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EFFECT OF THE COMBINED TREATMENTS OF LOW X-RAYS DOSES AND BIOBRAS-16 ON TOMATO (*Solanum lycopersicum* L.) cv. VYTA FOR SALINITY CONDITIONS

Efecto combinado de bajas dosis de rayos X y Biobras-16 en plantas de tomate (*Solanum lycopersicum* L.) cv. Vyta en condiciones de salinidad

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ABSTRACT. Salinity is one of the adverse environmental condition that cause high affectations to agricultural crops. The objective was to evaluate the combined effect of low doses of X-rays and different concentrations of Biobras-16 on tomato plants grown in saline conditions. The experiment was carried out at "Jorge Dimitrov" Agricultural Research Institute during the years 2009 to 2012. Fresh tomato seeds of variety cv. Vyta were exposed to X-ray doses of 20, 25 and 30 Gy and 15 days after transplantation the plants were sprayed with two concentrations (1,5 and 2,0 mg L^{-1}) of Biobras-16. Two substrates were used, one consisting of a Vertisol soil salinized with electrical conductivity of 6 dS m^{-1} extract of soil saturation and Vertisol soil no salinized. The best combination of treatments was observed for 30 Gy +2,0 mg L^{-1}, which increased significant ($p \leq 0,05$) the salt tolerance in plants, the tomato yield and its components.

Key words: brassinosteroids, saline stress, radiostimulation, tolerance

RESUMEN. La salinidad es una de las condiciones adversas del medio que mayores estragos causa a los cultivos agrícolas. El objetivo del trabajo fue evaluar el efecto combinado de bajas dosis de rayos X y diferentes concentraciones de Biobras-16 en plantas de tomate, cultivadas en condiciones de salinidad. El experimento se desarrolló en el Instituto de Investigaciones Agropecuarias "Jorge Dimitrov", en los años 2009 hasta el 2012. Se irradiaron semillas frescas de tomate cv. Vyta, con bajas dosis de rayos X (20, 25 y 30 Gy) y a los 15 días después del trasplante. Las plantas crecidas se asperjaron con dos concentraciones (1,5 y 2,0 mg L^{-1}) de Biobras-16. Se emplearon dos sustratos, uno constituido por un suelo Vertisol salinizado con conductividad eléctrica de 6 dS m^{-1} del extracto de saturación y un suelo Vertisol no salino. Se observó que la mejor combinación de tratamientos fue 30 Gy+2,0 mg L^{-1}, la cual incrementó significativamente ($p \leq 0,05$) la tolerancia a la salinidad, el rendimiento y sus componentes en las plantas de tomate.

Palabras clave: brasinosteroides, estrés salino, radioestimulación, tolerancia

INTRODUCTION

Soil salinity is among abiotic factors affecting agricultural crops. Approximately 800 million ha of land area is damaged by salinity levels, which sometimes exceed traditional species tolerance (1).

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This stressful factor causes considerable yield losses in many crops, including tomato (2). The main salinity effects on plants are water deficit, cell damage and nutritional imbalance (3). It also decreases photosynthesis and increases oxidative stress.

All these disorders involve serious effects on crop growth and development, thereby decreasing yield (4, 5).

In recent years, several authors have confirmed the stimulating effect of low doses of X-rays on many plant species, so corroborating that its effectiveness not only depends upon the whole radiation dose absorbed by the organism, but on a series of interrelated biological or environmental factors that can enhance or diminish such stimulating effect (6).

Furthermore, it was proved that low doses of ionizing radiation combined with growth hormones maximize the stimulating effect. In this sense, plant response to such combination of ionizing radiation with some plant hormones was studied and a synergistic response of auxins, gibberellins, cytokinins, abscisic acid and ethylene was observed with low doses of X-rays (7).

On the other hand, brassinosteroids have aroused great interest among the international scientific community, because some formulations with Biobras-16 (BB-16), a brassinosteroid analogue, as an active ingredient can be used to stimulate economically important crop yields (8-10).

Therefore, the study of the combined effect of ionizing radiation and brassinosteroids, considered the sixth plant growth hormone, is of great importance from the theoretical and practical viewpoint, even if considering that the possible synergistic effect of such plant hormone with low doses of X-rays could induce some degree of tolerance and mitigate the negative effect of salinity on tomato plants.

Thus, the aim of this study was to evaluate the combined effect of low doses of X-rays applied to seeds and spray different Biobras-16 concentrations to tomato plants.

MATERIALS AND METHODS

The study was developed at the experimental areas from "Jorge Dimitrov" Agricultural Research Institute of Granma province, during the optimal vegetable production months of November to February, 2009 to 2012.

The best combinations of different Biobras-16 (BB-16) concentrations and low doses of X-rays were used in the experiment, which had been evaluated and selected in earlier experiments (Table I). Seeds were treated during morning hours by Philips X-ray equipment, using an aluminum filter of 0,75 mm, a working speed of 55 KV and 30 mA as well as a power dose of 11,47 Gy/min at a temperature of 24±1 °C.

Table I. X-ray doses and BB-16 concentrations evaluated in the experiment

Varieties	Treatments	X-rays (Gy)	BB-16 (mg L^{-1})
Vyta	Control (non-saline)	-	-
Vyta	T0 (saline)	-	-
Vyta	T1	20	2,0
Vyta	T2	30	2,0
Vyta	T3	25	1,5

Two substrates were used in the experiments, one consisting of a saline Vertisol with predominant neutral salts, such as sodium, calcium and magnesium chlorides and sulfates, pH<7,6 for a total soluble salt concentration of 3565 ppm and mean values of electrical conductivity at the saturation extract of 6 dS m^{-1}, with S3 salinity degree, according to Cuban pattern (2009), where treatments T0, T1, T2 and T3 were seeded, meanwhile the other consisted of a non-saline Vertisol where the treatments and control were planted (11).

Seedlings from the nursery were transplanted to semi-controlled conditions in December, when the necessary requirements were collected, according to the Technical manual for organoponics, intensive orchards and semi-protected organoponics (12). Pots of 23 cm wide x 29 cm deep were employed; 20 seedlings were transplanted per replicate, following a randomized complete design with four replicates per treatment.

Different BB-16 concentrations were applied at a rate of 2 mL per plant (Table I) within flowering time (15 days after transplanting). Leaves were sprayed during early morning hours until they were fully wet by a 16-L-backpack with a previously calibrated conical spray nozzle.

The following indicators were evaluated: stem and root length and diameter, leaf area, dry matter percentage, bunch number per plant, fruit number per bunch, polar and equatorial diameter (cm), average fruit weight (g) and yield per plant (kg $plant^{-1}$).

Growth was assessed 20 days after transplanting, whereas yield and its components at the end of crop cycle in 80 plants per treatment. Data constitute the mean of three years, since there were no significant differences between them.

Plant tolerance to salinity was calculated with these data using the formula described (13):

$$ST= 100\ (SI/CI)$$

where:

ST: salinity tolerance

SI: mean of indicators evaluated under salinity conditions

CI: mean of indicators evaluated under normal conditions

Data recorded on growth, yield and its components, as well as salinity tolerance were processed through a one-way classification variance analysis and once significant differences were proved, Tukey mean multiple comparison test was used for 5 % error probability.

RESULTS AND DISCUSSION

When assessing the combined effect of low doses of X-rays and BB-16 in tomato plants under salinity conditions, a significant stimulation ($p\leq0{,}05$) was observed in some growth indicators, which allowed minimizing stress effect, since there was a similar response to seedlings grown under normal conditions (Table II).

In general, the best response was obtained in treatment T2, which significantly stimulated all growth indicators evaluated.

Stem length (SL) increased significantly ($p\leq0{,}05$), except in treatment T3, which did not show any difference in relation to control.

The greatest response of this indicator was observed in treatments T1 and T2, corresponding to the combined treatments of 20 Gy and 2,0 mg L^{-1} as well as of 30 Gy and 2,0 mg L^{-1}.

Regarding stem diameter (SD), significant differences were found ($p\leq0{,}05$) with respect to control in treatment T2, meanwhile root length and diameter (RL and RD) were stimulated in all treatments without significant differences between them.

Leaf area (LA) showed significant stimulation ($P\leq0{,}05$) in the three treatments and the highest increases were coincidentally observed in treatment T2.

An increase of this indicator could enable to assimilate solar energy during photosynthesis process and involve a higher photoassimilate production, largely contributing to plant growth and development (14).

Such increase of these growth indicators has been reported by other authors after they used these methods. A growth response has been shown in lettuce plants (*Lactuca sativa* L.) by spraying Biobras-16 to leaves, increasing both leaf development and root growth (15).

On the other hand, root length increased in tomato seedlings of Amalia cv. after applying Biobras-16 to a saline medium, which suggests the ability of this brassinosteroid analogue to induce NaCl tolerance mechanism activation in roots (16).

In addition, a highly significant stimulation (30 %) of leaf area has been reported in four tomato varieties when treated with low doses of X-rays[A].

[A] Ramírez, R. Efecto del tratamiento de semillas con dosis estimulantes de rayos X en el cultivo del tomate (*Lycopersicum esculentum* Mill.). [Tesis de Doctorado], Instituto Nacional de Ciencias Agrícolas, La Habana, Cuba, 2006, 130 p.

Table II. Combined effect of low doses of X-rays and BB-16 in tomato plants for some growth indicators under salinity conditions

Treatments	SL (cm)	SD (cm)	RL (cm)	RD (cm)	LA (cm)
T0 (saline control)	34,3 b	2,22 c	10,14 b	0,07 b	4,59 c
T1 (20 Gy+2,0 mg L^{-1})	36,40 a	2,92 b	13,21 a	0,10 a	6,51 b
T2 (30 Gy+2,0 mg L^{-1})	36,42 a	3,74 a	13,32 a	0,21 a	8,22 a
T3 (25 Gy+1,5 mg L^{-1})	34,2 b	2,21 c	13,12 a	0,10 a	5,83 b
SE±	0,99	0,05	0,2	0,01	0,01

In columns, distinct letters indicate significant differences between treatments for $p\leq0{,}05$

SL and SD: stem length and diameter RL and RD: root length and diameter LA: leaf area

Other authors observed increased growth indicators in *Arabidopsis thaliana* Landsberg (Ler) plants after applying low doses of gamma rays (100 Gy), also stimulating the number of flowers and siliques (17).

In this sense, root length increased in *Pterocarpus marsupium* Roxbcon plants compared to control when applying low doses of gamma radiation (18).

Regarding dry matter percentage, significant differences (p≤0,05) were observed in treatments T1 and T2 with respect to control, whereas treatment T3 showed no significant differences (Table III). This is a very important aspect, because other authors consider it is an accurate tolerance indicator in other species, tomato (19) and wheat (20).

Similar results were obtained by spraying 24-epibrassinolide to tomato leaves, observing an increased biomass accumulation and photosynthetic activity (21, 22).

Other authors noted increases in growth, dry matter and root length in *Arabidopsis thaliana* plants when stimulated with X-ray doses of 10 Gy (23).

Table III. Combined effect of low doses of X-rays and BB-16 on dry weight percentage of tomato plants cv. Vyta grown under saline conditions

Treatments	Entire plant (%)	Aerial part (%)	Root (%)
T0 (saline control)	8,30 c	10,30 c	6,34 c
T1 (20Gy+2,0 mg L^{-1}	10,58 b	12,83 b	7,32 b
T2 (30 Gy+2,0 mg L^{-1})	11,70 a	13,35 a	8,40 a
T3 (25 Gy+1,5 mg L^{-1})	8,35 c	10,48 c	6,15 c
SE±	0,02	0,03	0,01

In columns, distinct letters indicate significant differences between treatments for p≤0,05 according to Tukey test

SE±: standard error of the mean

Data are means of 80 plants per treatment

These results show the effectiveness of combining low doses of X-rays and BB-16 sprayed on increased tomato plant growth of Vyta cv. under salinity conditions.

On the one hand, this response may be related with the hormesis induced by low doses of ionizing radiations shown by an activation and increase of oxidative processes, as a result of which the general chemical metabolism of cells increases, leading to energy transformations in plants along their life cycle (24).

According to this theory, it was noted that increased oxidative processes promote certain vital functions of plant organism, related to variations of certain enzyme activity at protoplasmic aggregation state and its colloidal properties, which are shown by a generalized growth of chemical metabolism (25).

On the other hand, brassinosteroids have the ability of accelerating plant growth and maturity; besides, its induced effects cannot be considered in isolation, since these compounds interact with other endogenous plant growth regulators and are very sensitive to environmental signs, particularly with solar radiation quality, a typical response of other plant hormones (26).

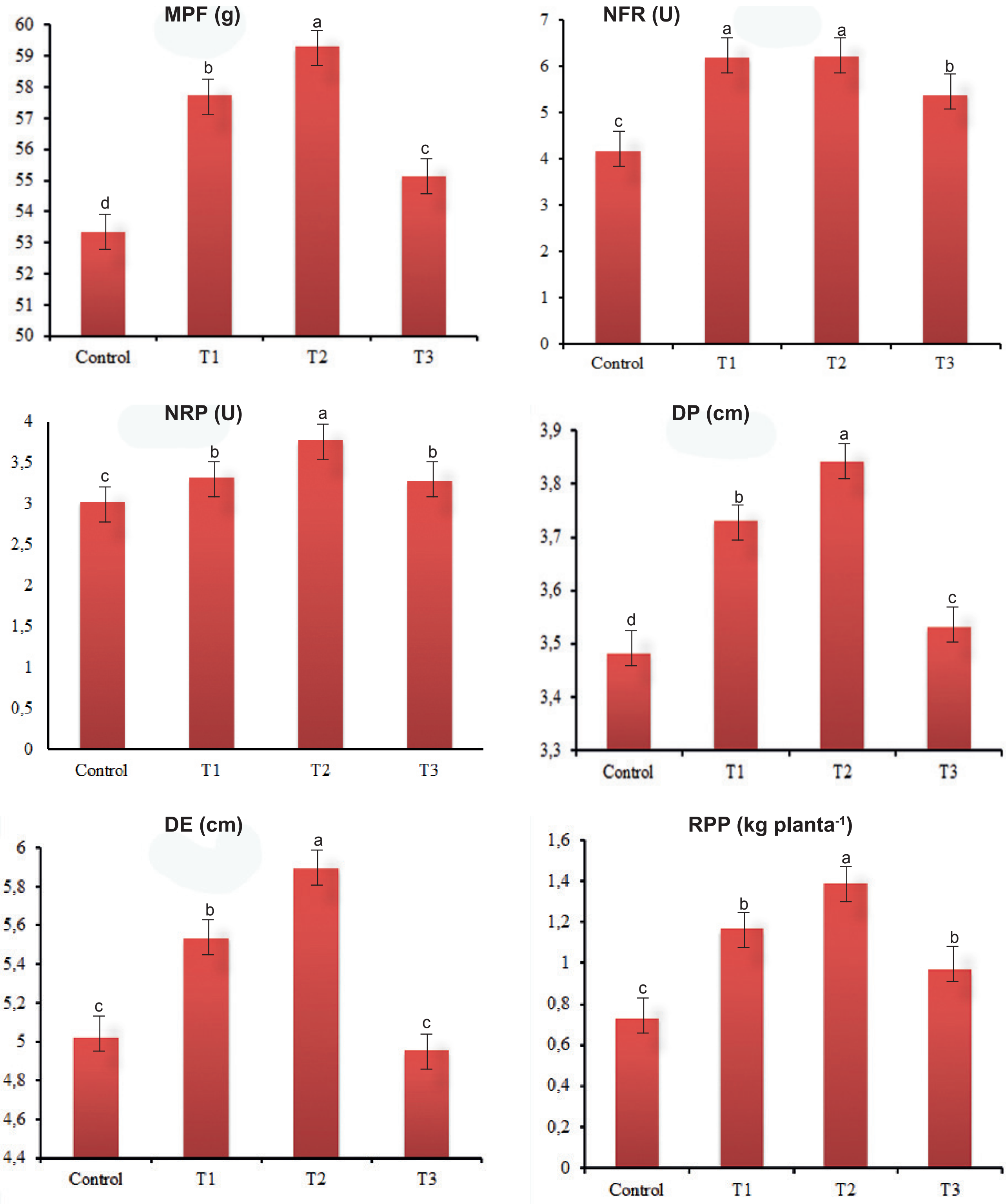
In this sense, hormonal activity could be influenced by low doses of X-rays and generate a stimulating effect on tomato plant growth indicators under saline conditions, leading to significant yield increases.

In addition, a significant stimulation (P≤0,05) of yield and its components was observed (Figure). The response of fruit and bunch number per plant showed significant differences (P≤0,05) between the treatments and control, T1 and T2 being remarkable in the first indicator whereas T2 in the second one.

The increased fruit number per plant is considered very important, since this variable is a direct indicator of agricultural yield and crop adaptation to soil and climatic conditions (27); thus, other authors recommend using it to select varieties adapted to adverse environmental conditions (28).

Despite the negative effect of salinity on plants, average fruit weight was also significantly stimulated (p≤0,05) in all combined treatments (T1-T3), T2 showing the best response by achieving significant stimulating values of 60 g in this indicator. Such result might indicate the possibility of using the combined effect of low doses of X-rays and Biobras-16 to induce salt tolerance in this crop.

Concerning equatorial and polar fruit diameter, significant stimulating increases (p≤0,05) were recorded. Treatment T2 showed the highest values compared to control; except equatorial diameter in T3, the other combined treatments induced a positive response to salinity conditions.



NFR: fruit number per bunch NRP: bunch number per plant MPF: average fruit weight
DP: polar fruit diameter DE: equatorial fruit diameter RPP: yield per plant
In columns, distinct letters indicate significant differences between treatments for p≤0.05 by Tukey test
SE±: standard error of the mean. Data are means of 80 plants per treatment

Effect of combined action of low doses of X-rays and BB-16 on yield and its components under salinity conditions

Regarding yield per plant, significant stimulating values (p≤0,05) were observed in all treatments (T1-T3), ranging between 0,97 and 1,39 kg $plant^{-1}$.

These results corroborate what was observed by other authors, who indicate the feasibility of using both ionizing radiations (29) and brassinosteroids (30) to increase plant tolerance to abiotic stress. In this sense, 40 % yield was stimulated in tomato cv. Campbell-28 after treating its seeds in a saline medium with low doses of X-rays (31). A significant increase was also found in bunch number per plant, fruit number per plant and yield, with doses of 20 and 25 Gy with INCA-9-1 cv. and Maybel as well as Domi mutants[B].

According to these authors, this response can be attributed to the fact that both methods generally trigger a series of adaptive defense reactions of plant organism against damages caused by adverse environmental conditions. It is stated that such stimulators decrease protoplasm penetrability and slow down toxic ion accumulation in plants, leading to increased stress tolerance (30, 31).

A theoretical analysis of the subject suggests that either X-rays or brassinosteroids significantly increase enzyme activity of RNA and DNA polymerases, RNA and DNA synthesis as well as proteins. Influenced by both stimulants, changes in enzymatic activities appear to affect nucleic acid metabolism, so that RNA, DNA and protein levels accumulated in the tissue increase during plant growth (32, 33).

When analyzing salinity response in tomato plants cv. Vyta, observed by the relative tolerance index, a differentiated effect was recorded in yield and its components. The three treatments combined with X-rays and Biobras-16 are the same or significantly exceed the relative tolerance index shown by control (Table IV).

Salinity tolerance index calculated in fruit number per bunch shows the combined effect of both stimulating methods to significantly increase Vyta tolerance, which reached maximum values in treatments T1 and T2, respectively.

Bunch number per plant was similar to the previous indicator, with respect to tolerance degree. Treatment T2 reached the best response to tolerance induction, showing values of 97,16 %. Furthermore, plants from treatment T1 demonstrated a similar response to T3.

Average fruit weight was less affected by salinity; however, tolerance indices increased significantly, so that T2 showed the best responses under these stress conditions.

Regarding tolerance indices in polar and equatorial fruit diameter, a similar response was observed, treatments T1 and T2 being remarkable by such increase, although T3 also showed significant differences with the control.

Yield per plant in the three combined stimulating treatments apparently induced a high degree of tolerance, treatment T2 being notable, which reached an index of 93,15 %.

It should be pointed out that salinity affects yield and its components. Other authors agree that plants affected under stress conditions are due to variations occurring at the concentration and relationship of growth and development promoting and inhibiting hormones, noting that this hormonal imbalance plays an important role in regulating plant response to salinity, mainly controlling organic compound synthesis processes (34).

Values recorded by salinity tolerance, yield and its components show the ability of brassinosteroid analog and X-rays to induce tolerance, even if considering that from the agricultural viewpoint, salinity tolerance is defined as plant ability to survive and reach economic yields under stress conditions, which is expressed as the relationship between yield of one variety under saline conditions with respect to its yield under normal conditions.

Brassinosteroids are considered strong plant growth regulators of steroidal nature. These hormones have pleiotropic effects, such as: stimulation of cell elongation and protoplast dedifferentiation, cell wall regeneration, regulation of treachery element differentiation and increased biomass as well as yield in different plant species (35).

[B] Ojeda, C. M. Incremento de la tolerancia a la salinidad en plantas de tomate (*Solanum lycopersicum* L), mediante el tratamiento de semillas con radiaciones ionizantes. [Tesis de Maestría], Facultad de Ciencias Agrícola, Universidad de Granma, Bayamo, Cuba, 2009, 69 p.

Table IV. Combined effect of low doses of X-rays, Biobras-16 and relative tolerance index to salinity in tomato plants cv. Vyta

Treatments	NFR ITR %	NRP ITR %	MPF ITR %	DE ITR %	DP ITR %	RPP ITR %
T0 (saline control)	64,34 c	83,89 b	84,75 c	79,55 c	78,11 c	57,21 d
T1 (20Gy+2,0mg L^{-1})	95,70 a	87,10 b	93,08 ab	91,22 a	90,81 a	87,14 b
T2 (30 Gy+2,0 mg L^{-1})	96,28 a	97,16 a	9,62 a	93,24 a	91,17 a	93,15 a
T3 (25 Gy+1,5 mg L^{-1})	83,25 b	86,05 b	89,90 b	88,12 b	85,24 b	71,94 c
SE±	1,93	2,03	2,01	1,99	1,85	1,14

NFR: fruit number per bunch NRP: bunch number per plant MPF: average fruit weight
DP: polar fruit diameter DE: equatorial fruit diameter RPP: yield per plant
ITR: relative tolerance index percentage to salinity
In columns, distinct letters indicate significant differences between treatments for p≤0,05 by Tukey test
SE±: standard error of the mean.
Data are means of 80 plants per treatment

Several authors also refer to the protective effect of brassinosteroids to different abiotic stress conditions, such as high and low temperatures, drought and salinity; moreover, they diminish stress effects caused by the lack of nutrients and excessive heavy metals, also increasing resistance to herbicides and pathogens (36).

Furthermore, in general, low doses of ionizing radiations are considered strong stimulating agents and X-rays more efficiently, taking into account the shorter wavelength that reduces the probability of causing or inducing mutations (29, 37).

Various authors reported the most significant advantages of radiostimulation or radio-hormesis related to increased yields (10-40 %), seed germination, carotene and vitamin C contents in some vegetables as well as protein and fat in cereals (38); their effectiveness has also been proved by increasing tolerance to environmental abiotic factors as salinity (31).

In addition, other authors observed a stimulating and protective effect against environmental biotic and abiotic factors, as well as an increase of antioxidant enzyme activity and poly (DNA-ribose) polymerase activation, a regulating enzyme to stress response, as a sign of tolerance (39, 40).

Simultaneously, brassinosteroids have a marked impact on plant growth under saline conditions. These experiments proved the effectiveness of brassinosteroid spirostanic analogue (BB-16) to reverse inhibition caused by salinity on seedling growth of two rice genotypes (41).

CONCLUSIONS

Treatments combined with low doses of X-rays and Biobras-16 can be used to achieve a stimulating effect on salinity tolerance in tomato plants of Vyta cv. and significant increases in growth, yield and its components.

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