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Stress Due To Soil Flooding And Differential Physiological Responses To Initial Growth Of Wheat Plants

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ABSTRACT

Background: Flooding in areas with poor drainage, can restrict the growth of cultured species and reduce productivity and quality of seeds**.** This work aimed to evaluate the physiological performance of initial growth and isoenzymeexpressionof wheat plants subjected to floodingsoil**. Methods**Thus, seeds of wheat cultivar FundacepVigore were sown in trays of black polyethylene, containing as substrate Planosol soil type. The treatments were six times by flooding (24, 48, 72, 144, 168 and 192 hours) + soil kept at field capacity. Length and dry mass of roots, stems and shoots, number of leaves, leaf area and leaf area ratio, leaf mass ratio, net assimilation rate, specific leaf area ratio of shoot/root and expression of isoenzymes glutamate oxaloacetate transaminase, malate dehydrogenase, esterase and acid phosphatase**. Results:** Flooding during the early growth of wheat plants reduces the dry biomass, leaf area ratio, the ratio of leaf mass and leaf area. However, the ratio increases air/root, does not affect the net assimilation rate and the expression of isozymes glutamate oxaloacetate transaminase, malate dehydrogenase, esterase and acid phosphatase.**Conclusions:**Flooding during the early growth of wheat plants and as the period of flooding adversely affects the initial growth characteristics of wheat plants, related to the allocation of carbon.

INTRODUCTION

Wheat (*Triticumaestivum* L.) occupies approximately 20% of the cultivated area worldwide (Maia *et al.*, 2007). During the 2016 crop, approximately 2.212,3thousand hectares will be cultivated in Brazil. The average yield reached 2,3 t ha^{-1} and the production surpassed 5,53 million tons. During this period, Rio Grande do Sul state had the largest cultivated area, reaching a production of 1,46 million tons and an average yield of 1,9 t ha^{-1} (Conab, 2016).

There are approximately 5,4 million hectares of land subject to flooding in Rio Grande do Sul state (Pinto *et al.* 1999). From this total, one million hectares are cultivated with rice (Irga, 2013) and the remainder is used for pasturing, with a great underutilized area for the production of grains (Schollesand Vargas, 2004).In this sense, the increase of use in these areas as annual grain-producing crops such as wheat might be a feasible alternative cultivation.

Flooding is a result of water saturation and causes changes in the physiology of plants, negatively altering energy metabolism due to hypoxia or anoxia (Liao& Lin, 2001). In flooded areas or areas exposed to frequent

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flooding, changes in the diffusion of gases may occur (Jackson andColmer, 2005) when the growth capacity and the distribution of inorganic nutrients, water and phytohormones define the survival of plants (Henry *et al.*, 2009). Thus, flooding may adversely affect growth and development of plants by reducing genetic potential expression and productivity (TaizandZeiger, 2013).

Such effects on growth and on the development of plants caused by flooding are a result of lactate and ethanol accumulation, resulting in a reduced energy production, protein synthesis (Zabalza*et al.*, 2009) and decreased growth rates (Van Dongen*et al.*, 2011). Some species show a reduced growth in flood situations, such as *Brachiariamutica*, *B. humidicola*, *B. decumbens*, *B. brizantha* (Mattos*et al.*, 2005), *B. decumbens* (Haddade*et al.*, 2002), *Lithraeamolleoides* (Medri*et al.*, 2007) and *Glycine max* (Fante*et al.*, 2010). However, other species are more tolerant to flooding, such as *Setariaanceps*(Haddade*et al.*, 2002), and potentially some genotypes of other cultivated species.

The identification of species with an economic importance able to survive to flooding and provide a higher performance and a higher economic return makes feasible the exploitation of areas subject to flooding (Fante*et al.*, 2010). The study on early growth characteristics is an important assessment tool regarding the genotype performance under different conditions and handling means, including stress due to hypoxia or anoxia. It can be used as a means of determining genotype tolerance to adverse environment conditions (Benincasa, 2003; Pedó*et al.*, 2013). In addition, the evaluation of isoenzymatic expressions is used as a biochemical marker of plant response to environmental stimuli. It changes differently under different environmental conditions and can be a way to efficiently assess genotype responses to certain abiotic stresses (Bensch*et al.*, 2009).

In this context, this study aimed to evaluate the physiological initial growth performanceand the isoenzymatic expression of wheat plants subjected to flooding.

MATERIAL AND METHODS

The experiment was conducted at the Campus Capão do Leão of the Federal University of Pelotas. It is located at 31°48'15" S and 52°24'55" W, in the protected environment of Capela model greenhouse, covered with polypropylene boards and automation for temperature control and relative humidity. The climate is temperate with a well distributed rainfall and a hot summer, classified as Cfa by Köppen.

Wheat seeds of the cultivar FUNDACEPVigore were seeded in plastic trays filled with sieved soil and collected from the A1 horizon of aHaplic Eutrophic solodicPlanosol, belonging to the Pelotas mapping unit (Streck*et al.*, 2008), previously corrected according to previous soil analysis and recommendations for the mentioned species (Cqfs RS/SC, 2004).

The experimental design was randomized blocks, seven treatments and four replications. The treatments were six soil flooding times (24, 48, 72, 144, 168 and 192 hours) applied 21 days after sowing. The flooding in each tray was made by fitting a second black polyethylene tray without holes in its base and placed beneath the tray containing soil and plants. At the same time, plants were collected and kept at field capacity, corresponding to each flooding time, and the soil was kept in such condition by manual irrigation. To determine field capacity, the tension table methodology was used and the maintenance of field capacity was made as recommended (Embrapa, 1997).

The collections of primary growth data were made from the 21st day after sowing at regular intervals,being collected plants of each flooding time and plants kept at field capacity. After collection, the plants were separated according to organs (shoots and roots). Roots were washed on a fine mesh sieve under running water. To determine the physiological characteristics of growth, the following parameters were used:

Root length and shoot length: four subsampleswith 10 seedlings for each flooding time were used. The shoot length was obtained by measuring the distance between the insertion of the basal portion of the primary root and the shoot top. The primary root length was measured by the distance between its apical and basal parts. The results were expressed as mm.seedling^{-1}.

Dry root, stem and leaf matter: estimated from the dry matter of four subsamples with 10 seedlings at each flooding time.Shoots and roots were packaged in brown paper envelopes and dried in a forced air ventilation oven at 70°C±2°C until constant weight. The values forshoots and roots dry matter were obtained by a precision scale and the results were expressed in mg.organ^{-1}.

Number of leaves and leaf area: the number of leaves was determined by directly counting the number of leaves per plant, and the leaf area was measured with a LI-3100 area meter using four subsamples with 10 plants. The number of leaves at each collection time was expressed as number of leavesplant^{-1}, and the leaf area in cm^{2}plant^{-1}.

Leaf area and net assimilation rate ratios were estimated from four subsamples with 10 plants collected at each flooding time through the equations $F_a=A_f/W_t$ and $E_a=1/A_f.d_W/d_t$, where A_f corresponds to leaf area, W_t indicates total dry matter of seedling, d_w refers to the derivative of total dry matter and d_t refers to the derivative with respect to time (Radford, 1967).

Shoot ratio and leaf weight ratio: determined from four subsamples with 10 plants collected at each flooding time using the equations $P_w=W_{pa}/W_r$and$F_w=W_f/W_t$, where W_{pa} refers to the dry matter allocated toshoots,W_r indicates the dry matter allocated to roots, W_f refers to total leaf dry matter,and$W_t$total dry matter.

Isoenzyme expression: for the determination of isoenzymes, samples were collected from leaf plant tissue of plants exposed at each flooding time. The samples were stored in an ultrafreezer at -72°C until analysis. The expression of glutamate oxaloacetate transaminase, malate dehydrogenase, esterase and acid phosphatase isoenzymes was determined by vertical electrophoresis in polyacrylamide gel.For this, plants were separately ground in a porcelain mortar in an ice bath. Then, 200 mg of the macerated material from each sample were transferred to micro-centrifuge tubes and added to the extraction solution (Lithium Borate 0.2 M at pH 8.3 + Tris Citrate+0.2M at pH 8.3)+0.15% of 2-mercaptoethanol in an1:2 ratio (*w/v*). The electrophoresis was performed in 7% polyacrylamide gels by applying 20 µL of each sample. The coloring systems used were those described by Scandálios (1969) and Alfenas (1998).

The data on initial growth characteristics of plants were subjected to analysis of variance and, when 5% significant, analyzed by polynomial regression. The interpretation of the results related to the expression of isoenzymes was performed by visual analysis of the gels considering the presence or absence of expression and the intensity of the bands.

RESULTS AND DISCUSSION

Flooding did not affect shoot length, primary root length, number of leaves and net assimilation rate. No difference between flooding times and the control was observed.

The dry matter of roots, stems and leaves of plants not subjected to flooding was higher if compared to plants subjected to flooding. The amount of dry matter allocated toleaves was higher than the other organs in all treatments (Figure 1a, 1b, 1c). However, in plants exposed to longer flooding periods, the dry matter of roots, stems and leaves showed an increase in the amount of carbon allocated when compared to plants submitted to shorter flooding periods. Such change in root growth may guard a relation to an acclimatization attempt as noted by the increase in the preference of this structure as a strong drain.

Through mechanisms of tolerance to flooding, plants may resume growth after the first periods of hypoxia (Batista *et al.*, 2008). Some plant species develop survival mechanisms to survive floods, as seen in maize plants, wherein ethylene synthesis is stimulated and results in death and disintegration of root cortex cells, allowing this space be filled by air and facilitating the diffusion of O_2 (TaizandZeiger, 2013).

Flooding may indirectly affect the production of biomatter, which is dependent on the photosynthetic rate (Pezeshki, 2001; Medri*et al.*,2012). Plants sensitive to stress due to oxygen deficiency may have a reduced stomatal conductance, resulting in a decreased photosynthetic capacity (Dias-FilhoandCarvalho, 2000) and synthesis and allocation of carbon. Thus, Medri*et al.* (2007) observed a decrease in total dry matter of *Lithraeamolleoides* plants subjected to flooding compared to the control. Fante*et al*. (2010), evaluating soybean cultivars' response to flooding, observed that the decrease in dry matter varies among cultivars and plant development stages. In *Brachiaria*, it was noted that different accesses respond differently to flooding, differing or not from the control (Dias-Filho, 2002).

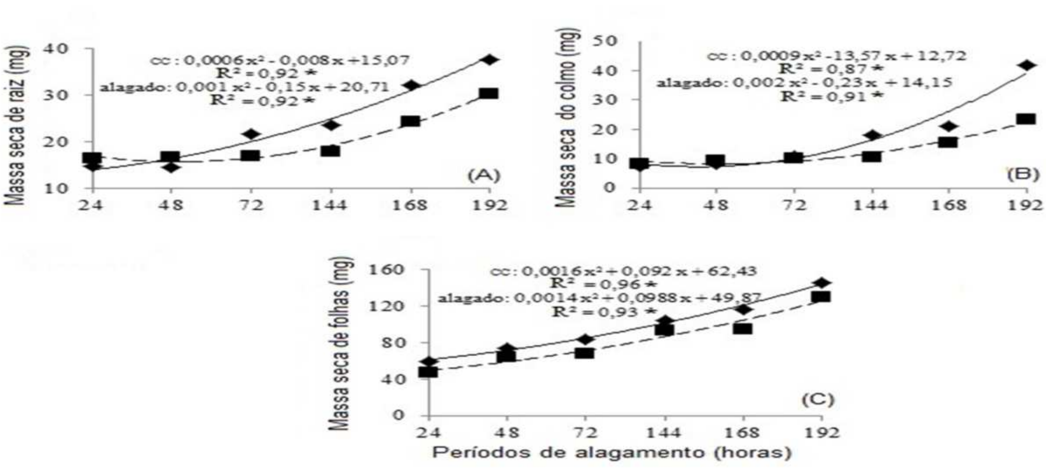

Fig. 1: Root dry mass (a), stem (b) and leaves (c) wheat plants under the effect of the flooding time (24, 48, 72, 144, 168 and 192 hours) and maintained soil at field capacity (s). cc = field capacity; = flooded= soil flooding times.

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The leaf area ratio, leaf matter ratio and leaf area were higher in plants not exposed to flooding compared to those subjected to such abiotic stress (Fig. 2a, 2b, 2c). However, the shoot and root ratio was increased by the increase in the flooding regime up to 192 h. The leaf area presented a quadratic tendency, with an elevation up to 157 h of flooding in plants subjected to flooding and in plants in non-flooded areas. As the number of leaves is related to leaf area and was not significant in this work, it is possible that the higher expansion of leaf area may have resulted in a greater interception of solar radiation, facilitating the photosynthetic process and a greater accumulation of dry matter in plants not subjected to flooding (Streck*et al.*, 2005).

Under environmental hypoxia, the roots do not support physiological processes, of which shoots are dependent (TaizandZeiger, 2013). Roots under oxygen deficiency result in hormonal imbalance (Grandis*et al.*, 2010) and strive to absorb nutrients and transport ions to shoots, a fact that results in leaf senescence.

The shoot/root ratio showed higher values in plants subjected to flooding compared to those kept at field capacity (Figure 2d). However, in plants subjected to flooding and in plants kept at field capacity, the shoot/root ratio presented a quadratic tendency, with an increase in growth until 144 h of flooding.

Roots in hypoxia are not able to meet the nutrient needs, resulting in deficit in shoots (Marenco and Lopes, 2009). According to Henry *et al.* (2005), flooding may cause a reduction in the rateof carbohydrate translocation from leaves to roots, slowing the growth and decreasing the metabolic activity of the root system,which thenrequires fewer carbohydrates, consequently accumulating starch in the leaves. This may induce a decrease in photosynthesis (Marenco and Lopes, 2009). However, the higher carbon investment in roots can be an attempt of the species to survive a certain stressful condition of the medium, as already noted(Figure 2).

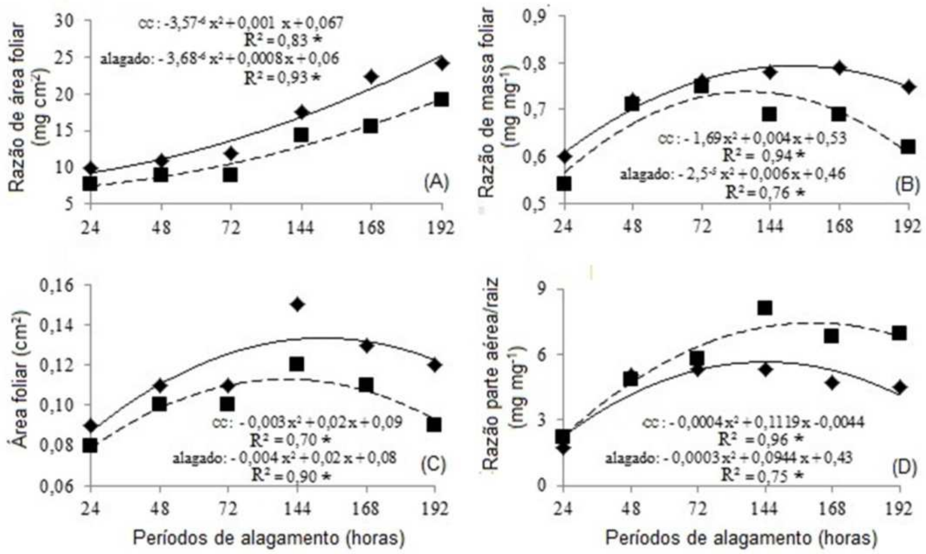

Fig. 2: Leaf area ratio (a) mass (b), leaf area (c) and ratio of shoot / root (d) of wheat plants under the effects of the flooding times (24, 48, 72, 144, 168 and 192 hours) and soil maintained at field capacity (s). cc = field capacity; = flooded= soil flooding times.

As already mentioned, the net assimilation rate showed no significant difference among treatments. This demonstrates that despite the decrease in the parameters related to the uptake of solar radiation, plants at different flooding times showed a certain plasticity in fixing or distributing carbon across the mentioned abiotic stress.

The isoenzymatic expression varied between flooding times evaluated and plant structures analyzed (Figure 3). The isoenzyme oxaloacetate transaminase had a higher glutamate expression in shoots and a lower expression in roots. Similar were the results between plants kept at field capacity and at other flooding times (Figure 3a). In relation to the isoenzyme malate dehydrogenase (MDH), it was inhibited by longer flooding times (Figure 3b). In shoots, there was no difference regarding the intensity of expression of the allele MDH2 in plants exposed to longer flooding times. However, in roots, changes in the enzyme expression started markedly from 48 h of flooding, and sharply inhibited expression at 144 h after the application of the stress.

Glutamate oxaloacetate transaminase is an enzyme that participates in the synthesis and degradation of amino acids, catalyzing the reverse reaction of glutamate and oxaloacetate to form aspartate + α-ketoglutarate. It is also a key enzyme connecting the Krebs cycle to the metabolism of amino acids and is associated with MDH to ensure the transfer of electrons through the mitochondrial membrane (Simon *et al.,* 1989). The reduction in theexpression of the MDH enzyme as days of flooding advance may be due to the anaerobic respiration of roots under hypoxic conditions, considering that the Krebs cycle is reduced under these conditions.

When wheat seedlings were subjected to flooding, there was an increase in the intensity of the esterase isoenzyme expression in shoots of seedlings subjected to up to 72 hours of flooding (Figure. 3c). In roots, two new bands appeared from 144 h of flooding.

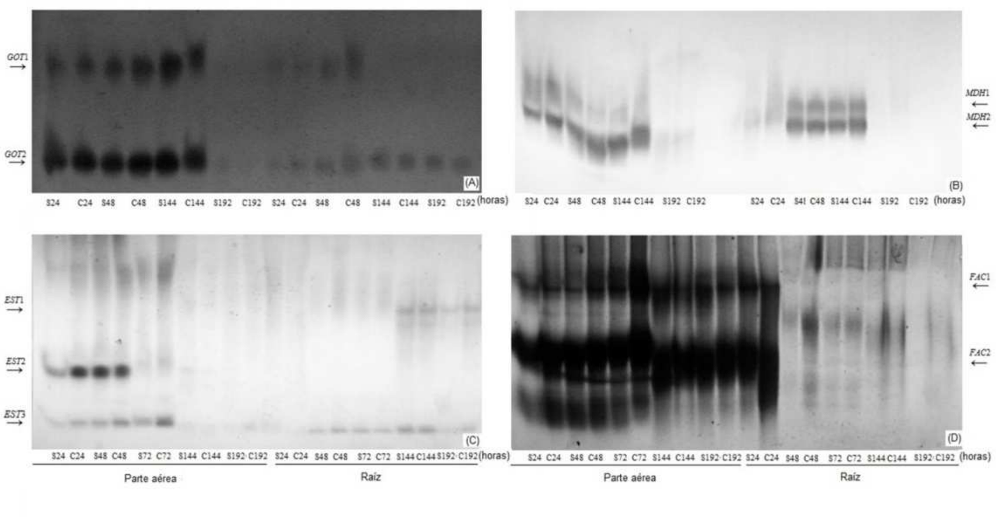

Fig. 3: Isozyme expression Glutamate oxalocetato transaminase (a), Malato dehydrogenase (b), Esterase (c) and acid phosphatase (d) in the shoot and root of wheat submit to flooding (C) for 24, 48, 72, 144 and 192 hours and maintained at field capacity (S).

Flooding did not affect the expression of esterase compared to the condition at field capacity. The enzyme esterase is involved in estershydrolysis reactions and is closely related to lipid metabolism (Aumonde*et al.,* 2013). It may be involved in lipid peroxidation processes and may accumulate free radicals (Lawlor, 1995) that lead to oxidative stress, cell damage and negative changes in plant growth.

The pattern of expression of the isoenzyme acid phosphatase in shoots of plants exposed to different flooding times was similar between flooded plants and the control (Figure 3d). Initially, the expression of the bands of this isoenzyme was increased in the root, and decreased over flooding times. Acid phosphatase participates in the hydrolysis of esters and possibly acts on the membrane system, taking part of the power supply and phosphate availability for seedling growth. Longest flooding times decreased the activity of this isoenzyme in roots, which may have resulted in a lower dry matter accumulation compared to plants kept at field capacity (Figure 1a).

In general, flooding reduced the early growth of wheat seedlings and modified the isoenzymatic expression. Physiological changes caused by soil flooding on the accumulation of dry matter reflected in the worst performance of wheat seedlings. However, while flooding did not affect number of leaves and net assimilation rate, the superiority of plants kept at field capacity compared to those kept in the flooded soil may partly be related with leaf area ratio.

In this sense, synthesis of amino acids, lipid metabolism, phosphate availability, synthesis of new proteins and chemical energy could have resulted in a decreased growth of seedlings subjected to flooding in relation those kept at field capacity. Thus, variations in isoenzymatic expression may be related to changes in its activity and with a specific sensitivity to the causative agent of the stress (Vieira *et al.,* 2000), in this case excess water in the soil.

Conclusion:

Flooding during the early growth of wheat plants reduces dry matter, leaf area ratio, leaf matter ratio and leaf area. However, it increases shoot and root ratio and does not affect net assimilation rate. The expression of the isoenzymes glutamate oxalocetate transaminase, malate dehydrogenase, esterase and acid phosphatase is variable according to the assessed organ and to the stress time caused by flooding.

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