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Tolerance of soybean cultivars to flooding stress in vegetative growth stages

Abstract – The objective of this work was to evaluate the tolerance of soybean (*Glycine max*) cultivars to flooding stress at different growth stages. The experiment was arranged in a 2x2x5 factorial arrangement, with two sowing dates (October and November), two soybean cultivars (TECIRGA 6070RR and NA 5909 RG), and five growth stages (SE–EM, EM–VC, VC–V2, V2–V4, and V6–V8) in the 2018/2019 and 2019/2020 crop years. The experimental design was completely randomized with four replicates. For both evaluated soybean cultivars, the growth stage most sensitive to soil flooding was SE–EM. After seedling emergence, the highest reductions in leaf area and shoot dry matter were observed in the V2–V4 stage, regardless of cultivar, sowing date, and crop year. From the V4 stage onwards, soybean shows a higher tolerance to flooding conditions. However, after seedling emergence, the TECIRGA 6070RR cultivar has a higher tolerance to flooding stress than NA 5909 RG. Sowing in October tends to reduce the impact of flooding stress on the plants.

Index terms: *Glycine max*, phenology, rice-soybean rotation, soil flooding, sowing dates.

Tolerância de cultivares de soja ao estresse por alagamento em estádios de crescimento vegetativo

Resumo – O objetivo deste trabalho foi avaliar a tolerância de cultivares de soja (*Glycine max*) ao estresse por alagamento, em diferentes estádios de crescimento. O experimento foi realizado em arranjo fatorial 2x2x5, com duas épocas de semeadura (outubro e novembro), duas cultivares de soja (TECIRGA 6070RR e NA 5909 RG) e cinco fases de desenvolvimento (SE–EM, EM–VC, VC–V2, V2–V4 e V6–V8), nos anos agrícolas 2018/2019 e 2019/2020. O delineamento experimental foi inteiramente casualizado com quatro repetições. Para ambas as cultivares avaliadas, o estádio de crescimento mais sensível ao alagamento do solo foi o SE–EM. Após a emergência das plântulas, as maiores reduções de área foliar e matéria seca de brotos foram observadas no estádio V2–V4, independentemente de cultivar, época de semeadura e ano agrícola. A partir do estádio V4, a soja apresenta maior tolerância às condições de alagamento. No entanto, após a emergência das plântulas, a cultivar TECIRGA 6070RR apresenta maior tolerância ao estresse por inundação do que a NA 5909 RG. A semeadura em outubro tende a reduzir o impacto do estresse por inundação nas plantas.

Termos para indexação: *Glycine max*, fenologia, rotação de arroz e soja, alagamento do solo, datas de semeadura.

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Introduction

In the last ten years, due to the increased pressure of weeds and higher costs for rice (*Oryza sativa* L.) production, farmers in Latin America started introducing soybean [*Glycine max* (L.) Merr.] to the traditional system of continuously flooded irrigated rice production (Theisen et al., 2017; Ulguim et al., 2018; Ribas et al., 2021a, 2021b). In Brazil, the adoption of the rice-soybean crop rotation system increased exponentially from 10 thousand hectares in the 2009/2010 crop year to 408 thousand hectares in 2021/2022, covering approximately 55% of the current flooded irrigated rice area in the state of Rio Grande do Sul (Pereira, 2018).

However, the average soybean yield in this crop rotation system is 1.8 Mg ha^{-1}, lower than that of 3.4 Mg ha^{-1} reported in Brazilian highlands (Irga, 2018; Conab, 2022). The main challenge for the rotation of soybean with flooded irrigated rice are the paddy fields cultivation, which have a reduced natural drainage and are subject to temporary flooding, especially after heavy rains (Sartori et al., 2016; Zanon et al., 2018) as those that usually occur in Rio Grande do Sul at the beginning of spring, coinciding with the start of soybean sowing, which explains why many producers need to replant the crop (Bortoluzzi et al., 2021b; Tagliapietra et al., 2021).

Under flooding conditions, plants show biochemical and physiological alterations a few hours after the stress begins and morphological alterations as they attempt to acclimatize to it, a capacity that varies according to cultivar and growth stage and that can be irreversible (Taiz et al., 2017). The intensity of flooding stress is determined by meteorological elements, which maximize or delay its effect by influencing the evapotranspiration system of the plants (Timm et al., 2014).

In the case of soybean, Dhungana et al. (2019) found that plant tolerance to excess water varies depending on soil texture, concluding that cultivars responded differently in lowland (clayey) and upland (sandy) soils. For this reason, according to the same authors, cultivars adapted to different edaphoclimatic conditions and used locally by farmers should be further researched. However, studies on the effects of soil flooding on the development of soybean cultivars at different phenological stages are still scarce.

For the identification of more tolerant cultivars and efficient management strategies, it is important to understand the ecophysiology of soybean plants in environments prone to flooding, which will allow to mitigate the damages caused by this stress in the most sensitive growth stages, improving the soybean-rice rotation system. The evaluation of the growth and development of soybean cultivars subjected to flooding stress under different environmental conditions and vegetative growth stages may also provide insights for other systems worldwide based on rice, aiming an increase in yield and profit, as well as a reduced environmental impact.

The objective of this work was to evaluate the tolerance of soybean cultivars to flooding stress at different growth stages.

Materials and Methods

The experiment was conducted during 105 days in the 2018/2019 and 2019/2020 crop years, each with two sowing dates: October 12 and November 28 in 2018, and October 12 and November 30 in 2019. The following two soybean cultivars were used: NA 5909 RG, one of the most grown in the South of Brazil in the last ten years, classified into relative maturity group (RMG) 6.2; and TECIRGA 6070RR, characterized as tolerant to flooding stress and classified into RMG 6.3 (Irga, 2018). The experiment was carried out in the experimental area of the Department of Plant Sciences of Universidade Federal de Santa Maria, located in the municipality of Santa Maria, in the state of Rio Grande do Sul, Brazil (29º43'S, 53º49'W, at 90 m above sea level).

The experimental design was completely randomized with four replicates, each consisting of one plant grown in a plastic pot filled with 7.0 dm^3 soil. The used soil was taken from the superficial layer (0–20 cm) of an Argissolo Bruno-Acinzentado Ta Alumínico típico (Santos et al., 2018), with 22% of clay, which corresponds to a Typic Albaqualf (Soil Survey Staff, 2022). Nutrient deficiency and soil acidity correction were conducted according to soil analysis results and local recommendations for soybean crops in order to avoid nutritional limitations and toxicity to plants (Manual..., 2016).

The evaluated treatments were the five following soybean growth stages, as well as a control: SE–EM, sowing-emergence; EM–VC, emergence-cotyledons;

VC–V2, cotyledons-first fully expanded trifoliate leaf; V2–V4, first fully expanded trifoliate leaf-third fully expanded trifoliate leaf; V6-V8, fifth fully expanded trifoliate leaf-seventh fully expanded trifoliate leaf; and control, pots at the same phenological stage as those of each treatment under flooding stress, with 90% available water capacity (AWC).

A 2x2x5 factorial arrangement was used, with two sowing dates (October and November), two soybean cultivars (NA 5909 RG and TECIRGA 6070RR), and the five growth stages (SE–EM, EM–VC, VC–V2, V2–V4, and V6–V8); the latter were chosen because they showed higher losses by flooding according to farmers, consultants, and researchers (Irga, 2018). In the first three growth stages, the crop was initially established and the number of plants per area (the main component of soybean yield) was determined, and, in the last two, branches and floral primordium were differentiated (Zanon et al., 2018).

The experiment was carried out inside a 150 m^2 greenhouse, covered with low-density (200 µm) polyethylene, where 160 pots with five seeds each were placed on a bench 70 cm above ground level. There was no temperature and humidity control, only pot irrigation control. Seeds were previously treated and inoculated with *Bradyrhizobium elkanii* (Fuhrmann & Wollum, 1985) and, then, sown at a 3.0 cm depth; after seedling emergence, only one plant was kept per pot. The space between pots in a row was 10 cm, and between rows, 40 cm.

For flooding stress, the pots with plants were placed individually inside larger containers, to which water was added, keeping a constant water column of 5.0 cm above soil level. Four replicates were also conducted for each growth stage in the control treatment, with 90% field capacity. For the control plants, maintenance irrigation was performed through the weighing method: the pot was weighed when 100% field capacity was reached, after which it was weighed daily to quantify the consumption of water, which was replaced every day to reach at least 90% AWC.

During the experimental period, air temperature data were collected using the HT-500 thermo-hygrometer (Instrutherm: Instrumentos de Medição Ltda, São Paulo, SP, Brazil), with an attached mini data logger, kept inside a small instrument shelter at the site. Solar radiation data was collected at an automatic meteorological station of Instituto Nacional de Meteorologia, at approximately 200 m from the experimental area. The incidence of daily global solar radiation on plants was corrected based on 80% transmission through plastic shading. Minimum and maximum temperatures, as well as solar radiation and photoperiod, are shown in Figure 1.

Before flooding stress and on the last day of the experiment, the following variables were evaluated: plant height, measured from the stem at soil level up to the last visible node; leaf area, calculated using the length and width of the central leaflet of all leaves of each plant, through the equation (length × width) × 2.0185 of Richter et al. (2014); and shoot dry matter, obtained by drying the plants in a forced-air oven, at 65ºC, until constant weight.

In the analysis and interpretation of the results, increases in the values of all variables of each treatment due to flooding stress were compared with those of the control. This was done by subtracting the value registered on the first day of the experiment from that obtained on the last, generating a coefficient. When the coefficients were higher than 1, the values of the variables were considered higher than those of the control, but, when the coefficients were lower than 1, lower.

The presuppositions of the model, specifically normality, randomness, and homogeneity of variances, were tested through the tests of Shapiro-Wilk, Breusch-Pagan, and Durbin-Watson, respectively. Then, the data were analyzed through the analysis of variance using the metan package of the R software (Olivoto & Lúcio, 2020). The graphs were built using the ggplot2 (Wickham, 2016) and emmeans (Lenth et al., 2022) packages. Means were compared by Tukey's test, at 5% probability.

Results and Discussion

In both crop years, the plants sown in October were exposed to a lower average air temperature than those sown in November, possibly resulting in a lower evaporative demand. Therefore, under flooding conditions, there was a direct effect of air temperature and solar radiation on plant morphological responses (growth and development). The minimum air temperature was 10.0ºC on 12/03/2018 and 10.6ºC on 10/25/2019, whereas the maximum air temperature was 38.6ºC on 12/11/2018 and 37.9ºC on 12/29/2019 (Figure 1 A and C). The photoperiod to which the soybean plants were exposed to ranged from 13.45 to 14.96 hours, and the periods with a higher solar radiation incidence were the end of October and November, as well as the beginning of December (Figure 1 B and D).

The most sensitive plant growth stage to flooding stress was SE–EM since seeds did not germinate in both sowing dates (Figure 2 A and B). This could be explained by an increase in the respiration rate and enzymatic activity after the first peak of seed imbibition, causing a high O_2 demand, which increases seed damage (Taiz et al., 2017). Similar results were found by Zhou et al. (2021), who concluded that flooding decreased seed vigor and germination due to a decrease in sugar contents and an increase in cell conductivity and ethanol content.

After emergence, no plant death was observed at any stage. However, under flooding conditions, the plants underwent morphophysiological changes such as the formation of superficial roots and cracking of the stem base due to aerenchyma development (Figure 2 C and D), which are strategies to capture O_2 in order to meet the minimum requirements for root respiration. Similar results have been reported for wheat (*Triticum aestivum* L.) by Araki et al. (2012) and sunflower (*Helianthus annuus* L.) by Loose et al. (2017).

Leaf area was significantly affected by the interaction between sowing season, cultivar, and treatment in the 2018/2019 crop year according to the analysis of variance (Figure 3). For soybean cultivars and sowing dates, flooding stress reduced leaf area in all plant growth stages. Likewise, Ludwig et al. (2016) found a decreased number of nodes on the main stem, plant height, and chlorophyll content index after eight days of flooding. Since leaf growth depends mainly on cell division and expansion, the growth rate of leaves is inhibited during their initial development (Taiz et al., 2017). Considering sowing dates, cultivars, and crop years, a leaf area similar to that of the control was observed in the EM–VC stage, which was the least affected by flooding stress probably due to its short duration (about three days) and to the presence of photoassimilates that promote growth, mainly of cotyledons.

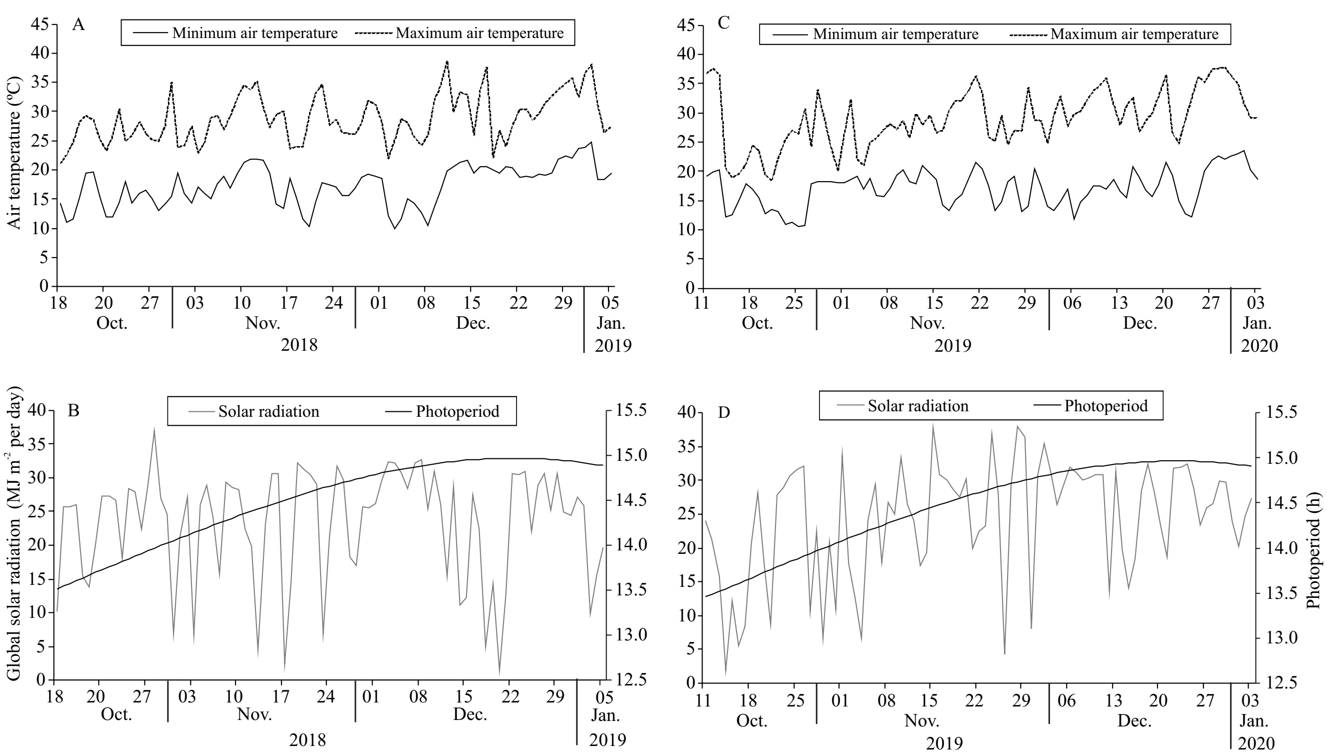

Figure 1. Minimum (A) and maximum (C) air temperatures and global solar radiation (B) and photoperiod (D) inside the greenhouse during the experiment in the 2018/2019 and 2019/2020 crop years, in the municipality of Santa Maria, in the state of Rio Grande do Sul, Brazil.

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In the same crop year, cultivar TECIRGA 6070RR showed a significantly reduced leaf area at the V2–V4 stage when sown in October and at the V6–V8 stage when sown in November. Garcia et al. (2020) also observed changes in the physiology and metabolism of the roots and leaves of five soybean genotypes during and after flooding stress, including late flowering and a significant reduction in leaf gas exchanges (photosynthesis, stomatal conductance, and leaf transpiration).

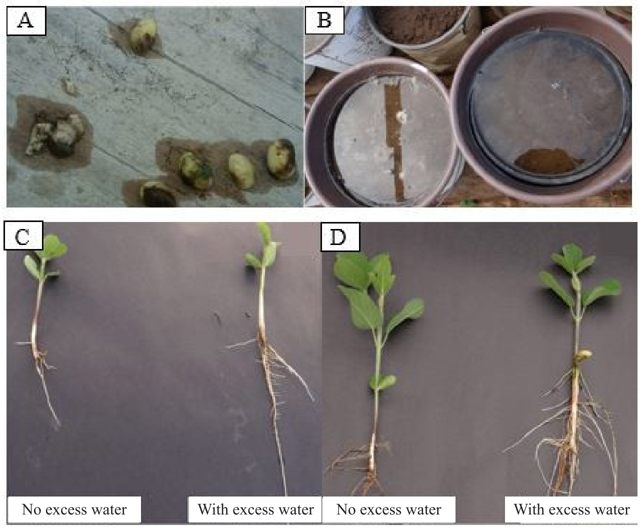

Figure 2. Images showing soybean (*Glycine max*) seeds damaged after soil flooding (A) and pots with no seedling emergence (B) in the sowing-emergence stage, as well comparing soybean plants from the control treatment with those subjected to flooding in the emergence-cotyledon (C) and cotyledon-first fully expanded trifoliate leaf (D) stages. Photos by Patrícia Carine Hüller Goergen.

In the 2019/2020 crop year, leaf area was not significantly affected by factors and their interactions. The increase in leaf area, compared with that of the control plants, was lower for the NA 5909 RG cultivar at all stages, but slightly higher for TECIRGA 6070RR at the V6–V8 stage in both sowing dates (Figure 4 A). This latter response to flooding stress can be explained by the genetic tolerance of the cultivar and by the growth stage, characterized by larger plants with greater root and shoot structures, which favor a better plant development in a stressful environment. Similarly, Kirkpatrick et al. (2006) found that seven days of flooding at the V4 stage were not enough to reduce plant stand, decreasing shoot dry matter in only one of the three study years.

In the two crop years, plant height was not significantly affected by factors and their interactions. The mean increase in plant height, compared with that of the control plants, was lower for cultivar NA 5909 RG in both sowing dates in 2018/2019 and for TECIRGA

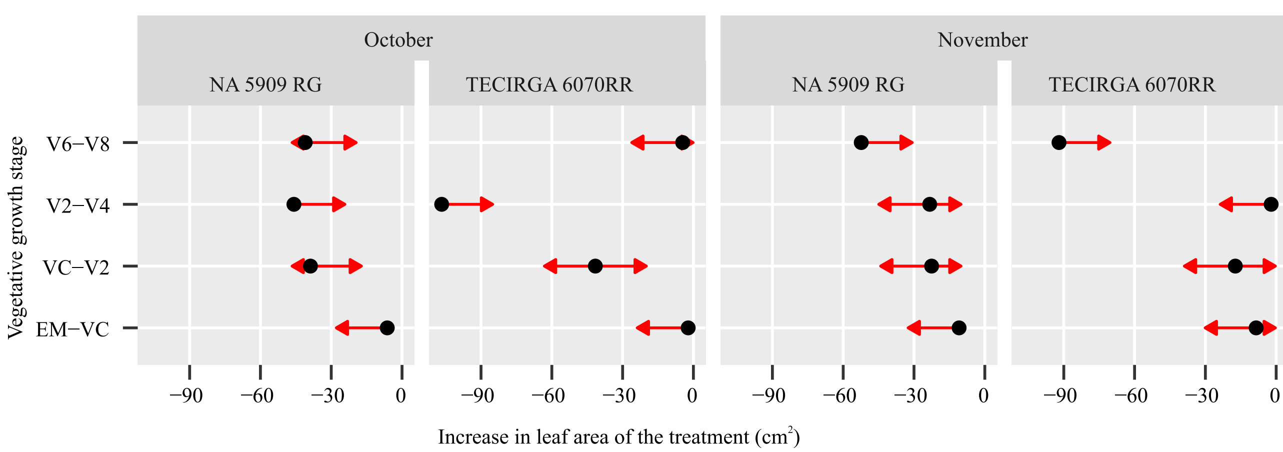

Figure 3. Increase in the leaf area of the NA 5909 RG and TECIRGA 6070RR soybean (*Glycine max*) cultivars determined by Tukey's test, at 5% probability, at four growth stages under soil flooding conditions in the October and November sowing dates in the 2018/2019 crop year. Arrows show the minimum significant difference according to Tukey's test. Treatments containing overlapping arrows are assumed to have no significant differences in mean values. EM–VC, emergence-cotyledons; VC–V2, cotyledons-first fully expanded trifoliate leaf; V2–V4, first fully expanded trifoliate leaf-third fully expanded trifoliate leaf; and V6–V8, fifth fully expanded trifoliate leaf-seventh fully expanded trifoliate leaf.

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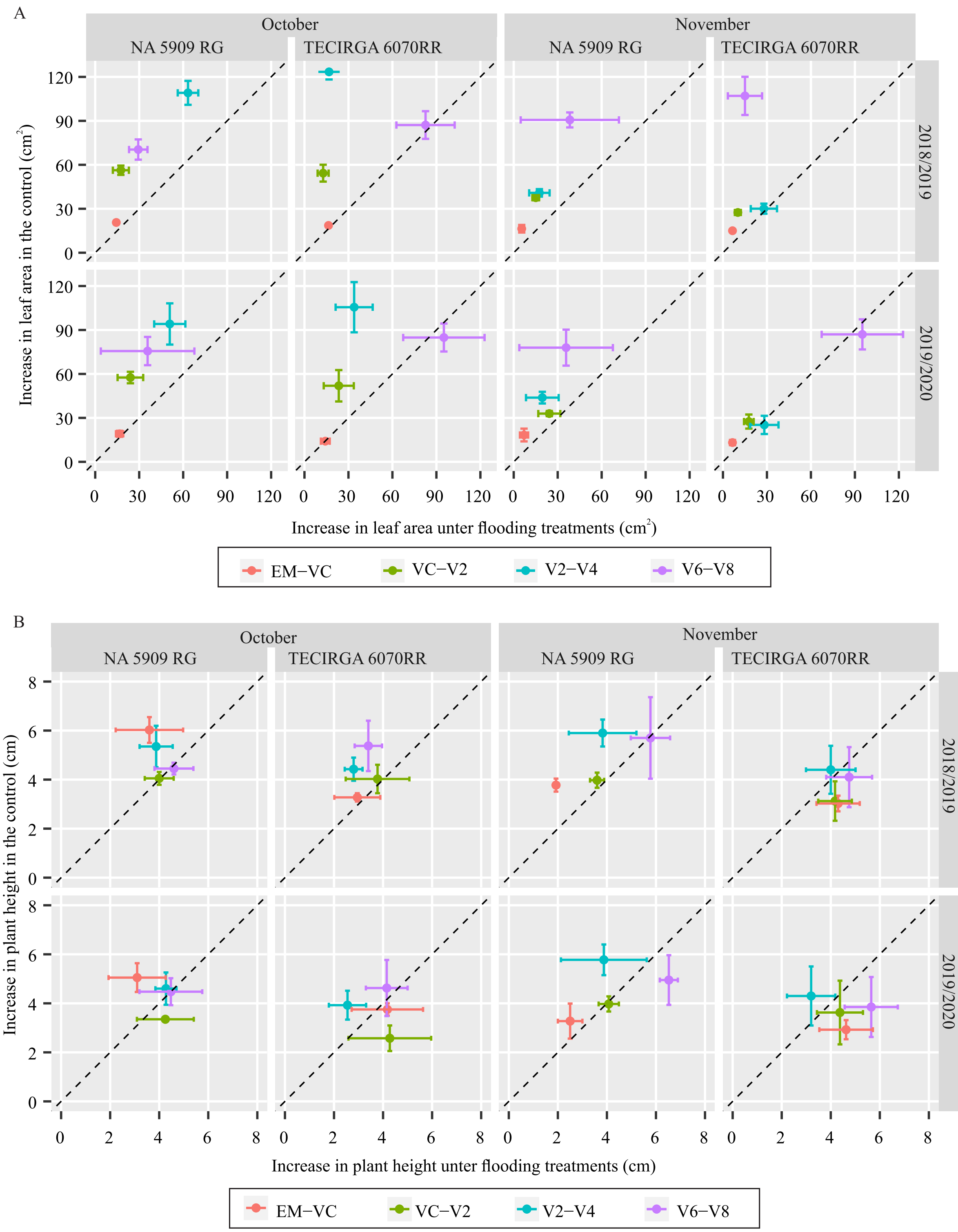

Figure 4. Mean increases in leaf area (A) and plant height (B), including average standard deviation, of the NA 5909 RG and TECIRGA 6070RR soybean (*Glycine max*) cultivars at four growth stages under soil flooding conditions in the October and November sowing dates in the 2018/2019 and 2019/2020 crop years, when compared with the control plants (without excess water). EM–VC, emergence-cotyledons; VC–V2, cotyledons-first fully expanded trifoliate leaf; V2–V4, first fully expanded trifoliate leaf-third fully expanded trifoliate leaf; and V6–V8, fifth fully expanded trifoliate leaf-seventh fully expanded trifoliate leaf.

6070RR sown in October (Figure 4 B). This lower plant growth is a result of the low-aeration environment generated by flooding, which favors the dissemination of diseases, oxidative damage to root cells, and nutrient loss by leaching (Taiz et al., 2017). Coutinho et al. (2018) pointed out that flooding stress affects both the primary and secondary metabolisms of soybean plants, as well as changes in carbon and nitrogen metabolisms and the phenylpropanoid pathway.

Cultivar TECIRGA 6070RR sown in November showed a good tolerance to flooding stress, i.e., a greater plant growth at the EM–VC, VC–V2, and V6–V8 stages due to the higher air temperature during these periods, which might have accelerated their already short duration of, on average, only a few days. In 2019/2020, the NA 5909 RG cultivar had a higher plant height at the VC–V2 and V6–V8 stages, respectively, on the first and second sowing dates. Moreover, cultivar TECIRGA 6070RR grew more than the control at the VC–V2 stage, on the first sowing date, and at all stages, except at V2–V4, in the second. Zhou et al. (2020) concluded that a greater stem growth allows of the plant to capture more light for photosynthesis and to increase the energy storage for primary life activities under flooding conditions, contributing to resistance to extended water stress.

In the 2018/2019 crop season, shoot dry matter was influenced by the interaction between sowing date, cultivar, and treatment, being affected by flooding stress in all cases. The exception was cultivar TECIRGA 6070RR at the V6–V8 stage in the second sowing date (Figure 5 A). As previously highlighted, at this stage, plants have larger root and shoot structures, which favor a better development under stressful conditions. The obtained results can also be attributed to the higher genetic tolerance to flooding stress of the TECIRGA 6070RR cultivar (Irga, 2018).

In 2019/2020, shoot dry matter was only significantly affected by the interactions between season and treatment and between cultivar and treatment. Flooding stress reduced plant growth at all stages and in both crop years, as shown by the values obtained for shoot dry matter (Figure 5 B). According to Zhou et al. (2020), soybean plants present a lower biomass production under flooding or hypoxia conditions. In the present study, the TECIRGA 6070RR cultivar sown in October was the exception, showing a higher increase in the studied variable at the V6–V8 stage, which is an indicative of its better performance under flooding conditions when compared with the control plants.

In relation to the control plants under field capacity conditions, cultivar NA 5909 RG showed a reduced growth at all stages, whereas TECIRGA 6070RR presented a better growth, enduring well the flooding conditions at the V6–V8 stage (Figure 5 C). Under flooding stress, plants respond via two primary alterations: low-oxygen quiescence syndrome and low-oxygen escape syndrome through phytohormone-mediated pathways involved in plant waterlogging stress (Sharma, 2018; Zhou et al., 2020). According to Sharma (2018), the second syndrome includes fast stem, internode, and petiole growth under flooding conditions, which allows of plants, such as those of rice, to reach the water surface quickly, reestablishing gas exchanges between their tissues and the atmosphere.

Problems related with plant stand have also been reported, explaining why soybean yield in lowlands (1.8 Mg ha^{-1}) is 30% lower than that in highlands (3.4 Mg ha^{-1}) (Pereira, 2021; Conab, 2022). Therefore, in lowlands, soybean producers must invest in drainage systems to reduce the risks associated with excess soil water, especially at the VE–V4 initial stages, considered the most susceptible. Although all plants survived after emergence in the present study, under field conditions, the cracking of the epidermis due to aerenchyma formation contributes to soil-fungi infection, which is responsible for plant death in the crop rotation system with soybean and flooded irrigated rice (Irga, 2018; Goulart et al., 2020; Bortoluzzi et al., 2021a). From the V4 stage onwards, even though soybean showed a higher tolerance to flooding stress, significant losses in yield potential were caused by the anaerobic environment, which induced the plant to adopt a metabolism with a lower energy gain (Taiz et al., 2017).

Flooding stress was lower for soybean sown in October due to the lower air temperatures registered (Figure 1 A and C). Taiz et al. (2017) concluded that the availability of solar radiation and the predominant air temperature during a period of soil saturation may delay or maximize the effects of flooding stress on the plant. In plants under flooding conditions, respiration is already affected by O_2 absence or deficiency in the soil and respiratory rates are increased through membrane and cell organelle disruption at higher air

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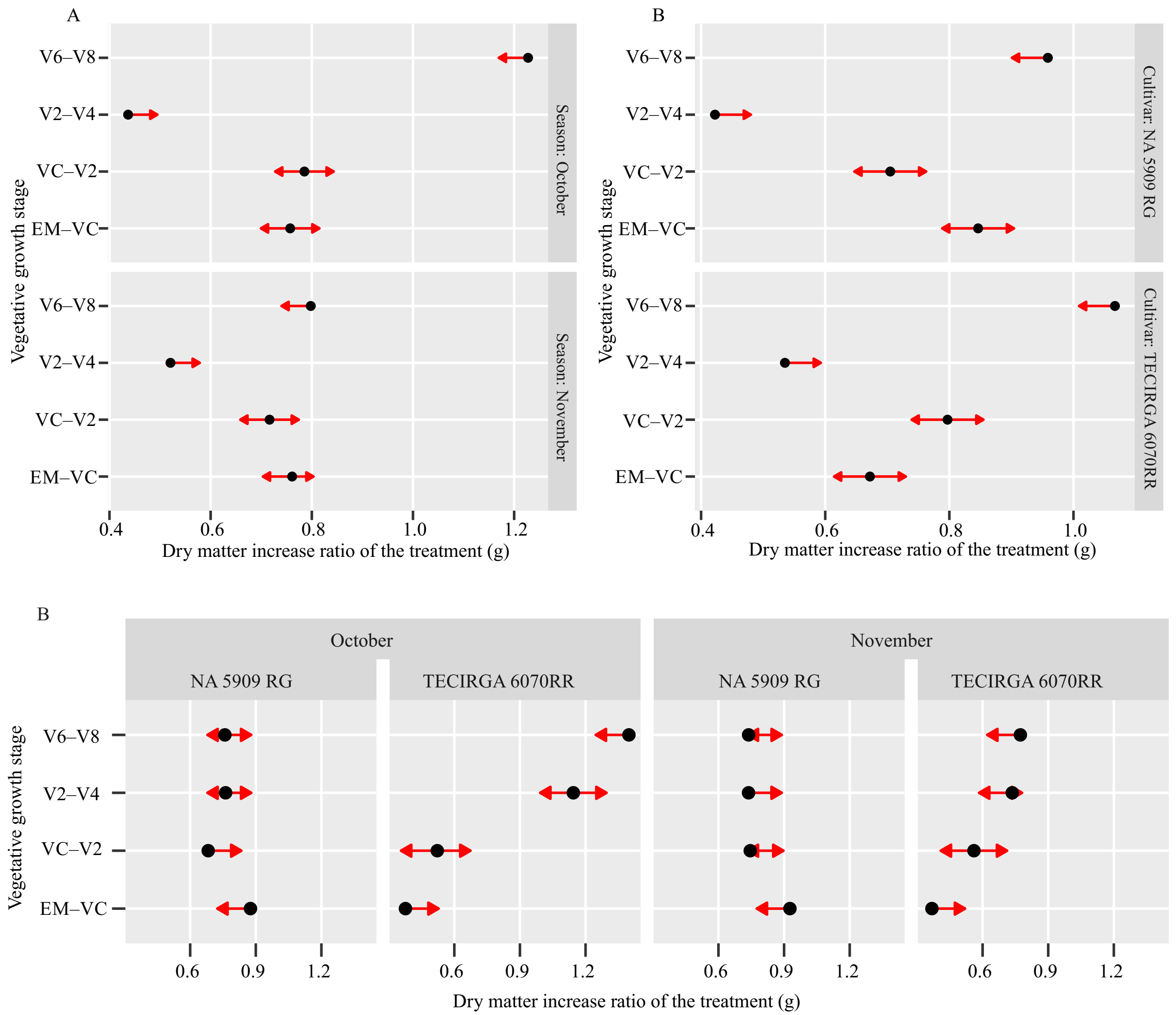

Figure 5. Mean comparison by Tukey's test, at 5% probability, for: dry matter increase ratio of the NA 5909 RG and TECIRGA 6070RR soybean (*Glycine max*) cultivars at four growth stages under soil flooding conditions in the October and November sowing dates in the 2018/2019 crop year (A); and interactions between season and treatment (B) and between cultivar and treatment (C) for soybean dry matter in 2019/2020. Arrows show the minimum significant difference according to Tukey's test. Treatments containing overlapping arrows are assumed to have no significant differences in mean values.

temperatures, a process that requires a greater amount of energy, leading to a reduced growth, development, and, consequently, yield (Taiz et al., 2017; Rajendran & Lal, 2021).

Therefore, to apply the results obtained in the rice-soybean rotation system, farmers must take into account meteorological broadcasts and the possibility of soil flooding, avoiding sowing during periods of excess rain because, regardless of the crop year, there will be problems related to soybean germination and emergence in anaerobic environments (Bortoluzzi et al., 2021a).

Conclusions

1. For the NA 5909 RG and TECIRGA 6070RR soybean (*Glycine max*) cultivars, the growth stage most sensitive to soil flooding is sowing-emergence.

2. After seedling emergence, the highest reductions in leaf area and shoot dry matter for all evaluated cultivars, sowing dates, and crop years are observed in the first fully expanded trifoliate leaf-third fully expanded trifoliate leaf stage.

3. After seedling emergence, cultivar TECIRGA 6070RR shows the highest tolerance to flooding stress from the third fully expanded trifoliate leaf stage onwards.

4. Sowing in October tends to reduce the impact of flooding stress on soybean plants due to the lower air temperatures registered.

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