



Analysis of seed dormancy breakage and seedling growth in sweet sorghum (*Sorghum bicolor* L.) through the electrical stimulation method: a scientific perspective aimed at promoting bioethanol production

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INFO

Keywords

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ABSTRACT

This pioneering study meticulously focused on the underexplored realm of sweet sorghum (*Sorghum bicolor* L.), utilizing commercial seeds to mitigate potential biases. The research intricately interwove the intricate facets of electrical stimulation, delineating its profound implications on the estimative bioethanol production of sweet sorghum, with potential extrapolation to future investigations involving sugarcane. Executed within the controlled confines of a greenhouse, after seed treatment procured from a local market, the commercial seeds underwent an electrifying metamorphosis. In the pursuit of enhancing the fresh biomass output of sweet sorghum, a nuanced interplay with electrical currents transpired. Noteworthy findings surfaced, elucidating that the attainment of optimal outcomes necessitated meticulous control over the applied electrical current. The identified optimal parameter advocated maintaining a conservative threshold of 50 mA during a seed treatment duration spanning approximately 15 min. However, as the experimental cadence intensified, the narrative evolved with a bold revelation, indicating that exploration of higher electrical currents, peaking at 150 mA, could yield favorable results under the condition of a truncated seed treatment time, notably as brief as 5 min. The delicate equilibrium achieved in this electrical choreography presented a compelling prospect for redefining the production paradigm of sweet sorghum. After analyzing the germination results, the estimative production projections derived from treatment #3 paint a vivid picture of the sorghum biomass production potential. A visionary forecast materialized, envisioning an impressive 25 t/ha of sweet sorghum, coupled with a remarkable total conversion rate into bioethanol reaching 1.5 million cubic meters. This transcendence transcends the boundaries of a conventional study; it signifies a leap into the future, pushing the envelope of conventional wisdom in the relentless pursuit of innovation in sustainable energy.

RESUMO

*Análise da quebra de dormência de sementes e do crescimento das plântulas de sorgo sacarino (*Sorghum bicolor* L.), por meio do método de estímulo elétrico: uma visão científica voltada para o incentivo da produção de bioetanol*

Este estudo pioneiro concentrou-se meticulosamente no domínio pouco explorado da produção de sorgo sacarino (*Sorghum bicolor* L.), utilizando sementes comerciais para mitigar possíveis vieses. A pesquisa entrelaçou de maneira elaborada as facetas complexas da estimulação elétrica, delineando suas profundas implicações na estimativa de produção de bioetanol do sorgo sacarino, com possíveis extrapolações para futuras investigações envolvendo a cana-de-açúcar. Executado dentro dos limites controlados de uma estufa, após o tratamento das sementes adquiridas em um mercado local, as sementes comerciais passaram por uma metamorfose eletrizante. Na busca por aprimorar a produção de biomassa fresca do sorgo sacarino, uma interação sutil com correntes elétricas transcorreu. Descobertas notáveis emergiram, esclarecendo que a obtenção de resultados ótimos demandava um controle meticuloso da corrente elétrica aplicada. O parâmetro ótimo identificado preconizava a manutenção de um limiar conservador de 50 mA durante um período de tratamento das sementes com duração de aproximadamente 15 min. Entretanto, à medida que a cadência experimental se intensificava, a narrativa evoluía com uma revelação audaciosa, indicando que a exploração de correntes elétricas mais elevadas, atingindo 150 mA, poderia gerar resultados favoráveis sob a condição de um tempo de tratamento de sementes truncado, notadamente tão breve quanto 5 min. O equilíbrio delicado alcançado nessa coreografia elétrica apresentava uma perspectiva convincente para redefinir o paradigma de produção do sorgo sacarino. Após análise dos resultados de germinação, as projeções estimativas de produção derivadas do tratamento #3 nos trazem um quadro vívido do potencial de produção de biomassa de sorgo. Uma previsão visionária se concretizou, projetando impressionantes 25 toneladas por hectare de sorgo sacarino, acompanhadas de uma notável taxa de conversão total em bioetanol atingindo 1,5 milhão de metros cúbicos. Esta transcendência vai além de um estudo convencional; ela representa um salto para o futuro, empurrando os limites da sabedoria convencional na incessante busca por inovação em energia sustentável.

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INTRODUCTION

Brazilian agriculture relies heavily on imported fertilizers, constituting approximately 85% of its fertilizer consumption, totaling 39.2 million t, and positioning the country as the fourth-largest global consumer, accounting for around 8% of the worldwide consumption (Ogasawara et al., 2013). In the 2021/22 crop year, Brazil witnessed a significant uptick in sweet sorghum production, reaching approximately 3 million metric tons, marking a substantial 43% increase compared to the preceding year (Ozbun, 2022). The Brazilian sorghum crop exhibits remarkable productivity, serving diverse roles as a food and feed source, and a key component in ethanol production (Jardim et al., 2020). Notably, Rezende and Richardson (2017) posit that sugarcane cultivation yields a risk premium benefit of around US\$ 8.7 thousand per year in average annual net cash income. Their research suggests that integrating sweet sorghum into the crop mix, serving as a raw material for ethanol production in sugarcane mills, could reduce costs by spreading fixed expenses. Consequently, mitigating the cost of producing sweet sorghum seedlings emerges as a viable strategy for large-scale fuel ethanol production.

It is noteworthy in contemporary times the substantial investment in technological innovation, particularly in the field of energy transition. A vast number of calls are opened each year to foster scientific and technological research in the quest for sustainable technological solutions, both from an environmental and economic perspective, to promote sustainable energy generation from renewable sources. Among the great success stories, Brazil has been producing immeasurable volumes of biofuel from sugarcane since the 1970s (Gonçalves et al., 2023; Vega et al., 2024). Bioethanol is a globally recognized success for providing clean and liquid bioenergy for combustion engines (Eaglin, 2022). However, while the production costs of biofuels in Brazil remain among the lowest in the world, the expenses associated with acquiring synthetic fertilizers through the importation from a limited number of countries that possess extensive deposits and mines of key minerals (potassium and phosphate) have increasingly restricted access to the essential minerals for chemical fertilizers (Abay et al., 2023; Bair and Mahutga, 2023).

Currently, researchers have published papers detailing advancements in biofertilizer production or enhancements in nutrient assimilation efficiency by plants, particularly C4 plants, the foundation for bioenergy production worldwide (such as corn, sorghum, or sugarcane) (Altaf et al., 2023; Wasi et al., 2023; Alqahtani, 2023). Traditionally, the global economy is based on increasing crop production, especially food and feed, by the large-scale use of

pesticides and fertilizers. Since the consumption profile has been more critical regarding the residual presence of these chemicals, which can negatively affect their health, public food safety agencies have sought alternatives to the use of fertilizers, for example. The availability of deposits of basic elements (nitrogen, phosphorous, or potassium) for the production of fertilizers in an area of armed conflict has also been another reason for the search for large-scale crop production using alternative techniques to stimulate plant growth. According to Amante (2019), in the Philippines, the agriculture fuels economy is significant to study innovations like electro culture to create more efficient ways to grow their crops or as reported by Christianto and Smarandache (2021) to reduce dependence on fertilizers.

Meanwhile, little has been spoken or published about a technology extensively studied years ago – electroculture. The stimulation of seed germination or plant propagation through electrical stimulation has not been in the headlines within scientific circles recently. The investigation into biopotentials, bioelectricity, or bioelectrogenesis was a vast domain of scientific exploration, with multiple facets yet to be fully elucidated (Vodeneev et al., 2015; Li et al., 2021; Mohan et al., 2016). In 1746, the Scottish scientist Dr. Von Maimbray conducted an experiment where he subjected two myrtles to electrification, observing a remarkably accelerated growth of branches, a phenomenon previously unreported (Manquiam et al., 2019). Capron (1883) documented that those trees exposed to the aurora borealis exhibited enhanced growth rates. Given the inherent potential difference across cellular membranes, characterized by a negative interior and positive exterior, it is hypothesized that this potential arises from the asymmetric distribution of ions, notably Na^+ , K^+ , Cl^- , and HPO_4^{2-} .

Living organisms, as part of their adaptive strategies, acquire environmental information and manifest it through physiological processes to optimize performance within the prevailing edaphoclimatic conditions, thereby maintaining homeostasis. Techniques involving electric current, magnetism, monochromatic light, and sound can influence the germination and growth of plants. Notably, electrical stimulation stands out as a technology utilized for various applications, including seed treatment and seedling growth (Acosta-Santoyo et al., 2018; Sánchez et al., 2018; Xu et al., 2022; Yuan et al., 2021).

Electrical stimulation manifests as either endogenous or exogenous phenomena. Endogenous occurrences are triggered by environmental stressors, such as fluctuations in temperature, which play a pivotal role in plant physiology (Lee and Oh, 2023;

Kim and Oh, 2023). This involves the regulation of key processes like respiration, transpiration, stomata opening, protein and hormone synthesis, and the gene expression of enzymes and ribosomal proteins. Exogenous electrical stimulation influences cellular metabolism by interacting with the physical and chemical components of plant structures. This interaction can yield both positive and negative effects, impacting aspects such as germination, biomass yield, fruit quality, and resilience to extreme events like frost (Li et al., 2019; Chen et al., 2019a; Chen et al., 2019b).

In this groundbreaking investigation, we push the envelope by delving into the revolutionary realm of directly applying electric current to saline solutions housing ungerminated sweet sorghum seeds. Our objective is to scrutinize the profound influence of this novel approach on seed development, including germination time, biometric parameters encompassing root and shoot length, and the resultant biomass yield arising from the experimental treatments. This avant-garde exploration seeks to unravel the untapped potential and redefine the conventional boundaries in the domain of seed germination and plant growth modulation. Based on the obtained results, we have shed light on the biomass production potential of sweet sorghum, a prominent C4 plant utilized as a renewable source of bioenergy. This is achieved through an estimated biomass production calculation and, consequently,

grounded in scientific agronomic data, the potential conversion into liquid energy, specifically biofuel in the form of bioethanol.

MATERIAL AND METHODS

The experiment was carried out in an indoor cultivation greenhouse installed at the Hugot Sugar Technology laboratories at the University of São Paulo, Brazil. Experiments were carried out controlling some variables such as lighting, water availability, and ambient temperature to optimize the culture performance, according to the appropriate agronomic characteristics of sweet sorghum. The cultivation was carried out in a plastic vase with 200 g of inert substrate, in sufficient numbers for the statistical design adopted. Two Shopped LED tube lamps (20 W) were used for lighting, with 1480 Lumens, totaling 2960 Lumens (i.e., $40.0 \mu\text{mol m}^{-2} \text{s}^{-1}$). Electric current was provided by a switched source, providing a different working current adapted to an e-Stimulator[®] current distribution donated by GS4|Science Consulting and Solutions (Figure 1). An Amanco irrigation system was used to supply potable water for irrigation. The system was controlled by a hydraulic interrupt coil driven and an analog Rohdina timer, set to irrigate 15 min every 8 h, resulting in a water depth of 150 mL per vase.

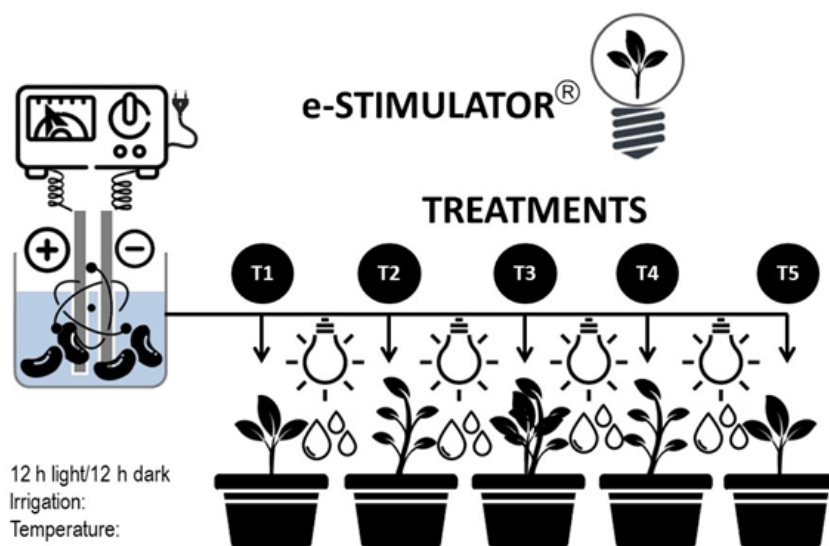


Figure 1 - Illustration of the experimental site for seedling electrical stimulation of sorghum seeds (*Sorghum bicolor* L.)

In each vase according to experimental design (Table 1), 30 g of healthy seeds of sweet sorghum (*Sorghum bicolor* L. Moench) was used per vase, totaling 8 treatments (7 tests and one control). The seeds were submitted to different current ranges (0;

50; 100; and 150 mA) for different times (0; 5; 10; and 15 min) into KCl-based solution at 1% wt in distilled water. The ratio of seeds to water was 1:10 (w/v).

Table 1 - Experimental design and experimental data matrix for seedling electrical stimulation of sorghum seeds (*Sorghum bicolor* L.) and real values of shoot and root lengths and biomass weight at 21 days after planting (n = 7)

Runs	I (mA)	Time (min)	Shoot Length (mm)	Root Length (mm)	Biomass Weight (g)
#1	50	5	108.3 ± 30	43.4 ± 12	3.10 ± 0.3
#2	150	5	176.4 ± 34	87.5 ± 9	3.94 ± 0.2
#3	50	15	149.4 ± 39	95.4 ± 12	5.10 ± 0.2
#4	150	15	94.6 ± 23	16.8 ± 3	0.92 ± 0.1
#5	100	10	156.3 ± 39	33.9 ± 8	2.05 ± 0.3
#6	100	10	124.0 ± 14	30.8 ± 4	0.67 ± 0.1
#7	100	10	127.7 ± 22	30.4 ± 5	1.64 ± 0.2

Data were collected weekly (biometrics: root and shoot length) for 21 days after planting (DAP) and then used for further statistical analyses. All the tests performed were tabulated, containing the data referring to the analyses, allowing to evaluate and discuss through the Minitab Statistical software (free trial version), by surface analysis of response to the current and time variations, making it possible to verify the germination difference from one trial to another. The data also were analyzed by principal component (PCA) and hierarchical clustering (HCA) analyses to confirm the most important variables within each cluster using MetaboAnalyst online tools (Chong *et al.*, 2019).

RESULTS AND DISCUSSION

Effect of electrical stimulation on sweet sorghum growth

After each electrical stimulation treatment, the sorghum seeds were planted in vases and their development profiles were monitored weekly using a caliper to measure the plants' lengths. As described in the Material and Methods, ten plants from each vase were randomly collected so that shoot and root length measurements were taken.

The results presented in Table 1 served as the foundation for the subsequent multivariate and cluster analyses. Given that it involves a 2² full factorial design with three repetitions at the central point, the outcomes enabled a comprehensive analysis of the interactions between the applied current (50, 100, or 150 mA) and the seed treatment time at these currents. The aim was to seek the optimal interaction between the current and its application time on sweet sorghum seeds, or at the very least, initially determine if there were statistically significant differences among the treatments. The response variables (biometric measurements of the seedlings) were utilized as reference indicators to ascertain whether there was a significant effect among the experimental trials. As evident, each trial yielded a distinct combination of responses following the seed treatments.

According to the original measures data and by the cluster analysis (Figure 2A) and hierarchical analysis (Figure 2B) it was possible to verify that the original repetitions data presented great values dispersion. Thus, for correct data analysis, a Discriminant Analysis was performed to identify the closest repetition values. These normalized values were used for statistical analysis and data discussion.

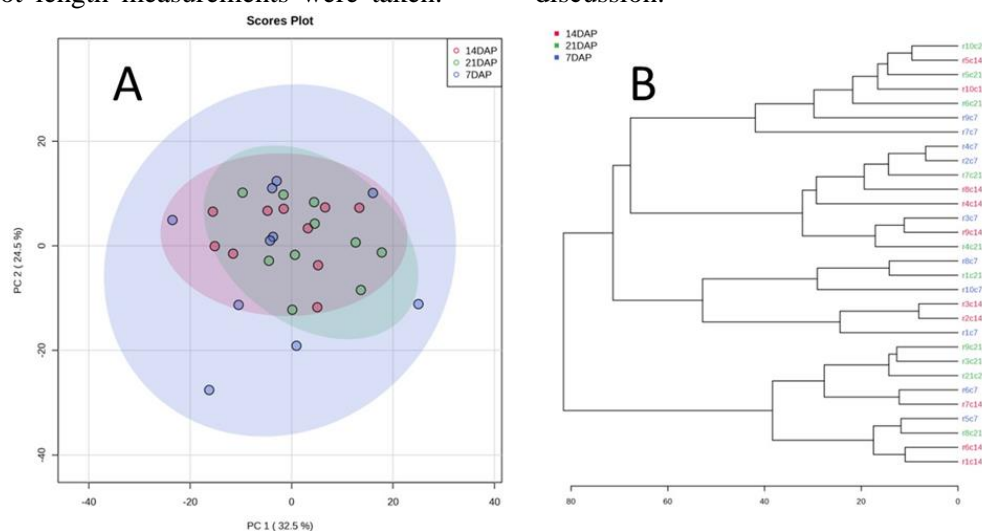


Figure 2 - Principal component analysis (A) and hierarchical analysis (B) of original data collected from shoot lengths of sorghum seedlings at 7, 14, and 21 days after planting (DAP)

Figure 3 shows the profile of those seedlings. It is possible to note some subtle differences between the sorghum seeds' development. The difference was the result of the current applied and the time at which the seeds were exposed to the current, as

shown in Table 1.

There is a great variability in the plant's lengths for the same treatment (Figure 3), i.e., the electrical stimulation effect has a generally negative impact on plant development.



Figure 3 - Random growth profile of sweet sorghum after treatment by electrical stimulation of seeds

After previous statistical analysis of original data, we noted that the electrical stimulation by direct transfer of electrons from graphite electrodes into the soil, and the seedling of sweet sorghum is very different (Figures 4 and 5). There is a significant effect of current intensity and applied time on the germination capacity of the seeds under the same cultivation conditions (i.e., light radiation, nutrients, and water supply). It is even possible to

notice the difference in plant development between the treatments.

During germination, we could notice that the current, which at the beginning was the factor with the greatest weight by the Pareto analysis (Figure 6), following the interaction between current and treatment time had the greatest impact on the vegetative development of the plants.

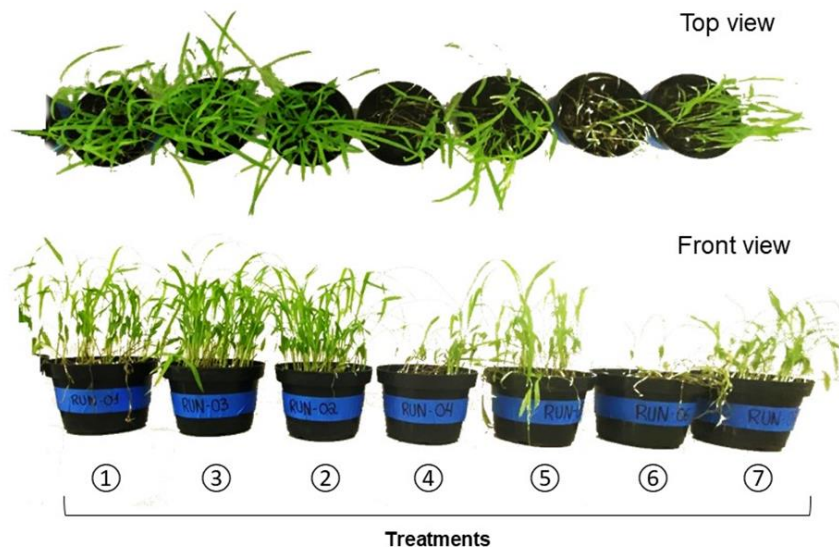


Figure 4 - Seed germination profile among sweet sorghum electrical stimulation treatments

It is plausible that an increase in seed treatment time may impact the vegetative biochemistry of the seedlings, resulting in a lower rate of vegetative development in sorghum plants. Despite treatment #3, where the combination of factors was 150 mA and 15 min of seed treatment, proving to be one of the most favorable for the vegetative development of sorghum seedlings, it also exhibited statistical similarity to treatments #2 and #5 when considering 21 days after planting (DAP). In treatment #2, the re-

sults, in absolute terms, achieved the most significant stimuli in the development of sorghum seedlings. Statistically, the treatments were largely similar. However, absolute differences between the results and treatments were discernible, suggesting that electrical stimulation with a lower current and longer treatment time, as well as a higher current and shorter treatment time, could potentially be viable options for sorghum seed electrical stimulation (Figure 5).

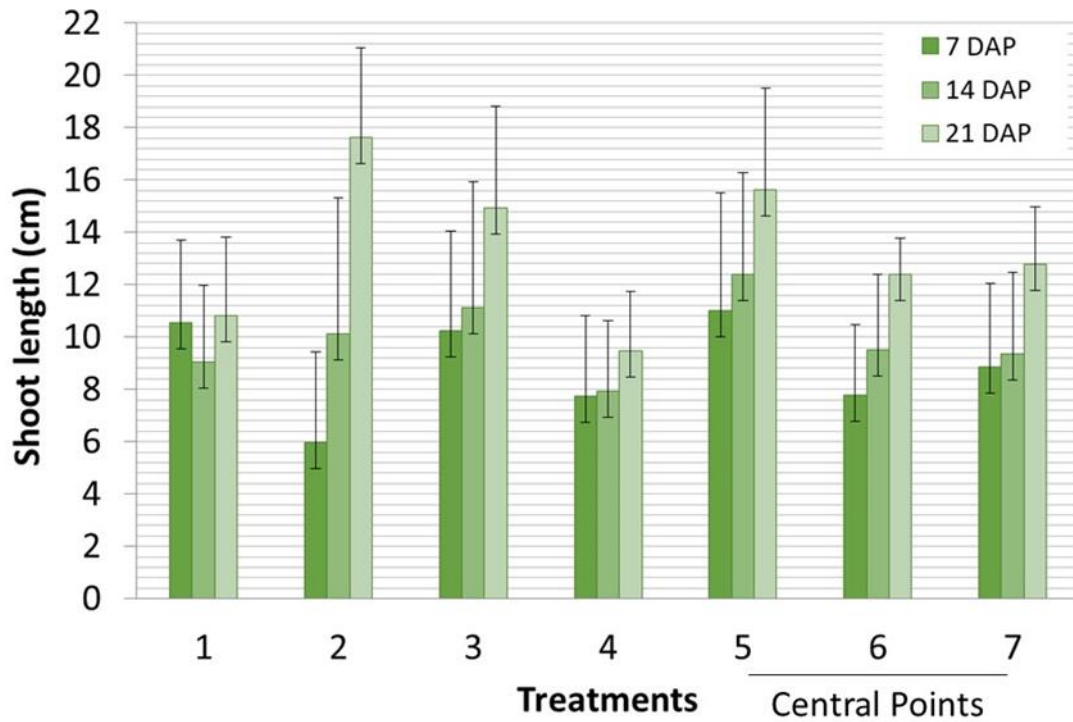


Figure 5 - Vegetative profile of sweet sorghum under electrical stimulation after 7, 14 and 21 days after planting (DAP)

Pareto charts illustrate that current, within the experimental conditions, was the most significant factor, but rather that the exposure time of sorghum seeds to those currents was important to promote seedling germination and also significant. Current

(Factor A) is an important factor, since the A×B interactions (current versus treatment time) were also important, although seed treatment time (Factor B) was not statistically significant (Figure 6).

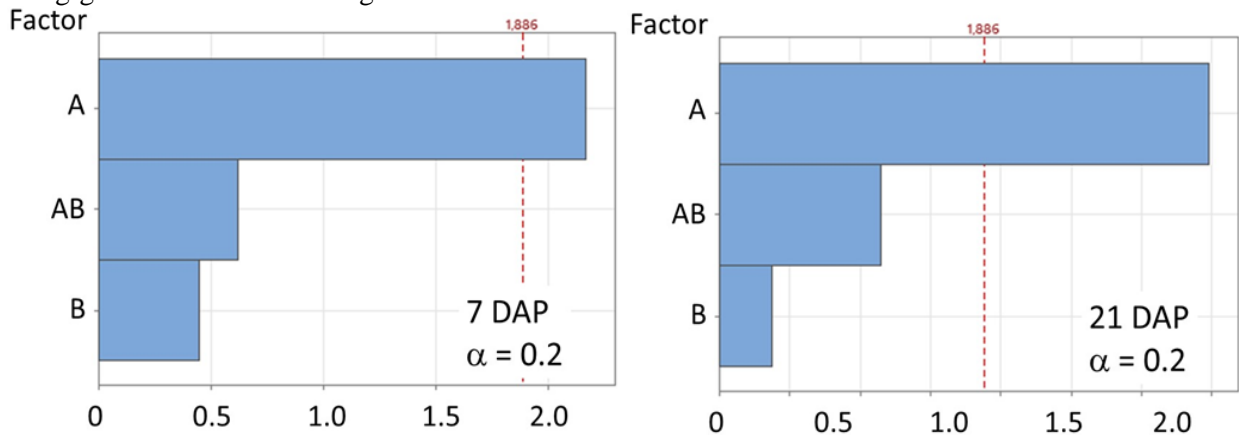


Figure 6 - Standardized Pareto chart of multivariate analyze of sweet sorghum vegetative development under electrical stimulation. The red line shows the F-value for shoot lengths. DAP: days after planting of seeds in the vases

The combination of current and seed treatment time resulted in interesting results regarding sweet sorghum germination. By analyzing the main effects and interactions, it is possible to notice that 7-DAP, the behavior of the vegetative development is different from the plant development after 21 days. It is so interesting to observe that after 7 days, there is a rapid development of the plants in some treatments, while in others, especially in those where the current was higher (150 mA), it was not favorable. The vegetative development after 7 days had a better performance in those treatments where the current was lower (50 mA) and with a longer exposure time of the seeds to electrical treatment. If we analyze the results of 14-DAP, we still have that the treatment with lower current is favorable, although

we begin to notice that the treatment time has no effect. The same can be observed by analyzing the factors' interactions, in which the current of 50 mA and the longer treatment time (15 min) were the conditions that most favored the initial sorghum plant developments. However, the longer the vegetative development time, the vegetative behavior of the plants demonstrates that for those treatments where the current was higher (150 mA), good results can also be obtained as long as the time of exposure to high currents is shorter (i.e., 5 min). Thus, the choice of current intensity will depend on the seed exposure time to that current, and it must be inversely proportional, that is, the higher the current, the shorter the exposure time and vice versa (Figure 7).

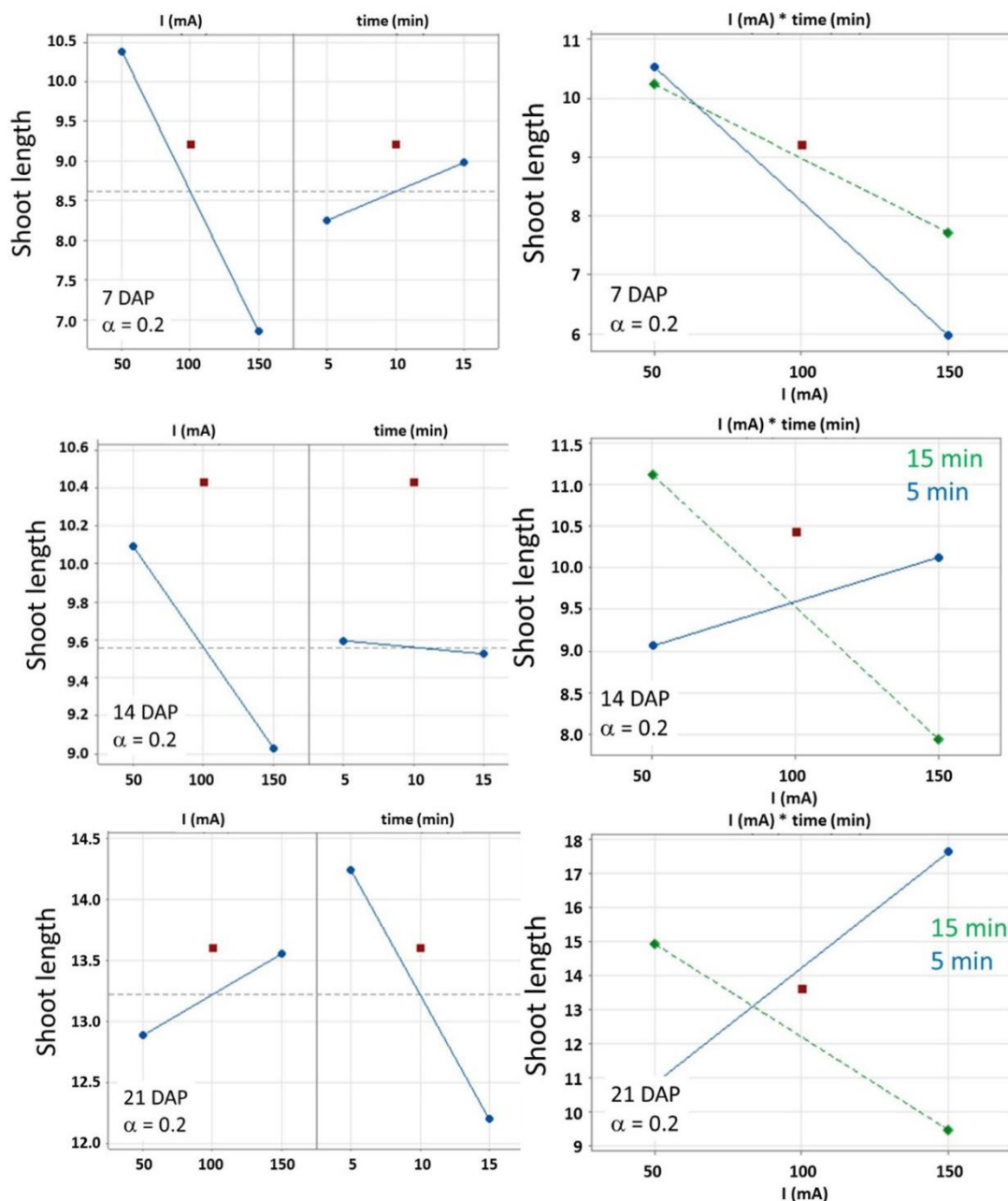


Figure 7 - Main and interaction effects of the two factors analyzed for shoot length of sweet sorghum

Since the application of current in biological organisms in a saline solution directly impacts the biochemical reactions in the embryo and, therefore, in the organism's physiology, the application of high currents for prolonged times can negatively affect the plant's vegetative cycle. Black *et al.* (1971) reported that electrical stimulation of tomato seeds increased the assimilation of ions, in particular calcium, potassium, and phosphorus. In addition, Songnuan *et al.* (2015) reported that under high-energy fields, *Arabidopsis thaliana* seeds had higher levels of gene expression at nearly tenfold (PAD3 and PR1) compared with untreated seeds.

Hepler (2005) reported that Ca^{2+} is a crucial regulator, working as a second messenger in plant cell growth and development and controlling membrane structure and function. According to Lee and Oh (2021), kale submitted to a current of 50 mA resulted in an increase in fresh mass, with greater active transport of ions by the roots, which in-

creased the calcium content by 72%, phenolic compounds by 57% and by 70% the antioxidant capacity of the plants. We also observe the effects of current and time on the development of sweet sorghum roots (Table 1). The current with less intensity favored the root development, in the same way in the time factor, where a shorter exposure period of seeds to the current also favored the root development. Taking into account the fact that the smaller the root system, the smaller the exposure area to nutrient absorption, such as calcium, which consequently alters receptor proteins in the plant cell membrane, impairing the assimilation of ions from the soil.

Thus, the electrical stimulation effect on sweet sorghum development is defined by a combination of factors, such as seed treatment time and the intensity of the applied current. Therefore, the best way to analyze the results is through multifactor analysis, such as response surface analysis.

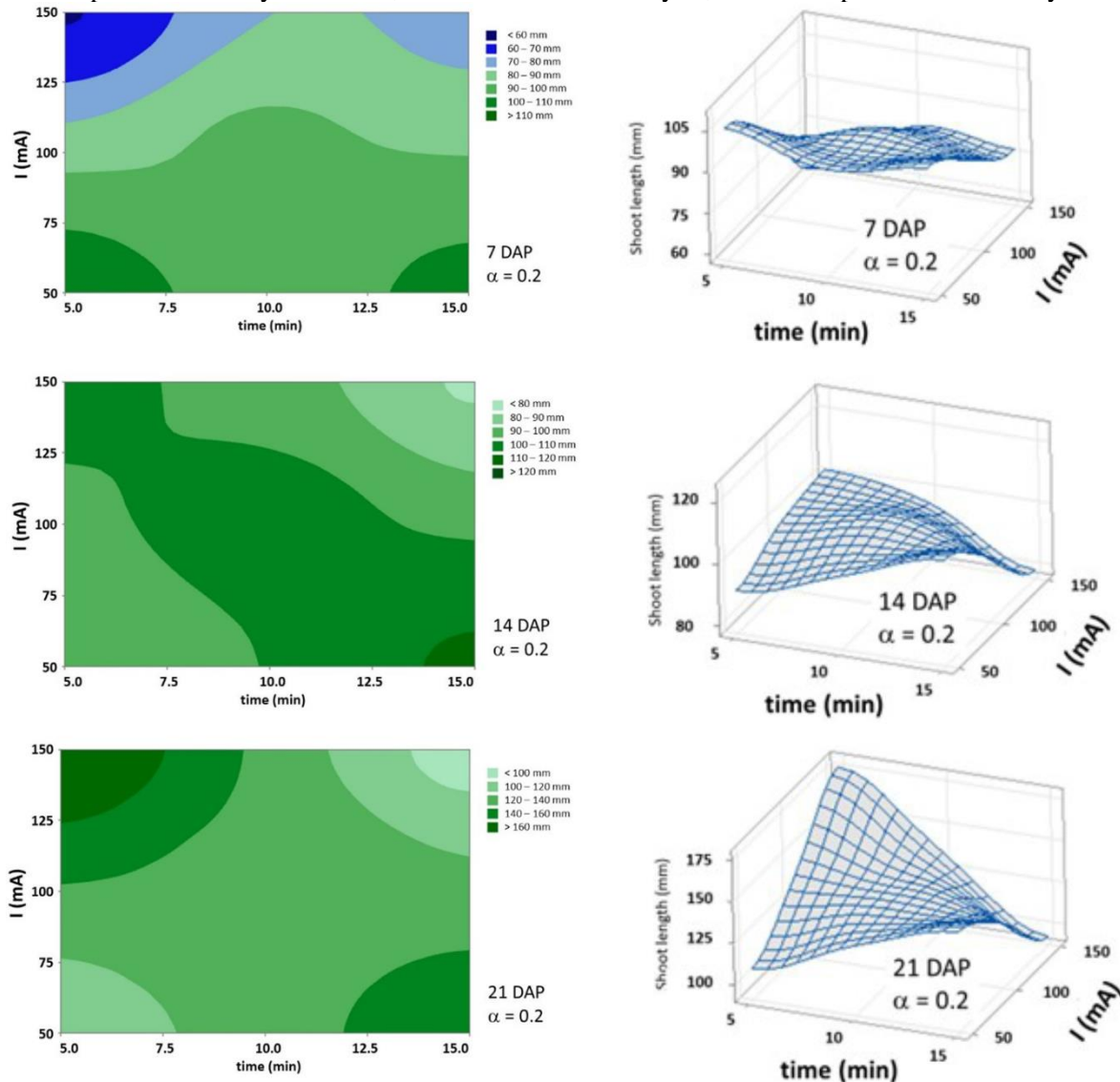


Figure 8 - Contour and surface plots of shoots development of sweet sorghum after electrical stimulation treatment

Bioethanol estimative production by biomass weight

After 21 days of cultivation, all plants in each vase were collected and the fresh mass was measured on an analytical balance with an accuracy up to 0.01 g. The electrical stimulation treatments that most promoted the vegetative development, in biomass weight, were treatments #3 and #2, with 5.1 ± 0.2 g and 3.94 ± 0.2 g, respectively. Again, we have a dichotomous profile in the vegetative development of sweet sorghum under electrical stimulation, i.e., if high current was used, the shorter exposure time is favorable and vice versa. Treatment #3 was carried out with a current of 50 mA and 15 min of exposure to the electrical treatment, while treatment #2 was carried out at 150 mA and 5 min to the treatment (Figure 8 and Table 1).

According to Assis and Morais (2014), the potential for generating ethanol from sweet sorghum in Brazil is $3.2 \text{ m}^3 \text{ ha}^{-1}$ to a biomass productivity of up to 55 t ha^{-1} , which would result in an ethanol yield of 58.6 L t^{-1} . During our experimental trials, polypropylene vases with a 14 cm diameter were used for planting, which resulted in a planting area of approximately 22.0 cm^2 of planted area ($= 2.2 \times 10^{-7} \text{ ha}$).

Based on these area proportions and the values obtained, in grams, of fresh biomass, for Treatment #3, the estimated production of sweet sorghum would be 25 t/ha, with a total conversion rate into bioethanol of $1.5 \times 10^9 \text{ L}$ ($= 1.5 \text{ million m}^3$ of bioethanol), as long as the proportions are maintained. For the calculations, harvest losses due to pests and diseases, among other factors, were not considered.

CONCLUSIONS

Undoubtedly, the preceding electrical stimulation applied to sweet sorghum seeds induces a shift in physiological behavior, significantly impacting fresh biomass production. To optimize the increase in fresh biomass, it is advisable to regulate the current at its minimum, approximately 50 mA, with a seed treatment duration of around 15 min. Intriguingly, noteworthy results can also be achieved at higher currents, such as 150 mA, provided the seed treatment time is minimized, for instance, to 5 minutes. Nonetheless, the current study unequivocally illustrates the discernible interference in the vegetative development of sweet sorghum, emphasizing the critical role of the duration of seed exposure to a specific electric current. Extended seed treatment may impact seedling biochemistry, lowering the sorghum plant's vegetative development rate. Some treatments had favorable outcomes with 150 mA and 15 min of seed treatment, while it was

statistically resembled to other treatments at 50 mA (at 21 DAP). Results yielded the most significant stimuli in sorghum seedling development. While statistically similar, noticeable differences suggest that electrical stimulation with lower current and longer treatment or higher current and shorter treatment could be potential options for sorghum seed stimulation. Therefore, the electrical stimulation of sorghum seeds could shorten the germination and establishment time of the sorghum crop for biomass production and the potential production of bioenergy by approximately 1.5 million cubic meters of bioethanol.

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