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RESEARCH ARTICLE

Physiological and production performance of maize and soybean in crop-livestock-forest integration system under hydrogel rates and nitrogen and potassium topdressing

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ABSTRACT

Water availability affects nutrient absorption and transport in plants, which is a limiting factor for production of annual crops in crop-livestock-forest integration system. Thus, the objective of the present work was to evaluate the effects of hydrogel rates combined with topdressing in a crop-livestock-forest integration system on physiological and production variables of maize and soybean plants grown intercropped with forage (*Panicum maximum* cv. BRS Kenya) and eucalyptus (*Eucalyptus urograndis*). The experiments were conducted at the School Farm of the Federal Institute Goiano, Iporá campus, Goias, Brazil, in a Typic Dystrudept. A randomized block experimental design was used, in a split-plot arrangement with four replications. The plots consisted of five hydrogel rates based on the rate recommended by the manufacturer: 0 kg ha^{-1} (0%), 7.5 kg ha^{-1} (50%), 15.0 kg ha^{-1} (100%), 22.5 kg ha^{-1} (150%), and 30.0 kg ha^{-1} (200%). The subplots consisted of presence and absence of nitrogen topdressing for maize, and potassium for soybean, with application of 120 kg ha^{-1} of nitrogen (urea) and 60 kg ha^{-1} of K_2O (potassium chloride). The crop systems were arranged in two experiments; experiment I consisted of eucalyptus intercropped with maize grown simultaneously with forage; experiment II consisted of eucalyptus intercropped with soybean, and the forage species grown in succession (post-harvest of soybean). Physiological variables of maize and soybean plants at the pre-flowering stage were analyzed. The treatments for maize crop affected the stomatal conductance, flavonoids, proline, and silage dry weight. The treatments for soybean crop had significant effects on relative water content, flavonoids, proline, and number of plants. The hydrogel rate of 7.5 kg ha^{-1} (50%) resulted in positive results for both crops.

Keywords: Potassium Chloride; Water Availability; Nitrogen; Recycling of Nutrients; Integrated System

INTRODUCTION

Brazilian agriculture is facing problems with soil degradation, which can be characterizing as a dynamic process of degeneration or decrease in yield of pastures and crops (Patrizi et al., 2018), which consequently compromise the sustainability of agricultural production. In this context, the adoption of alternatives for soil uses that aggregate economic and environmental returns is necessary (Gil et al., 2015), such as the crop-livestock-forest integration systems (CLFIS). CLFIS are practices that associate such characteristics by combining agricultural, livestock, and forest activities in a same area, resulting in a sustainable alternative that can be adopted by farmers (Moraes et al., 2014).

Integration systems can be classified in four main types: i- crop-livestock integration system (CLIS), which integrates agriculture and livestock by rotation (intercrop or succession); ii- livestock-forest integration system (LFIS), which integrates livestock (pasture and animal) and forest by intercropping; iii- crop-forest integration system (CFIS), which integrates forest and agriculture by intercropping arboreal species with agricultural crops (annual or perennials); and iv- crop-livestock-forest integration system (CLFIS), which integrates agriculture, livestock, and forest by rotation (intercrop or succession) in a same area (Balbino et al., 2011). The most common intercropping system are composed of eucalyptus intercropped with annual crops in the two first years, such as soybean and maize under intercrop or succession with grass pasture (Magalhães et al.,

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2019). Maize crops have been used due to their capacity to compete with forage species (Vilela et al., 2011). Soybean crop is used to recover degraded areas, in an arrangement of rotation with pasture (De Andrade et al., 2020). Both crops have presented good economic results in many regions of Brazil (Costa et al., 2018).

One of the synergism forms of CLFIS is the decomposition of organic matter (litterfall), benefiting the cycling of nutrients for the components of this system (Calil et al., 2016). Plant and animal residues from CLFIS impact soil nutrient stocks (Assmann et al., 2014). Nitrogen (N) is one the most required macronutrients and an element that participates in several important metabolic processes of plants, such as protein synthesis and composition of molecules, such as ATP, NADH, NADPH, and chlorophyll, denoting that N is directly correlated to plant development and yield (Demari et al., 2016). Potassium (K) is also an important nutrient, which participates in several functions, such as enzymatic activation, regulation of opening and closure of stomata, and osmotic control (Korber et al., 2017).

The homeostasis of these nutrients in plant tissues can be compromised by the lack of water (Mazloom et al., 2020), which affects nutrient absorption and transport in the plant (Ling et al., 2017). Therefore, water availability is a limiting factor for production of annual crops in CLFIS (Andrea et al., 2018). This limitation has been intensifying due to climate changes, which generate long periods without rainfalls associated with high temperatures (Langner et al., 2019) and, thus, inducing decreases in water potential of plant tissues, promoting metabolic disturbances and possible negative effects to plant growth and development (Lavinsky et al., 2015; Ávila et al., 2021).

An alternative that improves water availability and nutrient absorption by plants is the maintenance of soil moisture by using hydrogels. Hydrogels, also known as water retainers, are regulators of water availability (Kraisig et al., 2018). When hydrogels are incorporated into the soil, they withhold large quantities of water and nutrients, which are released according to the plant demand (Islam et al., 2011). Hydrogels can absorb salts present in the soil, decreasing the salinity around the roots and improving plant performance (El-Rehim, Hegazy and El-Mohdy, 2004). Hydrogels have been used in forest plantations, such as eucalyptus (Teixeira et al., 2019), native species used for recovery of degraded areas and reforestation (Fonseca et al., 2017), vegetables (Santos et al., 2015), fruit tree species, such as citrus (Ferreira et al., 2014), and coffee crops (Conte et al., 2014). However, important lacunas are found in studies, mainly due to the lack of tests of development of plants grown in CLFIS, which is an environment with high competitiveness for water and nutrients (Sarto et al., 2020). In this context, the objective of the present study was to evaluate the effects of hydrogel rates combined with topdressing in a CLFIS on physiological variables of maize and soybean intercropped with a forage (*Panicum maximum* cv. Kenya) and eucalyptus (*Eucalyptus urograndis*) species.

MATERIAL AND METHODS

Location and climate

The experiments were conducted at the School Farm of the Federal Institute Goiano, Iporá campus, Goiás, Brazil (16°25'26.91"S, 51°9'5.23"W, and 595 m altitude). Rainfall depths and temperatures recorded during the maize and soybean crop cycles are shown in Fig. 1.

The experimental area was cultivated with soybean in the 2016/2017 crop season, with pasture sown in the period between crop seasons. A triple planting was carried out in the 2017/2018 crop season using maize, pigeon pea, and a grass species (*Brachiaria brizantha* cv BRS Xaraés) to produce silage. Subsequently, the area was used during the second crop and between crop seasons for grazing of beef cattle. The crop-livestock-forest integration system (CLFIS) was implemented in October, 2018.

The spatial arrangement of trees consisted of planting of two eucalyptus clones (*Urograndis* - i144 and *Urocam* - VM01), with spacing of 1.5 × 10 m.

The crop systems were arranged in two experiments, conducted in different and adjacent areas with the same use and management history: experiment I consisted of eucalyptus intercropped with maize grown simultaneously with the forage species; experiment II consisted of eucalyptus intercropped with soybean, and the forage species was implemented in succession (post-harvest of soybean).

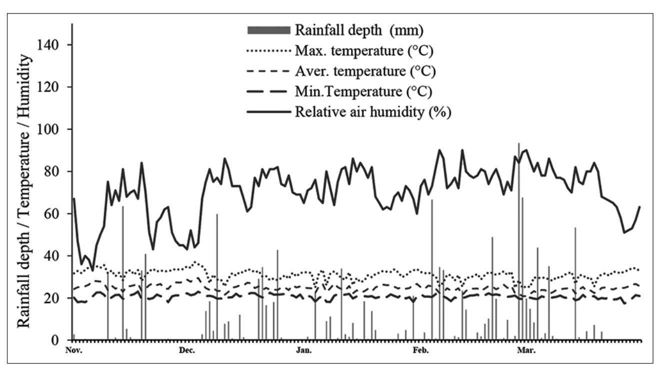

Fig 1. Daily rainfall depth, maximum temperature, average temperature, minimum temperature, and relative air humidity in the experimental area from November, 2020 to March, 2021. Source: Brazilian National Institute of Meteorology, Iporá, GO, Brazil.

The soil of the experimental area was classified as Typic Dystrudept (Cambissolo Háplico distrófico) (Santos et al., 2018). Before the implementation of the experiments, samples of the soil 0 to 0.20 m layer were collected for chemical and granulometric (texture) characterizations; they were analyzed according to methodologies described by Teixeira et al. (2017) and presented the following results: pH = 4.8; organic matter = 1.2%; P (Mehlich I) = 2.0 mg dm^{-3}; H + Al = 2.7 $cmol_c$ dm^{-3}; Ca = 1.4 $cmol_c$ dm^{-3}; Mg = 0.4 $cmol_c$ dm^{-3}; K = 0.37 $cmol_c$ dm^{-3}, and cation exchange capacity = 4.87 $cmol_c$ dm^{-3}. The soil presented 250, 60, and 690 g kg^{-1} of clay, silt, and sand, respectively.

Liming was carried out before the sowing of the crops, based on results from the soil analysis, with application of 2.0 Mg ha^{-1} of dolomitic limestone, broadcasted and incorporated into the soil using a plow, to correct the soil acidity and raise the base saturation to 60% (Sousa and Lobato, 2004).

Species used and experimental design

A randomized complete block experimental design was used, in a split-plot arrangement with four replications. The plots consisted of five hydrogel rates: 0.0, 7.5, 15.0, 22.5, and 30.0 kg ha^{-1}, respectively, corresponding to 0%, 50%, 100%, 150%, and 200% of the rate recommended by the manufacturer (Hydroplan-EB, 2019). The subplots consisted of presence and absence of topdressing using urea as source of nitrogen (N) (120 kg ha^{-1}) for the maize crop, and potassium chloride (KCl) as source of potassium (K) (60 kg ha^{-1} of K_2O) for the soybean crop. The plot area was 9 × 2.5 m and the subplot area was 4.5 × 2.5 m with 5 rows for both crops.

The granules of the hydrogel used in the experiments (Hydroplan®-EB) was applied together with the maize and soybean seeds at the time of sowing (Hydroplan-EB, 2019).

Seeds of the maize hybrid P4285VYHR (Pioneer®) were sown using 3.8 seeds per linear meter of furrows, totaling a density of 76,000 plants ha^{-1}. The forage species (*Panicum maximum* cv. BRS Kenya) was sown between the maize rows at the same time of maize sowing, using 5.0 kg ha^{-1} of viable pure seeds, with 80% cultural value.

Soybean seeds of the variety 8579IPRO (Brasmax Bônus®) were sown using 12.5 seeds per linear meter for a density of 250,000 plants ha^{-1}. Seeds of *P. maximum* (cv. BRS Kenya) were sown at the post-harvest of soybean plants.

Topdressing was carried out at 30 days after sowing (DAS). Other cultural practices were carried out following the recommendations for the crop species, considering the soil chemical characteristics and the economic damage level from pests and diseases.

Variables analyzed

Experiment I: Maize

The following physiological variables were evaluated in maize plants at the pre-flowering stage:

Water potential: determined using a Scholander pressure chamber at the mid-day (Ψ_{md}).

Anthocyanins and flavonoid contents: determined using the Dualex Scientific™ sensor (Force-A, Orsay, France), based on the chlorophyll fluorescence excitation spectra (Cerovic et al., 2012), and expressed as mg Rutin equivalents per gram of dried extract (mg RE g^{-1}).

Chlorophyll contents: determined by quantifying chlorophyll *a* (CLR *a*), Chlorophyll *b* (CLR *b*), and total chlorophyll (CLR *t*), using a ClorofiLOG® device (1030, Falker, Brazil) (Falker, 2008).

Gas exchange: evaluated to record photosynthetic rate (A, μmol m^{-2} s^{-1}) and transpiration rate (E, mmol m^{-2} s^{-1}), stomatal conductance (Gsw, mol H_2O m^{-2} s^{-1}), internal (Ci), and external (Ca) CO_2 concentration, which were evaluated using an infra-red gas analyzer (IRGA – Infrared Gas Analyzer) model LI 6400 (LICOR, Lincoln, NE, EUA), equipped with a fluorometer (LI-6400-40, LI-COLOR Inc). The measurements were carried out between 09:00 and 11:00 a.m., under artificial photosynthetically active radiation (RFA) of 1,500 μmol photons m^2 s^1 in the leaves with 21% O_2 and 400 μmol CO_2 mol^{-1} air.

Extraction and quantification of biomolecules of the primary metabolism: carbohydrates were extracted according to the methodology proposed by Rabelo et al. (2019). Starch was quantified using the anthrone method, based on the glucose standard curve (Yemm and Willis, 1954). Total soluble sugars (TSS) and saccharose contents were determined using the anthrone method (Yemm and Willis, 1954). Reducing sugar contents (RS) in leaves were estimated by the difference between TSS and saccharose contents.

Extraction and quantification of proline: determined based on the methodology proposed by Bates et al., (1973).

Yield: determined by cutting all forage plants (maize and forage) at the farinaceous grain stage (100 DAS) in the evaluation area of each plot. The harvested material was used to determine the fresh matter weight, which was packed in paper bags and dried in a forced air circulation oven at 55 ºC until constant weight. The material was then weighed in a precision balance to determine the dry matter yield (DMY; Mg ha^{-1}).

Experiment II: Soybean

The following physiological variables were analyzed in soybean plants at the pre-flowering stage:

Relative water content (RWC): determined according to the methodology proposed by Barrs & Weatherley (1962), by weighing the fresh matter (FM), turgid matter (TM), and dry matter (DM) of leaf discs (2 × 2 cm), using the formula: RWC = (FM - DM)/(TM - DM) × 100.

Anthocyanin and flavonoid contents: determined using the Dualex Scientific™ sensor (Force-A, Orsay, France), based on the chlorophyll fluorescence excitation spectra (Cerovic et al., 2012), and expressed as mg Rutin equivalents per gram of dried extract (mg RE g^{-1}).

Chlorophyll contents: determined by quantifying chlorophyll *a* (CLR *a*), Chlorophyll *b* (CLR *b*), and total chlorophyll (CLR *t*), using a ClorofiLOG® device (1030, Falker, Brazil) (Falker, 2008).

Gas exchange: evaluated to record photosynthetic rate (A, μmol m^{-2} s^{-1}), transpiration rate (E, mmol m^{-2} s^{-1}), stomatal conductance (Gsw, mol H_2O m^{-2} s^{-1}), internal CO_2 concentration (Ci), and external CO_2 concentration (Ca), which were evaluated using an infra-red gas analyzer (IRGA – Infrared Gas Analyzer) model LI 6400 (LICOR, Lincoln, NE, EUA), equipped with a fluorometer (LI-6400-40, LI-COLOR Inc). The measurements were carried out between 09:00 and 11:00 a.m., under artificial photosynthetically active radiation (RFA) of 1,000 μmol photons m^2 s^1 in the leaves with 21% O_2 and 400 μmol CO_2 mol^{-1} air.

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Extraction and quantification of proline: determined based on the methodology proposed by Bates, Waldren, and Teare (1973).

Yield: the number of plants was determined by counting the plants in the evaluation area of each plot. The plants in the evaluation area of each plot were manually harvested and, then, threshed in stationary thresher machine; the resulting grains were cleaned and weighed. The data were transformed into Mg per hectare (Mg ha^{-1}). The one thousand grain weight (1000GW) was determined using grain weights corrected to 13% moisture, wet basis.

Statistical analysis

The data were subjected to the Shapiro-Wilk test for normality and analysis of variance by the F test at 5% probability level. When the means were significant, they were subjected to linear and quadratic polynomial regression analyses for the hydrogel rates; and compared by the Tukey's test at 5% probability level for the factor topdressing. The tests were carried out using the Sisvar® program (Ferreira, 2011). Main component analysis (PCA) was carried out to assess the correlation between the treatments and the main variables that contributed to the similarity of the sample, using the R program.

RESULTS AND DISCUSSION

Maize

No effect of the treatments on water potential, anthocyanins, and silage dry weight was found for maize plants (Table 1). This absence of effects can be connected to the fact that, at the time of analyses, the rainfall accumulation was enough to maintain the maize plant tissues well hydrated (Fig. 1). These are similar results to those found by Hernández et al. (2015), who evaluated the effect of water deficit and N supply on maize plants and found no significant effect on water potential.

Regarding the gas exchange, the interaction hydrogel rates × nitrogen topdressing was significant for stomatal conductance; and the factor topdressing had significant effect on photosynthesis (Table 1). Plants with nitrogen topdressing had higher photosynthesis values (mean of 40.56 μmol m^{-2} s^{-1}) than those found in plants without nitrogen topdressing. Flavonoids were affected by the interaction hydrogel rate × nitrogen topdressing (Table 1). The factor nitrogen topdressing affected chlorophyll *a*, chlorophyll *b*, and total chlorophyll of maize plants grown in CLFIS (Table 1). Plants with nitrogen topdressing presented higher chlorophyll contents than those without nitrogen topdressing.

N is important for plants because it acts directly on the photosynthesis; is part of structure of chlorophylls and all plant proteins, including Rubisco, which is the most abundant enzyme in leaves; and acts in the plant cell division and expansion processes (Xu et al., 2019). Thus, N deficiency inhibits chlorophyll synthesis and decreases photosynthetic rates (Mortate et al., 2018). In addition, this effect on the photosynthesis decreases CO_2 assimilation,

Table 1: Analysis of variance for for physiological and productive variables of maize plants grown under hydrogel rates and nitrogen topdressing in crop-livestock-forest integration system (CLFIS)

Variables	Hydrogel	Nitrogen topdressing	Hydrogel × Nitrogen topdressing	Coefficient of variation 1 (%)	Coefficient of variation 2 (%)
	Degrees of freedom				
	4	1	4		
	P value				
Potential water	0.09	0.35	0.75	14.98	13.36
Transpiration	0.98	0.52	0.78	0.59	0.48
Photosynthesis	0.44	0.00**	0.83	12.27	13.44
CO_2 concentrations external	0.98	0.15	0.94	0.73	1.31
CO_2 concentrations internal	0.41	0.06	0.58	17.11	13.06
Stomatal conductance	0.83	0.03*	0.00**	13.21	14.21
Anthocyanins	0.48	0.34	0.05	15.07	8.75
Flavonoids	0.00**	0.40	0.00**	4.40	4.40
Chlorophyll *a*	0.41	0.00**	0.78	6.87	7.74
Chlorophyll *b*	0.27	0.00**	0.92	7.64	14.19
Chlorophyll *total*	0.41	0.00**	0.87	8.73	12.32
Total soluble sugars	0.42	0.00**	0.46	17.25	14.18
Reducing sugars	0.57	0.00**	0.23	28.25	27.98
Saccharose	0.02*	0.00**	0.03*	9.24	8.64
Starch	0.00**	0.01*	0.59	0.01	0.02
Proline	0.00**	0.66	0.23	9.5	11.97
Fresh forage weight	0.00**	0.44	0.4	5.3	11.28
Dry forage weight	0.24	0.04*	0.74	20.39	21.67

** and * = significant at 1% and 5% probability, respectively, by the F test at 5% probability

hindering plant growth and development, resulting in low leaf area indexes and, consequently, low yields (Mortate et al., 2018).

The hydrogel rates of 7.5 and 30 kg ha^{-1} combined with nitrogen topdressing resulted in higher stomatal conductance (Gsw), with means of 0.30 and 0.25 mol H_2O m^{-2} s^{-1}, respectively, compared to plants without nitrogen topdressing (Fig. 2). However, the rate of 22.5 kg ha^{-1} without nitrogen topdressing resulted in higher Gsw (0.28 mol H_2O m^{-2} s^{-1}) when compared to plants with nitrogen topdressing (0.20 mol H_2O m^{-2} s^{-1}). This may be connected to a response to drought by decreasing opening of stomata, which improves water use efficiency and decreases transpiration, keeping the photosynthesis high (Yang et al., 2018). Islam et al. (2011) evaluated the effect of application of hydrogel and irrigation and found increases in stomatal conductance in maize plants treated with hydrogel.

No increase in stomatal conductance was found in the present study as the hydrogel rates were increased, but the hydrogel rate of 7.5 kg ha^{-1} combined with nitrogen topdressing resulted in the highest Gsw. This is explained by the fact that nitrate is accumulated in guard cells, acting as an influx for potassium (K^+) during the stomatal opening (Fang-Qing et al., 2003).

The hydrogel rates of 15.0 and 22.5 kg ha^{-1} combined with nitrogen topdressing resulted in the higher flavonoid

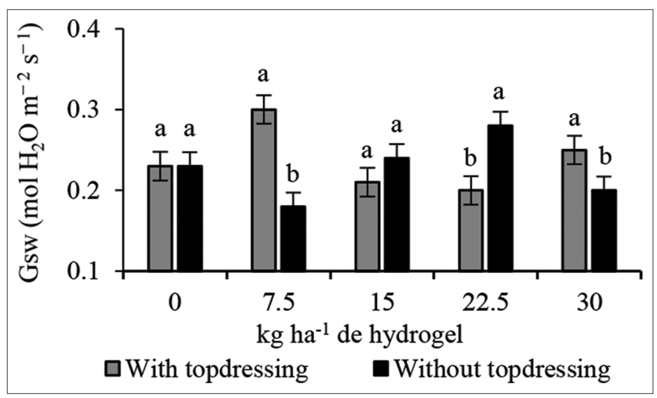

Fig 2. Statistical breakdown of the interaction hydrogel rates × nitrogen topdressing for stomatal conductance (Gsw) in maize plants grown in crop-livestock-forest integration system (CLFIS). Bars with equal letters within the same hydrogel rate are not different from each other by the Tukey's test at 5% probability.

contents, when compared to treatments without nitrogen topdressing (Fig. 3). The hydrogel rates of 0.0 and 30.0 kg ha^{-1} without nitrogen topdressing resulted in the highest flavonoid contents. Water shortage can increase flavonoid contents in plants (Stagnari et al., 2014), which is an important mechanism of defense to droughts (Senad et al., 2018). Other studies found decreases in total phenol contents in maize plants grown under water stress (Ali et al., 2010; Ali and Ashraf, 2011). Giordando et al. (2018) found that high N rates can decrease flavonoid contents. In the present study, it was found only for treatments with nitrogen topdressing and hydrogel rates of 15.0 and 22.5 kg ha^{-1}.

The factor nitrogen topdressing affected TSS and RS (Table 1); treatments with nitrogen topdressing resulted in higher TSS and RS when compared to treatments without nitrogen topdressing. This may be due to the fact that these plants have metabolic adjust capacity, altering the quantity and quality of primary metabolites, such as carbohydrates, in leaves and roots as a response to nutrient shortage (Bhargava and Sawant, 2013). In addition, high quantity of sugars is connected to high photosynthetic activity, resulting in high incorporation in biomass, increasing the forage weight of plants, as found in the present study.

Saccharose contents were affected by the interaction hydrogel rate × nitrogen topdressing; however, the data did not fit to the statistical model. Considering the nitrogen topdressing within the hydrogel rates, the rates of 0.0; 15.0 and 22.5 kg ha^{-1} resulted in significantly higher saccharose contents in plants without nitrogen topdressing (Fig. 4). Carbohydrate accumulation in plant tissues assists plants under water shortage, acting as compatible osmoregulatory agents (Mohammadkhani and Heidari, 2008). Water shortage affects the transport of nutrients in plants by compromising water exchange between xylem and phloem, causing accumulation of carbohydrates in the leaves (Bhargava and Sawant, 2013). This increase in carbohydrates, found in the plants without nitrogen topdressing in the present study, may have a protective effect against reactive oxygen species (Sami et al., 2016). Luo et al. (2009) found that *Populus euphratica* plants grown under hydrogel application present higher soluble sugar contents; this result was not found in the present study.

Starch contents were affected by the factor hydrogel rates, with values fitting to the second-degree polynomial equation. The estimated hydrogel rate of 19.43 kg ha^{-1} resulted in the lowest starch contents (129.85 μmol of glucose g^{-1} DM) (Fig. 5). Considering the factor nitrogen topdressing, plants without nitrogen topdressing resulted in higher starch contents than those with nitrogen topdressing. N is connected to protein structures; thus, N deficiency compromises the synthesis of enzymes and proteins that act on transport of sugars, causing accumulation of saccharose and starch in the leaves (Julius et al., 2017).

The factor hydrogel rates affected the proline contents of maize plants grown in CLFIS, whit values fitting to the second-degree polynomial equation. The lowest proline contents (1.77 μmol g^{-1} DM) were found for the estimated hydrogel rate of 20.93 kg ha^{-1}. Mazloom et al. (2020) found higher proline contents in maize plants that underwent water stress than in plants treated with hydrogel (lignin and synthetic basis). High hydrogel rates may not result in positive responses for physiological variables of plants,

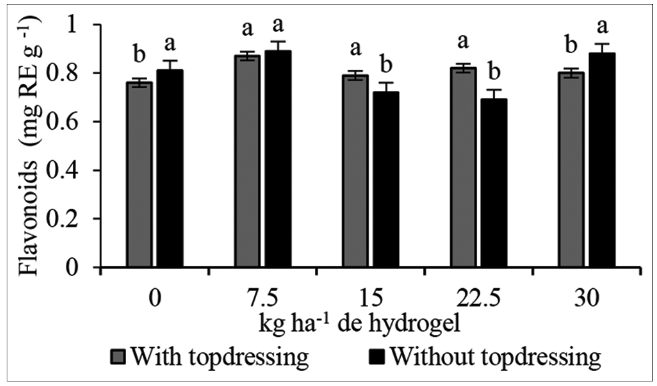

Fig 3. Statistical breakdown of the interaction hydrogel rates × nitrogen topdressing for flavonoid contents in maize plants grown in crop-livestock-forest integration system (CLFIS). Bars with equal letters within the same hydrogel rate are not different from each other by the Tukey's test at 5% probability.

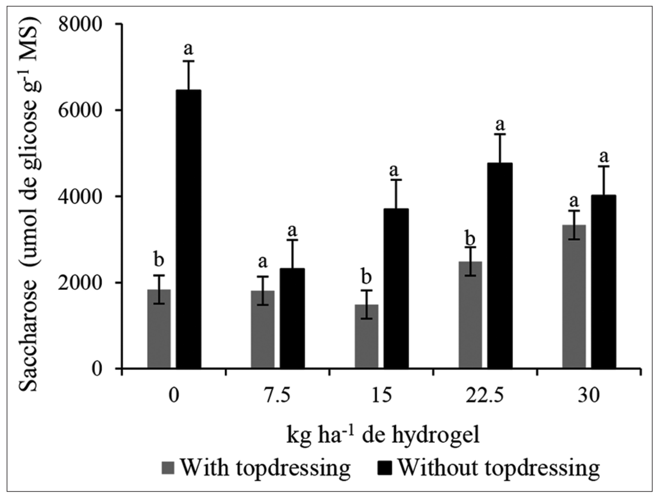

Fig 4. Statistical breakdown of the interaction hydrogel rates × nitrogen topdressing for saccharose contents in leaves maize plants grown in crop-livestock-forest integration system (CLFIS). Bars with equal letters within the same hydrogel rate are not different from each other by the Tukey's test at 5% probability.

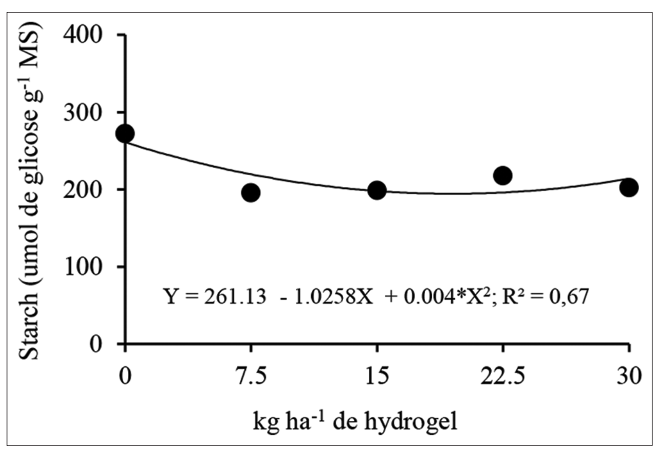

Fig 5. Starch contents in leaves of maize plants grown in crop-livestock-forest integration system (CLFIS) as a function of hydrogel rates.

since the soil act as a barrier, preventing and limiting the expansion of polymers and water retention (Vale et al., 2005), which may explain the results found in the present study.

DFW was affected by the factor nitrogen topdressing. FFW was affected by the factor hydrogel rates (Table 1), with values fitting to the second-degree polynomial equation. The highest FFW was 0.84 kg per plant, which was reached when using the estimated hydrogel rate of 16.08 kg ha^{-1} (Fig. 7). The nitrogen topdressing resulted in a mean DFW of 0.29 kg per plant, whereas the plants without nitrogen topdressing had a mean DFW of 0.25 kg per plant. The use of hydrogel combined with fertilizers allows a significant amount of nutrients to be stored in the hydrogel structure, which is slowly released according to the plant demand (Eneji et al., 2013). It may result in a better use of fertilizers, with positive effects on growth and biomass accumulation in maize plants (Islam et al., 2011). El-Asmar et al., (2017) found that hydrogel applied at 0.4% of the amount of soil in the pot improved the fresh and dry weights of maize plants. De Mamann et al. (2017) found increase in biomass of wheat plants due to application of N combined with hydrogel, and that the biomass of maize plants increased in 53% when treated with hydrogel and the standard fertilizer rate.

The spatial multivariate analysis carried out for the evaluated variables, considering the treatments (hydrogel rates combined with presence or absence of nitrogen topdressing), showed that two principal components explained 64.4% of the total variance observed, 44.0% for component 1 (DIM1) and 20.4% for component 2 (DIM2) (Fig. 8).

For the maize crop, SAD0 - without nitrogen topdressing + 0.0 kg ha^{-1} of hydrogel, SAD1 - without nitrogen topdressing + 7.5 kg ha^{-1} of hydrogel, and SAD4 - without nitrogen topdressing + 22.5 kg ha^{-1} of hydrogel were grouped in DIM 1 -/DIM 2 + with the variables: proline, starch, saccharose, and internal CO_2 rate. The treatments CAD4 - with nitrogen topdressing + 30 kg ha^{-1} of hydrogel, CAD1 - with nitrogen topdressing + 7.5 kg ha^{-1} of hydrogel, and CAD0 - with nitrogen topdressing + 0 kg ha^{-1} of hydrogel were grouped in the DIM 1 +/DIM 2 + with the variables: anthocyanins, flavonoids, water potential, total soluble sugars, reducing sugars, and photosynthesis. Whereas external CO_2 rate was found in the DIM 1 -/DIM 2 + connected to the treatments SAD2 - without nitrogen topdressing + 15.0 kg ha^{-1} of hydrogel and SAD3 - without nitrogen topdressing + 22.5 kg ha^{-1} of hydrogel. The variables: stomatal conductance, chlorophyll *a*, chlorophyll *b*, total chlorophyll, transpiration, and fresh and dry forage

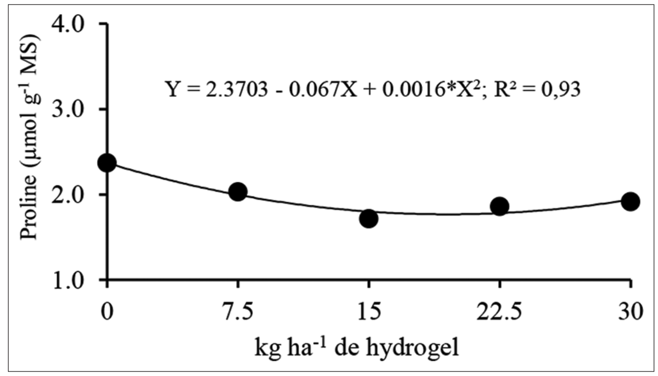

Fig 6. Proline contents in leaves of maize plants grown in crop-livestock-forest integration system (CLFIS) as a function of hydrogel rates.

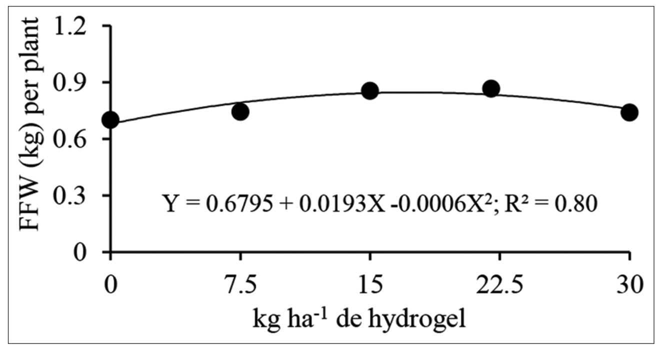

Fig 7. Fresh forage weight (FFW) of maize plants grown in crop-livestock-forest integration system (CLFIS) as a function of hydrogel rates.

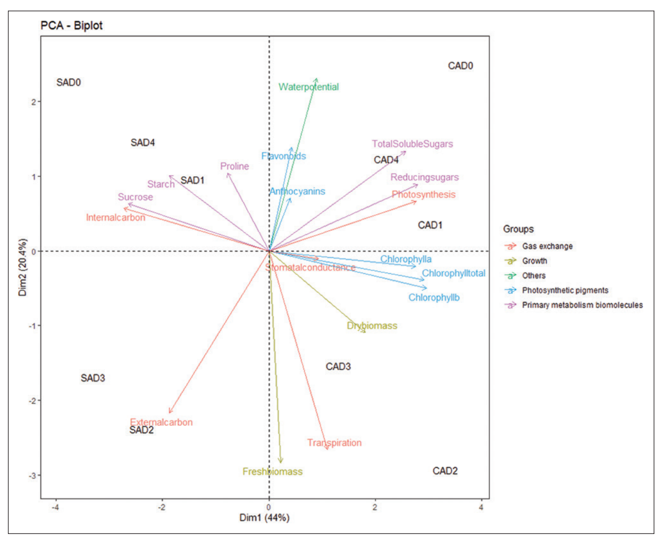

Fig 8. Scores of first and second principal components for physiological and production variables of maize plants grown under the following treatments: CAD0: with nitrogen topdressing + 0 kg ha^{-1} of hydrogel; CAD1: with nitrogen topdressing + 7.5 kg ha^{-1} of hydrogel; CAD2: with nitrogen topdressing + 15.0 kg ha^{-1} of hydrogel; CAD3: with nitrogen topdressing + 22.5 kg ha^{-1} of hydrogel; CAD4: with nitrogen topdressing + 30 kg ha^{-1} of hydrogel and SAD0: without nitrogen topdressing + 0 kg ha^{-1} of hydrogel; SAD1: without nitrogen topdressing + 7.5 kg ha^{-1} of hydrogel; SAD2: without nitrogen topdressing + 15.0 kg ha^{-1} of hydrogel; SAD3: without nitrogen topdressing + 22.5 kg ha^{-1} of hydrogel and SAD4: without nitrogen topdressing + 22.5 kg ha^{-1} of hydrogel.

weights were grouped in the DIM 1 +/DIM 2 - with the treatments CAD2 - with nitrogen topdressing + 15.0 kg ha^{-1} of hydrogel and CAD3 - with nitrogen topdressing + 22.5 kg ha^{-1} of hydrogel.

Anthocyanins, flavonoids, and water potential were, in general, inversely proportional to FFW. Anthocyanins and flavonols are compounds of the metabolism that belong to the group of flavonoids. Flavonoids are involved in a series of processes in plants, including plant-pathogen interactions, pollination, light search, and seed development. Many biosynthetic genes of flavonoids are induced under stress conditions and, consequently, the levels of flavonoids increase during the exposure to biotic and abiotic stresses, such as wounds, droughts, toxicity by metals, and nutrient shortage (Hernández et al., 2009). They are molecules of secondary metabolism connected to defense and are opposite to FFW, since its synthesis is carried out against the energy metabolism for formation of structural molecules.

This same situation is valid for proline, which is opposite to DFW. Proline is a multifunctional amino acid highly responsive to water stress in leaves and roots (Szabados and Savouré, 2010). Its synthesis occurs mainly from glutamate, but can also occur from ornithine, as an alternative pathway under osmotic stress conditions, such as drought. The biosynthetic pathway of proline is a reducing process; thus, during droughts, its biosynthesis in the chloroplasts can significantly contribute to NADPH consumption and surplus energy dissipation, preventing the formation of reactive oxygen species (Ronde et al., 2004; Sharma et al, 2011). Despite this is an important process during stressful conditions, the high consumption of reducing power during proline synthesis changes the dynamics of energy metabolism of plant tissues, assisting the plant in the stress, but limiting its growth, which is common in stress conditions.

Gas exchanges presented a clear negative correlation to photosynthesis and internal CO_2 concentration, denoting a consumption of internal carbon by the photosynthetic process.

Transpiration was inversely correlated to leaf water potential, denoting that, under these experimental conditions for maize, the higher the transpiration, the lower the leaf water potential, and that the plant hydraulic system is not able to replace all transpiration water. Photosynthesis was negatively correlated to saccharose and starch contents, which may be connected to the negative feedback of the product, since the synthesis of the final product can control the metabolic feedback in the short-term through recycling of inorganic phosphate (Paul and Foyer, 2001). In addition, carbohydrate accumulation in leaves occurs when there is an imbalance between the source and drain in the whole plant, which may lead to decreases in expression of photosynthetic genes and acceleration of leaf senescence (Dabu et al., 2019).

Considering the results found for the maize crop, the hydrogel rates of 7.5 and 15.0 kg ha^{-1} presented the best results. Nevertheless, the conduction of new studies in different soils and climate is recommended to validate the results found in the present research.

Experiment II: Soybean

The interaction hydrogel rates × potassium topdressing affected the relative water content (RWC), flavonoids, and number of soybean plants grown in CLFIS (Table 2). No effect of the treatments was found for gas exchange, anthocyanins, chlorophyll contents, total soluble sugars, reducing sugars, starch, yield, and 1000-grain weight of soybean plants grown in CLFIS (Table 2). Interactions that occur at the crop implementation process, such as environmental factors, pathogens, and pests, can directly affect different parameters in the plant, which may explain the absence of effects on these variables. Gales et al. (2012) found increases in chlorophyll contents in plants treated with hydrogel.

The hydrogel rate of 30 kg ha^{-1} with potassium topdressing resulted in a higher RWC when compared to plants without potassium topdressing (Fig. 9). RWC indicates the plant hydrological conditions, i.e., the water content present in the leaves. The intensification of deficit water makes plants to undergo a dehydration of protoplasm, which may hinder vital processes for cell growth (Ferrari et al., 2015). Therefore, hydrogel can be used as a possible solution in drought periods, since its application result in higher water availability from the physiological development up to the beginning of the reproduction period of soybean plants (De Pelegrin et al., 2017).

Flavonoid contents were affected by the interaction hydrogel rate × potassium topdressing (Table 2). Considering the hydrogel rates within the potassium topdressing, the results found for soybean plants fitted to the second-degree polynomial equation. The plants with potassium topdressing presented the lowest flavonoid contents (0.84 mg RE g^{-1}), found for the estimated hydrogel rate of 16.15 kg ha^{-1} (Fig. 10A). Considering the potassium topdressing within the hydrogel rates, significant differences were found in the hydrogel rates of 15.0 and 22.5 kg ha^{-1}; higher flavonoid contents were found for plants with potassium topdressing (Fig. 10B). The metabolism of flavonoids follows a complex pathway, and some environmental factors, such as temperature, water status, light conditions,

Table 2: Analysis of variance for physiological and productive variables of soybean plants grown under hydrogel rates×potassium topdressing in crop-livestock-forest integration system (CLFIS)

Variables	Hydrogel	Potassium topdressing	Hydrogel×Potassium topdressing	Coefficient of variation 1 (%)	Coefficient of variation 2 (%)
	Degrees of freedom				
	4	1	4		
	P value				
Relative water content	0.01*	0.64	0.03*	13.72	16.41
Anthocyanins	0.67	0.31	0.28	14.96	11.34
Flavonoids	0.00**	0.00**	0.00**	6.80	4.38
Chlorophyll *a*	0.42	0.77	0.95	4.97	3.52
Chlorophyll *b*	0.57	0.91	0.21	7.63	6.04
Chlorophyll *total*	0.59	0.81	0.68	4.73	3.88
Transpiration	0.97	0.30	0.79	29.14	13.92
Photosynthesis	0.64	0.11	0.34	17.70	14.16
CO_2 concentrations external	0.66	0.11	0.55	0.60	0.50
CO_2 concentrations internal	0.77	0.38	0.54	4.82	3.14
Stomatal conductance	0.74	0.20	0.97	30.97	25.33
Total soluble sugars	0.13	0.44	0.92	16.67	17.42
Reducing sugars	0.13	0.44	0.92	16.68	17.42
Saccharose	0.08	0.04*	0.45	11.17	15.47
Starch	0.54	0.85	0.12	16.36	16.34
Proline	0.00	0.19	0.00	12.78	9.38
Number of plants	0.00**	0.33	0.00**	2.86	5.94
Yield	0.15	0.87	0.41	24.42	24.78
One thousand grain weight	0.79	0.57	0.64	4.00	5.00

** and * = significant at 1% and 5% probability, respectively, by F test at 5% probability

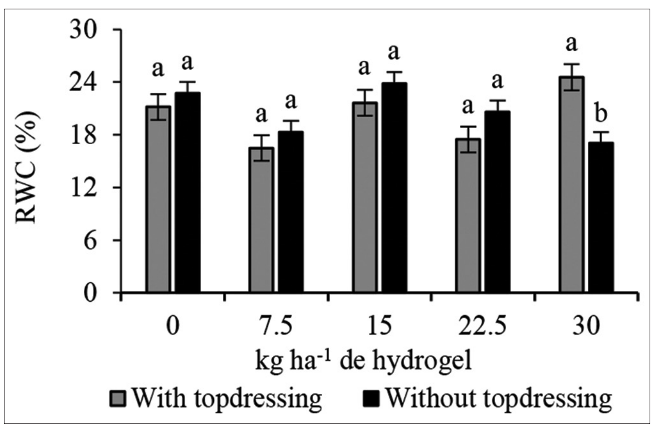

Fig 9. Statistical breakdown of the interaction hydrogel rates × potassium topdressing for water relative content in soybean plants grown in crop-livestock-forest integration system (CLFIS). Bars with equal letters within the same hydrogel rate are not different from each other by the Tukey's test at 5% probability.

and nitrogen affect their concentration in plants (Pource et al., 2007). In addition, low rainfall depths are connected to flavonoid contents, indicating that water status is an important factor that affects flavonoid accumulation (Yuan et al., 2012).

Regarding the saccharose, there was significant difference only for the isolate effect of potassium topdressing (Table 2). The plants without potassium topdressing resulted in higher saccharose contents, with a mean of 15.70 umol of glucose g^{-1} DM. Plants tend to increase the quantity of sugars in tissues as an alternative mechanism under water shortage conditions, which affects absorption and transport of nutrients (Da Silva et al., 2011). Nascimento et al. (2019) evaluated the same type of hydrogel at the rates 0.0, 5.0, 10.0, 15.0, and 20.0 kg ha^{-1} and found no difference in soluble carbohydrates in soybean grains.

Regarding the effect of the interaction hydrogel rates × potassium topdressing for the proline in soybean plants, the values fitted to the second-degree polynomial equation. The estimated hydrogel rate of 17 kg ha^{-1} combined with potassium topdressing resulted in the highest proline contents (0.007 μmol g^{-1} DM) (Fig. 11A). Considering the potassium topdressing within the hydrogel rates, the rates of 7.5, 15.0, and 30.0 kg ha^{-1} without potassium topdressing resulted in the highest proline contents (Fig. 11B). The rate of 22.5 kg ha^{-1} resulted in higher proline contents when combined with potassium topdressing. Proline acts as a biomarker of water stress, maintaining the cell turgor, protecting it against reactive oxygen species and, thus, preventing cell dehydration in plants (Ashraf, Foolad, 2007). Ferrari et al. (2015) found higher proline accumulation in soybean plants that underwent water stress when compared to plants under no water stress. The soil physical characteristics may affect plant responses to the use of hydrogels, which may explain the proline contents found with the rates of 15.0 and 22.2 kg ha^{-1} in the present

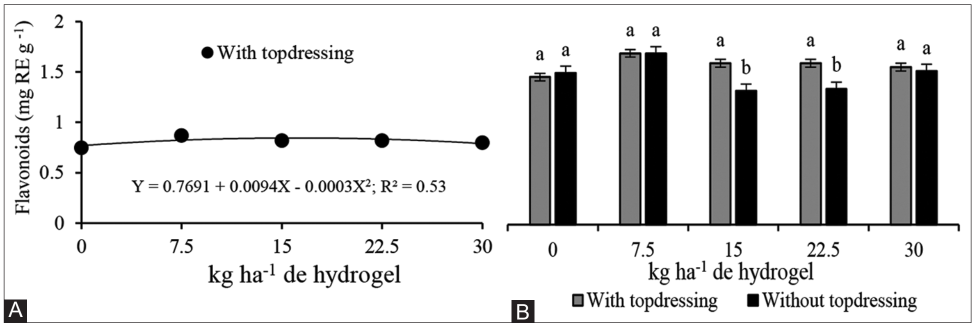

Fig 10. Statistical breakdown of the interaction between hydrogel rates × potassium topdressing within hydrogel rates (A) and statistical breakdown of potassium topdressing within hydrogel rates (B) for flavonoid contents in soybean plants grown in crop-livestock-forest integration system (CLFIS). Bars with equal letters within the same hydrogel rate are not different from each other by the Tukey's test at 5% probability.

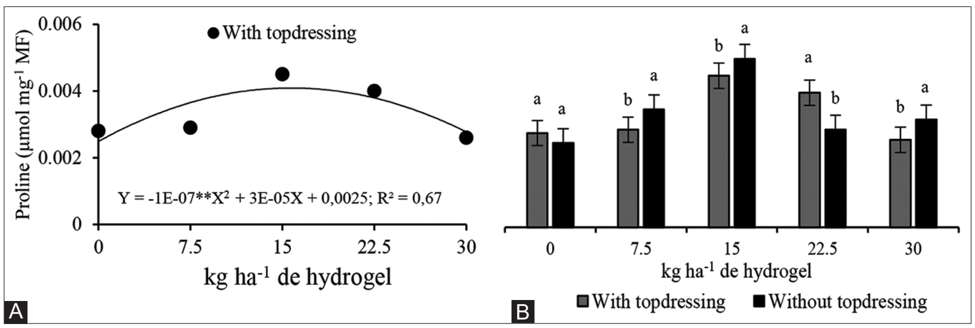

Fig 11. Statistical breakdown of the interaction between hydrogel rates × potassium topdressing within hydrogel rates (A) and statistical breakdown of potassium topdressing within hydrogel rates (B) for proline contents in soybean plants grown in crop-livestock-forest integration system (CLFIS). Bars with equal letters within the same hydrogel rate are not different from each other by the Tukey's test at 5% probability.

study. The results also show that the hydrogel rate of 7.5 kg ha^{-1} can result in low proline contents, denoting that half of the recommended rate is enough to provide an adequate water status for soybean plants.

The soybean yield and 1000GW presented no significant difference between treatments. Only the number of plants was affected by the interaction hydrogel rates × potassium topdressing, with values fitting to the second-degree polynomial equation. The lower number of plants was found for the estimated hydrogel rate of 70.2 kg ha^{-1} combined with potassium topdressing (Fig. 12). The results for plants without potassium topdressing fitted to a linear equation, with a decrease of 0.75 plants when the hydrogel rate was increased. However, high hydrogel rates not always have positive responses from some soybean variables (Mendonça et al., 2013). Abraão et al. (2020) found that the hydrogel rate of 20 kg ha^{-1} increased the number of plants in approximately 4%. Gales et al. (2012) found increases in soybean yield subjected to application of hydrogel, confirming the results found for the hydrogel treatments combined with potassium topdressing in the present study.

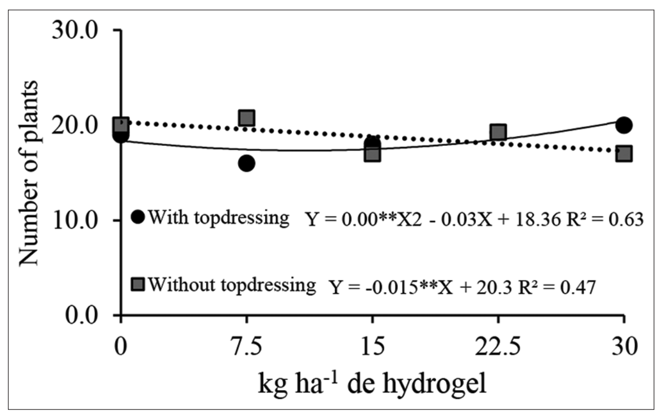

Fig 12. Statistical breakdown of the interaction hydrogel rates × potassium topdressing for number of soybean plants grown in crop-livestock-forest integration system (CLFIS).

The spatial multivariate analysis carried out for the evaluated variables, considering the treatments (hydrogel rates combined with presence or absence of potassium topdressing), showed that two principal components explained 52.6% of the total variance observed, 29.9% for component 1 (DIM1) and 22.7% for component 2 (DIM2) (Fig. 13).

For the soybean crop, CAD2 - with potassium topdressing + 15.0 kg ha^{-1} of hydrogel was grouped in DIM 1 -/DIM 2 + with the variables: stomatal conductance, transpiration, relative water content, and external CO_2 concentrations. DIM 1 +/DIM 2 – grouped the treatments SAD2 - without potassium topdressing + 15.0 kg ha^{-1} of hydrogel, CAD0

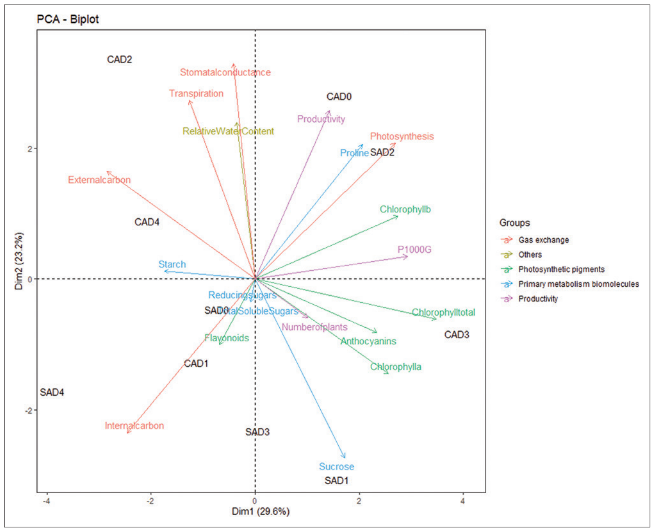

Fig 13. Scores of first and second principal components for physiological and production variables of soybean plants grown under the following treatments: CAD0: with potassium topdressing + 0 kg ha^{-1} of hydrogel; CAD1: with potassium topdressing + 7.5 kg ha^{-1} of hydrogel; CAD2: with potassium topdressing + 15.0 kg ha^{-1} of hydrogel; CAD3: with potassium topdressing + 22.5 kg ha^{-1} of hydrogel; CAD4: with potassium topdressing + 30 kg ha^{-1} of hydrogel and SAD0: without potassium topdressing + 0 kg ha^{-1} of hydrogel; SAD1: without potassium topdressing + 7.5 kg ha^{-1} of hydrogel; SAD2: without potassium topdressing + 15.0 kg ha^{-1} of hydrogel; SAD3: without potassium topdressing + 22.5 kg ha^{-1} of hydrogel and SAD4: without potassium topdressing + 22.5 kg ha^{-1} of hydrogel.

- with potassium topdressing + 0 kg ha^{-1} of hydrogel, and CAD3 - with potassium topdressing + 22.5 kg ha^{-1} of hydrogel with the variables: proline, chlorophyll *b*, and total chlorophyll. The treatments SAD0 - without potassium topdressing + 0 kg ha^{-1} of hydrogel, SAD4 - without potassium topdressing + 22.5 kg ha^{-1} of hydrogel, CAD1 - with potassium topdressing + 7.5 kg ha^{-1} of hydrogel and CAD4 - with potassium topdressing + 30 kg ha^{-1} of hydrogel were grouped in the DIM 1 -/DIM 2 - with the variables: total soluble sugars, reducing sugars, starch, flavonoids, internal CO_2 concentrations. DIM 1 +/DIM 2 – grouped the treatments SAD1 - without potassium topdressing + 7.5 kg ha^{-1} of hydrogel and SAD3 - without potassium topdressing + 22.5 kg ha^{-1} of hydrogel with the variables: anthocyanins, chlorophyll *a*, number of plants, and saccharose.

The PCA for soybean variables showed that photosynthesis is inversely proportional to internal CO_2 concentrations and that chlorophyll is inversely proportional to external CO_2 concentration. Changes in CO_2 concentrations affect the photosynthetic rates because photosynthesis is performed with consumption of CO_2; thus, the higher the photosynthetic rate, the lower the CO_2 concentration (Paul and Foyer, 2001). Chlorophyll contents are used to estimate the photosynthetic potential of plants due to its connection with absorption and transference of light energy (Buttery and Buzzell). A plant with high chlorophyll content can reach high photosynthetic rates.

Proline is inversely proportional to total soluble and reducing sugars. Proline acts as a compatible solute that adjusts the osmotic potential in the plant cytoplasm; it is produced immediately after the perception of water stress, protecting the plasma membrane (Amirjani, 2010). The high production of proline for osmotic regulation in plant tissues changes the synthesis of other amino acids, which affects sugar production (Kaviani, 2008).

A negative correlation between transpiration and saccharose content was found, denoting that high transpiration rates cause a mobilization of compounds, such as saccharose, to other plant parts, such as roots, working as a drain of photoassimilates (González et al., 1995).

Flavonoid content is inversely proportional to yield. Flavonoids encompass a large family of phenolic chemical substances widely distributed in plants, which work as inhibitors of enzymes, pigments, defense against disturbances by phytopathogens and herbivores, and protection front ultraviolet radiation. In addition, the synthesis of flavonoids is connected to increases in absorption of nutrients from the soil, which favors increases in biomass (Jones and Hartley, 1999).

The PCA showed the variables that contributed the most to the variation of the data of the analyzed treatments, and served as a tool to facilitate the selection of the most important physiological variables within those analyzed.

Based on the results, the hydrogel rates of 7.5 and 15.0 kg ha^{-1} can provide positive results for soybean crops. Nevertheless, the conduction of new studies in different soils and climate is recommended to validate the results found in the present research.

CONCLUSION

The application of hydrogel rates and topdressing can improve some physiological and production parameters of maize and soybean crops grown in crop-livestock-forest integration system (CLFIS). The hydrogel rates of 7.5 and 15.0 kg ha^{-1} resulted in the best results for most variables analyzed in both crops. Nevertheless, the conduction of new studies in different soils and climate is recommended to validate the results found in the present research.

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CONTRIBUTIONS OF AUTHORS

Caroliny Fatima Chaves da Paixão: implemented the field study, collected data, and wrote and revised the manuscript. Leonardo Nazário Silva dos Santos: designed the field study and revised the manuscript. Marconi Batista Teixeira: designed the field study. Frederico Antonio Loureiro Soares: carried out statistical analyses. Vitor Marques Vidal: carried out statistical analyses. Roniel Geraldo Ávila: carried out the data collection and revised the manuscript. Edson Cabral da Silva: revised the manuscript. Estenio Moreira Alves: implemented the field study and revised the manuscript. Fabiano Guimarães Silva: designed the field study.

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