

Screening for tolerance to waterlogging in forage plants

Moacyr Bernardino Dias-Filho¹ and Monyck Jeane dos Santos Lopes²

1 Eng. Agrônomo, Ph.D., Pesquisador da Embrapa Amazônia Oriental, C. Postal 48, CEP 66017-970, Belém, PA, Brazil. moacyr@diasfilho.com.br

2 Eng. Agrônoma, Mestranda em Botânica Tropical, Museu Paraense Emílio Goeldi, Belém, PA, Brazil

Introduction

The search of plant genotypes that are tolerant to waterlogging is becoming increasingly important due to the recurrence of this condition in many agricultural and natural areas throughout the globe. In addition to the perspective of extreme climatic events, such as excessive rains due to global climatic change (IPCC, 2007), it is possible to preview that, in a near future, waterlogging will become a more common condition in agricultural areas. This prospect is particularly true for animal production areas in the tropics, because many tropical pastures are located in marginal areas, not always suitable for agricultural activities and many of those areas have low soil drainage capacity (Dias-Filho, 1998).

According to Dias-Filho (2005), in cultivated and natural pastures, waterlogging may have a natural cause, such as during long rainy periods, or when soils have naturally poor drainage, or during seasonal events of groundwater elevation. Waterlogging in pastures may also be of an anthropogenic origin, due to pasture management practices that lead to soil compaction. For example, because of the excessive cattle trampling in areas with no vegetation cover, or the constant movement of vehicles or farm machinery, or because of the frequent tillage that causes the appearance of compacted sub superficial soil layers. So, even on pastures areas where waterlogging might not naturally be expected, it is possible that current of past management practices may contribute to make this stress even more frequent.

During the past few years, the death syndrome of *Brachiaria brizantha* cv. Marandu, a problem that is closely related to waterlogging in pastures (Dias-Filho, 2006), is rapidly becoming a major cause of pasture degradation in the north of Brazil (Dias-Filho, 2011). This particular situation has increased even more the need for the search for forage grasses genotypes with some degree of tolerance to waterlogging.

Here, we will summarize the major responses of plants to excess soil water, as well as the morphoanatomical and physiological characteristics of plants, in general, and of forage grasses, in particular, associated with tolerance to waterlogging. Based on this information, some strategies for screening forage plants for tolerance to waterlogging will be discussed.

Plant responses to excess soil water

The deficit in the oxygen availability (hypoxia and anoxia) is the main stress factor in waterlogged soils. In plants, the impact of excess soil water is felt directly by roots and indirectly by the shoots. In shoots, the excess soil water can induce chlorosis, premature wilting, decreased photosynthetic capacity, decreased water potential and nutrient concentration in leaves and decreased growth (Visser et al. 2003). Because they are essentially aerobic organs (Visser et al., 1996), roots are particularly sensitive to oxygen deficiency (reviewed by Armstrong et al., 1994), being directly affected by this stress. Because of the hypoxic environment generated in the rhizosphere, in intolerant plants, waterlogging reduces water absorption and stomatal conductance, leading to wilting, a response similar to that observed during water deficit (Jackson & Drew, 1984). Moreover, the excess water in the root zone inhibits aerobic respiration with simultaneous loss of mitochondrial ATP synthesis, reducing the energy levels of the plant and hence the capacity to absorb and transport water and nutrients to the shoot (Liau and Lin, 2001).

The responses of plants to excess soil water are determined by time, duration and intensity (depending on water depth) of the stress and the plant genotype (Caetano & Dias-Filho, 2008, Dias-Filho, 2002; de Oliveira & Joly, 2010, Grimoldi et al. 1999; Mahelka et al. 2006; Manzur et al. 2009; Mollard et al. 2008). Moreover, the stage of plant development at the time of imposition of stress also determines the pattern of response to excess water in the soil (reviewed by Bailey-Serres & Voesenek, 2008; Colmer & Voesenek, 2009).

Under relatively longer periods of flooding or waterlogging, one of the most common morphological and anatomical responses of plants to hypoxia and anoxia is the formation of aerenchyma and adventitious roots, which capture and transport oxygen to submerged tissues (Armstrong et al. 1994). Aerenchyma formation in plants facilitates the gas exchange between the shoot and root system (and vice versa), improving the diffusion of gases through the plant, mainly oxygen and ethylene (Suralta & Yamauchi, 2008, Yin et al. 2010). The oxygen provided

to the roots can be supplied directly from photosynthesis or from the atmosphere (Evans, 2003). The formation of adventitious roots as an adaptive response to excess soil water is directly related to the accumulation of ethylene in plants (Yin et al. 2009). In situations of excess water in the root zone, or when the plant is submerged, the diffusion of the volatile hormone ethylene is impaired. Under this condition, there is an increase in the concentration of endogenous ethylene in the roots in waterlogged soils, or in the roots and shoots, when the plant is completely submerged (Yin et al. 2009), triggering, the various plant responses to this stress.

Under flooding or waterlogging conditions, intolerant or low tolerant plants suffer a sharp decline in the photosynthetic capacity (Dias-Filho & Carvalho, 2000). The reduction in stomatal opening is a major cause of the initial decline in photosynthetic capacity of waterlogged or flooded plants (reviewed by Liao and Lin, 2000). This is because, in many plants under excess soil water, one of the first observed ecophysiological responses is stomatal closure (eg, Chem et al., 2002). The stomatal closure during episodes of flooding or waterlogging is a regulatory behavior of the water balance, and a critical response to prevent leaf dehydration in species susceptible to excessive soil water (Mollard et al. 2008). In flooded plants, stomatal closure increases the likelihood of plant survival, as well as reducing the demand for water and nutrients; also, it restricts the absorption of potentially toxic ions (Jackson, 1994).

Under excess soil water, several plant species show an increase in the concentration of starch in leaves (reviewed by Irfam et al. 2010). The explanation for this phenomenon is that photosynthesis in leaves would be processed at higher rates than respiration. Also, as in the submerged roots the demand for carbohydrates for respiration would be suspended, the transport of carbohydrates from leaves to roots would be minimal or nonexistent. This would reduce carbon accumulation in the leaves and the progressive exhaustion of carbohydrates in the roots (reviewed by Liao and Lin, 2001). The accumulation of starch in leaves has been suggested as an additional factor responsible for the decline in photosynthetic rate in plants under excess soil water, due to the phenomenon of downregulation (Araya et al. 2006).

Plant tolerance to flooding and waterlogging

Just like animals, plants are obligate aerobic beings, however, because of their relative immobility; they had to develop adaptive responses to deal with episodes of excess soil water (Mollard et al. 2008). The efficiency of these adaptive strategies will determine the plant's tolerance to periods of hypoxia and anoxia (Yin et al. 2009). Plant tolerance to flooding or waterlogging depends on the development of biochemical, physiological, anatomical and

morphological responses for survival in conditions of hypoxia or anoxia (Armstrong et al. 1994, Bailey-Serres & Voesenek, 2008, Colmer & Flowers, 2008; Colmer & Voesenek, 2009, Sairam et al. 2008). Ensure the internal transport of oxygen between the plant part not submerged to the submerged (usually from shoot to the roots) is a major mechanism for the maintenance of basic physiological processes within the plant, aiming for tolerance to periods of excess water in soil (Armstrong et al. 1994). Therefore, the degree of tolerance of a given plant genotype to flooding or waterlogging is directly related to its ability to develop biochemical, physiological, morphological and anatomical response mechanisms that ensure efficient maintenance of growth, or at least the maintenance of vital functions to survival under conditions of anoxia or hypoxia (Sairam et al. 2008; Mollard et al. 2008; Yin et al. 2009; Colmer & Voesenek, 2009).

In genotypes that are intolerant to excess soil water, the lack of oxygen affects the plant metabolism, triggering a series of processes that lead to severe physiological damage (Jackson & Drew, 1984). In tolerant species, resistance to hypoxia or anoxia is acquired via various adaptive strategies. The survival strategies of plants under anoxic stress are influenced by the duration and severity of stress (reviewed by Colmer & Voesenek, 2009). Among the strategies for tolerance to flooding and waterlogging, the formation of aerenchyma (Evans, 2003; Insausti et al. 2001; Mollard et al. 2008; Yin et al. 2010) and the development of a vigorous adventitious root system (Jackson & Drew, 1984) are of a major importance. The main objective of these strategies is to improve the capture and transport of oxygen to submerged tissues, aiding in the oxidation of the rhizosphere. Contributing also to restore and maintain aerobic respiration, or at least keep the alcoholic fermentation at the expense of lactic fermentation, as a source of energy, also removing toxic products that accumulate in the rhizosphere (Evans, 2003, Visser et al. 2000; Yin et al. 2010). With similar objectives, an effective tolerance strategy, useful in environments in which plants would be subject to prolonged periods of submersion, is the ability to quickly extend the shoot in order to restore contact of leaves with the atmosphere (Voesenek & Bailey-Serres, 2008; Banach et al. 2009; Sakagami et al. 2009).

Although the formation of adventitious roots is viewed as an indicator of the presence of adaptive mechanism in plants tolerant to excess soil water (Jackson & Drew, 1984), this mechanism per se may not necessarily guarantee full tolerance of the plant to this stress. In *B. brizantha* cv. Marandu, for example, a grass genotype with little tolerance to excess soil water (Dias-Filho & Carvalho, 2000), plants under this condition are able to develop adventitious roots in abundance (Dias-Filho, 2002), indicating that other adaptive strategies to this stress would also be necessary to increase plant tolerance.

In *Hymenachne amplexicaulis*, a C₃ grass known to be tolerant to excess soil water (Dias-Filho, 2005; Gordon & Feo, 2007), the mechanisms of flooding tolerance are based on the ability of this species to rapidly elongate culms and form adventitious roots (Kibble & Bahnisch, 1999). In addition, aerenchyma are present in the tissues of culms, leaves and roots of this species, both on flooded and in unflooded plants (Kibble & Bahnisch, 1999). Besides aiding in the movement of gases, in *H. amplexicaulis* the aerenchyma also assists in plant flotation.

In *P. dilatatum*, a number of morphoanatomical and physiological responses to excess soil water, such as increased root porosity, aerenchyma formation in leaves and higher rate of leaf extension and elongation of tillers, allow increased occurrence of this species over long periods of flooding in the Argentinean Pampa (Insausti et al. 2001; Vasellati et al. 2001). In *Paspalum modestum* and *P. wrightii*, typical species of wetland environments, the production of aerenchyma occurs in the cortical parenchyma of roots, rhizomes and stems, and in the mesophyll of the leaf blades, both in flooded and non-flooded plants (Fabbri et al. 2006). According to Fabbri et al. (2006), the soil flooding stimulates the formation of additional aerenchyma of both species; however, with contrasting responses to the different organs, this behavior has been interpreted by the authors as adaptations to situations of permanent or seasonal flooding.

Because most of the ATP to maintain cellular metabolism in plants is generated during respiration, by oxidative phosphorylation, a process that requires O₂ as the final electron receptor, an important stress to the plant under anoxia is the collapse in energy generation. This condition affects important metabolic processes in the plant. Thus, anoxia tolerance is closely related to the plant ability to manage this "energy crisis" (Colmer & Voesenek, 2009). Although anoxic catabolism under stress can produce some anaerobic ATP to meet the energy demand of the plant (Igamberdiev & Hill, 2009), yet there may be an energy deficit. Therefore, to effectively tolerate situations of flooding or waterlogging, the plant should be able to effectively regulate the distribution of the little energy available for use in essential metabolic processes (Gibbs and Greenway 2003, Greenway and Gibbs 2003). Thus, a particular species or genotype with the highest carbohydrate concentration of in the roots and higher levels of ATP will be better able to cope with the lack of oxygen. For genotypes of *Vigna radiata*, Kumutha et al. (2008) report that flooding tolerance depends on the availability of sugar reserves in the roots, the activity of sucrose synthase to provide sugars for the reduced glycolytic activity and ADH for recycling NADH for glycolysis maintenance (the principal source of energy in anoxia).

The accumulation of secondary metabolites in the soil in response to hypoxia or anoxia is an important event related to flooding or waterlogging (Banach et al. 2009). These toxic metabolites when absorbed by the plant, can, among other adverse effects, delay plant growth, reduce leaf size and cause wilting and necrosis of shoots (Becker and Asch, 2005). Therefore, the ability of waterlogged or flooded plants to control the transport of ions across cell membranes would be of great importance in determining the tolerance of these plants to soil flooding or waterlogging (Pang & Shabala, 2010).

Another important mechanism of plant tolerance to excess soil water is the presence of an efficient oxidative defense (reviewed by Colmer & Voesenek, 2009). This mechanism would be particularly useful in situations of short-duration recurrent flooding or waterlogging. Such cyclical situations of excess soil water may be relatively common during the rainy season in pastures grown on soils with poor drainage (Dias-Filho, 2005). The "reoxygenation damage" can occur in tissues (roots, for example) under hypoxia, or those under anoxia, re-exposed to O₂ after soil drainage (Blokchina et al. 2003). In these situations, oxidative stress resulting from increased generation of reactive oxygen species (ROS), such as hydrogen peroxide (H₂O₂) in plant cells, and the reduced plant ability to detoxify ROS (Blokchina et al. 2003).

Evaluation of the tolerance of tropical grasses to excess soil water

Scientific studies comparing the tolerance of tropical forage grasses to excess soil water are relatively scarce. In a pioneer study, Baruch (1994a, b) compared the morphophysiological responses to flooding of four grass species: two of known tolerance (*Brachiaria mutica* and *Echinochloa polystachya*) and two intolerant (*Hyparrhenia rufa* and *Andropogon gayanus*). Under flooding, *A. gayanus* and *H. rufa* rapidly close their stomata, reducing stomatal conductance and net photosynthesis. The activity of alcohol dehydrogenase (ADH) in *A. gayanus* increases under flooding, indicating its greater sensitivity to this stress. However, in *Brachiaria mutica* and *E. polystachya*, stomatal conductance, net photosynthesis and ADH activity did not differ between flooded and unflooded plants. The authors concluded that both *B. mutica* and *E. polystachya* have adaptive structures to flooding, such as the development of adventitious roots and aerenchyma tissues that allow the exchange of gases between the shoot and root, contributing to greater tolerance of these species to soil flooding.

In one of the first studies published in Brazil, comparing the tolerance to flooding of *Brachiaria* species (*B. brizantha* cv. Marandu, *B. decumbens* and *B. humidicola*), Dias-Filho & Carvalho (2000) concluded that *B. brizantha* cv. Marandu has low tolerance, *B. decumbens*, moderate tolerance and *B. humidicola* a greater tolerance to flooding. As part of this study, results published subsequently showed that the starch content measured in leaves, six days after the onset of flooding, was inversely proportional to the relative tolerance of these grasses to flooding (Dias-Filho, 2005, 2006). It is possible to infer that, as postulated for other species (Araya et al. 2006), the sharp decline in photosynthetic rate of *B. brizantha* cv. Marandu under flooding may, at least in part, be attributed to non-stomatal factors, such as the phenomenon of feedback inhibition, resulting from the accumulation of starch in the leaves of this cultivar. In the same study, both *B. brizantha* cv. Marandu and *B. humidicola* accumulated more carbohydrates in the roots when under flooding. This accumulation, however, was higher in *B. humidicola*. This behavior suggests that anaerobic conditions caused by soil flooding, restricted respiration of sucrose, which in grasses is the dominant form of carbohydrate in the phloem (Amiard et al. 2004). Thus, it is possible to infer that the greater accumulation of starch in leaves and the increased content of soluble sugars in the roots of *B. brizantha* cv. Marandu under flooding resulted from lower demand for carbohydrates due to the reduction of growth and metabolic activities in the roots. These responses also indicate that the level of sugars in the roots of *B. brizantha* cv. Marandu would not be critical to determine the tolerance of this cultivar to soil flooding.

Subsequent studies on the relative tolerance of *Brachiaria* genotypes to waterlogging (Dias-Filho, 2002; Caetano & Dias-Filho, 2008, Dias-Filho & Queiroz, 2003, Mattos et al. 2005) confirmed the low tolerance of the cultivar Marandu of *B. brizantha*, but also, interestingly, indicated that different genotypes within *B. brizantha* show differential tolerance to this stress (Dias-Filho, 2002; Caetano & Dias-Filho, 2008). Similarly, Silva et al. (2009) also found intraspecific variation in tolerance to soil flooding among seven genotypes of *Panicum maximum*. These findings suggest that the stress caused by excess soil water is able to select the populations of these grasses, resulting in the various gradients of tolerance to excess soil water reported in those studies. Similarly, Mollard et al. (2008), after comparing populations of *Paspalum dilatatum* from high and low areas, subject to periodic flooding, concluded that the populations from the lower areas (more prone to flooding) would have greater tolerance to soil flooding. These finding confirms that the stress caused by soil flooding can cause a selective pressure on the populations of forage grasses.

In comparing the tolerance to soil flooding of four cultivars of *Panicum maximum* (Massai, Mombaça, Milênio and Tanzania), Holanda (2004) observed that, in general, the performance of flooded plants is mostly affected in the cultivar Milênio and, to some degree in Mombaça. For example, under flooding, the relative growth rate was reduced by 31% in Milênio and 43% in Mombaça, while in Tanzania and Massai the reductions were 23 and 21%. The net photosynthesis, measured five days after the onset of flooding, decreased by 36% in the cultivar Milênio and 12, 15 and 24% in cultivars Massai, Mombaça and Tanzania. Root production was significantly decreased by flooding in all cultivars; however, the lowest decline occurred in Tanzania (41%), whereas in other cultivars the reduction ranged from 50 to 54%.

It is important to observe that even in waterlogged tolerant grasses this stress can decrease the performance of plants. For example, *Brachiaria mutica* and *Echinochloa polystachya* under soil flooding experience reductions in leaf area, dry matter production, leaf: culm ratio and number of tillers, compared to plants grown under field capacity (Costa, 2004). Similarly, the K, Ca, Mg and S contents are also reduced in the leaves of these species when under flooding (Costa, 2004).

Screening strategies of forage plants to excess soil water

Screening programs for tolerance to excess soil water in forage species should include strategies that reconcile the efficiency of the observed plant attributes as indicators of tolerance, the objectivity and practicality of the methods used for observing and measuring these attributes, and the reliability of the results. Thus, the effectiveness of a screening program for tolerance to excess soil water in forage species depends, first, on the identification of attributes that can be effectively used as indicators of plant tolerance to this stress. Once these attributes have been identified, the second step should be the search for effective and objective methods to measure these attributes in plants; this should be followed by strategies that are efficient in the use and interpretation of the data, in order to screen the evaluated genotypes. Once these conditions have been met, the rapid achievement of consistent results is ensured.

As previously mentioned, several morphological and physiological attributes have been used to screen the tolerance of forage plants to excess soil water, among them, the most common are: dry matter production of (leaves and roots), leaf elongation rate, gas exchange (net photosynthesis and stomatal conductance) and leaf chlorophyll content (SPAD). Other attributes have also been used, although to a lesser extent. Some of these are: the activity of alcohol

dehydrogenase (ADH), mineral concentration and starch content in leaves and soluble sugars in roots.

Dias-Filho & Carvalho (2000) proposed the leaf elongation rate as an efficient morphophysiological attribute for early detection of tolerance to excess soil water in grasses. Thus, the greater the decline in leaf elongation rate of a particular grass genotype in response to excess soil water, the less tolerant that genotype would be to that stress.

A morphological trait common to the various studies mentioned above is the evaluation of dry matter production (whole plant production or leaves and roots). Thus, the higher dry matter yield of a genotype under excess soil water, at first would indicate a greater tolerance of this particular genotype to this stress. However, a potential problem of a plain comparison of dry matter yield among genotypes under excess soil water is that, due to their natural morphological peculiarities, certain genotypes, even though with low tolerance, when grown under this stress still can attain higher absolute dry matter productions than more tolerant genotypes. For example, in two screening trials to assess the tolerance of *B. brizantha* genotypes to excess soil water, the dry mass production under waterlogged conditions of roots (Dias-Filho, 2002) and green leaves (Dias-Filho, 2002; Caetano, Dias-Filho, 2008) of cultivar Arapoty were lower than that of cultivar Marandu, although this cultivar is less tolerant to excess soil water than is the cultivar Arapoty (Dias-Filho, 2002; Caetano, Dias-Filho, 2008). Such inconsistency is the result of the natural differences (i.e., genetic) in size of these genotypes, even though they are of the same species. Marandu plants are naturally larger than those of Arapoty. So in this case, using only the absolute dry mass production as a screening decision tool for tolerance to excess soil water would lead to the discarding of the cultivar Arapoty as tolerant, or in an even more serious error, would promote the cultivar Marandu as tolerant to excess soil water.

One way to avoid this problem could be achieved by using the methodology described by Dias-Filho (2002). In that methodology, the tolerance of a grass genotype to excess soil water is assessed by comparing this genotype with itself, under ideal conditions. Thus, what would be measured and then compared among genotypes would not simply be the absolute dry matter production (or any other parameter) under stress, but how much this production has been affected by stress. So in this case, the percentage of decrease (or increase) in dry mass production (relative yields), caused by waterlogging, would be the tolerance meter to excess soil water, and not the absolute dry matter production per se. This logic can also be applied to other morphological and physiological parameters (Dias-Filho, 2002; Caetano, Dias-Filho, 2008).

Another relatively common event in trials for screening forage species to excess soil water, that can cause inconsistent results, is related to methodological flaws in planning. Thus, in experiments where forage grasses are grown in pots, for relatively long periods, it is possible that the performance of plants grown without stress (i.e., control plants) decreases with time. The explanation for this phenomenon is the excessive growth of plant roots in the restricted environment of the pots of the control treatment. This excessive growth would make these plants more susceptible to nutritional stress and water deficit, which could become more intense with time. This particular situation would cause a sharp drop in the performance of these plants, confounding the interpretation of the results. This type of situation is particularly evident and common among genotypes with high ability for root growth, as the cultivar Marandu of *B. brizantha*. For example, in a trial in which it was studied the photosynthetic rate and stomatal conductance of *B. brizantha* cv. Marandu under excess soil water and field capacity (control treatment), Mattos et al. (2005) found no difference between treatments for these parameters. However, by observing the results of this experiment, it is possible to infer that the mean photosynthetic rate of control plants ($9 \text{ mol m}^{-2} \text{ s}^{-1}$) is below of that expected for a C_4 grass, grown under theoretically ideal conditions. Therefore, it is possible to infer that in that particular situation, the control plants lacked the ideal conditions (environmental and physiological) to express their full photosynthetic potential. This situation probably also interfered with the other parameters measured in that study. The alternative to avoid this relatively common methodological problem is planning experiments with shorter periods of evaluation. Another alternative, not always completely effective and practical, is to use higher-capacity pots.

Bibliography

AMIARD, W.; MORVAN-BERTRAND, A.; CLIQUET, J.B.; BILLARD, J.P.; HUAULT, C., SANDSTROM, J.P.; PRUD'HOMME, M.P. Carbohydrate and amino acid composition in phloem sap of *Lolium perenne* L. before and after defoliation. *Can. J. Bot.* v.82, p.1594-1601, 2004.

ARAYA, T.; NOGUCHI, K.; TERASHIMA, I. Effects of carbohydrate accumulation on photosynthesis differ between sink and source leaves of *Phaseolus vulgaris* L. *Plant Cell Physiol.* v.47, p.644-652, 2006.

ARMSTRONG, W.; BRÄNDLE, R.; JACKSON, M.B. Mechanisms of flood tolerance in plants. *Acta Botanica Neerlandica.* v.43, p.307-358, 1994.

DIAS-FILHO, M.B.; LOPES, M.J.S. Screening for tolerance to waterlogging in forage plants. In: III SIMF - International Symposium on Forage Breeding, 3., 2011, Bonito, MS [Proceedings...]. Brasília, DF: Embrapa, 2011. 1 CD-ROM.

BAILEY-SERRES, J.; VOESENEK, L.A.C.J. Flooding stress: acclimations and genetic diversity. *Annual Review of Plant Biology*. v.59, p.313-339, 2008.

BANACH, K.; BANACH, A.M.; LAMERS, L.P.M.; DE KROON, H.; BENNICELLI, R.P.; SMITS, A.J.M.; VISSER, E.J.W. Differences in flooding tolerance between species from two wetland habitats with contrasting hydrology: implications for vegetation development in future floodwater retention areas. *Annals of Botany*, v.103, p.341-351, 2009.

BARUCH, Z. Responses to drought and flooding in tropical forages grasses. I. Biomass allocation, leaf growth and mineral nutrients. *Plant and Soil*. 164: 87-96, 1994a.

BARUCH, Z. Responses to drought and flooding in tropical forages grasses. II. Leaf water potential, photosynthesis rate and alcohol dehydrogenase activity. *Plant and Soil*. 164: 87-96, 1994b.

BECKER, M.; ASCH, F. Iron toxicity in rice - conditions and management concepts. *Journal of Plant Nutrition and Soil Science*, v.168, p.558-573, 2005.

BLOKHINA, O.; VIROLAINEN, E.; FAGERSTEDT, K.V. Antioxidants, oxidative damage and oxygen deprivation stress: a review. *Annals of Botany*, v.91 p.179-94, 2003.

CAETANO, L.P.S.; DIAS-FILHO, B.M. Responses of six *Brachiaria* spp. accessions to root zone flooding. *R. Bras. Zootec.*, v.37, n.5, p.795-801, 2008.

CHEN, H.; QUALLS, R.G.; MILLER, G.C. Adaptive responses of *Lepidium latifolium* to soil flooding: biomass allocation, adventitious rooting, aerenchyma formation and ethylene production. *Environmental and Experimental Botany*, v. 48, p.119-128, 2002.

COLMER, T.D.; FLOWERS, T.J. Flooding tolerance in halophytes. *New Phytologist*, v. 179, n. 4, p. 964-974, 2008.

COLMER, T.D.; VOESENEK, L.A.C.J. Flooding tolerance: suites of plant traits in variable environments. *Functional Plant Biology*, v. 36, p. 665-681, 2009.

COSTA, M.N.X. da. Desempenho de duas gramíneas forrageiras tropicais tolerantes ao estresse hídrico por alagamento em dois solos glei húmicos. Piracicaba: ESALQ/USP, 2004. 89p. Tese, Doutorado.

DIAS-FILHO, M.B.; LOPES, M.J.S. Screening for tolerance to waterlogging in forage plants. In: III SIMF - International Symposium on Forage Breeding, 3., 2011, Bonito, MS [Proceedings...]. Brasília, DF: Embrapa, 2011. 1 CD-ROM.

DE OLIVEIRA, V.; JOLY, C. Flooding tolerance of *Calophyllum brasiliense* Camb. (Clusiaceae): morphological, physiological and growth responses. *Trees - Structure and Function*, v.24, n.1, p.185-193. 2010.

DIAS-FILHO, M.B. Pastagens cultivadas na Amazônia oriental brasileira: processos e causas de degradação e estratégias de recuperação. In: DIAS, L.E.; MELLO, J.W.V. (Ed.) *Recuperação de áreas degradadas*. Viçosa: UFV, Departamento de Solos; Sociedade Brasileira de Recuperação de Áreas Degradadas, 1998. p.135-147.

DIAS-FILHO, M.B. Tolerance to flooding in five *Brachiaria brizantha* accessions. *Pesquisa Agropecuária Brasileira*, v.37, p.439-447, 2002.

DIAS-FILHO, M.B. Opções forrageiras para áreas sujeitas a inundação ou alagamento temporário. In: PEDREIRA, C.G.S.; MOURA, J.C. de; DA SILVA, S.C.; FARIA, V.P. de (Ed.). *Teoria e prática da produção animal em pastagens*. Piracicaba: FEALQ, 2005, p.71-93.

DIAS-FILHO, M.B. Respostas morfofisiológicas de *Brachiaria* spp. ao alagamento do solo e a síndrome da morte do capim-marandu. In: BARBOSA, R.A. (Ed.) *Morte de pastos de braquiárias*. Campo Grande: Embrapa Gado de Corte, 2006b. p. 83-101.

DIAS-FILHO, M. B. Degradação de pastagens: processos, causas e estratégias de recuperação. 4. ed. rev., atual. e ampl. Belém: Ed. do Autor, 2011. 215p.

DIAS-FILHO, M.B.; de CARVALHO, C.J. Physiological and morphological responses of *Brachiaria* spp. to flooding. *Pesquisa Agropecuária Brasileira*, Brasília, v.35, p.1959-1966, out. 2000.

DIAS-FILHO, M.B.; QUEIROZ, R.J.B. Tolerância ao alagamento do solo em seis acessos de *Brachiaria brizantha*. In: REUNIÃO ANUAL DA SOCIEDADE BRASILEIRA DE ZOOTECNIA, 40., 2003, Sta. Maria, RS. Sociedade Brasileira de Zootecnia: Sta. Maria, 2003. 5 p. 1 CD-ROM.

EVANS, D.E. Aerenchyma formation. *New Phytologist*, v.161, p.35-49. 2003.

FABBRI, L. T.; RUA, G. H.; BARTOLONI, N. Different patterns of aerenchyma formation in two hygrophytic species of *Paspalum* (Poaceae) as response to flooding. *Flora - Morphology, Distribution, Functional Ecology of Plants*. v.200, p.354-360, 2005.

DIAS-FILHO, M.B.; LOPES, M.J.S. Screening for tolerance to waterlogging in forage plants. In: III SIMF - International Symposium on Forage Breeding, 3., 2011, Bonito, MS [Proceedings...]. Brasília, DF: Embrapa, 2011. 1 CD-ROM.

GIBBS, J.; GREENWAY, H. Mechanisms of anoxia tolerance in plants. I. Growth, survival and anaerobic catabolism. *Funct. Plant Biol.* v.30, p.1-47, 2003.

GORDON, E.; FEO, Y. Dinámica de crecimiento de *Hymenachne amplexicaulis* en un humedal herbáceo en el Estado Miranda (Venezuela). *Acta Bot. Venez.* v.30, 2007.

GREENWAY, H.; GIBBS, J. Mechanisms of anoxia tolerance in plants. II. Energy requirements for maintenance and energy distribution to essential processes. *Funct. Plant Biol.* v.30, p.999-1036, 2003.

GRIMOLDI, A.A.; INSAUSTI, P.; ROITMAN, G.G.; SORIANO, A. Responses to flooding intensity in *Leontodon taraxacoides*. *New Phytologist.* v.141, p.119-128, 1999.

HOLANDA, S.W. da S. Tolerância de quatro cultivares de *Panicum maximum* Jacq. ao alagamento. Belém: UFRA/MPEG, 2004. 38p. Dissertação Mestrado.

IGAMBERDIEV, A.U.; HILL, R.D. Plant mitochondrial function during anaerobiosis. *Annals of Botany*, v.103, n.2, p.259-268. 2009.

INSAUSTI, P.; GRIMOLDI, A.A.; CHANETON, E.J.; CASELLATI, V. Flooding induces a suite of adaptive plastic responses in the grass *Paspalum dilatatum*. *New Phytologist.* v.152, p.291-299, 2001.

IPCC, 2007: Summary for Policymakers. In: *Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change* [Solomon, S., D. Qin, M. Manning, Z. Chen, M. Marquis, K.B. Averyt, M. Tignor and H.L. Miller (eds.)]. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA. Disponível em: <http://bit.ly/2X3F>. Acesso em out 2011.

IRFAN, M.; HAYAT, S.; HAYAT, Q.; AFROZ, S.; AHMAD, A. Physiological and biochemical changes in plants under waterlogging. *Protoplasma*, v.241, p.3-17, 2010.

JACKSON, M. B. Root-to-shoot communication in flooded plants: involvement of abscisic acid, ethylene, and 1-aminocyclopropane-1-carboxylic acid. *Agronomy Journal*, 86, 775-782, 1994.

JACKSON, M, DREW, M. Effects of flooding on growth and metabolism of herbaceous plants. In: KOZLOWSKI, T., ed. *Flooding and plant growth*. London: Academic Press, p.47-128, 1984.

DIAS-FILHO, M.B.; LOPES, M.J.S. Screening for tolerance to waterlogging in forage plants. In: III SIMF - International Symposium on Forage Breeding, 3., 2011, Bonito, MS [Proceedings...]. Brasília, DF: Embrapa, 2011. 1 CD-ROM.

KIBBLER, H.; BAHNISCH, L. M. Physiological adaptations of *Hymenachne amplexicaulis* to flooding. Australian Journal of Agricultural Research, Callingwood, v.39, p.429-435, 1999.

KUMUTHA, D.; SAIRAM, R.K.; MEENA, R.C. Role of root carbohydrate reserves and their mobilization in imparting waterlogging tolerance in green gram (*Vigna radiata* (L.) Wilczek) genotypes. Indian J. Plant Physiol., v.13, n.4, p.339-346. 2008.

LIAO, C.T.; LIN, C.H. Physiological adaptation of crop plants to flooding stress. Proc. Natl. Sci. Counc. v.25, p.148-157, 2001.

MAHELKA, V. Response to flooding intensity in *Elytrigia repens*, *E. intermedia* (Poaceae: Triticeae) and their hybrid. Weed Research. v.46, p.82-90, 2006.

MANZUR, M.E.; GRIMOLDI, A.A.; INSAUSTI, P.; STRIKER, G.G. Escape from water or remain quiescent? *Lotus tenuis* changes its strategy depending on depth of submergence. Annals of Botany. v. 104, n. 6, p. 1163-1169, 2009.

MATTOS, J.L.S.; GOMIDE, J.A.; MARTINEZ Y HUAMAN, C.A. Crescimento de espécies do gênero *Brachiaria* sob alagamento em casa de vegetação. Revista Brasileira de Zootecnia, v.34, p.765-773, 2005.

MOLLARD, F.P.O.; STRIKER, G.G.; PLOCHUK, E.L.; VEGA, A.S.; INSAUSTI, P. Flooding tolerance of *Paspalum dilatatum* (Poaceae: Paniceae) from upland and lowland positions in a natural grassland. Flora - Morphology, Distribution, Functional Ecology of Plants, v.203, p.548-556, 2008.

PANG, J.; SHABALA, S. Membrane Transporters and Waterlogging Tolerance. In: MANCUSO, S.; SHABALA, S.; PANG, J., ed. Waterlogging Signalling and Tolerance in Plants: Springer Berlin Heidelberg, 2010. Membrane Transporters and Waterlogging Tolerance, p.197-219.

SAIRAM, R.; KUMUTHA, D.; EZHILMATHI, K.; DESHMUKH, P.; SRIVASTAVA, G. Physiology and biochemistry of waterlogging tolerance in plants. Biologia Plantarum, v.52, p.401-412, 2008.

SAKAGAMI, J.-I.; JOHO, Y.; ITO, O. Contrasting physiological responses by cultivars of *Oryza sativa* and *O. glaberrima* to prolonged submergence. Annals of Botany. v.103, p.171-180, 2009.

SILVA, A. S.; LAURA, V. A.; JANK, L. Soil Flood Tolerance of Seven Genotypes of *Panicum maximum* Jacq. Braz. arch. biol. technol., v.52, p.1341-1348. 2009.

DIAS-FILHO, M.B.; LOPES, M.J.S. Screening for tolerance to waterlogging in forage plants. In: III SIMF - International Symposium on Forage Breeding, 3., 2011, Bonito, MS [Proceedings...]. Brasília, DF: Embrapa, 2011. 1 CD-ROM.

SURALTA, R.R.; YAMAUCHI, A. Root growth, aerenchyma development, and oxygen transport in rice genotypes subjected to drought and waterlogging. *Environmental and Experimental Botany*, v.64, n.1, p.75-82. 2008.

VASELLATI, V.; OESTERHELD, M.; MEDAN, D.; LORETI, J. Effects of flooding and drought on the anatomy of *Paspalum dilatatum*. *Annals of Botany*. v.88, p.355-360, 2001.

VISSER, E.J.W.; VOESENEK, L.A.C. J.; VARTAPETIAN, B.B.; JACKSON, M.B. Flooding and Plant Growth. *Annals of Botany*. v.91, p.107-109. 2003.

VISSER, E.J.W.; COHEN, J.D.; BARENDSE, C.W.M.; BLOM, C.W.P.M.; VOESENEK, L.A.C.J. An ethylene-mediated increase in sensitivity to auxin induces adventitious root formation in flooded *Rumex pahstris* Sm. *Plant Physiology*, v.11, p.1687-1692, 1996.

YIN, D.; CHEN, S.; CHEN, F.; GUAN, Z.; FANG, W. Morphological and physiological responses of two chrysanthemum cultivars differing in their tolerance to waterlogging. *Environmental and Experimental Botany*. v. 67, p. 87-93, 2009.

YIN, D.; CHEN, S.; CHEN, F.; GUAN, Z.; FANG, W. Morpho-anatomical and physiological responses of two *Dendranthema* species to waterlogging. *Environmental and Experimental Botany*. v.68, p.122-130, 2010.