Physiological attributes, growth and expression of vigor in soybean seeds under soil waterlogging

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Soil waterlogging in lowland areas is detrimental to many rainfed crops in all regions of the world, affecting the growth and development of plants. Thus, this study aimed to evaluate the growth, assimilate partitioning and expression of vigor in the soybean cultivar BMX Potência when subjected to waterlogging. The treatments consisted of 2- and 4-day periods of waterlogging during the vegetative growth stage, V5. Control plants were maintained at the field capacity. At regular intervals of 14 days after sowing until the end of the cycle, plants were collected for the determination of dry mass and leaf area. We evaluated growth according to simple logistics, assimilate partitioning and the expression of seed vigor in each period. Plants in different periods of growth showed a reduced efficiency in converting solar energy over time in response to waterlogging, yielding seeds with an increased mass and vigor. Thus, it was observed that an increase in the period of waterlogging reduces plant growth but does not affect the vigor of soybean seeds.

Key words: Glycine max (L.) Merrill, dry matter, leaf area, seed vigor.

INTRODUCTION

Soybean (Glycine max (L.) Merrill) belongs to the family Fabaceae and is grown on an acreage of 8.3% over the previous crop, with a productivity of 2.9 t ha⁻¹ (Conab, 2014). The products and by-products of soybean used by the chemical and food industries exhibit great versatility (Fante et al., 2010), and soybean is therefore one of the main commodities in the southern region of Brazil.

In recent years, the Rio Grande do Sul has shown unfavorable environmental conditions influencing soybean yields, causing many problems during the vegetative and reproductive growth of cultivated plants. The state of Rio Grande do Sul encompasses an area of 5.4 million hectares of lowland soils (Embrapa, 2005), most of which is used for rice cultivation and as pasture. Thus, employing other cultures in lowland areas is essential to improve the use and the yield of these areas.

Soil waterlogging affects the growth and development of various organs of plants, causing variations in respiration and photosynthesis (Alaoui-Sosse et al., 2005). These effects are reflected in changes in the leaves (Bailey-Serres and Voesenek, 2008), stomatal conductance (Alaoui-Sosse et al., 2005), decreased root...
permeability and alterations of hormonal balance (Moura et al., 2008).

With soil waterlogging, there is a decrease in oxygen in the root system, activating anaerobic metabolism (Kumutha et al., 2008; Sairam et al., 2009) and resulting in morphological and anatomical changes (Fukao and Bailey-Serres, 2004; Yin et al., 2010). The reduction of oxygen levels in the soil affects nitrogen fixation and other minerals, reducing root growth (Amarante and Sodek, 2006), the growth of leaves and relative growth (Almeida et al., 2003). The occurrence of these factors can cause lower rates of production and the transport of assimilates (Parent et al., 2008) as well as cessation of vegetative and reproductive growth (Moura et al., 2008) and may affect seed vigor.

Growth analysis is the first step for interpreting crop production, and it is important to evaluate the effects of different environmental conditions on the morphophysiological attributes of plants during ontogeny (Pedó et al., 2013). Through the examination of leaf area and organ dry matter, this method evaluates the effect of genotype x environment interactions and the influence of agronomic practices on plant growth (Barreiro et al., 2006). Furthermore, the evaluation of attributes related to seed vigor is important to estimate their performance in adverse field situations (Peske et al., 2012).

This study aimed to evaluate growth, assimilate partitioning and expression of vigor in soybean seeds subjected to periods of soil waterlogging.

MATERIALS AND METHODS

The experiment was conducted in a chapel model greenhouse, arranged in the north-south direction, coated with polycarbonate and equipped with temperature and relative humidity controls, at a geographical location of 31°52' S and 52°21' W, at the Federal University of Pelotas. The climate of the region is characterized as temperate with well-distributed rainfall and hot summers, corresponding to Köppen classification Cfa.

Sowing was performed on 01/06/2014, employing seeds from the BMX Potência soybean cultivar, which were allowed to germinate and develop in black polyethylene pots with a volume of 12 L, containing the A1 horizon of a Planossolo Haplic Eutrophic Solodico as a soil substrate, which was previously corrected in accordance with a prior soil analysis based on a fertilization manual (Qgfs RS / SC 2004).

The experiment was set up as a factorial experiment with a completely randomized 3x10 design (three periods and 10 collection times), with three replications. The treatments consisted of periods of flooding of two and four days during the vegetative stage - V5. To enable the establishment of treatments, holes were drilled at the bottom of polyethylene pots containing soil to facilitate the drainage of excess water and maintenance of the field capacity. The field capacity was determined using the voltage table method (Embrapa, 1997), maintaining a 20 mm blade of water on the soil surface by fitting a second black polyethylene vessel, without perforation of the vessels containing soil, seeking to ensure gas exchange and soil aeration. For drainage, the soaked soil was carried in the superimposed perforated vessel and imperforated vessel, allowing water to drain to the field capacity. As a control treatment, a set of plants that were maintained at the field capacity throughout the experimental period was used.

Data on the maximum, minimum and average temperature and solar radiation were obtained with a mercury thermometer and pyranometer, respectively, installed at a height of 1.5 m inside the greenhouse; the results are shown in Figure 1a and b. Evaluations were performed through the collection of primary growth data in successive harvests at regular intervals of 14 days after sowing, throughout the crop development cycle. At each harvest, the plants were cut close to the ground and divided into different organs (leaves, stems, roots and pods), which were packed separately in brown paper envelopes. To determine dry matter contents, the material was placed in a forced air oven at a temperature of 70 ± 2°C until constant weight.

Leaf area (A) was determined with a Licor LI-3100 model area meter, and the leaf area index (LAI) was estimated with the formula LAI = A/S, where A is the leaf area, and S is the vessel surface area occupied by the plant. The raw data for the accumulated total dry matter (W) were adjusted using the simple logistic equation Wt = Wm / (1 + Ae-δt), where Wm is an asymptotic estimate of the maximum growth; A and B are constants used for adjustment; e is the base natural logarithm (Naperian logarithm); and t is the time in days after sowing (Richards, 1969). The primary of leaf area (A) data were adjusted by means of orthogonal polynomials (Richards, 1969).

Instantaneous values for the dry matter production rate (C) were obtained from the derivative of the equation for total dry matter and the relative growth rate (Rd) using the equation Rd = 1/W, dW/dt, and instantaneous values for the net assimilation rate (Em) and leaf area ratio (Fo) and leaf weight (Fl) were estimated with the equations Em = 1/AdW/dt, Fo = Ao/2, and Fl = W/Wm, respectively (Radford, 1967). The conversion efficiency of solar energy (δ) was determined using the equation ξ (%) = (100Cp·C)/(Rm), where Rm is the mean value of the incident solar radiation (cal m² day⁻¹) recorded over fourteen days prior to the corresponding C, and the calorific value (δ) of 4460 cal g⁻¹ cited by Silva Neto et al. (1991) was used.

The dry matter partitioning between different plant structures (roots, stems, leaves and pods) was determined separately from the measurement of the weight allocated to each plant structure, followed by transformation of the primary mass allocation data for each organ to a percentage.

From the harvested seeds, the thousand seed weight in grams was determined from eight repetitions in which the mass of 1000 seeds was measured with an analytical balance, as indicated by the Seed Analysis Rules guidelines (Brasil, 2009). Seedling emergence was carried out using eight replicates of 50 seeds per treatment, which were allowed to germinate in black polyethylene trays containing characterized above ground, kept at field capacity according to the methodology described above in a greenhouse environment. The seedling emergence speed index was determined from the daily count of the number of seedlings that emerged from the substrate, as proposed by Nakagawa (1994). Leaf area and total dry matter were measured based on the mass of eight samples from 10 seedlings at the end of the emergence test.

Data on the emergence and the emergence speed index of the seedlings, the leaf area and the area of organs were subjected to analysis of variance, and for significant F values, we applied Tukey’s test at 5% probability. Primary data on total dry matter, the leaf area and the dry matter contents of the leaves, roots, stems and pods were subjected to analysis of variance, with data for the growth analysis being analyzed with a simple logistic equation (Radford, 1967). The statistical program used was Winstat.

RESULTS AND DISCUSSION

The production total dry matter (W) in the soybean plants
Figure 1. Maximum and minimum temperature (a), solar radiation (b), total dry matter (c), dry matter production rate (d), relative growth (e), leaf area index (f), rate of net assimilation (h) and leaf area ratio (g) in soybean plants exposed to flooding. Control (--), two (---) and four (-----) days of soil waterlogging.

showed a logistic trend, with a high coefficient of determination ($R^2 \geq 0.94$). Growth was slow until 28 days after sowing (DAS), and the maximum was observed at 98 DAS (Figure 1c). The increased periods of soil waterlogging reduced the production of total dry matter in the soybean plants compared with the control plants. According to Pires et al. (2002), in soybean, the level of tolerance to soil waterlogging can be represented by the dry matter content.

The increase in growth during ontogeny can be explained by the increase in the leaf area and the consequent increase in the net production of assimilates (Aumonde et al., 2011). However, hypoxic conditions reduce stomatal conductance, reducing the photosynthetic rate, consequently leading to reduced growth of plants subjected to soil waterlogging.

The dry matter production rate ($C_t$), representing the accumulation of dry matter as a function of time, peaked at 63 DAS in the control plants, whereas in the flooded plants, the maximum dry matter production rate was obtained at 70 DAS (Figure 1d). The plants subjected to two and four days of soil waterlogging exhibited a higher dry matter production rate up to 98 DAS, showing that this type of stress increases the growing season of
plants. Among the crop species, soybean is particularly susceptible to stress due to flooding (Komatsu et al., 2012). In the early stages of growth, soybean plants show differential regulation of hormone and carbohydrate metabolism (Nanjo et al., 2010, 2011), which contributes to the growth retardation of this species in soil waterlogging conditions.

The relative growth rate ($R_w$) presented the highest value in the early growth stages (14 and 28 DAS), with a further decrease being observed by the end of the crop cycle in the soybean plants (Figure 1e). However, at 65 DAS, there was a significant reduction of the relative growth rate in the plants that were not waterlogged compared with those subjected to soil waterlogging. In this case, the stress caused by soil waterlogging did not influence $R_w$ at the end of the cultivation cycle. The high photosynthetic capacity observed in young fully expanded leaves contributes to the high $R_w$ observed in early development (Aumonde et al., 2011), in addition to increasing the respiratory activity of other growth bodies (Barreiro et al., 2006), as the consequent decrease in this variable may be due to self-shading (Lopes et al., 1986).

The leaf area index (LAI), a variable dependent on the leaf area (Melges et al., 1989), showed a high coefficient of determination ($R^2 \geq 0.96$). The maximum growth occurred at 63, 70 and 56 DAS in the control plants and the plants subjected to two and four days of flooding, respectively (Figure 1f). An increase in the LAI results in greater interception of solar radiation, which may be reflected in the net photosynthesis (Heiffig et al., 2006). A decrease in the LAI is expected at the end of the cycle due to the increase in leaf senescence (Aumonde et al., 2011), and in soybean plants, this is enhanced by seed maturation.

The net assimilation rate ($E_a$) observed in control plants was higher compared with the plants subjected to periods of soil waterlogging (Figure 1g). The maximum values of the net assimilation rate were obtained at the beginning of vegetative growth (14 DAS) and subsequently decreased. A second peak occurred at 84 DAS, and the highest values were obtained in the plants subjected to two and four days of soil waterlogging, followed by the control plants. At the beginning of the development cycle, when self-shading is reduced, $E_a$ tends to present high values (Gondim et al., 2008). The second peak can be explained by the establishment of the reproductive phase (Lopes et al., 1986), which demands greater photosynthetic activity for the maintenance of new bodies.

Although it is only an estimate, $E_a$ express the rate of photosynthesis in terms of the dry matter produced, for the leaf area and time (Benincasa, 2003). Under soil waterlogging, the permeability of the root system decreases, and the absorption of water and minerals is therefore also impaired. Then, stomatal closure can be observed, accompanied by decreasing net photosynthesis (Liao and Lin, 2001). Under favorable conditions, plants with a high net assimilation rate tend to exhibit fast initial growth and greater interception of solar radiation (Vivian et al., 2013), which may have resulted in greater total dry matter production in the control plants.

The relationship between the leaf area and the total dry weight or the leaf area ratio ($F_a$) corresponds to the useful area for photosynthesis (Aumonde et al., 2011). The maximum values were obtained at 28 DAS in all treatments (Figure 1h), with a subsequent decrease being observed until the end of the crop cycle (98 days). Similar to $E_a$, the behavior of $F_a$ can be explained in part by the development of new leaves and the consequent self-shading (Peixoto and Peixoto, 2009; Barreiro et al., 2006). The high initial $F_a$ of plants under soil waterlogging may be due to the higher efficiency of the photosynthetic apparatus, regardless of the leaf area (Campos et al., 2008).

The leaf weight ratio ($F_w$), representing the estimated fraction of assimilates retained in the leaves and not exported to other organs of the plant, was high under all periods of soil waterlogging at 28 DAS. The control plants showed greater allocation of dry matter in the leaves at 28 DAS and at the end of the crop cycle compared with the soil waterlogging treatments. However, during ontogeny, the dry matter content tends to decrease due to an increase in the source/sink ratio (Linzmeyer Junior et al., 2008), whereas increased leaf senescence increases the need for assimilates to produce pods and for seed filling.

The solar energy conversion efficiency ($\xi$), which is related to the photosynthetic process and consequently the synthesis of assimilates, showed increasing curves up to 70 DAS (Figure 2b), with maximum values of 1.9, 1.4 and 1.2% being obtained for the control plants and the plants subjected to two and four days of soil waterlogging, respectively, with a subsequent decline being observed until the end of the crop cycle. This decrease is a result of the leaf senescence rate (Melges et al., 1989). Similar trends for $\xi$ were reported in soybean by Melges et al. (1989) and Silva Neto et al. (1991).

The dry matter partitioning between different structures in the soybean plants was modified during the development of the plants, under all periods of soil waterlogging (Figure 2). The control plants exhibited the greatest partitioning of dry matter in the leaves up to 28 DAS, followed by the stems and roots (Figure 2c). Beginning at 42 days, dry matter accumulation was observed in the pods, which increased at 70 DAS, reducing the allocation to the leaves and roots.

In the plants subjected to two days of soil waterlogging, dry matter accumulation in the leaves showed a maximum at 14 DAS and was reduced by the end of the crop cycle (Figure 2d). At 56 DAS, dry matter accumulation in the pods was increased, while that in the leaves and stem was reduced. A similar trend in the partitioning of dry matter between different organs was
observed in the plants subjected to four days of flooding (Figure 2e). However, at 42 DAS, there was an increase in dry matter accumulation in the leaves, which had decreased at 98 days. Thus, it was observed that the conditions of soil waterlogging increased the vegetative growth stage of the soybean plants, causing a delay in the early reproductive stage.

Soil waterlogging can reduce the rate of carbohydrate translocation to the leaves, causing accumulation of assimilates in the form of starch (Liao and Lin, 2001). The delay in dry matter accumulation in the pods of plants subjected to two and four days of flooding could be a result of reduced production and translocation of assimilates from the leaves to other organs and chemical changes in the soil caused by the flooding of the soil (Yordanova et al., 2004; Parent et al., 2008). The number of pods was greater in the control plants compared with those subjected to flooding. These results reflected the thousand seed weight, which was lower in the control plants (Table 1). The lower pod production observed in plants grown under soil waterlogging conditions may have been a reflection of the retardation of the vegetative cycle of the plants, which resulted in the lowest solar energy conversion efficiency (Figure 2b) for the production of pods. However, the increase of $E_a$ detected in plants under soil waterlogging during seed filling (84 DAS) may have resulted in a greater thousand seed weight compared with control plants.

Obtaining a good yield in soybean plants depends on a large intake of carbohydrates, which is in turn dependent on the photosynthetic rate guaranteed by the leafiness of plants (Pereira, 1989). The weight of seeds can be used as an indicator of physiological conditions, where seeds with a high weight exhibit greater amounts of reserve
substances and a consequent increase in the probability of success in seedling establishment (Peske et al., 2012). When seedling emergence was evaluated, it was found to be greater in seeds produced under flooding (Table 1). Similarly, the leaf area was greater in the plants obtained from seeds produced under four and two days of soil waterlogging, followed by the control. However, the plants from seeds produced under four days of soil waterlogging exhibited a higher total dry matter content, possibly due to the greater seed weight (Table 1), which is indicative of seeds with adequate reserves that may contribute to the increased expression of seed vigor and superior performance of seedlings (Peske et al., 2012).

The analysis of variance for the primary growth data is shown in Table 2. From the analysis of the mean squares for the leaf area and dry matter data for organs, it was observed that there was a high degree of significance between the conditions of soil waterlogging and the seasons of collection.

### Conclusions

Soil waterlogging affects the growth and partitioning of dry matter in soybean plants. Moreover, it reduces the efficiency of solar energy conversion over time and increases the thousand seed weight and the expression of seed vigor.

### Conflict of Interest

The authors have not declared any conflict of interest.

### REFERENCES


