

# *In Vitro* Priming of Sugarcane Varieties (RB966928 and RB867515)

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## Abstract

Sugarcane, as a glycophyte, shows sensitivity to saline soils at various stages of its growth. The aim of this study was to evaluate the *in vitro* priming response in two sugarcane varieties (RB966928 and RB867515). Micropropagated plants, from meristems, received priming treatments by adding the salts (NaCl and KCl) in different concentrations (0.0; 12.5; 25.0 and 50.0 mM), in the MS medium. Subsequently, the plants were cultivated in rooting medium without addition of salts, acclimatized and submitted to gradient *ex vitro* saline stress with 20 → 40 and 60 mM, of each salt, for 30 days. The analyzed variables were dry matter of shoot and root, number of tillers and estimation of chlorophyll content. The experiment was carried out in a 2 × 2 × 4 factorial arrangement, in a completely randomized experimental design. Twenty replicates were used throughout the experiment. Data were submitted to analysis of variance and regression and the means were compared by Tukey's test, at a 5% probability level. The priming treatments presented a significant effect, with triple interaction, in the chlorophyll index. In the treatment with NaCl, the variety RB966928 showed an increase in the chlorophyll index with the increase of treatment levels, up to an optimal limit of 31.47 mM. On the other hand, the variety RB867515 showed decreasing in chlorophyll index. In contrast, in KCl treatment, the variety RB867515 presented the increase at the chlorophyll index with the maximum point of 25 mM. For the variables, shoot dry matter (SDM) and root dry matter (RDM) there was a significant difference ( $p < 0.05$  and  $p < 0.01$ , respectively) only between the varieties. The variety RB966928 presented higher SDM and RDM in relation to the variety RB867515. Studies are recommended with increasing the duration of the priming treatments and more detailed study of the culture throughout its productive cycle.

## Keywords

Salinity Stress, Micropropagation, *Saccharum officinarum* L.

## 1. Introduction

Sugarcane (*Saccharum officinarum* L.) is one of the world's major crops, grown in more than 100 countries. Brazil is not only the largest producer of sugarcane, but also the first in sugar production and the second in ethanol production, attracting increasingly the foreign market, with the use of biofuel as an alternative of energy. It was estimated that in the 2017/18, shall be harvested, an area in Brazil, from sugarcane destined to the sugar-alcohol activity, of 8.77 million hectares [1].

With 25% of the total area cultivated with sugarcane in Brazil, the variety RB867515 is the result of the polycrossing between the variety RB722454, which in turn, can be considered the first variety, with real contribution to the program of varieties RB, fertilized with pollen of several other varieties. It presents a habit of erect growth and easy removing straw. The tillering is medium with stems of medium diameter and high uniformity. The highlight features are productivity and rusticity; even if there is blooming, the productive potential is not compromised. It performs best in light textured soils and average fertility. The restrictions are related to the susceptibility to red rot (*Fusarium moniliforme*) and breaking the pointers, if the harvest is late, after the recommended period from July to September.

The variety RB966928 was obtained from the crossing of the variety RB855156 with pollen from RB815690. It has a slightly decumbent growth habit. It comprises 8% of the total cultivated area. It is considered as an alternative when it is sought to obtain high yields of stalks and sucrose content between the months of April and July for the Center-South region of Brazil. It is also prominent in relation to cane sprouting and sanity. The constraints are based on growing environments, being more demanding on soil fertility (high ionic exchange capacity) and medium to high water availability [2].

One of the major limitations in crop productivity is soil salinity, due to its negative effect on plant growth, ion balance and hydric relations [3]. The high concentration of salt in agricultural soils, especially those that are irrigated, is of great concern, not only in Brazil, but also in the world. In Brazil, the problem of salinity is higher in the arid and semi-arid regions, since these naturally present high concentrations of salts [4]. The use of saline soils is becoming increasingly necessary, due to the growth of the world population and the extension of urban areas [5].

By being a glycophyte, sugarcane presents sensitivity to saline soils at various stages of its growth, with reduction in yield of 50% or less, of its true potential. In addition, sugarcane decreases its sucrose yield, through the effect on biomass and juice quality [6]. As the methods of genetic improvement in sugarcane present some problems ranging from the influence of the environment, a long period to achieve results and even the risk of varietal mixing, micropropagation emerges as a way to improve these processes [7].

Micropropagation is an alternative to the conventional process of vegetative

propagation through stalks, which can provide high rates of multiplication of sugarcane with numerous advantages in relation to multiplication in the field [8]. Under controlled conditions, this technique presents efficient methods that lead to uniformity in production, generating seedlings of physiological and sanitary quality, besides producing large numbers of seedlings, in reduced time and space [9].

To support sugarcane production and improve productivity, conventional and biotechnological methods need to be integrated in order to solve some restrictions such as tolerance to biotic and abiotic stresses, nutrient management, and improvement in sugar recovery [5]. Certain molecules, environmental factors, microorganisms or their parts may pre-sensitize the cellular metabolism of plants. So that, after exposure to these factors (priming) the plants are able to respond faster, and to a higher degree than plants that were not exposed and therefore, to deal better with the constraints [10].

The application of priming methods is a way of providing greater tolerance to the abiotic stress factors, among them the saline stress, to promote a process of rustification of the plants. The use of priming with sodium chloride (NaCl) in sugarcane wheels decreases the inhibitory effects of salinity on planting and seedling growth [11]. In addition, the association of priming and micropropagation techniques also proved effective, simple, practical and capable of favoring a resistance to sugarcane plants [12].

The present study aimed to apply different priming concentrations with NaCl and KCl, separately, in sugarcane plants of varieties RB966928 and RB867515, micropropagated in semi-solid medium and cultivate the plants under saline stress after acclimatization, to evaluate the response of different priming treatments.

## 2. Material and Methods

The study was conducted from January to August 2016 at the Plant Biotechnology Laboratory, belonging to the Center for Biotechnology and Genetic Improvement of Sugarcane, located at the Federal University of Grande Dourados (UFGD).

In order to obtain the plants used in *in vitro* stress, it was followed by the meristematic extraction protocol established by [13]. Plants of the varieties RB966928 and RB867515 (**Appendix**), vegetatively propagated in a greenhouse, with one month old, were used to obtain the palm hearts (young leaves). The palm hearts, with approximately 5 cm long, were submitted to disinfestation by immersion in 70% (v/v) alcohol for 2 minutes, in sodium hypochlorite (2.5% of active chlorine) for 20 minutes and a triple wash (10 minutes each) with distilled and autoclaved water. With the aid of a stereomicroscope microscope, meristem extraction was performed in Petri dishes, containing ascorbic acid solution (50 mg·L<sup>-1</sup>) to avoid oxidation of the material.

The meristems were inoculated into glass jars, containing 30 mL of MS me-

dium [14] supplemented with 0.2 mg·L<sup>-1</sup> of 6-benzylaminopurine (BAP) and 0.1 mg·L<sup>-1</sup> of kinetin (KIN). The explants were kept in the dark for five days at 25°C ± 2°C and, thereafter, under photoperiod of 16 hours and at the same temperature. At 15 days, an exchange of culture medium of same formulation was performed, in order to avoid phenolic oxidation, remaining for another 15 days until explant development.

Aiming at the multiplication of plants, two replications were performed, on average every 30 days, using the same formulation of the medium. After the multiplication phase, the pre-conditioning treatment was performed during 24 hours. The plants were transferred to test tubes containing 15 mL of semi-solid MS medium, supplemented with the combination of two salts NaCl and KCl and different concentrations (0.0, 12.5, 25.0 and 50.0 mM), for each salt.

After passing by the saline stress treatments, the plants were submitted to the rooting process, using MS medium supplemented with 30 g·L<sup>-1</sup> of sucrose and 0.5 mg·L<sup>-1</sup> of 3-indolebutyric acid (IBA).

For evaluation the response to *in vitro* priming and before the application of *ex vitro* stress, the plants underwent acclimatization, being taken to the greenhouse, under 30% shading, planted in plastic bags of 30 mL, containing commercial substrate Bioplant®. Where for 30 days, they were irrigated by sprinkling, twice a day. After this period, the *ex vitro* stress was performed, using irrigation solution, added of NaCl or KCl. The plants, which received the NaCl priming treatment, were treated with NaCl containing irrigation solution and the plants with KCl priming treatment received irrigation solution containing KCl. The saline concentration in the irrigation solutions was started with 20 mM of salt and every 10 days, an increment of 20 mM occurred, to create a graduate saline stress up to 60 mM for 30 days.

The analyzed variables were dry matter of shoot and root, number of tillers and estimation of chlorophyll content. Chlorophyll estimation was obtained with chlorophyll meter (SPAD-502 Plus, Konica Minolta), with readings at three points of the first fully expanded leaf, avoiding the central vein. Aerial part and root samples were dried in an aeration oven at 70°C until constant weight to determine the dry matter. The experiment was carried out in a 2 × 2 × 4 factorial arrangement, consisting of two varieties (RB966928 and RB867515), two salts (NaCl and KCl) and four salt concentrations (0.0, 12.5, 25.0 and 50.0 mM), in a completely randomized experimental design. Twenty replicates were used throughout the experiment. Data were submitted to analysis of variance and regression, using the statistical program SISVAR [15] and the means were compared by Tukey's test, at a 5% probability level.

### 3. Results and Discussion

The analysis of variance revealed triple interaction between varieties, salts and salt concentrations, for the SPAD chlorophyll index variable (**Table 1**).

By proceeding the decomposition of the triple interaction, the unfolding of the

**Table 1.** Summary of variance analysis of the number of tillers (NT), shoot dry matter (SDM), root dry matter (RDM), and SPAD index of sugarcane plants submitted to salt stress under different types and concentrations of salts.

Variation source	Mean squares				
	DF	NT	SDM	RDM	SPAD index
Varieties	1	1	0.0724*	0.0631**	16.7077*
Salts	1	1	0.0034	0.0019	2.8477
Concentrations	3	2.0208	0.0182	0.0100	0.9505
Varieties*Salts	1	0.0625	0.0291	0.0019	0.9264
Varieties*Concentrations	3	0.5417	0.0167	0.0047	5.2477
Salts*Concentrations	3	1.0417	0.0024	0.0038	5.6780
Varieties*Salts*Concentrations	3	1.0208	0.0050	0.0015	38.9967**
Residue	48	3	0.0129	0.0057	137.7325
Variation Coefficient (%)		68.43	28.36	28.78	10.5

\*\*Significant at 1% probability, by F test; \*significant at 5% probability, by the test of F.

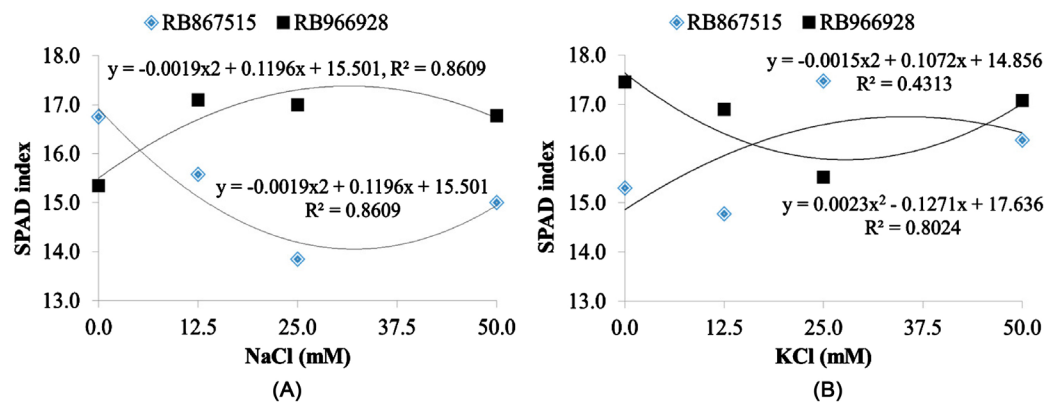
salt concentrations within each salt and variety proved to be significant. To explain the triple interaction, the quadratic regression model was the one that most appropriated to the data (Figure 1).

The interaction showed that the SPAD chlorophyll index was affected with the change in priming concentrations and that the two salts (NaCl and KCl) and the two varieties (RB966928 and RB867515) presented different responses.

In relation to NaCl, it can be observed that each variety responded differently (Figure 1(A)). Reference [4] has already demonstrated that each sugarcane cultivar can respond differently to salt stress. For the variety RB966928, it was observed that the chlorophyll index increased with the increasing of the priming treatment levels, up to an optimum limit of 31.47 mM, with a reduction from that limit. Reference [16] explains that the intensity of priming must be carefully manipulated, so that there is no negative response in metabolism, plant growth and development, but a mild and stimulating stress condition.

About the variety, RB867515 submitted to the priming treatment, with NaCl, it was observed that the addition and the increase of priming concentration led to a reduction in the chlorophyll index. Even at 50.0 mM concentration, inducing an increase of this level and indicating an adaptation of the plant, the chlorophyll index was still lower than the control (Figure 1(A)). Reference [17] also showed that the variety RB867515 presents a greater reduction in chlorophyll content and even better adaptation to the salinity conditions, when compared to other varieties. This response is a plant defense mechanism to better adapt to salt stress.

For stress with KCl, the two varieties also responded differently (Figure 1(B)). With the addition and increasing of the priming concentration, the plants RB966928 presented a decrease in the chlorophyll index, up to the concentration of 25.0 mM, and at the concentration of 50.0 mM, the plants showed an increase



**Figure 1.** Estimation of chlorophyll by SPAD index of sugarcane plants RB966928 and RB867515, from the application of priming with 0.0; 12.5; 25.0 and 50.0 mM of (A) NaCl and (B) KCl, submitted to salt stress treatment under greenhouse conditions.

in the chlorophyll index. For plants RB867515, even with the unsatisfactory adjustment of the trend line, it can be seen, that the concentration of 25.0 mM induced an increase in chlorophyll content.

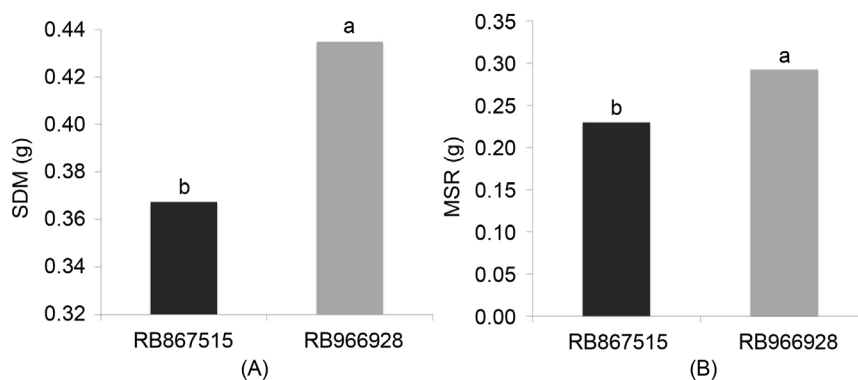
Several studies have already shown that the content of photosynthetic pigments is affected in plants susceptible to salinity, and the effect on chlorophyll content depends on the concentration of the stressor agent, as well as, of the plant species [18]. Therefore, the SPAD chlorophyll index becomes a simple way of comparing plant response to salinity.

Studies with other species of plants such as sesame, pea and soybean show that the decrease in chlorophyll content, in plants under salt stress is due to the increase in chlorophyllase degradation, since the presence of excess salts stimulates the activity of chlorophyllase that degrades chlorophyll molecules. In addition, the plant under saline stress presents a decrease in the synthesis of these pigments [19].

For the variables: shoot dry matter (SDM) and root dry matter (RDM) there was a significant difference only between the varieties (Table 1). The variety RB966928 presented higher SDM and RDM in relation to the variety RB867515, regardless of the treatment used (Figure 2).

The low growth is an adaptive characteristic of the plant that grows under stress conditions, because it allows allocating its resources in a way that does not compromise its full development [20]. Even with the variation observed in the chlorophyll index, the other parameters (number of tillers, SDM and RDM) showed no significant difference between the priming treatments and were not significant within the interactions (Table 1).

According to [21], the most salient symptom of salinity is the reduction in plant growth. Thus, priming treatments had an effect on chlorophyll, but this effect was not enough to affect the plant in its development. According to [22], plants need to undergo stressful conditions to develop tolerance. Reference [23] complements that for the plants to tolerate a new cycle of stress, it is necessary to occur a process of rustification.



**Figure 2.** (A) Shoot Dry Matter—SDM; (B) Root Dry Matter—RDM of sugarcane plants (RB867515 and RB966928) from the priming application and subjected to saline stress under greenhouse conditions. Averages followed by different letters differ one another, by the Tukey test at 0.05 probability.

Reference [12] obtained contrasting results, where the *in vitro* priming characterized by 25.0 mM NaCl, for 24 hours, it was sufficient to prevent the deleterious effect of the salt on tillering and the reduction of biomass of sugarcane plants subjected to *ex vitro* saline stress. The difference in results may have occurred due to the use of different methodologies and varieties of sugarcane, since [12] used plants of the variety RB98710, micropropagated in a temporary immersion bioreactor and the present work made use of traditional methodology, based on semi-solid culture medium.

The temporary immersion bioreactors make use a liquid nutrient medium, allowing the renewal of air and nutrients during cultivation, resulting in higher plant growth and multiplication, when compared to semi-solid medium cultivation [24]. Furthermore, in bioreactors, the greater area of contact of plants with the culture medium, increases, considerably, their absorption, since the leaves, stems and roots can absorb the ions. In thesis, plants absorb more ions in the immersion system than in the traditional one [25].

In order to increase the efficiency of priming under micropropagation conditions in semi-solid medium, further studies should be carried out, regarding the intensity and duration of the treatments. Exposure to the aggressor agent over 24 hours, may be more effective in generating a stress capable of activating the cellular metabolism and increasing the physiological activity of the plant.

For priming, also applied in sugarcane, but in wheels cultivated in greenhouse, the concentration and the time of the treatments, with NaCl were superior to those used in micropropagated plants under a temporary immersion bioreactor. Reference [11] used 150 mM NaCl for 8 days and obtained satisfactory results, where the application of priming reduced the inhibitory effects of salinity on germination and seedling growth, in terms of fresh and dry matter production.

All evaluations of the experiment were conducted only in the initial phase of vegetative growth of the plants. As sugarcane is a long cycle crop, it is recom-



mended a more detailed study of the crop throughout its productive cycle, to better evaluate the priming treatments and the genetic potential of each variety of sugarcane.

#### 4. Conclusions

The varieties RB966928 and RB867515 responded differently to the priming treatments with different concentrations of NaCl and KCl.

The changes in the concentrations of the priming treatments had a significant effect under the chlorophyll index, and the two salts (NaCl and KCl) and the two varieties (RB966928 and RB867515) generated different responses.

Studies are recommended with increasing the duration of the priming treatments and more detailed study of the culture throughout its productive cycle.

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#### References

- [1] CONAB, Companhia Nacional de Abastecimento (2017) Accompaniment of the Brazilian Harvest: Sugarcane, Harvest 2017/2018. Third Collection Data, 1-81. [http://www.conab.gov.br/OlalaCMS/uploads/arquivos/18\\_01\\_08\\_09\\_08\\_38\\_cana\\_d\\_ezembro\\_novo.pdf](http://www.conab.gov.br/OlalaCMS/uploads/arquivos/18_01_08_09_08_38_cana_d_ezembro_novo.pdf)
- [2] Daros, E., Oliveira, R.A.de. and Barbosa, G.V.S. (2015) 45 Years of RB Varieties of Sugarcane: 25 Years of Ridesa. Graciosa, Curitiba.
- [3] Chen, J., Wang, W., Wu, F., He, E., Liu, X., Shanggua, Z. and Zheng, H. (2015) Hydrogen Sulfide Enhances Salt Tolerance through Nitric Oxide-Mediated Maintenance of Ion Homeostasis in Barley Seedling Roots. *Scientific Reports*, **5**, 1-19.
- [4] Sengar, K., Sengar, R.S. and Singh, A. (2013) Biotechnological and Genomic Analysis for Salinity Tolerance in Sugarcane. *International Journal of Biotechnology and Bioengineering Research*, **4**, 407-414. <http://www.ripublication.com/ijbbr.htm>
- [5] Munns, R. (2005) Genes and Salt Tolerance: Bringing Them Together. *New Phytologist*, **167**, 645-663. <https://doi.org/10.1111/j.1469-8137.2005.01487.x>
- [6] Suprasanna, P., Patade, V.Y., Desai, N.S., Devarumath, R.M., Kawar, P.G., Pagariya, M.C., Ganapathi, A., Manickavasagam, M. and Babu, K.H. (2011) Biotechnological Developments in Sugarcane Improvement: An Overview. *Sugar Tech*, **13**, 322-335. <https://doi.org/10.1007/s12355-011-0103-3>
- [7] Eldessoky, D.S., Ismail, R.M., Hadi-Abdel, A. and Abdallah, N. (2014) Establishment of Regeneration and Transformation System of Sugarcane Cultivar GT54-9 (C9). *GM Crops*, **2**, 126-134. <https://doi.org/10.4161/gmcr.2.2.17288>
- [8] Lakshmanan, P., Geijskes, R.J., Aitken, K.S., Grof, C.L.P., Bonnet, G.D. and Smith, G.R. (2005) Sugarcane Biotechnology: The Challenges and Opportunities. *In Vitro Cellular & Developmental Biology—Plant*, **41**, 345-363. <https://doi.org/10.1079/IVP2005643>
- [9] Geijskes, R.J., Wang, L., Lakshmanan, P., McKeon, M.G., Berding, N., Swain, R.S., Elliott, A.R., Grof, C.P.L., Jackson, J.A. and Smith, G.R. (2006) SMARTSETTM Seedlings: Tissue Cultured Seed Plants for the Australian Sugar Industry. In: Janick, J., Ed., *Proceedings Australian Society Sugar Cane Technologists*, John Wiley &

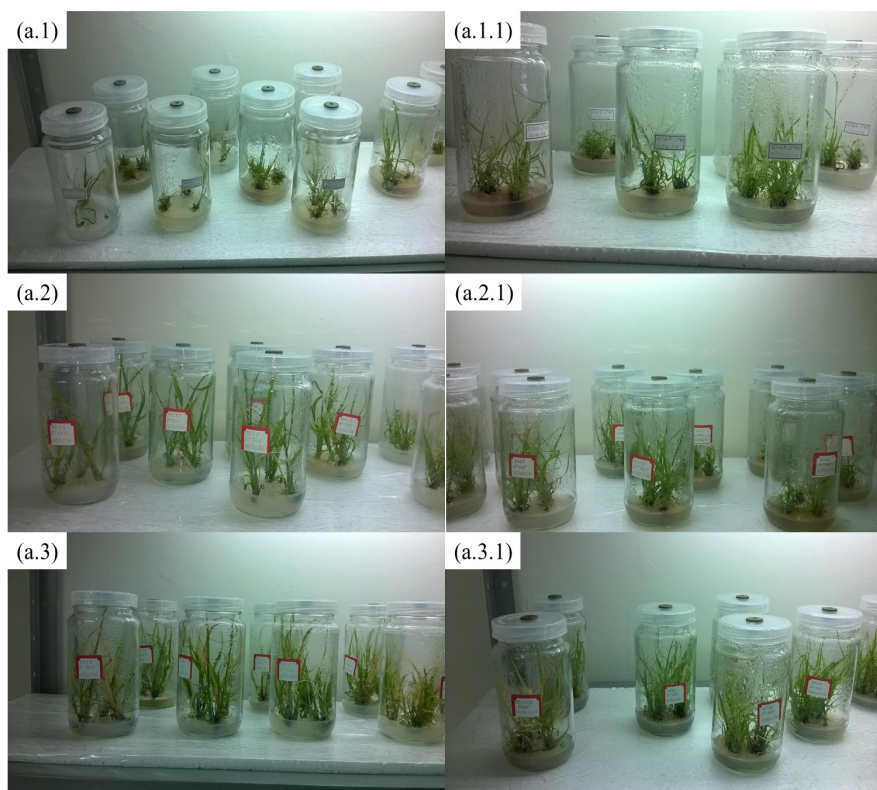


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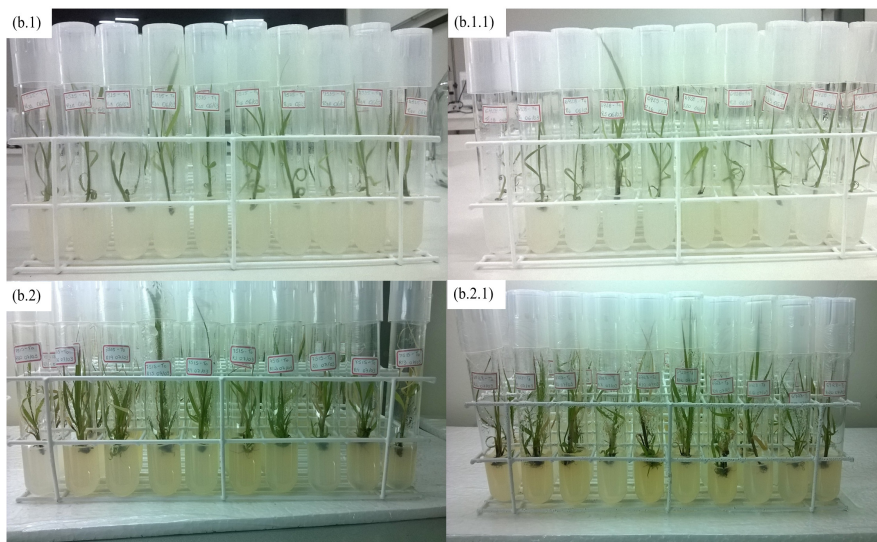
- [10] Conrath, U. (2009) Priming of Induced Plant Defense Responses. *Advances in Botanical Research*, **51**, 361-395. [https://doi.org/10.1016/S0065-2296\(09\)51009-9](https://doi.org/10.1016/S0065-2296(09)51009-9)
- [11] Patade, V.Y., Bhargava, S. and Suprasanna, P. (2011) Salt and Drought Tolerance of Sugarcane under Iso-Osmotic Salt and Water Stress: Growth, Osmolytes Accumulation, and Antioxidant Defense. *Journal of Plant Interactions*, **6**, 275-282. <https://doi.org/10.1080/17429145.2011.557513>
- [12] Melo, G.M. de, Barbosa, M.R., Dias, A.L.F., Willadino, L. and Camara, T.R. (2014) Preconditioning *in Vitro* of Sugarcane (*Saccharum* spp.) Seedlings to Salt Stress Tolerance. *Revista Brasileira de Engenharia Agrícola e Ambiental*, **18**, 27-33.
- [13] Dutra, L.F., Donini, L.P., Silva, S.D.A., Silva, N.D.G., Thiel, F.B., Vitória, J.M. and Zacarias, F.M. (2011) Micropropagation Protocol of Sugarcane. Embrapa Clima-Temperado, Pelotas, 8 p. <https://ainfo.cnptia.embrapa.br/digital/bitstream/item/67353/1/Circular-128.pdf>
- [14] Murashige, T. and Skoog, F.A. (1962) Revised Medium for Rapid Growth and Bioassays with Tobacco Tissue Cultures. *Physiologia Plantarum*, **15**, 473-497. <https://doi.org/10.1111/j.1399-3054.1962.tb08052.x>
- [15] Ferreira, D.F. (2000) SISVAR (Analysis of Variance System). Federal University of Lavras (Exact Sciences Department DEX), Lavras.
- [16] Lichtenthaler, H.K. (2004) Stress and the Measure of Stress in Plants. In: Reigosa, M.J., Pedrol, N. and Sánchez, A., Eds., *Plant Ecophysiology—A Science of Synthesis*, Thomson, Madrid, 59-111.
- [17] Willadino, L., Oliveira Filho, R.A., Silva Junior, E.A., Gouveia Neto, A. and Camara, T.R. (2011) Salinity Stress in Two Varieties of Sugar Cane: Enzymes of the Antioxidant System and Chlorophyll Fluorescence. *Revista Ciência Agronômica*, **42**, 417-422. <http://ccarevista.ufc.br/seer/index.php/ccarevista/article/view/1745/557>
- [18] Jamil, M., Rehman, S., Lec, K.J., Kim, J.M., Kim, H.S. and Rha, E.S. (2007) Salinity Reduced Growth PS2 Photochemistry and Chlorophyll Content in Radish. *Scientia Agrícola*, **64**, 111-118. <https://doi.org/10.1590/S0103-90162007000200002>
- [19] Majumdar, S., Ghosh, S., Glick, B.R. and Dumbroff, E.B. (1991) Activities of Chlorophyllase, Phosphoenolpyruvate Carboxylase and Ribulose 1,5 Bisphosphate Carboxylase in the Primary Leaves of Soybean during Senescence and Drought. *Physiologia Plantarum*, **81**, 473-480. <https://doi.org/10.1111/j.1399-3054.1991.tb05087.x>
- [20] Zhu, J.K. (2002) Salt and Drought Stress Signal Transduction in Plants. *Annual Review of Plant Biology*, **53**, 247-273. <https://doi.org/10.1146/annurev.arplant.53.091401.143329>
- [21] Munns, R. and Gilliham, M. (2015) Salinity Tolerance of Crops—What Is the Cost? *New Phytologist*, **208**, 668-673. <https://doi.org/10.1111/nph.13519>
- [22] Levitt, J. (1980) Responses of Plant to Environmental Stresses. Academic Press, New York.
- [23] Rivas, R., Oliveira, M.T. and Santos, M.G. (2013) Three Cycles of Water Deficit from Seed to Young Plants of *Moringa oleífera* Woody Species Improves Stress Tolerance. *Plant Physiology and Biochemistry*, **63**, 200-208. <https://doi.org/10.1016/j.plaphy.2012.11.026>
- [24] Ribeiro, J.M. and Bastos, D.C. (2008) Bioreactors: General Aspects and Their Use for Plant Tissue Culture. Embrapa Semi-Árido, Petrolina, 30 p. <http://ainfo.cnptia.embrapa.br/digital/bitstream/CPATSA-2009-09/40172/1/SDC214.pdf>

- [25] Murch, S.J., Liu, C., Romero, R.M. and Saxena, P.K. (2004) *In Vitro* Culture and Temporary Immersion Bioreactor Production of *Crescentia cujete*. *Plant Cell, Tissue and Organ Culture*, **78**, 63-68.  
<https://doi.org/10.1023/B:TICU.0000020397.01895.3e>

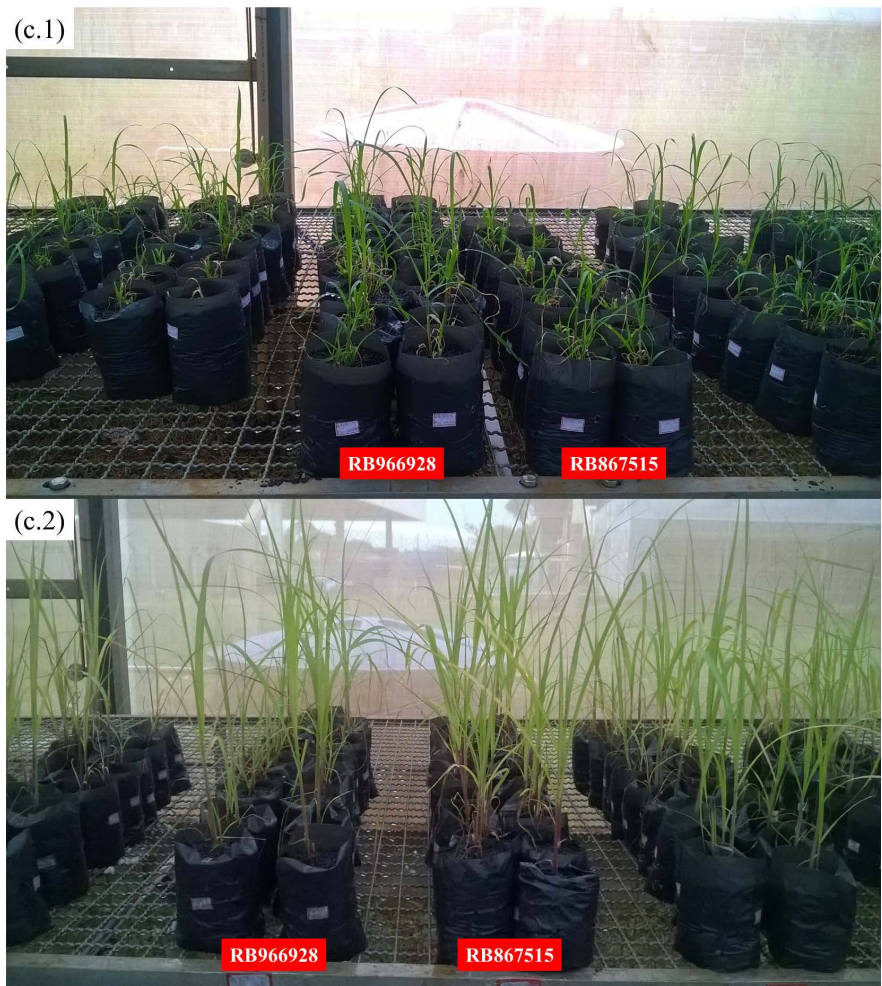
## Appendix



**Figure A1.** (a.1) Sugarcane meristems developed after 5 days of inoculation (5 DAI), variety RB867515; (a.1.1) Variety RB966928; (a.2) First exchange of culture medium (MS) with the same formulation, variety RB867515; (a.2.1) Variety RB966928; (a.3) Developed plants after the second replication (60DAI), variety RB867515; (a.3.1) Variety RB966928.



**Figure A2.** (b.1) Sugarcane plants replicated, under priming *in vitro* with semi-solid MS medium, containing the salts NaCl and KCl, at different concentrations (0.0, 12.5, 25.0 and 50.0 mM), for each salt. Variety RB867515; (b.1.1) Variety RB966928; (b.2) Sugarcane plants submitted to the rooting process, with MS medium supplemented with 0.5 mg·L<sup>-1</sup> of 3-indolebutyric acid (IBA), variety RB867515; (b.2.1) Variety RB966928.



**Figure A3.** (c.1) Sugarcane plants, varieties RB867515 and RB966928, after 30 days of acclimatization in greenhouse, under 30% shading, planted in plastic bags of 30 mL, containing commercial substrate Bioplant®; (c.2) Sugarcane plants, varieties RB867515 and RB966928, after 30 days of graduate saline stress, with irrigation solution (20 → 60 mM) of NaCl and KCl.