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An overview on drought induced changes in plant growth, water relations and photosynthesis

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Abstract

At a whole plant level the effects of drought is usually perceived as a decrease in growth and photosynthetic carbon assimilation. That is why this review is focused mainly on recent information about the effects of drought on plant growth, water relations and photosynthesis, as well as mechanisms of adaptation. It is shown that plants have evolved a great number of adaptive mechanisms that allow the biochemical systems to cope with increased water deficit. The literature analyzed in this review shows the complexity of tolerance to water deficit and supports the statements of many authors that the flexibility of cell metabolism and its fast acclimation to changes in environmental conditions is a first essential step in stress avoidance.

Key words: Drought, Growth, Osmotic adjustment, Photosynthesis, Plant water relations

Introduction

Under both natural and agricultural conditions plants are often exposed to various environmental stresses. Drought is one of most important environmental factors inhibiting photosynthesis and decreasing growth and productivity of plants. It is one of the major causes of crop loss worldwide, reducing average yields for most major crop plants by more than 50% (Bray et al., 2000; Wang et al., 2003). Under these stress conditions usually a water deficit in plant tissues develops. In the last years effects of water deficit were studied on different levels from ecophysiology to cell metabolism (Shinozaki and Yamaguchi-Shinogzaki, 1997; Dekov et al., 2000; Chaves et al., 2003).

The high level of organization of living organisms, including the plants, presupposes the presence of complex and multiple relations with the environment. The influence of the environment on the plant organism is determined both by the strength and duration of the corresponding factor and by the interaction between the factor and the genetic peculiarities of the plant. For each of the numerous physiological processes, constituting the live system, there always exists the so-called "stability limit". Each deviation of the environmental factors out of this stability limit of the live system results in stress, which to a different degree disturbs its structure and functional activity. The range and importance of these effects depend on the genetically determined plant capacity and sensitivity, as well as on the intensity and duration of the stress, when applied alone or in combination (Bhadula et al., 1998; Chaves et al., 2009). The acclimation capacity of the plant organisms depends on the presence of a certain buffer property, i.e. a given norm of reaction towards concrete external conditions. This means, that the higher acclimation capacity, and hence the greater resistance to a given stress factor, is determined by the plant capacity to maintain its physiological processes within the reaction norm, at a greater variation of this factor (Nilsen and Orcutt, 1996; Valladares et al., 2007).

Levitt (1982) defines stress as "any environmental factor capable of inducing a potentially injurious strain in living organisms". A biological strain is any change of physiological processes and functional activity (a shift in metabolism) of the plants subjected to stress. Larcher (1980) defines stress as "a state in which increasing demands made up to an initial destabilization of functions, followed by normalization and improved resistance. Stress contains both destructive and constructive elements and is a selection factor as well as a driving force for improved resistance and adaptive evolution".

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Lichtenthaler (1996) extended the Larcher's stress concept of plants by differentiating between eu-stress and dis-stress. Eu-stress is an activating, stimulating stress and a positive element for plant development. Dis-stress is a severe and real stress that causes damage, and thus negatively affects the plants and its development. Repair processes and adaptation not only lead to a restitution of the previous physiological functions, but also to hardening of plants by establishing a new physiological standard, which is an optimum stage of physiology under the changed environmental conditions.

Effects on plant growth

Drought is a multidimensional stress affecting plants at various levels of their organization (Yordanov et al., 2000; Wentworth et al., 2006). The dehydration process during drought is characterized by fundamental changes in water relations, biochemical and physiological processes, membrane structure, and ultrastructure of subcellular organelles (Tuba et al., 1996; Sarafis, 1998; Yordanov et al., 2003). The response to drought at the whole plant and crop levels is complex because it reflects the integration of stress effects and responses at all underlying levels of organization over space and time (Blum, 1996).

For agricultural crops in dry environments, both a high potential growth rate and efficient use of available water are desirable traits. The rates of growth and of water use are both influenced by the allocation of biomass to the different organs and by the physiological and morphological properties of these organs. The influence of physiological traits on water use efficiency depends on the balance between the effects on growth and on water use. Plant traits that increase water use efficiency may conflict with those that promote growth rate. Water use efficiency of individual plants is influenced by the relative growth rate (RGR_{pl}) and the rate of transpiration of the plant (T_{pl}). The RGR_{pl} can be divided into a morphological component, the leaf area ratio (LAR) and a physiological component, the net assimilation rate (NAR) (Van den Boogaard et al., 1997).

The allocation pattern that maximizes growth or water use efficiency depends on the availability of water. Under conditions of mild water deficit the relative allocation of biomass to roots usually increases (Hamblin et al., 1991; Gorai et al., 2010). The benefit of higher allocation of biomass to the roots is an increased capacity for water uptake. The costs of a larger root system are the costs of construction (possibly at the expense of construction of photosynthetic tissue) and the increased respiratory losses associated with its maintenance. So it can be hypothesized, that greater allocation of biomass to roots is associated with benefits in terms of water uptake capacity and with costs in terms of carbon.

Under favourable conditions for growth, the advantage of a high leaf area ratio is a greater photosynthesizing area and hence a higher growth rate. Interspecific variation in relative growth rate has been found to be mainly due to differences in LAR (Poorter and Remkes, 1990). A higher rate of transpiration per plant use may be a drawback of a large leaf area, when the availability of water is limited. Thus, it is likely that a higher allocation of biomass to leaf area is beneficial in terms of growth, but associated with higher water loss.

Not only the pattern of biomass allocation, but also differences in the rates of uptake and loss of carbon and water of the different plant organs will contribute to variation in growth and water use efficiency. For instance, a plant with a low leaf area but a high rate of photosynthesis may assimilate as much as a plant with a high leaf area and a low rate of photosynthesis. Likewise, rates of transpiration and water uptake need to be considered to understand variation of water use efficiency.

Soil drought inhibits plant growth and development. Ahmad et al. (2007) established dry matter reduction in wheat under water deficiency stress. Boutraa and Sanders (2001) established that mild water deficit inhibited RGRpl with 25%. The main reasons are changes in NAR and in photosynthetic rate. Changes in LAR are insignificant. The same changes are established by Poorter and Remkes (1990) for 24 wild species, Van den Boogard et al. (1997) for ten wheat cultivars, Lutts et al. (2004) for durum wheat and Berova and Zlatev (2002) for young bean plants.

Ramos et al. (1999) established that water deficit inhibits accumulation in fresh plant mass in greater extent than dry biomass. Relatively lower influence of drought on dry biomass than on fresh mass indicates a presence of disturbances in water relations. This is in accordance of results of Konings (1989) and Augé et al. (2001) for cowpea and common bean plants. Lazcano-Ferrat and Lovatt (1999) established decreasing of 14-27% in dry biomass in young bean plants subjected to drought and significantly increasing in ratio dry mass/fresh mass (DM/FM). It is considered that increased ratio DM/FM is a stress parameter at plant level (Baker, 1993; Augé et al., 2001).

Changes in plant water relations

Drought is one of the most important constrains for crop production but improvement of drought tolerance is very difficult because of the set of mechanisms involved. Among them, however, osmotic adjustment could play a primary role (Turner, 1986; Ludlow and Muchow, 1990).

Crop plants have developed many mechanisms to survive water deficit, including escape, tolerance, and avoidance of tissue and cell dehydration (Turner, 1986). Avoidance of stress includes rapid phenological development, increased stomatal and cuticular resistance, changes in leaf area, orientation and anatomy, among others (Morgan, 1984; Jones and Corlett, 1992). Plants tolerate drought by maintaining sufficient cell turgor to allow metabolism to continue under increasing water deficits. Tolerance to stress involves at least two mechanisms, osmotic adjustment and changes in the elastic properties of tissues (Munns, 1988; Savé et al., 1993).

Osmotic adjustment is generally thought to be the major mechanism to maintain cell turgor in many species as the water potential decreases, enabling water uptake and the maintenance of plant metabolic activity and therefore growth and productivity (Shackel et al., 1982; Parker and Pallardy, 1987; Gunasekera and Berkowitz, 1992; Martiınez et al., 2004). Osmotic adjustment (OA) is recognized as an effective component of drought resistance in several crop plants (Ludlow and Muchow, 1990; Kramer and Boyer, 1995; Martiınez et al., 2007). OA involves the net accumulation of solutes in a cell in response to a fall in the water potential of the cell's environment. As a consequence of this net accumulation, the osmotic potential of the cell is lowered, which in turn attracts water into the cell and tends to maintain turgor pressure. In fact, constitutive accumulation (by overexpression of the responsible gene) of a cellular osmolytes is regarded as a serious approach to increasing crop drought resistance by genetic engineering (Bohnert et al., 1995). Lowering of the osmotic potential of the cells accumulating solutes is considered to be due to real OA if the buildup of compounds is not merely the result of fast tissue dehydration (Bray, 1997).

Generally, OA contributes to turgor maintenance of both shoots and roots as plants experience water deficit. This allows turgor-dependent processes such as growth and stomatal activity to continue to progressively lower leaf water potential. The accumulated compatible solutes may also protect specific cellular functions, irrespective of turgor (Shen et al., 1997). Substantial genotype diversity for OA was observed in bread wheat (*Triticum aestivum* L.) (Morgan, 1995), sorghum (*Sorghum* spp.) (Basnayake et al., 1993; Tangpremsri et al., 1995), chickpea (*Cicer arietinum* L.) (Morgan et al., 1991), field pea (*Pisum sativum* L.) (Rodrigues-Maribona et al., 1992), black spruce [*Picea mariana* (Mill.) B.S.P.] (Tan and Blake, 1997), sunflower (*Helianthus annuus* L.) (Jamaux et al., 1997), common bean (*Phaseolus vulgaris* L.) (Zlatev, 2005) and various turfgrasses (Qian and Fry, 1997). The range of variation for osmotic adjustment in the plants subjected to water deficit was found to be about 0.5 to 0.6 MPa (Jamaux et al., 1997; Babu et al., 1999; Zlatev, 2005). Greater variation may be expected if a broader genetic base is examined.

Several reports suggest that plant metabolic processes are in fact more sensitive to turgor and cell volume than to absolute water potential (Jones and Corlett, 1992; Martiinez et al., 2007). Among the physiological mechanisms that act to maintain leaf turgor pressure, decreased osmotic potential resulting either from a decrease in osmotic water fraction or from an osmotic adjustment (net accumulation of solutes in the symplast) was pointed out (Jones and Turner, 1980). Changes in tissue elasticity in response to drought, which modify the relationship between turgor pressure and cell volume, might contribute to drought tolerance, as observed in black spruce (Blake et al., 1991), sunflower (Maury et al., 2000) and common bean (Zlatev, 2005; Martiınez et al., 2007). Leaf water relations data may provide a useful indication of the capacity of species to maintain functional activity under drought (White et al., 2000).

The analysis of pressure-volume (PV) curve data showed an active OA in bean leaves, in response to water stress imposed slowly, at a rate of about 0.15 MPa day^{-1} (Zlatev, 2005).

The capacity to maintain high relative water content (RWC) values under drought was observed in drought tolerant bean cultivars (Zlatev, 2005) and in *Astragalus gombiformis* Pom. and *Medicago sativa* L. (Gorai et al., 2010). For bean plants this could be explained by their capacity to accumulate great quantities of proline and other osmotic active compounds which participate in the reduction of Ψo and in osmotic adjustment (Zlatev, 2005). Stressed plants exhibited a higher value of the maximum leaf bulk elastic modulus as compared with well-watered plants both for primary and first

trifoliate leaves. However, this change does not appear to be a function of an alteration in wall structure, translated in the increase of the cell wall rigidity. Rather, it is a consequence of the lower solute potentials at full turgor which lead to greater maximum turgor potential. The relation between Ψo decrease and OA has already been observed by Teulat et al. (1997) in barley and durum wheat, by Rodrigues et al. (1993) in grapevine, and by Saeed Rauf and Sadaqat (2008) in sunflower.

Turgor loss point in the stressed leaves was reached at lower Ψ_w than in well-watered leaves. This indicates that they have an increased capacity to maintain turgor at lower water potentials. That parameter was higher in well-watered plants than in the stressed plants, in spite of the higher ε_{vmax} of the latter. These results are in accordance with the data obtained in grasses by Wilson et al. (1980) and in grapevine by the Rodrigues et al. (1993).

Variability for proline metabolism has been reported in various crop species, but it is not well known whether accumulation of this imino acid contributes to the susceptible or tolerant nature of the genotypes (Hanson, 1980; Iannucci et al., 2000). Naidu et al. (1992) and Iannucci et al. (2000) reported that proline levels were more closely related to the decrease in RWC than inΨ_w. Navari-Izzo et al. (1990) proposed that the metabolic differences among cultivars may reflect differences in water status achieved, rather than metabolic differences at a given water status. As indicated by Irigoyen et al. (1992) and Iannucci et al. (2000), such a relationship between turgor and proline accumulation could be useful as a possible drought-injury sensor.

Effects on photosynthesis

At a whole-plant level, soil drought and leaf water deficit lead to a progressive suppression of photosynthtesis, and is associated with alterations in carbon and nitrogen assimilation (Chaves, 1991; Mwanamwenge et al., 1999; Yordanov et al., 2000). Decreased photosynthetic rate is result of stomatal and non-stomatal (biochemical) limitations (Wise et al., 1992; Yordanov et al., 2003).

The ability to maintain the functionality of the photosynthetic machinery under water stress, 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, 1991, Flexas et al., 2006). The decrease in net photosynthetic rate (A_n) under drought stress observed in many studies is often explained by a lowered internal CO_2 concentration (C_i) that results in a limitation of photosynthesis at the acceptor site of ribulose-1,5-bisphospate carboxylase/oxygenase (Rubisco) (Cornic et al., 1992) or by the direct inhibition of photosynthetic enzymes like Rubisco (Haupt-Herting and Fock, 2000) or ATP synthase (Tezara et al., 1999; 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 PSII is inhibited (Chen and Hsu 1995; Chakir and Jensen, 1999). 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; He et al., 1995). The mechanism by which the water deficit inhibits this electron transport is still unclear.

However, many other studies have shown that the decreased photosynthesis under water stress can be attributed to the perturbations of the biochemical processes (Lauer and Boyer, 1992). There are several reports that underline the stomatal limitation of photosynthesis as a primary event, which is then followed by the adequate changes of photosynthetic reactions (Chaves, 1991; Zlatev and Yordanov, 2004). 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), or to inhibited functional activity of PSII. Concomitantly inhibition or damages in the primary photochemical and biochemical processes may occur (Lawlor, 2002). 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.

Drought reduces gas exchange, the maximal carboxylation efficiency and increases the CO_2 compensation point of young been plants. This treatment also changes the shape of CO_2 curves of photosynthesis (Zlatev and Yordanov, 2004). As compared with control plants, plants submitted to drought exhibited a noticeable decrease in both the

initial slope and plateau of these curves. 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. Drought treatment led also 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. According to Lawlor and Cornic (2002) decreased A_{max} under low RWC is caused by impaired metabolism (storage of ATP, limiting RuBP synthesis without or less inhibition of photosynthetic enzymes including Rubisco). 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 depressions in A_{max} occurring at the end of drought period were accompanied by such large changes in the relative quantum efficiency of electron flux through PSII (α). 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).

Zlatev and Yordanov (2004) established that despite of significant stomatal limitation of photosynthesis in young bean plants, this was not accompanied with reduction of C_i. In fact, there was a slight increase (10 – 14%) in C_i at C_a=350 μmol mol^{-1} in primary and first trifoliate leaves of the genotypes studied. One of the reasons for the slight increase 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. 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; and reduced α.

Both stomatal and non-stomatal factors contribute to a decreased photosynthetic rate, but their proportion changes significantly. The drought tolerance species control stomatal function to allow some carbon fixation at stress, thus improving water use efficiency or open stomata rapidly when water deficit is relieved (Lawlor, 2002). Stomatal conductance is more closely linked to soil moisture content than to leaf water status (Davies and Zhang, 1991). At the end of drought period the values of stomatal limitations are higher than the control plants, suggesting enhanced stomatal limitation.

As Baker and Horton (1987) mentioned, two distinct phenomena at least, are involved in producing the changes in the fluorescence parameters under unfavorable environmental conditions. One phenomenon results in an increase in minimal fluorescence level from dark-adapted leaves (F_0), possibly due to the reduced plastoquinone acceptor (Q_A^-), being unable to be oxidized completely because of retardation of the electron flow through PSII (Krause and Weis, 1991; Velikova et al., 1999), or to the separation of light-harvesting Chl a/b protein complexes of PSII from the PSII core complex (Cona et al., 1995). The second is responsible for the quenching both variable fluorescence level from dark-adapted leaves (F_v) and maximal fluorescence level from dark-adapted leaves (F_m). Preferential quenching of F_v would indicate more extensive damage to the reaction centers, such that charge recombination is prevented. The drop 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 (Aro et al., 1993). 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.

Table 1. Parameters of chlorophyll fluorescence in leaves of control and drought stressed bean plants (Zlatev and Yordanov, 2004).

Genotype	Variant	F0	Fm	Fv/Fm	Y	qP	qN
Control							
Plovdiv 10	Primary leaf	425±16	2083±82	0.796±0.028	0.485±0.021	0.773±0.031	0.573±0.028
	I trifoliate leaf	361±13	1900±77	0.810±0.031	0.514±0.026	0.811±0.039	0.569±0.027
Dobrudjanski ran	Primary leaf	484±19	2343±79	0.793±0.026	0.424±0.020	0.742±0.032	0.644±0.034
	I trifoliate leaf	385±13	2047±70	0.812±0.033	0.497±0.023	0.801±0.041	0.681±0.036
Prelom	Primary leaf	407±18	2157±74	0.811±0.035	0.491±0.028	0.788±0.035	0.572±0.032
	I trifoliate leaf	382±13	1900±66	0.799±0.029	0.534±0.031	0.816±0.043	0.546±0.027
Drought treated Plovdiv 10	Primary leaf	484±19*	1820±64*	0.734±0.025	0.262±0.013 ***	0.495±0.026 ***	0.802±0.042* **
	I trifoliate leaf	398±15	1780±74	0.776±0.027	0.324±0.017 ***	0.584±0.037 **	0.745±0.038* *
Dobrudjanski ran	Primary leaf	570±24*	1915±71*	0.702±0.021 *	0.107±0.011 ***	0.356±0.022 ***	0.969±0.051* **
	I trifoliate leaf	433±15*	1721±58*	0.748±0.024	0.204±0.014 ***	0.457±0.028 ***	0.984±0.053* **
Prelom	Primary leaf	451±19	1914±68*	0.765±0.023	0.397±0.019 *	0.559±0.036 **	0.670±0.041
	I trifoliate leaf	403±14	1850±67	0.782±0.028	0.465±0.024 *	0.668±0.039 *	0.607±0.033

* P<0.05; ** P<0.01; *** P<0.001

In the conditions of severe stress the increase of F_0 and decrease of F_m under drought occurred concomitantly to smaller decrease in F_v/F_m in the studied genotypes of bean plants (Zlatev and Yordanov, 2004, Table 1). That seems to indicate, to some extent, the occurrence of chronic photoinhibition due to photoinactivation of PSII centers, possibly attributable to D1 protein damage (Rintamäki et al., 1994; 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. Indeed, during illumination of *Zea mays* wilted leaves, a strong inhibition of PSII efficiency was observed even under moderate PPFD (Saccardy et al., 1998). Low relative leaf water content clearly predisposes the leaves to photoinhibitory damage (Björkman and Powles, 1984), and the inhibition of photosynthetic activity could in fact reflect an inactivation of PSII activity and the concomitant uncoupling of non-cyclic photophosphorylation, as shown in soybean (Younis et al., 1979) and *Nerium oleander* (Björkman and Powles, 1984).

The occurrence of photoinhibition is 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. Droughted plants 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 (Campos, 1998) and bean (Zlatev and Yordanov, 2004, Table 1) plants. These decreases may be due to a direct dehydration effect on Rubisco (Kaiser, 1987), reflecting an increase in Rubisco hydrolysis, since the amount of Rubisco largely determines photosynthesis (Evans, 1989), and/or a decline in its catalytic ability. In fact, changes in the ATP pool size (Seeman, 1989), or the tight binding of inhibitors and failure of the Rubisco activase to operate in stressed leaves (Lawlor, 2002) will decrease enzyme affinity for the substrate, and hence, influence its activity.

Similar effects on these Chl fluorescence parameters have been observed in different species and under various stress conditions. Vassilev and Manolov (1999) demonstrated a significant decrease of Y and photochemical quenching (qP) accompanied by an increase of non-photochemical quenching (qN) in cadmium treated plants. 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 (Öquist et al., 1992; Cechin et al., 2006). 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. qP presented a similar behaviour to Y. This means that under drought 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, qN values are low and, when 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 in all the species can be taken as indicative of a physiological regulation of electron transport by increasing excitation energy quenching process in the PSII antennae. At the other hand, lower decrease in Y 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 droughted bean leaves it has been shown that photorespiration does not act to protect the photosynthetic apparatus from photodamage (Brestic et al., 1995; 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 larger drought-induced decreases in qP 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 Dobrudjanski ran indicate 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, since no significant changes are found in F_v/F_m. Consequently, the drought induced decreases in Y that occur in these plants 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. According with the statement of Baker and Horton (1987), the bulk of quenching in the stressed leaves is due to reversible qN processes, since Q_A was maintained in a highly reduced state throughout the quenching.

Literature analyzed pointed out that drought produced large increases in stomatal limitation, which is the first step to cope with drought. The large increases in stomatal limitation accompanied the decreases in all photosynthetic parameters and, consequently, stomatal closure would appear to be a more important factor contributing to the depressed CO_2 assimilation. In conclusion, plants with maintained Fv/Fm, Y and qP significantly less affected, and with a lower increase in qN can be considered as drought tolerant in what concerns photosynthetic activity.

Oxidative stress and antioxidative defense system

Drought affects not only water relations, but also induces stomatal closure and decreases the photosynthetic rate and growth. Closure of stomata decreases CO_2 concentration in leaf mesophyll tissue and results in an accumulation of NADPH. Under such conditions, where NADP is a limiting factor, oxygen acts as an alternate acceptor of electrons from the thylakoid electron transport chain, resulting in the formation of superoxide radical ($O_2^{\bullet}$) (Cadenas, 1989). Superoxide radical and its reduction product H_2O_2 are potentially toxic compounds, and can also combine by the Haber-Weiss reaction to form the highly toxic hydroxyl radical (OH^-) (Sairam et al., 1998).

Under optimal conditions leaves are rich in antioxidant enzymes and metabolites and can cope with reactive oxygen species (ROS), thus minimizing oxidative damage. A large number of reports deal with the deleterious effects of ROS, which production is stimulated under water stress conditions (Malenčić et al., 2000, Blokhina et al., 2003, Foyer and Noctor, 2005). ROS cause lipid peroxidation and consequently membrane injuries, protein degradation, enzyme inactivation (Sairam et al., 2005), thus induce oxidative stress. Tolerant genotypes, therefore, should not only be able to retain sufficient water under drought, but should

also have a highly active system to protect against oxidative injury. Plants possess several tissue antioxidant enzymes for protection against ROS, such as superoxide dismutase (SOD, EC 1.15.1.1), ascorbate peroxidase (APOX, EC 1.11.1.11), guajkol peroxidase (GPOX, EC 1.11.1.7), glutathione reductase (GR, EC 1.6.4.2) and catalase (CAT). These enzymes either quench toxic compounds or regenerate antioxidants with the help or reducing power provided by the photosynthesis (Zacchini et al., 2003). During drought conditions high activities of antioxidant enzymes are associated with lower levels of lipid peroxidation, being connected to drought tolerance (Bowler et al., 1992). In fact, an increased metabolic capacity of these enzymes may be part of a general antioxidative system in plants involving regulation of protein synthesis or gene expression (Foyer et al., 1994, Scandalios et al., 1997). Low-molecular weight antioxidants are presented by carotenoids, tocopherols, glutathione and ascorbic acid. Apart their obvious role as enzyme substrates, they can react chemically with almost all forms of ROS. Among substances able to protect plant cell from oxidative attack, a specific role of polyamines in preventing photooxidative damages is reported (Tadolini, 1988; Løvaas, 1997).

Genotypes of the same species respond differentially to environmental stresses and oxidative injury, as a result of genetic based differences in their antioxidant systems. That provides us with an important tool to have an insight into the physiological mechanisms operative in stress tolerant genotypes (Sairam et al., 1998). According to Foyer et al. (1997) much of the injuries caused by exposure to biotic and abiotic stresses are associated with oxidative damage at a cellular level, the chloroplasts being an important site of H_2O_2 generation.

Zlatev et al. (2005) established that, at the end of drought period, an increased H_2O_2, and $OH^{\bullet}$ production was observed in young bean plants, therefore revealing a state of oxidative stress in cells (Table 2). H2O2 is a strong oxidant produced mainly as a result of scavenging of superoxide radical, and its higher concentration is injurious to cells, resulting in a localized oxidative damage, lipid peroxidation, and disruption of metabolic function and losses of cellular integrity at sites where it accumulates (Menconi et al., 1995; Velikova et al., 2000). It is well known that H_2O_2, similar to glutathione, has multi-functional interactive roles in the early stages of plant stress response. H_2O_2 can diffuse to relatively long distances, causing changes in the redox status of surrounding cells and tissues where, at relatively low concentrations, may trigger an antioxidative response (Foyer et al., 1997). Rather than just the scavenging capacity, a fine-tuning of H_2O_2 levels is essential for an efficient control. The rationale of this assmption is that H_2O_2, whilst deleterious to some cellular components, is essential to plants in various biosynthetic reactions and, as suggested by some authors, possibly also in signal transduction pathways, which could contribute to plant defense (Schreck and Baeuerle, 1991). In fact, the drought induced production of H_2O_2 in the mesophyll cells may be associated with changes in the cell wall structure (Scandalios et al., 1997). Furthermore, H_2O_2 is necessary for the peroxidase-mediated oxidative polymerization of cynnamil alcohols to form lignin, and several enzymatic systems have been proposed as responsible for hydrogen peroxide production, on the surface of plant cells (Lütje et al., 2000). It may be therefore suggested that the increased level of H_2O_2 observed by many authors in the drought treated plants is due to oxidative damages, but eventually may also have a signal function.

H_2O_2, $OH^{\bullet}$ and other ROS can be expected to be responsible for the lipid peroxidation (Douglas, 1996). The increase of MDA content indicates that the bulk oxidative lipid metabolism in leaves was enhanced by drought, suggesting a relationship between drought and oxidative stress (Munné-Bosch et al., 2001). A decrease in membrane stability reflects the extent of lipid peroxidation caused by reactive oxygen species. Premachandra et al. (1990) has reported that cell membrane stability is an indicator of drought tolerance. Lower LPO and higher membrane stability (lower electrolyte leakage) has also been reported in drought tolerant genotypes of maize (Pastori and Trippi, 1992) and wheat (Sairam et al., 1998).

Du and Klessig (1997) proposed that catalase may be inactivated by binding to salicylic acid or to other cellular components, but the relevancy of these data towards physiological conditions is difficult to assess. Increased APOX and CAT activity in drought tolerant genotypes of pea (Gillham and Dodge, 1987), tomato (Walker and McKersie, 1993), *Sorghum* (Jagtap and Bhargava, 1995) and bean (Zlatev et al., 2005, Table 3) have also been reported. The results are in accordance with other authors reporting similar patterns of APOX and CAT activities in different stress situations, such as As toxicity (Stoeva et al., 2003), and acid rain stress (Velikova et al., 2000).

Table 2. Content of H_2O_2 [μmol g^{-1} (f.m.)], OH• [mmol g^{-1} (f.m.)] and changes in lipid peroxidation [nmol (MDA) g^{-1} (d.m.)] and electrolyte leakage, expressed as injury index I [%], in the leaves of three bean (*Phaseolus vulgaris* L.) cultivars (Plovdiv 10, Dobrudjanski ran and Prelom) submitted to drought. Means ± SE, n=5. Different letters express significantly different results between control and drought stressed plants in the same genotype (a, b) or between cultivars within each treatment (r, s, t) (Zlatev et al., 2005).

Cultivar	Treatment	H_2O_2	OH•	MDA	I
Plovdiv 10	Control	4.23±0.21 a/r	0.135±0.011 b/r	114±8.5 b/r	
	Drought	4.65±0.24 a/s	0.208±0.013 a/t	169±9.4 a/s	28±1.8 s
Dobrudjanski ran	Control	4.46±0.19 b/r	0.143±0.009 b/r	147±9.6 b/r	
	Drought	5.91±0.27 a/r	0.483±0.022 a/r	284±12.7 a/r	48±3.1 r
Prelom	Control	3.41±0.17 b/s	0.158±0.012 b/r	124±8.6 b/r	
	Drought	4.53±0.19 a/s	0.301±0.017 a/s	189±10.4 a/s	35±2.3 s

Table 3. Changes in the antioxidant enzyme activities, in the leaves of three bean (*Phaseolus vulgaris* L.) cultivars (Plovdiv 10, Dobrudjanski ran and Prelom) submitted to drought. APOX - ascorbate peroxidase [μmol Asc mg^{-1} Chl min^{-1}]; SOD – superoxide dismutase [U mg^{-1} Chl min^{-1}]; CAT – catalase [μmol H_2O_2 mg^{-1} Chl min^{-1}]. Means ± SE, n=5. Different letters express significantly different results between control and drought stressed plants in the same genotype (a, b) or between cultivars within each treatment (r, s, t) (Zlatev et al., 2005).

Cultivar	Treatment	APOX	SOD	CAT
Plovdiv 10	Control	917±56 a/r	442.6±24.9 b/r	241.2±19.8 b/r
	Drought	1037±79 a/r	593.1±29.5 a/r	784.9±25.9 a/r
Dobrudjanski ran	Control	254±16 b/s	341.7±22.7 b/s	114.7± 8.7 a/s
	Drought	350±21 a/t	689.8±29.4 a/r	83.6± 4.2 b/t
Prelom	Control	296±11 b/s	438.6±21.8 b/r	138.4±10.2 b/s
	Drought	635±30 a/s	620.5±24.1 a/r	504.6±14.1 a/s

As reported by Sgherri and Navari-Izzo (1995), the increase in the activity of scavenging enzymes could be due either to an adaptive change in catalytic properties or to the transcription of the corresponding silent genes. This could be related to enhanced levels of free radicals or other ROS in plant cells and correlate with a temporal coordination of the production of H_2O_2 via SOD and destruction of this peroxide by APOX and CAT. Such coordinated responses are believed to promote plant tolerance to oxidative stress (Foyer et al., 1994; Aziz and Larher, 1998). It is also possible that increased SOD activity could alter the expression of other metabolic processes associated with water stress. Thus, Gupta et al. (1993) have demonstrated that enhanced activity of Cu,Zn SOD in transgenic plants was associated with increased activity of APOX. Some other authors also reported an increase in SOD activity in plants under oxidative stress (Gupta et al., 1993; Kang and Saltveit, 2002; Zlatev et al., 2005, Table 3).

It appears that relative tolerance of plant genotypes, as reflected by its lower lipid peroxidation and higher membrane stability, is related with the levels of its antioxidant enzymes activity. APOX, Cu, Zn-SOD and CAT are involved in overcoming of oxidative stress. The increased activities of antioxidant enzymes act as a damage control system and, thus, provide protection from oxidative stress, resulting in lower LPO and higher membrane stability in tolerant genotypes.

The literature analyzed in this review shows the complexity of tolerance of plants to water deficit and supports the statements of many authors that the flexibility of cell metabolism and its acclimation to changes in environmental conditions is a first essential step in stress avoidance (Yordanov et al., 2000). The wider the range of adaptation capacity of plants, the better they are protected against various stresses. The changes in program of plant development are always associated with changes in their physiological and biochemical program and activity.

In spite of intensive investigation of the problem of water deficit tolerance, many of its aspect remain to be explored. Water deficit induces expression of particular genes and this is associated in most cases with adaptive responses of stressed plants. The functions of many of them are still not established. One of valuable approaches to understand drought resistance mechanisms is to

identify the key metabolic steps that are most sensitive to drought.

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