaller aggregates prior fractionation, are exposed to mobile phase, and therefore more polar environment influences their spectroscopic properties.

On the other hand, E300/E400 ratio is increased for fractions, collected at 15 and 16 minutes (major contribution to fluorescence of unfractionated sample). The smallest size-fractions, eluted at the end of the HPSEC chromatographic separation are predominantly composed of oxidized carbons (Chen et al., 2003; Conte et al., 2007). This is in agreement with the loss of absorbance due to donor-acceptor interactions that are expected to red-shift and broaden near-UV absorbances giving long wavelength absorptivity (Richard et al., 2004).

Table 2. Absorbance at 300 nm divided with absorbance at 400 nm of unfractionated sample and fractions.

fractions, collected at (min)	unfractionated	13	14	15	16
E_{300}/E_{400}	3.0	2.8	2.8	36	3.7

Obviously we observe a dual behavior of HA regarding size: bigger aggregates exhibit intensive increased fluorescence and blue shift in absorbance spectra after fractionation/treatment, however smaller aggregates exhibit smaller fluorescence and red shift in absorbance spectra after fractionation /treatment, which is in accordance also with the results of NMR analysis, revealing that structure of DOM is significantly altered with size (Conte et al., 2006; Lam and Simpson, 2009; Woods et al., 2010).

Conclusions

The biggest HA aggregates do not fluoresce, because of fluorescence quenching. The reason for this behavior is not the property of a particular HA fraction, but a consequence of the local environment (which aggregates are nearby, which functional groups participate in molecular bonding etc.). When local environment changes, as in HPSEC fractionation, different response in secondary chromatograms of fractions on UV-VIS and fluorescence detector is observed indicating that HA have reorganized.

And changes of environment happen in soil all the time. It means that humic substances reorganize all the time, which can be a simple explanation of their environmental resistance, but even more, continuous reorganization can play an important role in nutrients soil plant mobility. And should be studied in more details from that perspective.

References

Alberts, J. J. and M. Takács. 2004. Comparison of the natural fluorescence distribution among size fractions of terrestrial fulvic and humic acids and aquatic natural organic matter. Org. Geochem. 35(10):1141–1149.

Aster, B., P. Burba and J. A. C. A. Broekaert. 1996. Analytical fractionation of aquatic humic substances and their metal species by means of multistage ultrafiltration. Fresenius J. Anal. Chem. 354(5):722–728.

Baigorri, R., M. Fuentes, G. González-Gaitano and J. M. García-Mina. 2007. Analysis of molecular aggregation in humic substances in solution. Coll Surf. A 302(1-3):301–306.

Boyle, E. S., N. Guerriero, A. Thiallet. R. D. Vecchio, and N. V. Blough. 2009. Optical properties of humic substances and CDOM: Relation to structure. Env. Sci. Technol. 43(7):2262–2268.

Buffle, J. and G. G. Leppard. 1995. Characterization of aquatic colloids and macromolecules. 2. Key role of physical structures on analytical results. Env. Sci. Technol. 29(9):2176–2184.

Burba, P., B. Aster, T. Nifant'eva, V. Shkinev and B. Y. Spivakov. 1998. Membrane filtration studies of aquatic humic substances and their metal species: a concise overview: Part 1. Analytical fractionation by means of sequential-stage ultrafiltration. Talanta 45(5):977–988.

Burba, P., V. Shkinev and B. Y. Spivakov. 1995. On-line fractionation and characterization of aquatic humic substances by means of sequential-stage ultrafiltration. Fresenius J. Anal. Chem. 351(1):74–82.

Chen, J., E. J. LeBoeuf, S. Dai and B. Gu. 2003. Fluorescence spectroscopic studies of natural organic matter fractions. Chemosphere 50(5):639–647.

Coelho, C., G. Guyot, A. ter Halle, L. Cavani, C. Ciavatta and C. Richard. 2010. Photoreactivity of humic substances: relationship between fluorescence and singlet oxygen production. Environ. Chem. Lett. 2010:1–5.

Conte, P., R. Spaccini and A. Piccolo. 2006. Advanced CPMAS-13 C NMR techniques for molecular characterization of size-separated fractions from a soil humic acid. Anal. Bioanal. Chem. 386(2):382–390.

Conte, P., R. Spaccini, D. Smejkalová, A. Nebbioso and A. Piccolo. 2007. Spectroscopic and conformational properties of size-fractions separated from a lignite humic acid. Chemosphere 69(7):1032–1039.

Cozzolino, A., P. Conte and A. Piccolo. 2001. Conformational changes of humic substances

induced by some hydroxy-, keto-, and sulfonic acids. Soil. Biol. Biochem. 33(4-5):563–571.

Di-Giovanni, C., J. R. Disnar, V. Bichet and M. Campy. 1998. Sur la présence de matières organiques mésocénozoïques dans des humus actuels (bassin de Chaillexon, Doubs, France). Comptes Rendus de l'Académie des Sciences - Series IIA - Earth and Planetary Science 326(8):553–559.

Engebretson, R. R. and R. von Wandruszka. 1999. Effects of humic acid purification on interactions with hydrophobic organic matter: Evidence from fluorescence behavior. Env. Sci. Technol. 33(23):4299–4303.

Gilbert, A., J. E. Baggott and P. J. Wagner. 1991. Essentials of molecular photochemistry. Blackwell Scientific Publications Oxford.

Hoque, E., M. Wolf, G. Teichmann, E. Peller, W. Schimmack and G. Buckau. 2003. Influence of ionic strength and organic modifier concentrations on characterization of aquatic fulvic and humic acids by high-performance size-exclusion chromatography. J. Chromatogr. A 1017(1-2): 97–105.

Hur, J. and M. A. Schlautman. 2003. Using selected operational descriptors to examine the heterogeneity within a bulk humic substance. Env. Sci. Technol. 37(5):880–887.

Kitis, M., T. Karanfil, A. Wigton and J. E. Kilduff. 2002. Probing reactivity of dissolved organic matter for disinfection by-product formation using XAD-8 resin adsorption and ultrafiltration fractionation. Water Res. 36(15):3834–3848.

Korshin, G. V., M. U. Kumke, C. W. Li and F. H. Frimmel. 1999. Influence of chlorination on chromophores and fluorophores in humic substances. Env. Sci. Technol. 33(8):1207–1212.

Kumke, M. U., C. H. Specht, T. Brinkmann and F. H. Frimmel. 2001. Alkaline hydrolysis of humic substances-spectroscopic and chromatographic investigations. Chemosphere 45(6-7):1023–1031.

Lam, B. and A. J. Simpson. 2009. Investigating aggregation in Suwannee River, USA, dissolved organic matter using diffusion-ordered nuclear magnetic resonance spectroscopy. Environ. Toxicol. Chem. 28(5):931–939.

Lee, Y. S. and R. J. Bartlett. 1976. Stimulation of plant growth by humic substances. Soil Sci. Soc. Am. J. 40(6):876–879.

Lin, C. F., Y. J. Huang and O. J. Hao. 1999. Ultrafiltration processes for removing humic substances: effect of molecular weight fractions and PAC treatment. Water Res. 33(5):1252–1264.

Lin, C.., T. Y. Lin and O. J. Hao. 2000. Effects of humic substance characteristics on UF performance. Water Res. 34(4):1097–1106.

Nardi, S., D. Pizzeghello, A. Muscolo and A. Vianello. 2002. Physiological effects of humic substances on higher plants. Soil Biol. Biochem. 34(11):1527–1536.

De Nobili, M. and Y. Chen. 1999. Size exclusion chromatography of humic substances: Limits, perspectives and prospectives. Soil Sci. 164(11):825.

Perminova, I. V. 1999. Size exclusion chromatography of humic substances: complexities of data interpretation attributable to non-size exclusion effects. Soil Sci. 164(11):834.

Piccolo, A. 1996. Humic Substances in Terrestrial Ecosystems. Elsevier. Amsterdam.

Piccolo, A., P. Conte and A. Cozzolino. 1999. Effects of mineral and monocarboxylic acids on the molecular association of dissolved humic substances. Eur. J. Soil. Sci. 50(4):687–694.

Piccolo, A., S. Nardi and G. Concheri. 1996a. Micelle-1ike conformation of humic substances as revealed by size exclusion chromatography. Chemosphere 33(4):595–602.

Piccolo, A., S. Nardi and G. Concheri. 1996b. Macromolecular changes of humic substances induced by interaction with organic acids. Eur. J. Soil. Sci. 47(3):319–328.

Richard, C., O. Trubetskaya, O. Trubetskoj, O. Reznikova, G. Afanas' Eva, J.P. Aguer and G. Guyot. 2004. Key role of the low molecular size fraction of soil humic acids for fluorescence and photoinductive activity. Env. Sci. Technol. 38(7):2052–2057.

Schaumann, G. E. 2006. Soil organic matter beyond molecular structure Part I: Macromolecular

and supramolecular characteristics. J. Plant Nutr. Soil Sci. 169(2):145–156.

Schimpf, M. E. and M. P. Petteys. 1997. Characterization of humic materials by flow field-flow fractionation. Coll. Surf. A 120(1-3):87–100.

Skoog, D. A., F. J. Holler and S. R. Crouch. 2007. Principles of instrumental analysis. Thomson Brooks/Cole.

Specht, C. H., M. U. Kumke and F. H. Frimmel. 2000. Characterization of NOM adsorption to clay minerals by size exclusion chromatography. Water Res. 34(16):4063–4069.

Stevenson, F. J. 1994. Humus chemistry: genesis, composition, reactions. John Wiley & Sons Inc.

Sutton, R. and G. Sposito. 2005. Molecular structure in soil humic substances: The new view. Environ. Sci. Technol. 39(23):9009–9015.

Swift, R. S. and A. M. Posner. 1971. Gel chromatography of humic acid. Eur. J. Soil. Sci. 22(2):237–249.

Valencia, S., J. M. Marín, G. Restrepo and F. H. Frimmel. 2013. Application of excitation–emission fluorescence matrices and UV/Vis absorption to monitoring the photocatalytic degradation of commercial humic acid. Sci. Total Env. 442: 207–214.

Del Vecchio, R. and N. V. Blough. 2004. On the origin of the optical properties of humic substances. Env. Sci. Technol. 38(14):3885–3891.

Win, Y. Y. M. U. Kumke, C. H. Specht, A. J. Schindelin, G. Kolliopoulos, G. Ohlenbusch, G. Kleiser, S. Hesse and F. H. Frimmel. 2000. Influence of oxidation of dissolved organic matter (DOM) on subsequent water treatment processes. Water Res. 34(7):2098–2104.

Woods, G. C., M. J. Simpson, B. P. Kelleher, M. McCaul, W. L. Kingery and A. J. Simpson. 2010. Online high-performance size exclusion chromatography−nuclear magnetic resonance for the characterization of dissolved organic matter. Env. Sci. Technol. 44(2):624–630.

Wu, F. C., R. D. Evans and P. J. Dillon. 2003. Separation and characterization of NOM by high-performance liquid chromatography and on-line three-dimensional excitation emission matrix fluorescence detection. Env. Sci. Technol. 37(16):3687–3693.

Zanardi-Lamardo, E., C. A. Moore and R. G. Zika. 2004. Seasonal variation in molecular mass and optical properties of chromophoric dissolved organic material in coastal waters of southwest Florida. Mar. Chem. 89(1-4):37–54.

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REGULAR ARTICLE

Physiological responses and membrane integrity in three *Vigna* genotypes with contrasting drought tolerance

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Abstract

In the present work some dehydration-induced responses at whole plant and cellular levels were evaluated in three *Vigna* genotypes (*V. glabrescens*, Vg; *V. unguiculata* cv. EPACE-1; *V. unguiculata* cv. 1183) from different origins. Changes in leaf stomatal conductance (g_s), membrane lipids composition and abscisic acid (ABA) content were determined under water stress. Membrane integrity was assessed through leaf discs dehydration with polyethylene glycol (PEG 8000, -1.3 MPa), and expressed as an injury index (I%).. In EPACE-1 and 1183, rapid stomatal closure at early stages of dehydration was related with the ability to accumulate endogenous ABA. On the contrary in Vg stomata closed at lower leaf relative water content as ABA increase was delayed. In 1183, high I% values occurred in PEG-dehydrated discs, and lipid degradation was observed even under moderate water stress (S1), becoming significant under more severe drought (S2). Also, along drought imposition this genotype presented a continuous leaf ABA increase that probably contributed to early visual tissue senescence. Vg and EPACE-1 presented lower I% values, probably denoting the ability to preserve membrane integrity. In EPACE-1 this was achieved through a quite stable lipid content, while in Vg new membrane lipids were synthesized. It is suggested that a higher protoplasmic tolerance in EPACE-1 and Vg might be related to stable and low endogenous ABA contents under increasing water deficit.

Key words: *Vigna*, Abscisic acid, Drought, Lipids, Membrane leakage, Stomatal conductance

Introduction

Legumes are agronomically important crops mainly due to their nitrogen fixing capacity and to their high protein content. *Vigna* species and varieties successfully grow in arid to semi-arid regions, and additionally constitute a valuable genetic resource in studies concerning drought, one of the main factors affecting crop productivity and quality (Van der Maesen and Somaatmadja, 1989).

Cell membranes are major targets of environmental stresses (Leshem et al., 1992) and *in vitro* screening tests for membrane permeability are frequently used to evaluate membrane tolerance to dehydration (Vasquez-Tello et al., 1990; Navari-Izzo et al., 1993; Campos et al., 1997). Altered membrane permeability may result in increased leakage from cells due to the appearance of heterogeneous membrane domains as regards lipid phases and configurations (Leshem, 1992), or to damage of membrane components, namely in the lipid matrix (Harwood, 1998). However there are changes in lipid composition of higher-plant membranes that may contribute to preserve membrane integrity and cell compartmentation (Kuiper, 1985), and hence to control metabolic activity (Bewley, 1993; Harwood, 1998). Such an adaptive role for qualitative and quantitative modifications of membrane lipids has been reported under different environmental stresses, namely high irradiance (Ramalho et al. 1998), cold (Öquist 1982; Partelli et al., 2011), heat (Dias et al., 2010) and drought (Pham Thi et al., 1990; Matos et al., 2010).

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On the other hand, plants respond to water deficit by accumulating abscisic acid (ABA) and genetic variation in the capacity to accumulate ABA is known among crop species (Pekic and Quarrie, 1988; Quarrie, 1991; Tuberosa et al., 1994). The rapid translocation of ABA in shoots via xylem flux and the increase of ABA concentration in plant organs correlate with the major physiological changes occurring under water stress conditions, playing a predominant role in drought avoidance through the induction of stomatal closure (Wilkinson and Davies, 2002). Genetic variation in shoot sensitivity to ABA imported from the root may be important in mediating the root signal effect (Blum and Sinmena, 1995). This growth regulator is also involved in triggering tolerance mechanisms at cellular level (Ingram and Bartels, 1996). Direct effect of ABA on membranes has also been reported, using both natural and artificial membrane systems. ABA can directly affect membrane physical properties, like microviscosity (Shripathi et al., 1997), fluidity (Stillwell et al., 1988), and permeability to ions and solutes (Stillwell and Hester, 1984). Some of these modifications seem to be related to changes in lipid composition of cellular membranes (Shripathi et al., 1997; Campos and Pham Thi, 1997).

The studied *Vigna* genotypes were previously compared as regards the effects of water deficit on photosynthetic performance and water relations (Campos et al., 1999a). The present work deals with whole plant and cellular responses underlying contrasting drought sensitivities in three *Vigna* genotypes from different origins, focusing the changes in stomatal conductance (g_s), ABA content, lipid composition and membrane integrity in leaves under dehydration conditions. Results are expected to contribute for the better understanding of physiological responses and/or constitutive features be relevant in the process of plants adaptation to drought.

Material and Methods

Plant material and experimental conditions

For this study we selected three genotypes belonging to the genus *Vigna* (*Phaseolidae*) with contrasting drought sensitivities. *V. glabrescens* (Vg) is a wild species originating from the Philippines (drought-resistant), *V. unguiculata* cv. 1183 (drought-sensitive) is cultivated in humid regions of China, and *V. unguiculata* cv. EPACE-1 (drought-resistant) is grown in the semi-arid North-East of Brazil. Seeds were provided by the University of Gembloux (Belgium) and the Agronomy Research Station of Ceará (Brazil). After germination, plants were grown in pots, in a mixture of vermiculite: Triohum-Tray substrat (4:5) and were irrigated with two fold micronutrients Hoagland and Snyder solution, as described in Campos et al. (1999a). Plants were kept in a greenhouse under natural irradiance (with a maximal photosynthetic photon flux density of *ca*. 800-900 µmol m^{-2} s^{-1}), mean diurnal temperatures of 30ºC and relative humidity values between 70% (morning) and 40% (late afternoon). Dehydration was progressively induced by withholding irrigation for 10 to 12 days in six weeks old plants. According to relative water content (RWC) values, two drought levels were considered: mild stress (S1, RWC 65-75%) and severe stress (S2, RWC < 60%). All determinations were carried out on recently fully expanded leaves.

Plant water status

Relative water content (RWC) was calculated as previously described (Campos et al., 1999a) from samples of 10 leaf discs of 0.5 cm^2, as RWC=(FW-DW/TW-DW)x100, where FW is the fresh weight, TW is the turgid weight after overnight water saturation of the discs in a humid chamber at room temperature, and DW is the dry weight after drying at 80ºC for 24 h.

Stomatal conductance measurements

Stomatal conductance for water vapour (g_s) was measured under a photosynthetic photon flux density of 1300-1600 µmol m^{-2} s^{-1}, in one attached leaf of three plants from each treatment, using a portable IRGA LI-6200 (LI-COR, Inc., Nebraska, USA). Measurements were performed between 10:00 and 11:00 a.m.

Quantification of leaf ABA content

Leaf samples (*ca.* 400 mg FW) were collected along the dehydration period, frozen in liquid nitrogen, freeze-dried and stored over silica gel in the dark, at *ca*. 20ºC. For ABA determinations, the freeze-dried samples were cut to a fine powder and extracted in deionized water at 5ºC overnight. Samples were centrifuged and the supernatant removed for analysis by radioimmunoassay (RIA), following Quarrie et al. (1988). The monoclonal antibody used (AFR MAC 62) is specific for (+)-ABA. Cross-reactivity of the assay with substances other than ABA was tested on *Vigna* genotypes by internal standardization and dilution analysis according to Jones (1987). The results (data not shown) indicated the absence of potentially interfering compounds in the aqueous leaf extracts of the three genotypes (Prof. W. Davies, personal communication).

Electrolyte leakage measurements

Fifteen leaf discs (0.8 cm^2) per sample were cut from well watered plants, rinsed three times with deionized water, and floated for 17 h on deionized water (control), or on a 30% (w/v) PEG MW 8000 solution (osmotic potential: -1.54 MPa). After washing, the discs were allowed to float on deionized water for 24 h. During this period leakage was monitored with a conductimeter (Crison 522, Crison Instruments, Spain). Injury index (I%) was calculated according to the formula I%=[1-(T-D/T-W)]x100, where D and W represent the electrolytes released by PEG-dehydrated and watered (control) samples, respectively, and T the total electrolyte content measured after heating the control samples at a temperature of 90ºC for 2 h, followed by cooling until *ca*. 20ºC, as described in Campos and Pham Thi (1997).

Lipid analysis

Leaf samples (2 g FW) were boiled 2 min in deionized water, in order to stop lipolytic activities. Lipids were extracted in chloroform/methanol /water (1/1/1, by vol.) according to Allen et al. (1966). After evaporation of chloroform under nitrogen, the lipophilic extracts were resuspended in ethanol:toluene (1/4, by vol.) and stored at -20ºC, for further analysis. Fatty acids were saponified, methylated with BF_3 (Merck) using heptadecanoic acid (C17:0) as an internal standard (Metcalfe et al., 1966), and analysed with a gas chromatograph (Unicam 610, Unicam Ltd., U.K.), equipped with a flame-ionization detector, as described in Campos et al. (2003). The fatty acid methyl esters were separated on a DB-Wax column, (J&W Scientific, U.S.A., 0.25 mm i.d. x 30 m, 0.25 µm) with programmed temperature. Carrier gas was hydrogen with a flow rate of 1 ml min^{-1}, at a split ratio of 1:100 of the sample.

For lipid class analysis, a mixture of three samples was prepared for each treatment and analysed in triplicate. Separation was performed by thin layer chromatography on G60 silicagel plates (Merck) in chloroform/acetone/methanol/acetic acid/water (50/20/10/10/5, by vol.) according to Lepage (1967), and subsequently in petroleum ether/diethyl ether/acetic acid (70/30/0.4, by vol.), according to Mangold (1961), to obtain a clear separation between MGDG and neutral lipids. After spraying the plates with primuline 0.01% in 80% acetone and visualisation under UV, the bands were scraped off, saponified, methylated and analysed by gas liquid chromatography, as described above.

Statistical analysis

The data were analysed statistically using a two-way ANOVA, applied to the various measured and calculated parameters, followed by a Tukey test for mean comparison between genotypes or degrees of dehydration (for a 95% confidence level).

Results

Stomatal conductance

Well-watered plants of the three genotypes presented similar g_s values (*ca*. 360-370 mmol H_2O m^{-2} s^{-1}), but their responses varied along the dehydration period (Figure 1). At early stages of water deficit (*ca*. 85% RWC), cvs. EPACE-1 and 1183 presented lower g_s values than Vg. Such difference of behavior was maintained for RWC values around 80%, when g_s remained high in Vg (*ca*. 300 mmol H_2O $m^{-2}s^{-1}$) while EPACE-1 and 1183 presented already g_s values below 100 mmol H_2O m^{-2} s^{-1}. In Vg similar lowered g_s values were observed only for RWC *ca*. 72-75%.

Leaf ABA content

Under control conditions, EPACE-1 and 1183 presented a somewhat higher ABA content than Vg, which remained low until 80% RWC (Figure 2). Furthermore, for higher dehydration levels Vg displayed a stable value of around 3 µg g^{-1} DW, whereas a contrasting response was observed for EPACE-1 and 1183. In fact, in these cvs. ABA content started to rise from the very early stages of dehydration (RWC between 90-80%). Maximal values (*ca*. 4-6 µg g^{-1} DW) were reached under moderate water deficits in EPACE-1, being maintained with severe drought. For cv. 1183 a continuous ABA accumulation occurred with the imposition of stronger water deficits, reaching *ca*. 11.5 µg g^{-1} DW under severe stress conditions (*ca*. 6 fold increase in relation to control).

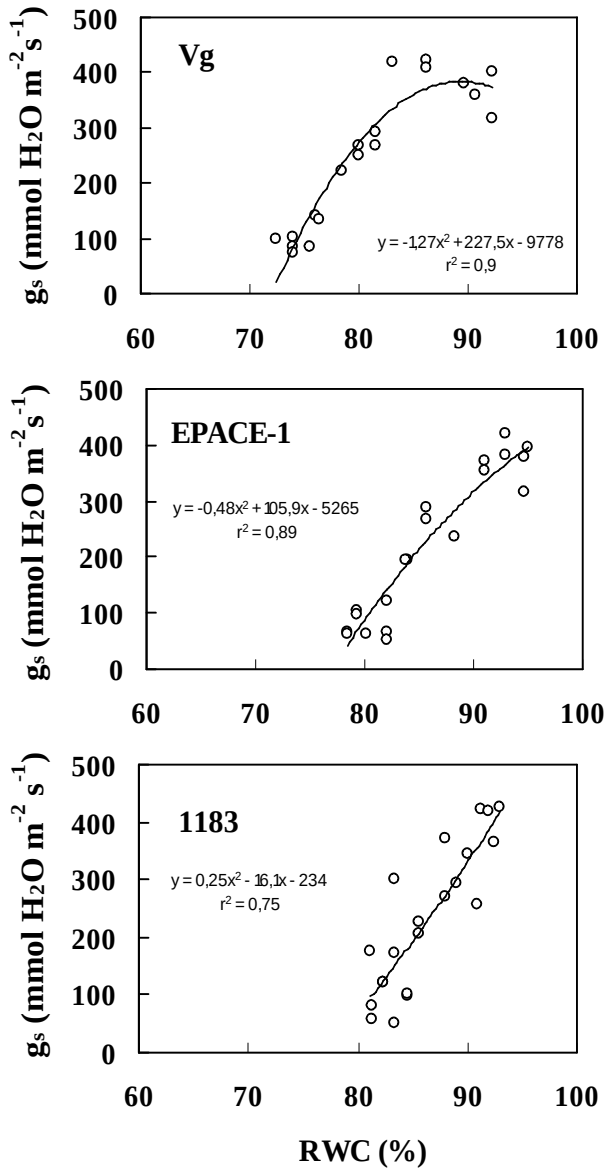


Figure 1. Relation between decreasing leaf relative water content (RWC) and stomatal conductance (g_s) in *V. glabrescens* (Vg) and *V. unguiculata* (cvs. 1183 and EPACE-1). Each value represents an individual measurement and lines correspond to the best fit regression.

Electrolyte leakage test

The three genotypes were affected differently by PEG-induced dehydration (Figure 3) from the beginning of measurements. After the 3rd hour, significant leakage differences were already observed when comparing cv. 1183 with Vg and EPACE-1. By the 5th hour I% values of 41%, 24%, and 19% were obtained respectively for 1183, EPACE-1 and Vg. Differences in I% values were further amplified after 24 h, reflecting a much stronger impact of dehydration on membrane structure and integrity in cv. 1183.

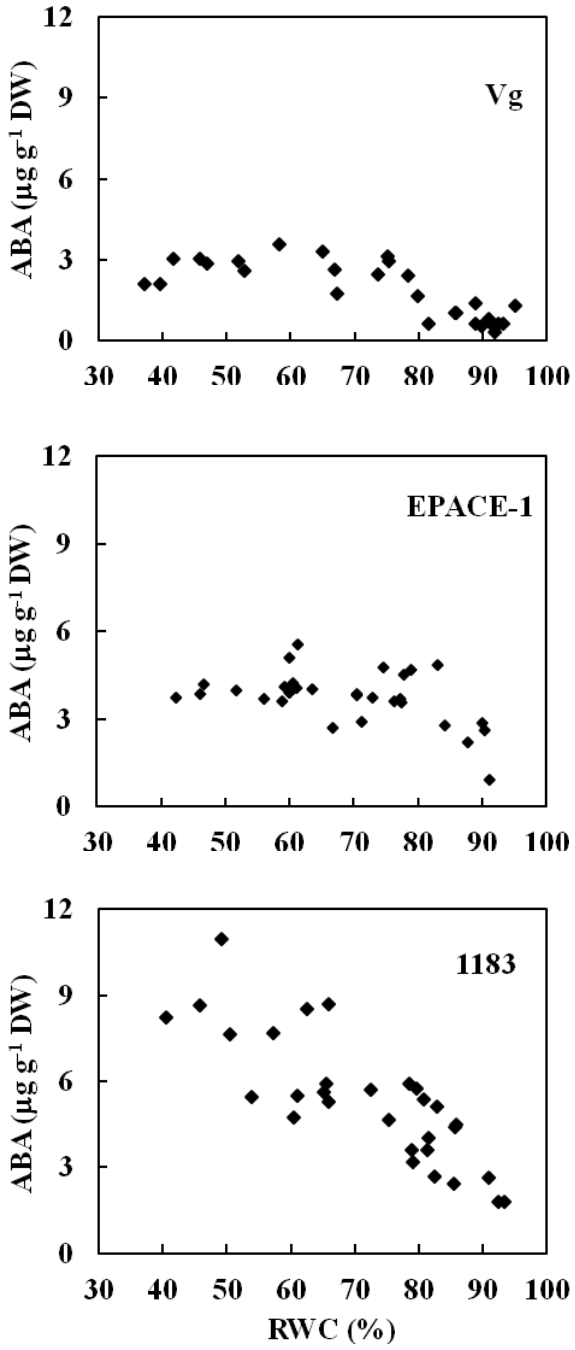


Figure 2. Changes in leaf endogenous abscisic acid (ABA) content in *V. glabrescens* (Vg) and *V. unguiculata* (cvs. 1183 and EPACE-1) with decreasing relative water content (RWC). Each value represents an individual measurement.

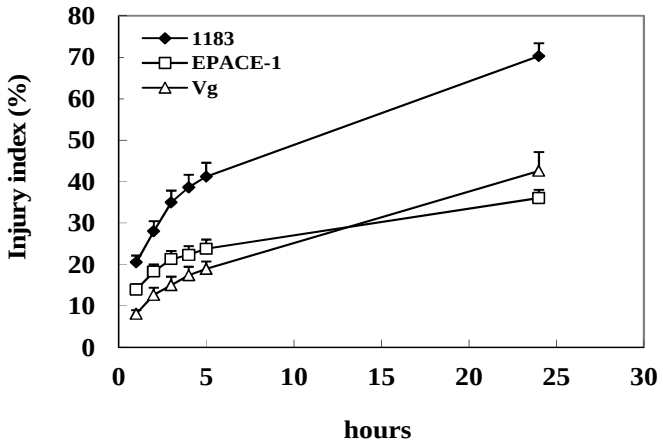


Figure 3. Electrolyte leakage, expressed as an injury index (I%), after leaf discs exposure to dehydration induced by PEG (PEG 8000, -1.54 MPa). Vg, *V. glabrescens*; 1183, *V. unguiculata* cv. 1183; EPACE-1, *V. unguiculata* cv. EPACE-1. Values are the mean ± SE (n=3 independent measurements).

Lipid analysis

Total fatty acid (TFA) content was similar in control plants of the three genotypes (Fig. 4). Under mild stress (S1, RWC = 65-75%), TFA sharply increased (45%) in Vg, due to increase in all polar lipid contents, particularly in phosphatidylethanolamine (PE), phosphatidyl-choline (PC) and phosphatidylcholine (PC), that showed increases of 167%, 96% and 82%, respectively, when compared to control plants (Figure 5). As regards EPACE-1, although TFA content remained unaltered under mild stress (Figure 4), a 27% decrease in MGDG occurred that was counterbalanced by an increase of the two main phospholipids PC (50%) and PG (42%), as well as in PI (43%). In the case of 1183, a gradual decrease of TFA was observed in parallel to the increase of drought severity. Under S1 conditions, drastic reductions were found for MGDG (56%), PC (36%) and PG (46%), that were only partially compensated by the increases in DGDG (6%), PE (128%) and PI (128%), therefore resulting in a TFA reduction of 20%.

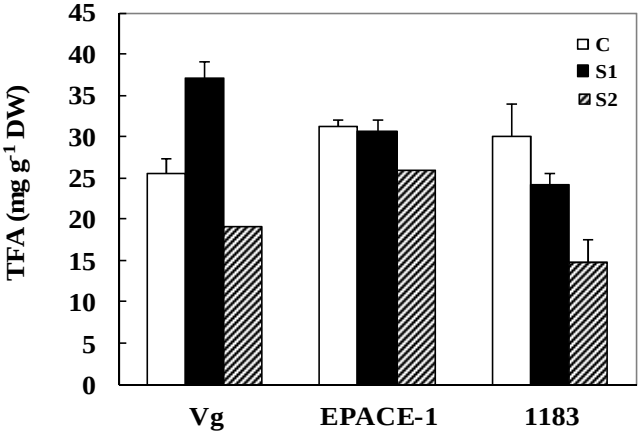
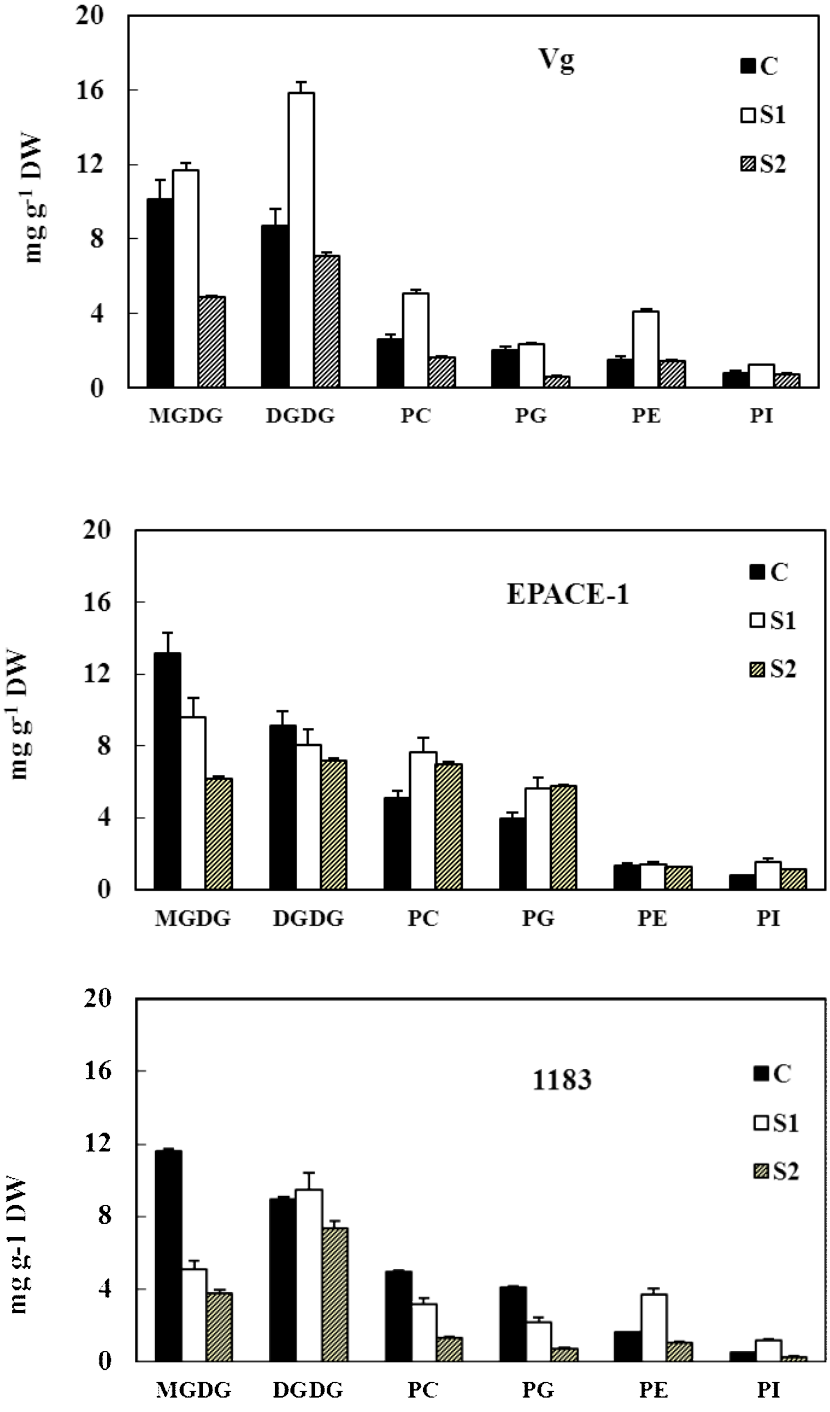


Figure 4. Impact of water stress on the total fatty acids (TFA) content of *V. glabrescens* (Vg) and *V. unguiculata* (cvs. 1183 and EPACE-1) leaves. C: control, RWC > 90%; S1: mild stress, RWC 75-65%; S2: severe stress, RWC < 60%. Values are the mean ± SE (n=3-6 independent measurements).



At severe water deficit (S2, RWC < 60%), a significant 24% TFA reduction was observed in Vg in relation to its control values (although that decrease reached 48% when compared to S1), whereas EPACE-1 showed decreases of 20% and 17%, when compared to control and S1, respectively (Figure 4). Cv. 1183 presented a gradual TFA decrease, reaching a 52% and 39% lower values in comparison to control and S1 stages, respectively. Under S2 conditions, the observed impact was linked to decreases in all lipid classes, except in EPACE-1 (Figure 5), where only galactolipids (mostly MGDG) content decreased. It should be noticed that MGDG was the most drought-susceptible lipid throughout the whole drought period and in all genotypes, showing reductions higher than 50% at S2 (Figure 5).

Figure 5. Changes in lipid classes content of *V. glabrescens* (Vg) and *V. unguiculata* (cvs. EPACE-1 and 1183) leaves in response to three water regimes: C: RWC > 90%; S1: RWC 75-65%; S2: RWC < 60%. Values are the mean ± SE (n=9). MGDG, monogalactosyl-diacylglycerol; DGDG, digalactosyl-diacylglycerol, PC, phosphatidylcholine, PE, phosphatidylethanolamine, PG, phosphatidylglycerol, PI, phosphatidylinositol.

Table 1. Effects of water stress on the fatty acid composition (mol %) of galactolipid classes monogalactosyl-diacylglycerol (MGDG) and digalactosyl-diacylglycerol (DGDG) in *V. unguiculata* (cvs. 1183 and EPACE-1) and *V. glabrescens* (Vg) leaves. Mean values (n = 3) followed by different letters express significant differences between dehydration levels within a genotype (a, b, c) or between genotypes with the same dehydration degree (r, s, t), based upon Tukey's HSD means separation test at $P < 0.05$; a and r represent the highest values. C: RWC > 90%; S1: RWC 75-65%; S2: RWC< 60%.

Genotype	Lipid	Treatment	C16:0	C16:1*c*	C18:0	C18:1	C18:2	C18:3
Vg	MGDG	C	3.03 $^{a/r}$	0.43	0.21	0.25	3.55 $^{b/r}$	92.53 $^{a/r}$
		S1	2.96 $^{a/r}$	0.36	0.86	0.45	6.09 $^{a/r}$	89.27 $^{a/s}$
		S2	3.38 $^{a/r}$	0.40	0.66	0.20	6.27 $^{a/r}$	89.09 $^{a/r}$
	DGDG	C	30.48 $^{a/r}$	4.19	2.20	0.74	7.95 $^{a/r}$	54.45 $^{b/s}$
		S1	21.82 $^{b/s}$	1.71	3.24	1.10	8.63 $^{a/r}$	63.51 $^{a/s}$
		S2	24.41 $^{b/s}$	3.71	2.88	1.00	7.43 $^{a/r}$	60.57 $^{a/s}$
EPACE-1	MGDG	C	1.95 $^{c/s}$	0.37	0.10	0.37	1.41 $^{c/s}$	95.81 $^{a/r}$
		S1	2.36 $^{b/r}$	0.13	0.48	0.15	3.17 $^{b/s}$	93.71 $^{a/r}$
		S2	3.37 $^{a/r}$	0.40	0.53	0.19	4.24 $^{a/rs}$	91.27 $^{a/r}$
	DGDG	C	16.63 $^{b/s}$	4.62	1.02	0.09	1.03 $^{c/s}$	76.61 $^{a/r}$
		S1	16.58 $^{b/t}$	1.90	2.11	0.34	2.33 $^{b/s}$	76.75 $^{a/r}$
		S2	19.77 $^{a/t}$	1.37	2.75	0.37	3.94 $^{a/s}$	71.81 $^{b/r}$
1183	MGDG	C	2.65 $^{a/r}$	0.30	0.23	0.15	1.66 $^{c/s}$	95.00 $^{a/r}$
		S1	2.86 $^{a/r}$	0.87	0.49	0.33	2.31 $^{b/s}$	93.14 $^{a/r}$
		S2	2.76 $^{a/r}$	3.67	0.46	0.42	3.19 $^{a/s}$	85.30 $^{b/s}$
	DGDG	C	20.02 $^{b/s}$	4.61	0.97	0.17	1.01 $^{c/s}$	73.22 $^{a/r}$
		S1	30.67 $^{a/r}$	1.22	2.52	0.80	6.21 $^{b/r}$	58.57 $^{b/t}$
		S2	31.58 $^{a/r}$	1.34	1.97	1.18	8.29 $^{a/r}$	55.62 $^{c/t}$

In parallel to the above presented class content modifications, the weight of the main FA within galactolipid classes also changed with drought implementation. In control plants, linolenic acid (C18:3) percentage in MGDG molecules did not differ between the three genotypes, but was significantly reduced by 10%, 5% and 4% for 1183, EPACE-1 and Vg, respectively, under severe drought (Table 1). As regards the C18:3 percentage in DGDG, Vg presented the lowest values in control plants (Table 1), which increased under S1 and S2 conditions. In 1183 the percentage gradually decreased under moderate and severe stress, while in EPACE-1 a reduction occurred only under S2. Such decreases occurred concomitantly to increases in the percentage of more saturated fatty acids, namely linoleic acid (C18:2) and palmitic acid (C16:0).

Concerning phospholipid classes, no noticeable change was found in their FA acid composition, except a decrease in the percentage of 16:1*t* in PG (data not shown).

Discussion

For the studied cvs., there appear to be two mechanisms in *Vigna* sp. to cope with drought in what concerns stomatal control to water loss. In the first, plants close their stomata at the early stages of dehydration, as was the case of *V. unguiculata* cvs. (1183 and EPACE-1). In the second plants tolerate a lower water availability without strong g_s reduction, as happened in *V. glabrescens.* Such stomatal closure at moderate dehydration (80% RWC), occurring in *V. unguiculata* cvs. 1183 and EPACE-1, was probably controlled by endogenous leaf ABA that increased in both genotypes.

Electrolyte leakage test showed that membrane integrity under PEG-induced dehydration was better preserved in *V. glabrescens* and *V. unguiculata* EPACE-1, suggesting that both genotypes present a higher protoplasmic tolerance to dehydration than cv. 1183.

Drought impact on membrane lipids further points to a higher sensitivity of cv. 1183 due to a large reduction of TFA. On the other hand, under S1, total lipid content of leaves remained unchanged in EPACE-1 and increased in Vg, being affected only in S2 conditions (and to a lower extent than cv. 1183). In Vg *de novo* lipid biosynthesis under S1 reflected a general rise in all lipid classes, particularly in DGDG. Such higher DGDG content may represent an important adaptive mechanism. Effectively, DGDG is a bilayer forming lipid involved in membrane stabilization, regulation of ionic permeability and preserving activity of membrane proteins (Webb and Green, 1991; Siegenthaler and Trémolières, 1998). Also, a reduction of the MGDG to DGDG

ratio, linked to DGDG synthesis, was reported in drought tolerant genotypes under mild water deficit (Torres-Franklin et al., 2007) and under cold (Harwood, 1998). Furthermore, the newly synthesized DGDG in Vg showed qualitative modifications, as it presented a raise of the highly unsaturated C18:3. This may contribute for the lower photosynthetic impairments previously observed in this genotype under drought (Campos et al., 1999a). It is generally accepted that preponderance of unsaturated fatty acids enhances fluid phase in membranes, maintaining biological activity under stressfull conditions (Leshem, 1992; Murata and Wada, 1995; Somerville, 1995; Partelli et al., 2011), as photosynthetic performance is tightly associated to membrane lipid composition (Horváth et al., 1987). Water stress favours membrane rigidity (Webb and Green, 1991; Leone et al., 1996), and a compensatory increase in acyl unsaturation may contribute to maintain lipid acyl motion in thylakoid membranes, therefore preserving the rate of plastoquinone diffusion and ensuring photosynthetic electron transport (Harwood, 1998; Siegenthaler and Trémolières, 1998).

V. unguiculata cv. 1183 was more susceptible to PEG-induced dehydration than the other genotypes, suggesting a loss of selectivity. That could have been related to TFA decrease which in turn was a result of PC, PG and, mostly MGDG decreases in S1 conditions, that was extended to other lipid classes in S2. Furthermore, linolenic acid (C18:3) weight in MGDG (and DGDG) decreased under severe drought. That suggested an inhibition of lipid biosynthesis and/or an acceleration of degradative phenomena particularly as regards chloroplast membranes, probably linked to oxidative and hydrolytic reactions already described in *Vigna* species (Monteiro de Paula et al., 1993; Ferrari-Iliou et al., 1994; Sahsah et al., 1998; Maarouf et al., 1999). Such a low capacity to withstand MGDG degradation agrees with our previous report on the presence of higher galactolipase activities in water stressed plants of 1183 in comparison with EPACE-1 (Sahsah et al., 1998; Matos et al., 2001) and Vg (Campos et al., 1999b). Therefore, the results point that in cv. 1183 membrane repair and/or protection mechanisms might be impaired or insufficient to cope with increasing dehydration, leading to poor membrane preservation, which in turn compromises cell compartmentation and functioning.

Drought induced increasingly higher ABA contents in 1183, which may have contributed to accelerate senescence, resulting in cell damage. A similar mechanism was suggested to occur in the drought-susceptible Mediterranean shrub *Lavandula stoechas* L. (Lopez-Carbonell et al., 1996). ABA effects on cell metabolism are multiple and sometimes contradictory (Wilkinson and Davies, 2002). Apart from eliciting stomatal closure and triggering cell signalling pathways (Himmelbach et al., 2003) which stimulate beneficial responses, ABA can also cause deleterious effects in plant development, due to its involvement in leaf senescence and abscission (Trewawas and Jones, 1991). Jiang and Zhang (2002) remarked that treatment of maize seedlings with low concentrations of ABA induced antioxidative defence response, whereas high ABA concentrations led to excessive generation of activated oxygen species.

More dehydration-tolerant membrane systems in Vg and EPACE-1 seem to be related to stable and lower ABA contents under increasing water deficit (RWC < 80%). The question raises if, in these tolerant genotypes, ABA could be involved in the preservation of membrane integrity, therefore contributing to enhance membrane tolerance. Our previous experiments carried out *in vitro* using *Vigna* leaf discs highlighted a protective effect of exogenously applied ABA against membrane lipid degradation under dehydration conditions (Campos and Pham Thi, 1997). Direct effects of ABA on artificial membrane systems have been reported, leading for example to increases in microviscosity (Shripathi et al., 1997) and permeability (Schauf et al., 1987). Membrane stability may be achieved through binding of ABA molecules to specific lipid components (Wassal et al., 1985; Leshem, 1992), or through ABA involvement in lipid metabolism (Aghofack-Nguemezi et al., 1991; Zou et al., 1995), although the mechanisms by which ABA act are still unknown. Some evidence has been reported concerning the effects of ABA on the regulation of genes coding for enzymes of lipid metabolism (Matos et al., 2001; Matsuda et al., 2001; Wang, 2002; Narusaka et al., 2003). Studies using cDNAs microarrays showed that ABA triggers expression of a large set of genes (Seki et al., 2002; Rabbani et al., 2003). Several of these genes are linked to lipid degradation, through generation of lipid second messengers by phospholipases (Sanchez and Chua, 2001; Wang, 2002). Long-term modifications of lipid metabolism may also result from these ABA-induced phenomena Aghofack-Nguemezi et al., 1991; Zou et al., 1995). A possible involvement of ABA in the induction of antioxidant enzymes

should not be excluded (Jiang and Zang, 2002), and could also result in lipids preservation.

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References

Aghofack-Nguemezi, J., A. Christmann, S. Frosch, A. Trémolières and E. Wagner. 1991. Contrasting photo and thermoperiod-induced changes in abscisic acid and lipid contents in leaves of mungbean seedlings. Physiol. Plant. 83:346-352.

Allen, C. F., P. Good, H. S. Davis, P. Chisum and S. D. Fowler. 1966. Methodology for the separation of plant lipids and application to spinach leaf and chloroplast lammelae. J. Am. Oil Chem. Soc. 43:223-230.

Bewley, J. D., T. L. Reynolds and M. J. Oliver. 1993. Evolving strategies in the adaptation to desiccation. In: T. J. Close, E. A. Bray (Eds.), pp. 193-201. Plant Responses to Cellular Dehydration during Environmental Stress, Washingon, U.S.A.: Am. Soc. Plant Physiol.

Blum, A. and B. Sinmena. 1995. Isolation and characterization of variant wheat cultivars for ABA sensitivity. Plant Cell Environ. 18:77-83.

Campos, P. S. and A. T. Pham Thi. 1997. Effects of an abscisic acid pretreatment on membrane leakage and lipid composition of *Vigna unguiculata* leaf discs subjected to osmotic stress. Plant Sci. 130:11-18.

Campos, P. S., J. Ramalho, M. J. Silva, J. A. Lauriano and M. C. Matos. 1999a. Effects of drought on photosynthetic performance and water relations of four *Vigna* genotypes. Photosynthetica 36:79-87.

Campos, P. S., J. Vieira da Silva and A. T. Pham Thi. 1999b. Effect of water stress on membrane integrity and enzymatic breakdown of galactolipids in *Vigna* sp. Agronomia Lusitana 47:185-196.

Campos, P. S., V. L. Quartin, J. C. Ramalho and M. A. Nunes. 2003. Electrolyte leakage and lipid degradation account for cold sensitivity in leaves of *Coffea* sp. plants. J. Plant Physiol. 160:283-292.

Dias, A. S., M. G. Barreiro, P. S. Campos, J. C. Ramalho and F. C. Lidon. 2010. Wheat cellular membrane thermotolerance under heat stress. J. Agr. Crop Sci. 196:100-108.

Ferrari-Iliou R., A. d'Arcy-Lameta, A. T. Pham Thi, Y. Zuily-Fodil and P. Mazliak. 1994. Effect of drought on photodynamic peroxydation of leaf total lipophilic extracts. Phytochem. 37:1237-1243.

Harwood, J. L. 1998. Involvement of chloroplast lipids in the reaction of plants submitted to stress. In: Siegenthaler, P.-A., Murata, N. (Eds.), vol. 6, pp. 287–302. Lipids in Photosynthesis: Structure, Function and Genetics, Series Advances in Photosynthesis, Dordrecht, The Netherlands, Kluwer Academic Publishers.

Himmelbach, A., Y. Yang and E. Grill. 2003. Relay and control of abscisic acid signalling. Curr. Opin. Plant Biol. 6:470-479.

Horváth, G., A. Melis, É. Hideg, M. Droppa and L. Vigh. 1987. Role of lipids in the organization and function of photosystem II studied by homogeneous catalytic hydrogenation of thylakoid membranes *in situ*. Biochem. Biophys. Acta 891:68-74.

Ingram, J. and D. Bartels. 1996. The molecular basis of dehydration tolerance in plants. Ann. Rev. Plant Physiol. Plant Mol. Biol. 47:377-403.

Jiang, M. and J. Zhang. 2002. Role of abscisic acid in water stress-induced antioxidant defense in leaves of maize seedlings. Free Radic. Res. 36:1001-1015.

Jones, H. G. 1987. Correction for non-specific interference in competitive immunoassays. Physiol. Plant. 70:146-154.

Kuiper, J. P. C. 1985. Environmental changes and lipid metabolism of higher plants. Physiol. Plant. 64:118-122.

Leone, A., A. Costa, S. Grillo, M. Tucci, I. Horvath and L. Vigh. 1996. Acclimation to low water potential determines changes in membrane fatty acid composition and fluidity in potato cells. Plant Cell Environ. 19:1103-1109.

Lepage, M. 1967. Identification and composition of turnip root lipids. Lipids 2:244-250.

Leshem, Y. 1992. Plant membranes: A Biophysical Approach to Structure, Development and Senescence. Kluwer Academic Publishers, Dordrecht, The Netherlands: p. 266.

Lopez-Carbonell, M., L. Alegre, A. Pastor, E. Prinsen and H. Van Onckelen. 1996. Variations in abscisic acid, indole-3-acetic and zeatin riboside concentrations in two Mediterranean shrubs subjected to water stress. Plant Growth Regulat. 20:271-277.

Maarouf, E. H., Y. Zuily-Fodil, M. Gareil, A. d'Arcy-Lameta and A. T. Pham-Thi. 1999. Enzymatic activity and gene expression of phospholipase D in two cultivars of *Vigna unguiculata* L.Walp differing in drought tolerance. Plant Mol. Biol. 39:1257-1265.

Mangold, H. K. 1961. Thin layer chromatography of lipids. J. Am. Oil Chem. Soc. 38:708-727.

Matos, A. R., A. d'Arcy-Lameta, M. França, S. Pêtres, L. Edelman, J. C. Kader, Y. Zuily-Fodil and A. T. Pham Thi. 2001. A novel patatin-like gene stimulated by drought stress encodes a galactolipid acyl hydrolase. FEBS Lett. 491:188-192.

Matos, M. C., P. S. Campos, J. A. Passarinho, J. N. Semedo, N. M. Marques, J. C. Ramalho and C. P. Ricardo. 2010. Drought effect on photosynthetic activity, osmolyte accumulation and membrane integrity of two *Cicer arietinum* genotypes. Photosynthetica 48:303-312.

Matsuda, O., C. Watanabe and K. Iba. 2001. Hormonal regulation of tissue-specific ectopic expression of an *Arabidopsis* endoplasmic reticulum-type omega-3 fatty acid desaturase (FAD3) gene. Planta 213:833-40.

Metcalfe, L. D., A. A. Schemitz and J. R. Pelka. 1966. Rapid preparation of fatty-acid esters from lipids for gas chromatographic analysis. Anal. Chem. 38:514-515.

Monteiro de Paula, F., A. T. Pham Thi, Y. Zuily-Fodil, R. Ferrari-Iliou, J. Vieira da Silva and P. Mazliak. 1993. Effect of water stress on the biosynthesis and degradation of polyunsaturated lipid molecular species in leaves of *Vigna unguiculata*. Plant Physiol. Biochem. 31:707-715.

Murata, N. and H. Wada. 1995. Acyl-lipid desaturases and their importance in the tolerance and acclimatization to cold of cyanobacteria. Biochem. J. 308:1-8.

Narusaka, Y., M. Narusaka, M. Seki, M. Fujita, J. Ishida, M. Nakashima, A. Enju, T. Sakurai, M. Satou, A. Kamiya, P. Park, M. Kobayashi and K. Shinozaki. 2003. Expression profiles of *Arabidopsis* phospholipase A IIA gene in response to biotic and abiotic stresses. Plant Cell Physiol. 44:1246-52.

Navari-Izzo, F., M. T. Milone, M. F. Quartacci and C. Pinzino. 1993b. Metabolic changes in wheat plants subjected to a water-deficit stress programme. Plant Sci. 92:151-157.

Öquist, G. 1982. Seasonally induced changes in acyl lipids and fatty acids of chloroplast thylakoids of *Pinus silvestris*. A correlation between the level of unsaturation of monogalatosyldiglyceride and the rate of electron transport. Plant Physiol. 69:869-875.

Partelli, F. L., P. Batista-Santos, P. Scotti-Campos, I. P. Pais, V. L. Quartin, H. D. Vieira and J. C. Ramalho. 2011. Characterization of the main lipid components of chloroplast membranes and cold induced changes in *Coffea* sp. Env. Exp. Bot. 74:194-204.

Pekic, S. and S. A. Quarrie. 1988. Abscisic acid in leaves of inbred lines and F1 hybrids of maize growing in the field and its relationship to diurnal and seasonal trends in leaf water potential. Ann. Bot. 61:669-678.

Pham Thi, A. T., J. Vieira da Silva and P. Mazliak. 1990. The role of membrane lipids in drought resistance of plants. Bull. Soc. Bot. Fr. 137:99-114.

Quarrie, S. A. 1991. Implications of genetic differences in ABA accumulation for crop production. In: W. J. Davies, H. G. Jones (Eds.), pp. 227-243. Abscisic Acid Physiology and Biochemistry. Bios. Scientific Publishers. Oxford, U.K.

Quarrie, S. A., P. N. Whitford, N. E. J. Appleford, T. L. Wang, S. K. Cook, I. E. Henson and B. R. Loveys. 1988. A monoclonal antibody to (S)-abscisic acid: its characterization and use in a radioimunoassay for measuring abscisic acid in crude extracts of cereal and lupin leaves. Planta 173:330-339.

Rabbani, M. A., K. Maruyama, H. Abe, M. A. Khan, K. Katsura, Y. Ito, K. Yoshiwara, M. Seki, K. Shinozaki and K. Yamaguchi-Shinozaki. 2003. Monitoring expression profiles of rice genes under cold, drought, and high-salinity stresses and abscisic acid application using cDNA microarray and RNA gel-blot analyses. Plant Physiol. 133:1755-1767.

Ramalho, J. C., P. S. Campos, M. Teixeira and M. A. Nunes. 1998. Nitrogen dependent improvement of antioxidant systems and changes in lipid composition of chloroplast membranes in *Coffea arabica* plants submitted to high irradiance. Plant Sci. 135:115-124.

Sahsah, Y., P. Campos, M. Gareil, Y. Zuily-Fodil and A. T. Pham Thi. 1998. Enzymatic degradation of polar lipids in *Vigna unguiculata* leaves and influence of drought stress. Physiol. Plant. 104:577-586.

Sanchez, J. P. and N. H. Chua. 2001. *Arabidopsis* PLC1 is required for secondary responses to abscisic acid signals. Plant Cell 13:1143-1154.

Schauf, C. L., B. Bringle and W. Stillwell. 1987. Membrane-directed effects of the plant hormones abscisic acid, indole-3-acetic acid and 2,4-dichlorophenoxyacetic acid. Biochem. Biophys. Res. Commun. 143:1085-1091.

Seki, M., J. Ishida, M. Narusaka, M. Fujita, T. Nanjo, T. Umezawa, A. Kamiya, M. Nakajima, A. Enju, T. Sakurai, M. Satou, K. Akiyama, K. Yamaguchi-Shinozaki, P. Carninci, J. Kawai, Y. Hayashizaki and K. Shinozaki. 2002. Monitoring the expression pattern of around 7,000 *Arabidopsis* genes under ABA treatments using a full-length cDNA microarray. Funct. Integr. Genomics 2:282-291.

Shripathi, V., G. S. Swamy and K. S. Chandrasekhar. 1997. Microviscosity of cucumber (*Cucumis sativus L.*) fruit protoplast membranes is altered by triacontanol and abscisic acid. Biochim. Biophys. Acta 1323:263–271.

Siegenthaler, P. A. and A. Trémolières. 1998. Role of acyl lipids in the function of photosynthetic membranes in higher plants. In: Siegenthaler, P.-A., Murata, N. (Eds.), vol. 6, pp. 145–173. Lipids in Photosynthesis: Structure, Function and Genetics, Ser. Adv. Photosynthesis, Kluwer Academic Publishers. The Netherlands.

Somerville, C. 1995. Direct tests of the role of membrane lipid composition in low temperature-induced photoinhibition and chilling sensitivity in plants and cyanobacteria. Proc. Natl. Acad. Sci. USA 92:6215-6218.

Stillwell, W., B. Brengle and S. R. Wassall. 1988. Abscisic acid enhances aggregation and fusion of phospolipid vesicles. Biochem. Biophys. Res. Commun. 156:511–516.

Stillwell, W. and P. Hester. 1984. Abscisic acid increases membrane permeability by interacting with phosphatidylethanolamine. Biochemistry 23:2187–2192.

Torres-Franklin, M. -L., A. Gigon, D. F. Melo, Y. Zuily-Fodil and A. -T. Pham-Thi. 2007. Drought stress and rehydration affect the balance between MGDG and DGDG synthesis in cowpea leaves. Physiol. Plant. 131:201–210.

Trewavas, A. J. and H. G. Jones. 1991. An assessment of the role of ABA in plant development. In: Abscisic Acid Physiology and Biochemistry. W. J. Davies and H. G. Jones (Eds.), pp. 169-188. Oxford, U.K., Bios. Scientific Publishers.

Tuberosa, R., M. C. Sanguineti and P. Landi. 1994. Abscisic acid concentration in leaf and xylem sap, leaf water potential and stomatal conductance in maize. Crop Sci. 34:1557-1563.

Van der Maesen, L. J. G. and S. Somaatmadja. 1989. Plant Resources of South-East Asia. N. 1, Pulses, Pudoc/Prosea, Wageningen, The Netherlands.

Vasquez-Tello, A., Y. Zuily-Fodil, A. T. Pham Thi and J. V. Silva. 1990. Electrolyte and inorganic phosphate leakages and soluble sugar content as physiological tests for screening resistance to water stress in *Phaseolus* and *Vigna* species. J. Exp. Bot. 41:827-832.

Wang, X. 2002. Phospholipase D in hormonal and stress signalling. Curr. Opin. Plant Biol. 5:408-414.

Wassall, S. R., P. Hester and W. Stillwell. 1985. Abscisic acid increases lipid bilayer permeability to cations as studied by phosphorus-31 NMR. Biochim. Biophys. Acta 815:519-522.

Webb, M. S. and B. R. Green. 1991. Biochemical and biophysical properties of thylakoid acyl lipids. Biochem. Biophys. Acta. 1060:133-158.

Wilkinson, S. and W. J. Davies. 2002. ABA-based chemical signals: the co-ordination of responses to stress in plants. Plant Cell Env. 25:195-210.

Zou, J., G. D. Abrams, D. L. Barton, D. C. Taylor, M. K. Pomeroy and S. R. Abrams. 1995. Induction of lipid and oleosin biosynthesis by (+)-abscisic acid and its metabolites in microspore-derived embryos of *Brassica napus* L. cv Reston. Plant Physiol. 108:563-571.

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REGULAR ARTICLE

Drought-induced changes and recovery of photosynthesis in two bean cultivars (*Phaseolus vulgaris* L.)

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Abstract

The effects of soil drought on photosynthesis and chlorophyll fluorescence in the leaves of two common bean (*Phaseolus vulgaris* L.) cultivars – Zarya and Tangra were studied, as well as recovery of photosynthesis after re-watering. Drought was imposed 14 days after the emergency by withholding water for 10 days in which soil water potential reached -0.9 MPa. Water stress led to a noticeable decrease in both the initial slope of the A_n/C_i curve and A_{max} in the primary leaf of the studied cultivars. The most marked reduction in leaf gas exchange was observed in cv. Tangra. α was reduced more than three folds and A_{max} - more than six folds. Exposure of bean plants to soil drought and provoked leaf water deficit resulted in a dramatic reduction (with an 84.17%) of A_n at normal ambient CO_2 concentration (C_a=370 μmol mol^{-1}). The lower reduction in leaf gas exchange parameters were observed in cv. Zarya. Drought stress induces an increase of F_0 accompanied by a decrease of F_m in the studied cultivars, being cv. Zarya less affected. The F_v/F_m ratio was significantly decreased in cv. Tangra and only showed a slight tendency to a decrease in cv. Zarya. Cv. Tangra presented a decrease of 56% in qP in the primary leaf, while in cv. Zarya qP decreased with 32%. Accordingly, Y strongly decreased in cv. Tangra, while in cv. Zarya Y was less affected. 3 days after re-watering photosynthesis of cv. Zarya was about 87% from the control plants, while in cv. Tangra photosynthesis was only 68% from control. Chlorophyll fluorescence parameters were recovered to the greater extent. On the basis of the data obtained we could arrange photosynthetic apparatus of cv. Zarya as relatively drought tolerant and that of cv. Tangra as drought sensitive.

Key words: Drought, Photosynthesis, Chlorophyll fluorescence, *Phaseolus vulgaris*, Recovery

Introduction

Maintaining growth and crop productivity under adverse stress environmental conditions is presumably the major challenge facing modern agriculture. To meet this challenge, it is necessary to understand the physiological and biochemical bases of plant acclimation to growth in stressed conditions, and the relationship between them and environment. Drought stress is one of the major causes of crop loss worldwide, reducing average yields for most major crop plants by more than 50% (Wang et al., 2003). Global climatic changes will probably make water shortage an even greater limitation to plant productivity across an increasing amount of land (Chaves et al., 2009). The limitation of plant growth imposed by low water availability is mainly due to reductions of plant carbon balance, which is largely dependent on leaf photosynthesis. For this reason, photosynthesis responses to water stress have been the subject of study and debate for decades, in particular, concerning which are the most limiting factors for photosynthesis under water stress (Flexas and Medrano, 2002; Lawlor and Cornic, 2002; Grassi and Magnani, 2005; Souza et al., 2004).

The ability to maintain the functionality of the photosynthetic machinery under water stress and extent of recovery, therefore, is of major importance in drought tolerance. The plant reacts to water deficit with a rapid closure of stomata to avoid further loss of water through transpiration (Cornic, 1994; Lawlor, 1995). As a consequence, the diffusion of CO_2 into the leaf is restricted (Chaves et al., 2003). The decrease in net photosynthetic rate under drought stress observed in many studies is often explained by a lowered internal CO_2 concentration that results in a limitation of photosynthesis at the acceptor site of ribulose-1,5-bisphospate carboxylase/oxygenase

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(Rubisco) (Cornic et al., 1992; Chaves et al., 2003) or by the direct inhibition of photosynthetic enzymes like Rubisco (Haupt-Herting and Fock, 2000) or ATP synthase (Nogués and Baker, 2000).

Despite of fact that photosystem II (PSII) is highly drought resistant (Yordanov et al., 2003) under conditions of water stress photosynthetic electron transport through PS II is inhibited (Chakir and Jensen, 1999, Zlatev et al., 2010). Several *in vivo* studies demonstrated that water deficit resulted in damages to the oxygen evolving complex of PSII (Lu and Zhang, 1999; Skotnica et al., 2000) and to the PSII reaction centers associated with the degradation of D1 protein (Cornic, 1994; Rivero et al., 2010). The mechanism by which the water deficit inhibits this electron transport is still unclear.

The aim of this study was to determine the effects of soil drought stress on leaf gas exchange and chlorophyll fluorescence parameters in leaves of two common bean (*Phaseolus vulgaris* L.) cultivars. Analyses of the response of net CO_2 assimilation to intercellular CO_2 concentration and chlorophyll fluorescence measurements allow evaluation of the relative limitations to leaf photosynthesis imposed by changes in stomatal conductance, carboxylation efficiency, capacity for regeneration of RuBP and PSII electron transport efficiency. The degree of recovery of photosynthesis is also analyzed.

Materials and Methods

Plant material and growth conditions

For this study two common bean (*Phaseolus vulgaris* L.) cultivars were used: cv. Zarya and cv. Tangra. Seeds were washed in distilled water, after that surface sterilized with a 2% sodiumhypochloride (NaOCl) solution for 5 min, and germinated on moist filter paper, in Petri dishes, maintained at 25°C, in the dark, for 3 days. After germination seedlings with well-developed roots and having approximately the same morphological aspect were selected and cultivated in pots as soil culture in a growth chamber. Dissolved nutrients were added to the soil 15 days before planting: 270 mg $Ca(NO_3)_2$ kg^{-1} dry soil, 190 mg KNO_3 kg^{-1} dry soil and 210 mg $NH_4H_2PO_4$ kg^{-1} dry soil. One seedling was maintained in each pot. The environmental conditions in the growth chamber were: photosynthetic photon flux density (PPFD) of 320 μmol m^{-2} s^{-1}, day/night temperature 25±2/15±2ºC, photoperiod of 14 h, and relative air humidity between 60-65%. Pots were watered daily to maintain control soil water content of 41% (0.410 g H_2O g^{-1} dry soil) corresponding to soil water potential (Ψ_{soil}) of -20 kPa. It is considered that soil is well watered and there is no water stress if Ψ_{soil} is above -30 kPa (Ali et al., 1999). Water stress was progressively induced in 14-day old plants by withholding water supply for 10 days in which soil water content reached 23% (0.230 g H_2O g^{-1} dry soil) corresponding to soil water potential of -0.9 MPa. After imposition of stress, re-watering period for 3 days was applied. The measurements were made at the end of stress period, and at the end of re-watering period, 2 h after the start of photoperiod on primary leaf, which was fully matured.

Gas exchange measurements

Gas exchange measurements were performed with a portable photosynthetic system LCA-4 (Analytical Development Company, Hoddesdon, UK) equipped with a PLCB-4 chamber. PPFD was 650 μmol m^{-2} s^{-1} generated by a metal halide lamp, leaf temperature was 27±2ºC and ambient CO_2 concentration (C_a) was 370 μmol mol^{-1}.

Maximal carboxylation efficiency (α) was calculated by the initial slope of the CO_2 curve representing the net CO_2 assimilation (A_n) versus intercellular CO_2 concentration (C_i), according to von Caemmerer and Farquhar (1981).

The following function was fitted to the experimental data:

$$A_n = a + b\, e^{(-Ci/c)}, \qquad [1]$$

where a is maximal CO_2 assimilation (A_{max}) at saturated zone; b is parameter which is used for the calculation of CO_2 evolved during the dark respiration (R) at A_{max} (R = a + b) (Nacheva et al., 2002); c is constant.

CO_2-compensation point (Γ) was calculated from [1] at y=0 as follow:

$$\Gamma = -c \ln(-a/b)\ [\mu mol\ mol^{-1}] \qquad [2]$$

The stomatal limitations of photosynthesis (SL) were calculated according Farquhar and Sharkey (1982) as: $SL = (A_{Ci} - A_{Ca})/A_{Ci}$, where A_{Ci} is the net photosynthetic rate at C_i = 370 μmol mol^{-1} and A_{Ca} is the net photosynthetic rate at ambient CO_2 concentration (C_a), C_a= 370 μmol mol^{-1}.

Chlorophyll fluorescence

Chlorophyll fluorescence parameters were measured using a pulse amplitude modulation chlorophyll fluorometer MINI-PAM (Walz, Effeltrich, Germany). Minimal fluorescence, F_0, was measured in 60 min dark-adapted leaves using weak modulated light of < 0.15 μmol m^{-2} s^{-1} and maximal fluorescence, F_m, was measured after 0.8 s saturating white light pulse (>5500 μmol m^{-2} s^{-1}) in the same leaves. Maximal variable fluorescence

(F_v=F_m–F_0) and the photochemical efficiency of PSII (F_v/F_m) for dark adapted leaves were calculated. In light adapted leaves steady state fluorescence yield (F_s), maximal fluorescence (F'_m) after 0.8 s saturating white light pulse (> 5500 μmol m^{-2} s^{-1}) and minimal fluorescence (F'_0) measured when actinic light was turned off, were determined. Photochemical (qP) and non-photochemical (qN) quenching parameters were calculated according to Schreiber et al. (1986), using the nomenclature of van Kooten and Snel (1990). The efficiency of electron transport as a measure of the total photochemical efficiency of PSII (Y) was calculated according to Genty et al. (1989).

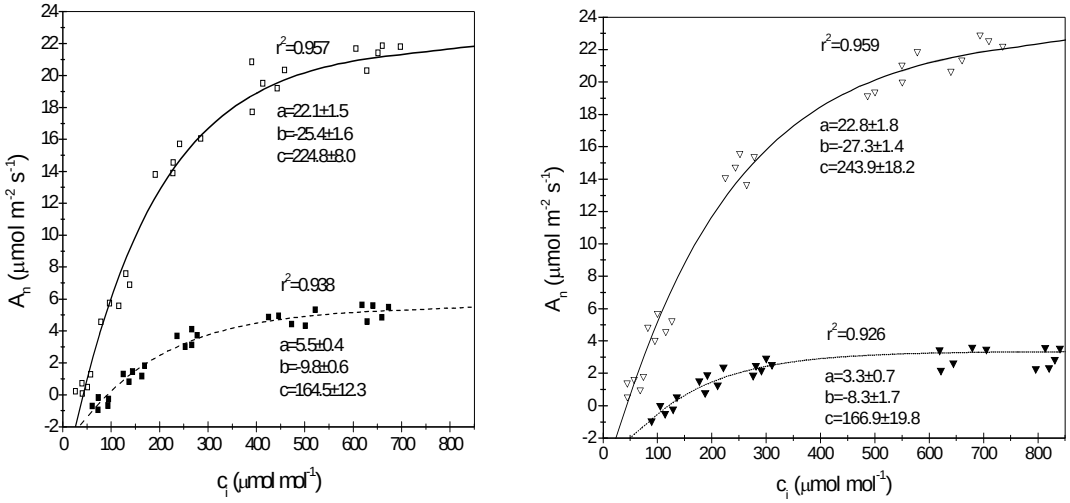
Statistical analysis

Values are the mean ± SE from three consecutive experiments, each including at least five replications of each variant. The Student's *t*-test was used to evaluate the differences between control and stressed variants.

Results

Effects of drought on photosynthetic rate at different intercellular CO_2 concentrations

The changes in net photosynthetic rate of bean leaves as a function of the intercellular CO_2 concentration were used to determine the role of stomatal limitations (SL) on A_n under drought stress. Leaf water deficit led to a noticeable decrease in both the initial slope of the A_n/C_i curve and A_{max} in the primary leaf of the studied cultivars (Fig. 1). A decline in the initial slope indicates a decreased Rubisco activity, while a low level of A_{max} at saturating CO_2 implicates a suppressed capacity for RuBP regeneration (von Caemmerer and Farquhar, 1981). The most marked reduction in leaf gas exchange was observed in cv. Tangra (Table 1). α was reduced more than three folds and A_{max} - more than six folds. Exposure of bean plants to soil drought and provoked leaf water deficit resulted in a dramatic reduction (with an 84.7%) of A_n at normal C_a (370 μmol mol^{-1}). CO_2 compensation point (Γ) increased more than three folds. SL increased slightly which suggests a stronger influence of non-stomatal (biochemical) factors. Lower reduction in leaf gas exchange parameters were observed in cv. Zarya. It is noteworthy that SL increased significantly in this cultivar, which suggests a stronger influence of stomatal factors.



A B

Figure 1. Changes of net photosynthetic rate to intercellular CO_2 concentration in primary leaf of control and drought stressed bean plants. **A** – cv. Zarya, control (□)) and drought stressed plants (■); **B** – cv. Tangra, control (∇) and drought stressed plants (▼). The function $A_n = a + be^{(-Ci/c)}$ was fitted to experimental data. The values of parameters a, b and c with their standard errors are given in the figure and they were used for calculation of the photosynthetic characteristics in Table 1.

Table 1. Effect of soil drought on leaf gas exchange parameters in primary leaf of control and drought stressed bean plants. α, maximal carboxylation efficiency; Γ, CO_2 compensation point; A_{max}, net photosynthetic rate at saturating CO_2; Ac_a=370, net photosynthetic rate at 370 μmol mol^{-1} ambient CO_2 concentration; A_{Ci}=370, net photosynthetic rate at 370 μmol mol^{-1} intercellular CO_2 concentration; SL, stomatal limitation of photosynthesis.

Variant	α (μmol m^{-2} s^{-1} mol^{-1})	Γ (μmol mol^{-1})	A_{max} (μmol m^{-2} s^{-1})	Ac_a=370 (μmol m^{-2} s^{-1})	A_{Ci}=370 (μmol m^{-2} s^{-1})	SL (%)
Control						
Zarya	0.105	31.3	22.1	14.13	18.76	24.7
Tangra	0.103	43.9	22.8	13.84	17.24	19.7
Drought stressed						
Zarya	0.045	95.0	5.5	2.61	4.62	43.5
Tangra	0.033	153.9	3.3	2.11	2.73	22.7

Chlorophyll fluorescence

Data in Table 2 show that drought stress induces an increase of F_0 accompanied by a decrease of F_m in primary leaf of the studied cultivars, being cv. Zarya less affected (Table 2). According Baker and Horton (1987) an increase in F_0 is characteristic of PSII inactivation, whereas a decline in F_v may indicate the increase in a non-photochemical quenching process at or close to the reaction center.

The F_v/F_m ratio, which characterizes the maximal quantum yield of the primary photochemical reactions in dark adapted leaves was decreased significantly in cv. Tangra, and only showed a slight tendency to a decrease in cv. Zarya.

Cv. Zarya presented a decrease of 18.85% in the proportion of energy driven to the photosynthetic pathway (qP) in the primary leaf, while in cv. Tangra qP decreased with 48.47%. Accordingly, Y strongly decreased - in cv. Tangra with 46.74% and in cv. Zarya with 27.83%.

By the end of drought period a significant increase was observed in the non-photochemical quenching (qN) of studied cultivars, thus denoting an increase in the energy dissipation through non-photochemical processes.

Recovery effects

Data in Table 3 show photosynthetic parameters after 3 day period of recovery. The lowered levels of all parameters under soil water deficit had a tendency to recover. The plants reached levels of α, Γ, and photosynthesis were similar to those in the control. SL was slightly reduced in studied cultivars. The photosynthetic parameters of cv. Zarya were recovered in greater extent.

Table 2. Parameters of chlorophyll fluorescence in leaves of control and drought stressed bean plants.

Variant	F_0	F_m	F_v/F_m	Y	qP	qN
Control						
Zarya	387±18	2014±82	0.808±0.039	0.539±0.031	0.732±0.038	0.493±0.025
Tangra	373±21	2010±79	0.814±0.041	0.522±0.029	0.751±0.036	0.474±0.031
Stressed						
Zarya	426±20 *	1716±64 *	0.752±0.037	0.389±0.023 *	0.594±0.029 *	0.732±0.041**
Tangra	448±25 *	1593±71 *	0.719±0.028 *	0.278±0.016 **	0.387±0.026 **	0.945±0.052***

* P<0.05; ** P<0.01; *** P<0.001

Table 3. Leaf gas exchange parameters in primary bean leaves after 3-day period of recovery. α, maximal carboxylation efficiency; Γ, CO_2 compensation point; A_{max}, net photosynthetic rate at saturating CO_2; Ac_a=370, net photosynthetic rate at 370 μmol mol^{-1} ambient CO_2 concentration; A_{Ci}=370, net photosynthetic rate at 370 μmol mol^{-1} intercellular CO_2 concentration; SL, stomatal limitation of photosynthesis.

	α (μmol m^{-2} s^{-1} mol^{-1})	Γ (μmol mol^{-1})	A_{max} (μmol m^{-2} s^{-1})	Ac_a=370 (μmol m^{-2} s^{-1})	A_{Ci}=370 (μmol m^{-2} s^{-1})	SL (%)
Control						
Zarya	0.117	41.5	21.9	13.78	17.89	23.0
Tangra	0.111	49.9	22.5	12.65	17.38	27.4
Recovery						
Zarya	0.097	58.2	18.3	12.53	15.30	18.1
Tangra	0.084	88.6	16.7	10.86	14.60	25.6

Table 4. Parameters of chlorophyll fluorescence in bean leaves after 3-day period of recovery.

Variant	F_0	F_m	F_v/F_m	Y	qP	qN
Control						
Zarya	411±19	1974±92	0.791±0.034	0.517±0.028	0.756±0.041	0.503±0.032
Tangra	402±21	2003±85	0.799±0.035	0.528±0.024	0.729±0.043	0.485±0.030
Recovery						
Zarya	437±22	1920±71	0.772±0.036	0.489±0.021	0.686±0.032	0.623±0.041*
Tangra	440±23	1825±91 *	0.759±0.032	0.476±0.023 *	0.673±0.029	0.682±0.044**

* P<0.05; ** P<0.01

Parameters of chlorophyll fluorescence are recovered to greater extent (Table 4). F_0 of stressed plants still remain higher than control plant, and F_m of primary leaf was decreased, being cv. Zarya recovered in a greater extent. F_v/F_m and qP show only tendency to decreasing, and Y was significantly lower in cv. Tangra. Only qN was significantly higher in leaves of studied cultivars.

Discussion

Soil drought and leaf water deficit lead to a permanent depression of photosynthtetic carbon assimilation (Yordanov et al., 2003; Chaves et al., 2009). Decreased photosynthetic rate is result of stomatal and non-stomatal (biochemical) limitations (Yordanov et al., 2000; Zlatev and Lidon, 2012).

There are many reports, which underline the stomatal limitation of photosynthesis as a primary event, which is then followed by the adequate changes of photosynthetic reactions (Chaves, 1991; Yordanov et al., 2000; Zlatev and Lidon, 2012). Today, there is a consensus that a decrease of photosynthesis due to water stress has been attributed to both stomatal and non-stomatal limitations (Shangguan et al., 1999). Non-stomatal limitation of photosynthesis has been attributed to reduced carboxylation efficiency (Jia and Gray, 2004), reduced ribulose-1,5-bisphospate (PuBP) regeneration (Tezara and Lawlor, 1995), reduced amount of functional Rubisco (Kanechi et al., 1995; Medrano et al., 2002), or to inhibited functional activity of PSII. Concomitantly inhibition or damages in the primary photochemical and biochemical processes may occur (Lawlor and Cornic, 2002; Subrahmanyam et al,. 2006). Since maximal CO_2 assimilation (A_{max}) reflex the result of those mesophyllic impairments, its determination under severe water stress allows us to evaluate non-stomatal limitations of photosynthesis and hence, the degree of drought tolerance of the photosynthetic machinery.

According to von Caemmerer and Farquhar (1981), the initial slope of the CO_2 curve is defined as the maximal carboxylation efficiency of Rubisco, whereas the rate of photosynthesis at high C_i reflects the capacity of the leaves to regenerate RuBP, which is connected with electron transport activity. In our study drought treatment led to a reduction of both Rubisco carboxylation activity and RuBP regeneration capacity, as indicated by the lowering of the initial slope and the plateau of saturation. This dependence is strongly expressed in leaves of cv. Tangra (Figure 1A). Thus, photosynthesis could be adjusted through a balance between Rubisco carboxylation capacity, RuBP utilization and its regeneration. It may be suggested that some of the reactions of Calvin cycle taking part in RuBP regeneration are inhibited. RuBP regeneration could be limited either by an inability to supply reductants and ATP from electron transport or by an inactivation or loss of Calvin cycle enzymes other than Rubisco (Baker et al., 1997; Nogués and Baker, 2000). The large depression in A_{max} occurring at the end of drought period was accompanied by such large changes in the relative quantum efficiency of electron flux through PSII-Y (Table 2). This suggests that decrease in the ability to regenerate RuBP can be attributed to a reduction in non-cyclic electron transport and the ability to produce ATP and reductants, as is the situation in sunflower where inhibition of RuBP regeneration induced by water stress has been attributed to decrease in ATP supply resulting from a loss of ATP synthase (Tezara et al., 1999). Decrease in α is likely to result from loss or inactivation of Rubisco (Allen et al., 1997).

Despite of significant stomatal limitation of photosynthesis in cv. Zarya determined by SL parameter (Table 1), this was not accompanied with reduction of C_i. One of the reasons for the slight decrease in C_i could be the increased mesophyllic resistance for CO_2 transport. Another reason could be the intensified respiratory processes that are implied by the enhanced value of the CO_2 compensation point. Our results are consistent with those presented by Stolf-Moreira et al. (2010), and

are connected also with a significant influence of non-stomatal factors. Restricted diffusion of CO_2 into the leaf might not be the only reason for decreased A_n under drought stress, because high external CO_2 concentrations (1500 μmol mol^{-1}) fail to restore A_n to values of control plant. Direct inhibition of biochemical processes by altered ionic or osmotic conditions, which affect, e.g. ATP synthase and Rubisco activity, might be another reason for decreased A_n under drought (Tezara et al., 1999; Haupt-Herting and Fock, 2000). The suggestion that biochemical factors are involved in the response of photosynthesis to drought stress is supported by the reduced rate of A_{max}, the occurrence of increasing CO_2 compensation points (Γ), and reduced α.

At the end of drought period the value of SL in the primary leaf of cv, Zarya is significantly higher than the control plants, suggesting enhanced stomatal limitation. In cv. Tangra SL show only slight tendency to increase; therefore, mainly non-stomatal factors determine the response of photosynthesis to drought.

The rise of F_0 under unfavorable environmental conditions is usually due to the reduced plastoquinone acceptor (Q_A^-), being unable to be oxidized completely because of retardation of the electron flow through PSII (Velikova et al., 1999), or to the separation of light-harvesting Chl a/b protein complexes of PSII from the PSII core complex (Komura et al., 2010). The decrease of F_m may be associated with processes related to a decrease in the activity of the water-splitting enzyme complex and perhaps a concomitant cyclic electron transport within or around PSII (Rochaix, 2011). Gilmore and Björkman (1995) have pointed out that increased non-radiative energy dissipation would be expected to be accompanied by a quenching of F_m.

In the present work the increase of F_0 and decrease of F_m under drought stress occurred concomitantly to significantly decrease in F_v/F_m (Table 2) in cv. Tangra. That seems to indicate, to some extent, the occurrence of chronic photoinhibition due to photoinactivation of PSII centers, possibly attributable to D1 protein damage (Campos, 1998). Photoinhibitory impact over PSII might be occurred in bean droughted leaves since, as previously noted by Verhoeven et al. (1997), a given light intensity (even at low PPFD) is potentially in greater excess under stress conditions, which usually limit photosynthetic activity.

In the studied cultivars the occurrence of photoinhibition was further highlighted by the significant decline of quantum yield of electron transport (Y), which is a measure of the total photochemical efficiency of PSII under photosynthetic steady-state conditions.

Cv. Tangra showed a greater decrease in the proportion of energy driven to the photosynthetic pathway (qP), what agrees with the most probable overreduction of the electron transport chain caused by the strong loss of PSI activity also, as shown in vigna plants (Campos, 1998).

Despite the decreases in the photochemical efficiency of PSII, cv. Zarya presented highest qP and Y, as well as the lowest energy dissipation (qN) values, what agrees with the higher photosynthetic capacity and carboxylation efficiency (Table 1). Similar effects on these Chl fluorescence parameters have been observed in different species and under various stress conditions. Velikova et al. (1999) established significant decrease in F_v/F_m, Y and qP in bean plants after simulated acid rain. Therefore, any factor that reduces the utilization of photosynthetic energy in carbon metabolism and affects high-energy-state-related qN, e.g. drought and water stress, will modify the rate of electron transport through PSII.

F_v/F_m reflects the maximal efficiency of excitation energy capture by "open" PSII reaction centers. A decrease in this parameter indicates down regulation of photosynthesis or photoinhibition (Hu et al., 2010). Primary leaves showed a slight decrease in this parameter (Table 2). This is the result of a large proportion of absorbed light energy not being used by the plants in the photosynthesis process, as shown by the increase in qN (Table 2). Photochemical quenching (qP) presented a similar behaviour to Y. This means that under our experimental conditions, Y is mainly dependent on the proportion of reaction centers which are photochemically "open" (expressed by qP), rather than on the efficiency with which an absorbed photon can reach a reaction centre.

High values of qP are related to the presence of Q_A in the oxidized state. In this situation, non-photochemical quenching (qN) values are low and, if light intensity increases to values close to light saturation, qN increases rapidly corresponding to high rates of energy dissipation (Plesnicar and Pancovic, 1991)

Decreases in Y are associated with increases in excitation energy quenching in the PSII antennae and are generally considered indicative of "down-regulation" of electron transport (Horton et al., 1996). Consequently, the decreases in Y exhibited during drought can be taken as indicative of a physiological regulation of electron transport by increasing excitation energy quenching process in

the PSII antennae. In leaves of the studied cultivars the capacity for CO_2 assimilation decreases significantly (Table 1). However, in the cv. Tangra Y decreases with 46.74% (Table 2). This suggests that a considerable greater rate of non-cyclic electron transport is occurring than is required to maintain CO_2 assimilatory. An alternative sink to CO_2 assimilation for electrons would be oxygen reduction by photorespiration and/or a Mehler reaction, although in drought bean leaves it has been shown that photorespiration does not act to protect the photosynthetic apparatus from photodamage (Nogués and Baker, 2000).

Decreases in qP are attributable to either decreases in the rate of consumption of reductants and ATP produced from non-cyclic electron transport relative to the rate of excitation of open PSII reaction centres or damage to PSII reaction centres. The large drought-induced decreases in qP in Tangra could to be due to a combination of both of these factors. The very large decreases in the gas exchange parameters that occur in young been plants under drought and relatively smaller decreases in F_v/F_m suggests that demand for reductants and ATP has decreased dramatically and this is a major factor in the closure of PSII reaction centres. The larges decreases in Y in leaves of Tangra indicating that either PSII reaction centres had been damaged or slowly relaxing quenching had been induced. Clearly, negligible photodamage to PSII occurs during drought in leaves of cv. Zarya since no significant changes are found in F_v/F_m. Consequently, the drought induced decreases in Y that occur in Zarya are attributable to "down-regulation" of electron transport. This study supports the contention that photodamage to PSII reaction centres is not a primary factor in the depression of CO_2 assimilation of the leaves induced by the water stress. However, photoinhibitory damage to PSII may be a secondary effect of drought in Tangra.

Photosynthetic parameters were almost recovered to the well-watered control level in both cultivars after 3 days of re-watering. Net photosynthetic rate of cv. Zarya returned near the well watered control level in greater extent than that for cv. Tangra, which could reflect the less severe damage in photosynthetic system during drought stress or less recovery required returning to non-stressed values.

At 3 days of re-watering, SL values of cv. Zarya decreased significantly below the levels at 10 days of drought, which may be the result of stomatal opening and resumption of metabolic activities from rehydration. Cv. Tangra had significantly higher SL than Zarya at 3 days of re-watering. The drought-induced decrease in photosynthetic capacity (A_{max},) was rapidly reversed following re-watering in cv. Zarya, indicating rapid recovery in Rubisco carboxylation and light saturated electron transport rates or less damages in both carbon fixation and light reaction of photosynthesis under drought stress in this cultivar. Our results are consistent with those presented by Hu et al. (2010) for *Poa pratensis* L. Decreased photochemical efficiency (F_v/F_m) after 10 days of drought and fast restoring to the control level also supporting the conception that the photosynthetic apparatus in leaves of cv. Zarya was maintained in a relatively high photoprotected state under drought stress and during recovery.

Conclusions

This study pointed out that drought produced significant increase of stomatal limitation in the primary leaf of cv. Zarya. This is accompanied by the decrease in all photosynthetic parameters and, consequently, stomatal closure would appear to be a more important factor contributing to the depressed CO_2 assimilation. In primary leaf of cv. Tangra non-stomatal limitations seem to assume a more important role in drought response. PSII activity in cv. Zarya was more efficiently protected than in cv. Tangra, as indicated by fluorescence measurements. After 3-days period of re-watering photosynthetic performances are recovered in greater extent in cv. Zarya.

In conclusion, cv. Zarya showed a higher drought tolerance in what concerns photosynthetic activity since F_v/F_m was maintained, Y and qP were significantly less affected than in the other genotype, and it presented a lower increase in qN. Photosynthetic apparatus in cv. Tangra can be considered as drought sensitive.

References

Ali, M., C. R. Jensen, V. O. Mogensen and A. Bahrun. 1999. Drought adaptation of field grown wheat in relation to soil physical conditions. Plant Soil 208:149-159.

Allen, D. J., I. F. Mc Kee, P. K. Farage and N. R. Baker. 1997. Analysis of the limitation to CO_2 assimilation to exposure of leaves of two *Brassica napus* cultivars to UV-B. Plant Cell Environ. 20:633-640.

Baker, N. R. and P. Horton. 1987. Chlorophyll fluorescence quenching during photoinhibition, In: D. J. Kyle, C. B. Osmond

and C. J. Arntzen (Eds.), pp. 85-94. Photoinhibition. Elsevier Scientific Publisher, Amsterdam.

Baker, N. R., S. Nogues and D. J. Allen. 1997. Photosynthesis and photoinhibition, In: P. J. Lumsden (Ed.). pp. 95-111. Plants and UV-B: responses to environmental change. Cambridge University Press, Cambridge.

Campos, P. S. 1998. Effects of water stress on photosynthetic performance and membrane integrity in *Vigna* spp. The role of membrane lipids in drought tolerance. Ph.D. dissertation, Universidade Nova de Lisboa, Lisboa.

Chakir, S. and M. Jensen. 1999. How does Lobaria pulmoria regulate Photosystem II during progressive desiccation and osmotic water stress? A chlorophyll fluorescence study at room temperature and at 77 K. Physiol. Plant. 105:257-265.

Chaves, M. M. 1991. Effects of water deficits on carbon assimilation. J. Exp. Bot. 42:1-16.

Chaves, M. M., J. Flexas and C. Pinheiro. 2009. Photosynthesis under drought and salt stress: regulation mechanisms from whole plant to cell. Ann. Bot. 103:551-560.

Chaves, M. M., J. P. Maroco and J. S. Pereira. 2003. Understanding plant responses to drought—from genes to the whole plant. Funct. Plant Biol. 30:239–264.

Cornic, G. 1994. Drought stress and high light effects on leaf photosynthesis, In: N. R. Baker and J. R. Boyer (Eds.), pp. 297-313. Photoinhibition of photosynthesis: from molecular mechanisms to the field. Bios Scientific Publishers, Oxford.

Cornic, G., J. Ghashghaie, B. Genty and J-. M. Briantais. 1992. Leaf photosynthesis is resistant to a mild drought stress. Photosynthetica 27:295-309.

Farquhar, G. D. and T. D. Sharkey. 1982. Stomatal conductance and photosynthesis. Annu. Rev. Plant Physiol. 33:317-345.

Flexas, J. and H. Medrano. 2002. Energy dissipation in C_3 plants under drought. Funct. Plant Biol. 29:1209–1215.

Gilmore, A. M. and O. Björkman. 1995. Temperature sensitive coupling and uncoupling of ATPase-mediated , nonradiative energy dissipation: Similarities between chloroplasts and leaves. Planta 197:646-654.

Genty, B., J. M. Briantais and N. R. Baker. 1989. The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. Biochim. Biophys. Acta 990:87-92.

Grassi, G. and F. Magnani. 2005. Stomatal, mesophyll conductance and biochemical limitations to photosynthesis as affected by drought and leaf ontogeny in ash and oak trees. Plant Cell Environ. 28:834–849.

Haupt-Herting, S. and H. P. Fock. 2000. Exchange of oxygen and its role in energy dissipation during drought stress in tomato plants. Physiol. Plant. 110:489-495.

Horton, P., A. V. Ruban and R. G. Walters. 1996. Regulation of light harvesting in green plants. Annu. Rev. Plant Physiol. Plant Mol. Biol. 4:655-684.

Hu, L., Z. Wang and B. Huang. 2010. Diffusion limitations and metabolic factors associated with inhibition and recovery of photosynthesis from drought stress in a C perennial grass species. Physiol. Plant. 139(1):93-106.

Jia, Y. and V. M. Gray. 2004. Interrelationships between nitrogen supply and photosynthetic parameters in *Vicia faba* L. Photosynthetica 41(4):605-610.

Kanechi, M., E. Kunitomo, N. Inagaki and S. Maekawa. 1995. Water stress effects on ribulose-1,5-bisphosphate carboxylase and its relationship to photosynthesis in sunflower leaves,. In: M. Mathis (Ed.). Vol. IV, pp. 597-600. Photosynthesis: from light to biosphere. Kluwer Academic Publisher, Dordrecht, London.

Komura, M., A. Yamagishi, Y. Shibata, I. Iwasaki and S. Itoh. 2010. Mechanism of strong quenching of photosystem II chlorophyll fluorescence under drought stress in a lichen, *Physciella melanchla*, studied by subpicosecond fluorescence spectroscopy. Biochim. Biophys. Acta 1797:331-338.

Lawlor, D. W. 1995. Effects of water deficit on photosynthesis, In: N. Smirnoff (Ed.). pp. 129-160. Environment and Plant Metabolism. Bios Scientific Publishers Ltd., Oxford.

Lawlor, D. W. and G. Cornic. 2002. Photosynthetic carbon assimilation and associated metabolism

in relation to water deficits in higher plants. Plant Cell Environ. 25:275–294.

Lu, C. and J. Zhang. 1999. Effects of waters stress on Photosystem II photochemistry and its thermostability in wheat plants. J. Exp. Bot. 50 (336):1199-1206.

Medrano, H., J.M. Escalona, J. Bota, J. Gulias and J. Flexas. 2002. Regulation of photosynthesis of C3 plants in response to progressive drought: stomatal conductance as a reference parameter. Ann. Bot. 89(7):895-905.

Nacheva, L., Z. Zlatev, K. Ivanova and P. Manolov. 2002. Possibilities for application of photoautotrophy in micropropagation of dzhanka 4 (*Prunus Cerasifera* Ehrt.) rootstock. Acta Hort. 577:199-205.

Nogués, S. and N. R. Baker. 2000. Effects of drought on photosynthesis in Mediterranean plants grown under enhanced UV-B radiation. J. Exp. Bot. 51(348):1309-1317.

Plesnicar, M. and D. Pancovic. 1991. Relationship between chlorophyll fluorescence and photosynthetic O_2 evolution in several *Helianthus* speciec. Plant Physiol. Biochem. 29:681-688.

Rivero, R. M., J. Gimeno, A. van Deynze, H. Walia and E. Blumwald. 2010. Enhanced cytokinin synthesis in tobacco plants expressing PSARK::IPT prevents the degradation of photosynthetic protein complexes during drought. Plant Cell Physiol. 51(11):1929-1941.

Rochaix, J. -D. 2011. Regulation of photosynthetic electron transport. Biochim. Biophys. Acta 1807:375-383.

Schreiber, U., U. Schliwa and W. Bilger. 1986. Continuous recording of photochemical and non-photochemical chlorophyll fluorescence with a new type of modulation fluorometer. Photosynth. Res. 10:51-62.

Shangguan, Z., M. Shao and J. Dyckmans. 1999. Interaction of osmotic adjustment and photosynthesis in winter wheat under soil drought. J. Plant Physiol. 154:753-758.

Skotnica, J., M. Matouškova, J. Nauš, D. Lazár and L. Dvořák. 2000. Thermoluminescence and fluorescence study of changes in Photosystem II photochemistry in desiccating barley leaves. Photosynth. Res. 65:29–40.

Souza, R. P., E. C. Machado, J. A. B. Silva, A. M. M. A. Lagôa and J. A. G. Silveira. 2004. Photosynthetic gas exchange, chlorophyll fluorescence and some associated metabolic changes in cowpea (*Vigna unguiculata*) during water stress and recovery. Env. Exp. Bot. 51:45–56.

Stolf-Moreira, R., M. E. Medri, N. Neumaier, N. G. Lemos, J. A. Pimenta, S. Tobita, R. L. Brogin, F. C. M. Guimarães, M. C. N. Oliveira, J. R. B. Farias, R. V. Abdelnoor and A. L. Nepomuceno. 2010. Soybean physiology and gene expression during drought. Gen. Mol. Res. 9(4):1946-1956.

Subrahmanyam, D., N. Subash, A. Haris, and A. K. Sikk. 2006. Influence of water stress on leaf photosynthetic characteristics in wheat cultivars differing in their susceptibility to drought. Photosynthetica 44(1):125-129.

Tezara, W. and D. W. Lawlor. 1995. Effects of water stress on the biochemistry and physiology of photosynthesis in sunflower, In: M. Mathis (Ed.), Vol. IV, pp. 625-628. Photosynthesis: from light to biosphere. Kluwer Academic Publisher, Dordrecht, London.

Tezara, W., V. J. Mitchell, S. D. Driscoll and D. W. Lawlor. 1999. Water stress inhibits plant photosynthesis by decreasing coupling factor and ATP. Nature 401:914-917.

Van Kooten, O. and J. F. H. Snel. 1990. The use of chlorophyll fluorescence nomenclature in plant stress physiology. Photosynt. Res. 25:147-150.

Verhoeven, A. S., B. Demmig-Adams and B. B. Adams. 1997. Enhanced employment of the xanthophylls cycle and thermal energy dissipation in spinach exposed to high light and N stress. Plant Physiol. 113:817-824.

Von Caemmerer, S. and G. D. Farquhar. 1981. Some relationships between the biochemistry of photosynthesis and the gas exchange of leaves. Planta 153:376-387.

Velikova, V., T. Tsonev and I. Yordanov. 1999. Light and CO_2 responses of photosynthesis and chlorophyll fluorescence characteristics in bean plants after simulated acid rain. Physiol. Plant. 107:77-83.

Wang, W., B. Vinocur and A. Altman. 2003. Plant responses to drought, salinity and extreme temperatures: towards genetic engineering for stress tolerance. Planta 218:1–14.

Yordanov, I, V. Velikova and T. Tsonev. 2000. Plant responses to drought, acclimation, and stress tolerance. Photosynthetica 38(1):171-186.

Yordanov, I., V. Velikova and T. Tsonev. 2003. Plant responses to drought and stress tolerance. Bulg. J. Plant Physiol. Sp. Issue 187–206.

Zlatev, Z., A. Vassilev, V. Goltsev and G. Popov. 2010. Drought-induced changes in chlorophyll fluorescence of young bean plants. Agr. Sci. 2(4):75-79.

Zlatev, Z. and F. C. Lidon. 2012. An overview on drought induced changes in plant growth, water relations and photosynthesis. Emir. J. Food Agric. 24(1):57-72.

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Alhadrami, G. A. 1991. Effect of preservatives and maturity on the nutritional value of alfalfa hay for lactating dairy cows. Ph.D. dissertation, University of Arizona, Tucson.

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