

## Residual effect of bur gherkin seed treatment with biostimulant under salt stress<sup>1</sup>

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**ABSTRACT** - The physiological quality of seeds used in agricultural production is one of the main factors that should be considered when implanting the culture. This study aimed at evaluating the quality of bur gherkin seeds, cv. Liso de Calcutá, coming from plants grown from seeds that were previously treated with biostimulant (0, 5, 10, 15 and 20 mL kg<sup>-1</sup> seeds) and cultivated in two irrigation water salinities (0.5 and 3.5 dS. m<sup>-1</sup>). Initially, the water content of seeds was determined and the following tests were performed: germination, accelerated aging, greenhouse emergence, emergence speed index, height and dry mass of seedling aerial part. It was found that the physiological quality of bur gherkin seeds coming from plants that were subjected to salt stress is negatively affected, and that the use of biostimulant, applied via seeds, provides beneficial effects on the physiological quality of seeds produced in the next generation.

Index terms: *Cucumis anguria*, salinity, bioregulator, vigor.

## Efeito residual do tratamento de sementes de maxixe com bioestimulante sob estresse salino

**RESUMO** - A qualidade fisiológica de sementes utilizada na produção agrícola é um dos principais fatores que deve ser considerado no momento da implantação da cultura. Objetivou-se avaliar a qualidade de sementes de maxixeiro, cv. Liso de Calcutá, provenientes de plantas oriundas de sementes previamente tratadas com bioestimulante (0, 5, 10, 15 e 20 mL kg<sup>-1</sup> de sementes) e cultivadas sob dois níveis de salinidade da água de irrigação (0,5 e 3,5 dS. m<sup>-1</sup>). Inicialmente, determinou-se o teor de água das sementes e realizou-se os testes de germinação, envelhecimento acelerado, emergência em casa de vegetação, índice de velocidade de emergência, altura e massa seca da parte aérea das plântulas. Constatou-se que a qualidade fisiológica de sementes de maxixeiro oriundas de plantas submetidas ao estresse salino é afetada de maneira negativa, e que o uso de bioestimulante, aplicado via semente, proporciona efeito benéfico na qualidade fisiológica das sementes produzidas na geração seguinte.

Termos para indexação: *Cucumis anguria*, salinidade, biorregulador, vigor.

### Introduction

The culture of bur gherkin (*Cucumis anguria* L.) is quite known in the northern and northeastern regions of Brazil. According to Medeiros et al. (2010), bur gherkin producers frequently use local seeds obtained from volunteers; since these seeds are produced without proper management, their physiological quality is normally lower than the one of those produced according to production standards and regulations, set by the Ministry of Agriculture, Livestock and Supplies - Brasil (2009).

The physiological quality of seeds is a very important factor, since it guarantees a quick and uniform seedling

establishment (Lopes et al., 2014), maximizing the action of the other inputs and production factors used in cultivation (Souza et al., 2007). Among the main factors in vegetable production, it is possible to mention the production of quality seedlings. Seedling development is one of the most important stages for the culture cycle; it directly influences the plant final performance, both from the nutritional and the productive point of view, since there is a direct relation between healthy seedlings and field production (Campanharo et al., 2006).

One of the serious problems that directly affect plants is salinity, since it acts damaging their metabolism, because the predominance of toxic ions during root growth may

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cause toxicity (Nobre et al., 2010). Moreover, according to these authors, this stress brings changes in the plant ability to absorb, transport and use the ions that are essential to its growth. Consequently, one of the alternatives to minimize the harmful effects of salts on plants is the use of plant regulators that reduce the intensity of these effects over plant growth, enabling the use of saline water (Oliveira et al., 2013).

Currently, plant regulators have been largely used in Brazilian agriculture, acting as mediators of physiological processes and increasing plant growth and development, cellular division and also being able to increase the absorption of water and nutrients by plants (Vieira and Castro, 2004).

Various studies have already been performed to evaluate the effects of treating seeds with biostimulants on the seed quality and seedling vigor within various cultures, such as watermelon (Silva et al., 2014), soybean (Moterle et al., 2011), rice (Elli et al., 2016), maize (Oliveira et al., 2016), and others. These authors report the good effect of seed treatment with bioregulators on plant development; however, these studies only evaluated the biostimulant direct effect, whereas there are only a few studies about the residual effect on the quality of produced seeds (Melo et al., 2015).

Recently, studies were developed to evaluate the effect of salinity on bur gherkin seed germination and seedling vigor (Oliveira et al., 2013; Reis et al., 2013; Alves et al., 2014). However, there is scarce information regarding the effect of salinity and seed treatment on the quality of those produced under these conditions.

Hence, the goal was to evaluate the physiological quality of bur gherkin seeds coming from plants that were cultivated under salt stress conditions, and whose seeds were treated with biostimulants.

## Material and Methods

The work was developed at the Seed Testing Laboratory of the Universidade Federal Rural do Semi-Árido (UFERSA), in Mossoró, Rio Grande do Norte state, Brazil. Bur gherkin seeds, West Indian gherkin cultivar, were used; obtained from fruits produced by plants whose seeds were treated with biostimulant (0, 5, 10, 15 e 20 mL kg<sup>-1</sup> seeds) and cultivated in two levels of irrigation water salinity (S1 = 0.5 dS. m<sup>-1</sup>, water coming from the UFERSA supply sector; and S2 = 3.5 dS. m<sup>-1</sup>, obtained by the dissolution of sodium chlorate (NaCl), diluted in water with saline level 0.5 dS. m<sup>-1</sup>). Thus, the 5 x 2 combination resulted, summing up to 10 treatments.

The biostimulant Stimulate<sup>®</sup> was used, a liquid product composed by three plant regulators, that is, 90 mg. L<sup>-1</sup> (0.009%) kinetin, 50 mg. L<sup>-1</sup> (0.0005%) gibberellic acid and

50 mg. L<sup>-1</sup> (0.005%) indolebutyric acid, as well as 99.981% inert ingredients (Stoller, 1998).

Fruits were harvested when they reached physiological maturity (35 to 40 days after anthesis), with transitional external color from yellowish to completely yellow, according to Medeiros et al. (2010) for the municipality of Mossoró, Rio Grande do Norte State, Brazil. After harvesting, the seeds were manually extracted, washed and placed to dry in the shade. Then, the seeds were homogenized, placed in kraft paper packages and stored in cool chamber (15 °C and 50% relative humidity of the environment) until the beginning of the experimental phase.

The seeds were evaluated by the following tests and determinations:

*Water content*: performed in an oven at 105 ± 3 °C, for 24 hours, according to the Rules for Seed Testing (RAS) (Brasil, 2009). The results were expressed in percentage (wet bases).

*Germination*: four replications of 50 seeds per treatment were seeded in paper towel (*germitest*), moistened twice the weight of dry paper and settled for germination at 25 °C, with no light. Counts were performed on day four and eight after seeding, according to RAS criteria (Brasil, 2009). The results were expressed in average percentage of normal seedlings for each treatment.

*Accelerated aging with NaCl saturated solution* - performed in transparent plastic box method (11.5 x 11.5 x 3.5 cm) (mini-chamber), with holders on the inside to support a metallic mesh. On the surface of each one, 250 seeds from each treatment were distributed; at the bottom of each gerbox there were 40 mL of NaCl saturated solution instead of water, providing an environment with 76% relative humidity, obtained through the proportion 40 g NaCl/100 mL water (Jianhua and McDonald, 1996). The closed boxes were kept in a *Biochemical Oxygen Demand* (B.O.D.) chamber, set at 42 °C, for 72 hours (Torres and Marcos-Filho, 2001). Subsequently, four sub-samples corresponding to replications of 50 seeds per treatment were settled for germination, following the same procedure used for the germination test. The evaluation was performed four days after seeding, calculating the percentage of normal seedlings for each treatment. In order to monitor this test, the water content of seeds after the aging period was determined.

*Seedling emergence*: it was performed in a greenhouse, using expanded polystyrene trays with 200 cells that contained commercial substrate composed by coconut fibers (Vida Verde<sup>®</sup>); they were kept under environmental conditions, with daily irrigation. For each treatment, four 50-seed replications were used; they were evaluated 12 days after planting, by counting normal seedlings emerged in a

germination test (Brasil, 2009). The results were expressed in average percentage of normal seedlings for each treatment.

*Seedling emergence speed index* - performed daily since the beginning of emergence and carried on until day twelve. The index was calculated by summing the ratios of the number of seedlings emerged in the period with the number of days from planting to emergence, using the formula proposed by Maguire (1962).

*Height and dry mass of seedling aerial part* - at the end of the seedling emergence test, these were measured with the help of a cm graduated ruler and they were measured from the lap to the apex of the leaf. Subsequently, seedlings from the usable area were cut near the substrate, placed in paper bags to be treated and set to dry in a forced air circulation oven at 60 °C for 72 hours. After this period, the aerial part of normal seedlings was weighed on a precision scale (0.01 g) and the results were expressed in grams.

The obtained results were submitted to analysis of variance, where the comparison of averages was performed by Tukey's test at 5% probability for the condition of salt stress and regression for the biostimulant doses. Data about the water content of seeds were not submitted to statistical analysis, only for monitoring purposes. The statistical analysis was performed through the SISVAR program (Ferreira, 2011).

## Results and Discussion

The initial water content of bur gherkin seeds, obtained by plants cultivated under salt stress conditions and coming from seeds previously treated with biostimulant doses, varied by 1.2 percentage points (p.p.) (9.2 to 10.4%) (Table 1). This variation lies within the tolerable limit, which is 2.5% maximum between samples (Marcos-Filho, 2015). According to this author, uniformity in the water content of seeds is essential to the assessment standardization and the acquisition of consistent results. In Table 1, it is possible to see that the water content after accelerated aging varied by 2.4 p.p., that is, within the tolerable limits.

Plants cultivated under saline conditions produced seeds with lower physiological quality, in terms of germination. It is particularly noted that this effects varies according to tested species and concentrations.

For first count results of germination and accelerated aging with NaCl saturated solution, it was verified that none of the biostimulant doses mitigated the negative effects of salinity; on the other hand, the highest values occurred for seeds coming from plants irrigated with less saline water (Table 2). In literature there are scarce reports about the vigor of seeds produced in plants irrigated with saline water;

however, various studies evaluated the effect of osmotic potential on the vigor of seeds from other species like broccoli (Lopes et al., 2014) and bok choy (Lopes and Macedo, 2008), and they showed that, under salt stress, there is a decrease during first count.

Table 1. Water content (WC) at the beginning and after the accelerate aging test with NaCl saturated solution (AASS) in bur gherkin seeds, West Indian Gherkin cv., cultivated under salt stress and with different biostimulant doses.

Salinity (dS. m <sup>-1</sup> )	Biostimulant dose (mL. kg <sup>-1</sup> of seeds)	Initial WC (%)	WC (%) after AASS
0.5	0	9.0	9.2
0.5	5	9.7	9.8
0.5	10	9.8	10.0
0.5	15	9.7	10.7
0.5	20	9.6	9.8
3.5	0	9.2	11.0
3.5	5	10.4	11.5
3.5	10	9.6	10.2
3.5	15	9.9	10.4
3.5	20	9.7	10.3

A slower germination process is due to the osmotic effect, where there is a higher difficulty in water absorption by seeds; it is also due to the toxic effect provoked by the absorption of Cl<sup>-</sup> and Na<sup>+</sup> ion in the embryo: they modify the soaking process, which is the first stage of germination (Tobe et al., 2000).

This reduction may affect the kinetics of water absorption by the seeds (osmotic effect), as well as raise the concentration of ions in the embryo to toxic levels (toxic effect) (Cramer et al., 1986; Tobe et al., 2000).

Emergence speed index and height presented significant and negative effects of salinity on seedlings originated from seeds of plants coming from seeds that were treated with 0 to 15 mL. kg<sup>-1</sup> doses. Plants resulting from seeds that were treated with 20 mL. kg<sup>-1</sup> biostimulant and cultivated under salt stress provided seeds with higher ESI performance; however, there was no salinity effect on seedling height for this dose (Table 2).

The development of shorter seedlings observed for the seeds coming from plants that were irrigated with saline water may be due, partially, to the probable accumulation of Cl<sup>-</sup> and Na<sup>+</sup> ions, as observed by Neves et al. (2008) in a study developed with cowpea.

In literature there are scarce studies about the physiological quality of seeds produced in plants submitted to salt stress; however, various studies demonstrated a decrease

in seedling height when the seeds are planted in a substrate dampened with saline solution or saline irrigation water, as shown in works developed with melon seeds (Kusvuran, 2012; Sivritepe et al., 2008; Kaya et al., 2007) and bur gherkin seeds (Oliveira et al., 2013).

As for dry mass accumulation of the aerial part (DM),

no salinity residual effects were observed for the seedlings coming from seeds that were produced with biostimulant seed treatment, in doses of 0 and 5 mL kg<sup>-1</sup>. However, there was salinity residual and negative effect for the other dosages, so that the highest values occurred in the absence of salt stress (Table 2).

Table 2. Germination (G) mean values, germination first count (GFC), accelerated aging with NaCl saturated solution (AASS), emergence speed index (ESI), height (H), and dry mass (DM) of aerial part of seedlings of bur gherkin, West Indian Gherkin cv., coming from plants generated by biostimulant-treated seeds, cultivated under salt stress.

Biostimulant doses (mL. kg <sup>-1</sup> )	Salt stress	Variables					
		G (%)	GFC (%)	AASS (%)	ESI	H (cm)	DM (g)
0	Without	54 a	34 a	36 a	5.37 a	2.49 a	13.0 a
	With	59 a	7 b	10 b	3.99 b	2.12 b	11.0 a
5	Without	70 a	36 a	40 a	5.89 a	2.71 a	13.0 a
	With	38 b	10 b	14 b	3.36 b	2.31 b	11.0 a
10	Without	66 a	31 a	44 a	6.09 a	2.83 a	15.0 a
	With	34 b	16 b	14 b	3.5 b	2.28 b	12.0 b
15	Without	75 a	31 a	46 a	6.82 a	2.68 a	14.5 a
	With	46 b	20 b	22 b	4.51 b	2.43 B	12.2 B
20	Without	90 a	48 a	59 a	4.95 B	2.73 a	16.0 a
	With	50 b	11 b	20 b	5.36 a	2.63 a	14.2 B
Averages	Without	71	36	45	5.82	2.68	14.3
	With	45	13	16	4.15	2.35	12.8

\* Averages followed by the same letter in the columns do not statistically differ among themselves by Tukey's test ( $p < 0.05$ ).

The salinity effect on the vigor of seedlings from produced seeds has been poorly studied over vegetables (Souza et al., 2014); the majority of already developed studies were meant to evaluate the direct effect of salt stress on the early development of seedlings, as shown in the work performed with bur gherkin seeds (Alves et al., 2014), as well as other vegetables, such as broccoli (Lopes et al., 2014) and bok choy (Lopes and Macedo, 2008).

Generally, a decrease in the quality of seeds produced in cultivated plants was observed with salt stress, regardless of the use of biostimulant on seeds that originated the respective plants; the greatest losses were verified in the results of germination, first count, accelerated aging with NaCl saturated solution and emergence speed index, with decreases of 36; 64; 65 and 28.7% respectively (Table 2).

These results partially support what was observed by Souza et al. (2014) in a study developed with okra seeds; they noted that the physical and physiological quality of okra seeds were negatively affected when the plants were irrigated with saline water.

According to the stress condition, there was an effect from the biostimulant used to treat precursor seeds of seed producing plants. The increase in biostimulant doses within seed treatment resulted in a linear and positive response in

the germination of seeds produced when plants were irrigated with non-saline water. In this case, higher doses provided a higher germination percentage (86%).

For the seeds produced in plants irrigated with saline water, increased doses of biostimulant used in seed treatment resulted in quadratic response, with the best result taking place with no biostimulant. When increasing doses, there was a decrease in germination, reaching 36% when the 10.5 mL. kg<sup>-1</sup> dose was used. On this basis, it was noted that germination increased with biostimulant increment, reaching 53% in the maximum dose. Despite presenting an increase in germination for a higher biostimulant dose (20 mL. kg<sup>-1</sup>), there was no significant gain compared to the seeds produced in plants coming from seeds that were not treated with biostimulant (Figure 1A). On Figure 1A, it is also verified that, in plants coming from seeds that were not treated with biostimulant, the plants that were irrigated with less saline water produced seeds that presented the highest germination percentages.

As a result proving biostimulant efficacy, it is possible to mention the one obtained in the survey performed by Melo et al. (2015) with peanut seeds; they observed that treating seeds with a biostimulant increased the germination of produced seeds.

During germination test, on the fourth day after

planting, the evaluation of the first count of germination was performed. For this, it was verified that the use of saline water in irrigation affected the results of this variable, so that in seeds coming from the absence of salinity, the average was 36% of germination, whereas the one resulting from salt stress was 13%; the harmful action of salinity on the results of germination first count test was evident (Table 2).

Regardless of the salinity of water used in plant irrigation, the number of seeds germinated in the first count was affected as quadratic by the increase in biostimulant doses used in

the treatment of seeds that originated the producing plants. It was also noted that when the plants were not submitted to salt stress, there was significant difference only in the plants coming from seeds treated with the highest biostimulant doses (20 mL. kg<sup>-1</sup> seeds), with better results (45.6%).

As for seeds obtained from plants that were cultivated with salinity, there was an initial increase in the values of first count germination, reaching the maximum value of 17% for the 12.4 mL. kg<sup>-1</sup> dose; over this dose, there was a decrease for this variable (Figure 1B).

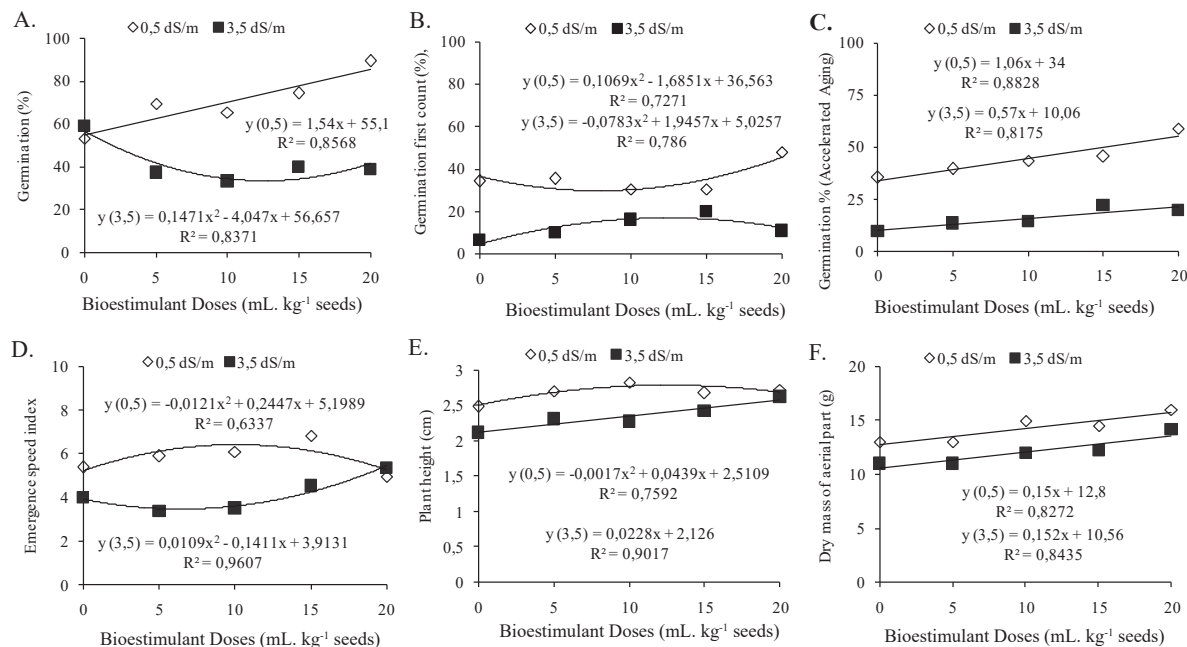


Figure 1. Germination (G) average values, germination first count (GFC), accelerated aging with NaCl saturated solution (AASS), emergence speed index (ESI), height (H), and dry mass (DM) in the aerial part of seedlings of bur gherkin, West Indian Gherkin cv., coming from plants generated by biostimulant treated seeds, cultivated under salt stress.

The negative effect of using saline water on plants, which promoted a significant decrease on the first count of germination, was probably provoked by plant difficulty in absorbing water, as well as by the input of ion in toxic concentrations, mainly of Cl<sup>-</sup> and Na<sup>+</sup>, as shown in the study performed by Neves et al. (2008) with the cowpea culture; they verified that plants irrigated with saline water presented an increase in the content of these ions in the produced seeds.

As for the effect of biostimulant on the first count of germination, studies point to the proportional increase of this variable in relation to the applied biostimulant dose, as in the survey performed by Melo et al. (2015), in the peanut culture.

With the results obtained for the test of accelerated aging with NaCl saturated solution, a significant difference was verified between the seeds obtained in plants that were

cultivated in the two saline conditions, highlighting that seeds produced in plants cultivated with lower salinity presented higher germination values, regardless of the biostimulant dose studied on the treatment of seeds that originated these plants (Table 2).

When analyzing the biostimulant effect within each saline level, it was verified for both salinities that the increase in the dose of biostimulant used in the treatment of seeds that originated the producing plants provided an increase in germination percentage; thus, in plants that were irrigated with 0.5 dS. m<sup>-1</sup> salinity water and which originated from seeds treated with a 20 mL. kg<sup>-1</sup> dose, 55% germination was obtained. On the other side, plants that were irrigated with more saline water (3,5 dS. m<sup>-1</sup>) and that originated from seeds treated with a 20 mL. kg<sup>-1</sup> biostimulant dose, produced seeds

that presented only 22% germination (Figure 1C).

For the emergence speed index (ESI), it was possible to verify that, as well as for the other analyzed variables, there was significant effect between the two conditions of salt stress (Table 2). Probably, the negative effect of salinity applied on plants over the emergence speed index observed in produced seeds, derived from the accumulation of  $\text{Cl}^-$  and  $\text{Na}^+$  ions in seeds coming from irrigation water (Neves et al., 2008).

Another probable reason for this fact may be related to the osmotic effect that occurs in plants that were submitted to irrigation with saline water, since salinity reduces the activity of solution ions and alters the processes of absorption, transport, assimilation and distribution of nutrients in the plant (Farias et al., 2009).

In literature there are scarce studies about the residual effect of salt stress in the quality of produced seeds; however, there are reports about the effect of water stress in the physiological quality of seeds. In this context, it is possible to mention the research performed by Freitas et al. (2013) in a study about cowpea; they reported that plants cultivated under salt stress produced seeds that, after germination test, presented lower emergence speed index when compared to the ones produced in plants cultivated without water stress. This was also observed by Silva et al. (2016) when evaluating the quality of sesame seeds that were produced in plants cultivated under salt stress.

As for the effect of biostimulant on the emergence speed index, it was verified that there were higher values in the seeds produced by plants coming from seeds that were treated with 10.1 mL.  $\text{kg}^{-1}$  (6.4) and 6.5 mL.  $\text{kg}^{-1}$  (3.5) biostimulant doses, when the plants were irrigated with waters having 0.5 dS.  $\text{m}^{-1}$  and 3.5 dS.  $\text{m}^{-1}$  salinities, respectively (Figure 1D).

In literature there are scarce studies about the interaction between salinity and biostimulant on the physiological quality of seeds; however, there are reports about the isolated effect of biostimulant in other cultures, for example, the work developed by Ávila et al. (2008) with soybean culture; they report that the best seeds were obtained by plants coming from biostimulant-treated seeds.

Plants originated by biostimulant-treated seeds produced seeds that originated seedlings which were statistically different for height variable in a different way, according to the salinity condition of the water used in plant irrigation. Considering the plants irrigated with less saline water, there were higher height values in seeds produced by plants coming from seeds that were treated with a 12.9 mL.  $\text{kg}^{-1}$  biostimulant dose (2.79 cm); there was a 11.34% increase compared to the value obtained in seeds produced by plants coming from non-treated seeds (Figure 1E).

On the other hand, when plant irrigation was performed with saline water, the highest seedlings were obtained from seeds produced in plants coming from seeds that were treated with the highest biostimulant dose (20 mL.  $\text{kg}^{-1}$ ); a maximum height of 2.58 cm was obtained, equivalent to a 21.7% increase, compared to the height that was observed in seeds coming from plants that were generated by non-treated seeds (2.12 cm) (Figure 1E).

The dry mass of the aerial part of seedlings was also affected by the applied treatments; there was linear and positive response to the increase in biostimulant doses applied in the treatment of seeds that originated seed producing plants, regardless of the salinity of the water used in plant irrigation. Thus, plants obtained from seeds that were treated with the highest biostimulant dose (20 mL.  $\text{kg}^{-1}$ ) and cultivated with and without salt stress, produced seeds that resulted in seedlings with the biggest dry mass in the aerial part, with 15.80 and 13.56 g respectively. These values correspond to a 23.44 and 28.41% increase, compared to the values obtained in seedlings coming from seeds that were collected in plants originated by non-biostimulant treated seeds (Figure 1F).

As mentioned before, in literature there are scarce studies about the residual effect of biostimulant application on the physiological quality of curcubitaceae seeds. However, there are study reports about the topic for other species of agronomic interest, especially for grains like soybean (Ávila et al., 2008) and peanut (Melo et al., 2015). According to Ávila et al. (2008), soybean plants coming from biostimulant-treated seeds produce seeds with a higher content of oil and proteins. For Melo et al. (2015), treating seeds with biostimulants results in plants with the ability of producing seeds with higher germination power; they present more vigorous seedlings and a higher quantity of normal seedlings.

The beneficial effect of biostimulant-treated seeds on the physiological quality of produced seeds may be related to the bigger accumulation of reserves in seeds, as related by Ávila et al. (2008) and Albrecht et al. (2010) with soybean culture; they observed that plants coming from seeds treated with the same biostimulant used in this work produce seeds with higher protein content. In this context, studies developed by other authors show that there is a direct relation between protein content and physiological quality of seeds (Bortolotto et al., 2008), since proteins catalyze chemical reactions or are used to create new tissues in the growth points of the embryo (Marcos-Filho, 2015). This is related to metabolism efficiency to formation speed and to the vigor of seedlings (Bortolotto et al., 2008).

## Conclusions

The physiological quality of bur gherkin seeds obtained from plants that were submitted to salt stress is negatively affected.

The use of biostimulant, applied via seeds, provides beneficial effects in the physiological quality of seeds produced in the following generation.

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