Criteria for the Selection of Vegetable Growth-Promoting Bacteria to be applied on Roselle Crop (*Hibiscus Sabdariffa* L.) and Bioremediation

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Abstract**—***In order to define which are the most important criteria for the selection of plant Growth-Promoting bacterial strains of the Hibiscus sabdariffa L. crop (Roselle), bacterial strains isolated from the roots of Roselle plants of two varieties (Creole and Spider) were used, collected in the community of Río de los Peces, municipality of Candelaria Loxicha, Oaxaca and seeds of the same varieties. To characterize the varieties, the following were determined: total germination percentage (TGP), germination speed (GS), the root length(RL), the stem length (SL), the dry root biomass (DRB), the dry stem biomass (DSB) and the chlorophyll content (CC). Three types of LED lamps were used to illuminate the seedlings. The seeds inoculated with cells of six selected bacterial strains were grown in a greenhouse to determine: the stem length (SL) at 3, 45 and 65 days after sowing (das). The treatments were distributed under a completely random design and comparison of means (Tukey, p = 0.05). The TGP, DSB and DRB parameters were not useful in the selection process of the strains that promoted plant growth to a greater degree. The GS and SL to be considered safe criteria or not, what is important is the relationship of what happens at the time of germination and development of the seedlings in the laboratory and greenhouse. The SL of the plants in the greenhouse showed differences between strains, but not regarding the control and also only observed in the first days of development (3 das). The CC did not prove to be a good selection criterion either. The lamp composed of 15% white light, 27% blue light and 58% red light was the one that most promoted root growth.*

Keywords—Plant Growth-Promoting bacteria strains, Hibiscus sabdariffa L, Bacterial strains selection criteria, LED lamps.

I. INTRODUCTION

The study of microorganisms that promote plant growth has gained importance worldwide because of the multiple advantages they represent (Ortiz-Texon et al., 2016). Currently, research is focused on the evaluation of various rhizosphere microorganisms, selecting those most efficient in inoculation experiments under controlled environmental conditions in the laboratory, greenhouse and in the field. This seeks to increase yield and reduce the amount of agrochemicals used (PazosRojas et al., 2016).

Plant Growth-Promoting Rhizobacteria (PGPR) are bacteria influenced by exudates from plant roots that can improve plant growth in the short term (Molina-Romero et al., 2017), through the production of plant Growth-Promoting substances, which are synthesized in different structures of the plant. These molecules exhibit effects on plant physiology, such as increasing root volume and root respiration rate, resulting in the absorption of soluble mineral elements (Molina-Romero et al., 2015). The beneficial bacteria applied to agricultural crops allow the phytostimulation and bioremediation of toxic compounds associated with plants; having a positive impact on human health and the environment (Sing and Trivedi, 2016; Pazos-Rojas et al., 2016). These can interact effectively with plants in contaminated agricultural soils, carrying out the degradation of pollutants and increasing the yield of crops (Báez-Rogelio et al., 2016).

Among the satisfactory results for the control of phytopathogenic microorganisms is the genus *Pseudomonas spp*. (Anguloa, et al., 2014). They are a group of bacteria that can exert a direct beneficial effect, through the synthesis of phytohormones and vitamins, stimulation of seed germination and emergence of seedlings, inhibition of ethylene synthesis, solubilization of inorganic phosphorus (P). Indirectly, they exercise the function of controlling pathogenic microorganisms through the synthesis of antibiotics and fungicides, competition for nutrients, production of siderophore or by inducing systemic resistance to pathogens (Alcarraz-Curi et al., 2019). For example, in *P. fluorescens* G20-18 the ability to efficiently control infection by *P. syringae* has been identified, which allows the maintenance of tissue integrity, reflecting on the biomass yield (Großkinsky et al., 2011). Some criteria commonly used to test a crop are:

Total germination: it is the maximum germination percentage got under previously defined conditions (Durán and PérezGarcía, 1984).

Germination speed: Maguire (1962) defines it as the ratio of the number of germinated seeds to the germination time.

$$M = \sum \left(\frac{n_i}{t}\right)$$

where M = germination speed, n = number of seeds germinated on day i, t = germination time from sowing to germination of the last seed.

1.1 Accumulation and distribution of dry matter

The accumulation of dry matter is commonly used as a parameter to characterize growth, because it usually has great economic significance (Ñústez et al., 2009). The distribution of dry matter plays an important role in the final yield of a crop, since it is given by the ability to accumulate biomass in the organs that are destined for harvest (Barrientos-Llanos et al., 2015).

1.2 Chlorophyll content

Allows us to relate it to the nutritional level of the plants, it also has a close relationship with the photosynthesis index and these two factors, considerably influence the performance of a plant, both in its development and in the final yield of harvest (López-Tolentino et al., 2016).

Roselle (*Hibiscus sabdariffa L.*), is a species belonging to the Malvaceae family (Ríos et al., 2013). It is native to India and Malaysia. It has been widely distributed in the tropics and subtropics of both hemispheres, in addition, it has become naturalized in many areas of the Antilles and Central America (Morton, 1987). During the colonial era, the Spanish were the ones who introduced Roselle to Mexico (Romano-Cadena et al., 2017). It is a crop that is currently gaining more importance in Mexico and is part of the sector of spices and medicinal plants (Sánchez-Prado et al., 2019). In recent years it has had a potential use for lowering cholesterol and hypertension, in addition, it is attributed diuretic and antipyretic properties (Caamal et al., 2020). These benefits are supported by various scientific investigations that relate them to compounds such as vitamins E and C, polyphenolic acids and antioxidants such as flavonoids and anthocyanins (Cid and Guerrero, 2012). However, the information devoted to the study of Plant Growth-Promoting Bacteria in this crop is almost nil. Considering the above, the present work was developed, with the aim of defining which are the most important criteria for the selection of bacterial strains that promote plant growth in seedlings of two varieties of Roselle.

II. MATERIALS AND METHODS

2.1 Biological material and experiment location

Roselle seeds (*Hibiscus sabdariffa* L.), Creole and Spider varieties, were obtained from the community of Río de los Peces, municipality of Candelaria Loxicha, Oaxaca. And the roots used in this research were also collected there.

2.2 Selection of plants to get root samples for the isolation of bacteria

The selection of the plants was carried out in a plot of approximately 1 hectare, choosing the largest plants, with more foliage and a healthy appearance, 5 of each variety (Spider and Creole) to collect the tips of 3 roots of each variety, about 10 cm long.

2.3 Getting root samples from Roselle plants

To get the bacteria present, the roots of each plant were placed in sterile test tubes and washed with 5 mL of sterile distilled water, shaking the tubes in a vortex for 2 minutes. An aliquot sample of 500 µL was taken from each of the 10 tubes, to make

dilutions of 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5}. The roots that remained in the test tubes were rinsed 4 times with sterile water, vortexing for 30 seconds and, after removing the water, were macerated with sterile glass rods. 2 mL of sterile water were added to each tube and 500 µL aliquots were taken from this liquid to make the 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5} dilutions.

2.4 Getting bacterial isolates

Aliquots of 100 µL were taken from each of the dilutions (from the series of the washing liquid and from the macerates) to spread them on the surface of King's B culture medium in Petri dishes. The Petri dishes were incubated at a temperature of 28-30°C to allow the development of the cultures for 48h and to perform the colony count using the plate count method of Allaert and Escolá (2002).

2.5 Identification and isolation of bacterial colonies

Petri dishes were observed under UV light to identify fluorescent colonies (Fig 3). With sterile wooden sticks, 5 samples of completely isolated colonies were taken from each of the Petri dishes, getting 200 isolates from the samples of the washings and the root maceration.

A bacterial bank prepared in sterile 2 mL Eppendorf tubes containing the bacterial suspensions in 1 mL of sterile distilled water was used to keep the 200 bacterial isolates got in the refrigerator, before making the bank in tubes with slanted agar of the selected isolates.

2.6 Identification of bacterial isolates that promote germination and vigor of Roselle seeds

From the temporary bacteria bank, the bacterial cultures were prepared to inoculate the seed samples of the 2 varieties of Roselle. The seed inoculation was carried out in 4 blocks. In the first block, 20 strains were tested, 4 from the root washing and 16 from the maceration; In the second block, 30 strains were tested, all from the root maceration; In the third and fourth block, 24 and 23 strains were tested respectively, all from the maceration. From the total of the 97 strains, 5 strains were selected for the Creole variety and 3 for the Spider variety. Subsequently, of the 5 previously selected strains, only 3 were chosen for the Creole variety, considering only the percentage of total germination as a criterion.

To carry out the seed inoculation, the bacterial cultures were prepared in Petri dishes containing King's B medium. From the cultures, after 24h of incubation at 28-30^{o}C, bacterial suspensions were prepared in sterile distilled water adjusted between 0.8 and 1 turbidity (660 nm).

The seeds were mixed with the bacterial suspension (25 seeds mixed with 400 µL of bacterial suspension), preparation that was left for 60 minutes at room temperature. Subsequently, the inoculated seeds were placed on two sheets of filter paper moistened with 4 mL of distilled water in 9 cm diameter plastic Petri dishes. The seeds were placed in a germination chamber set at 28-30^{o}C and the seeds that had germinated were counted daily (seeds in which the root tip was already visible) (Fig 4).

2.7 Lighting type

Three types of LED lamps were used to illuminate the Roselle seedlings previously inoculated with the 6 selected strains (3 for each variety Creole and Spider), to observe if the type of lighting also influenced the dry stem biomass (DSB), dry root biomass (DRB), stem length (SL), root length (RL) and chlorophyll content (CC). The lamps used were of LED light: one composed of 100% white light; the second composed of 15% white light, 27% blue light and 58% red light and the third composed of 29% blue light and 71% red light. The seedlings were placed at a distance of approximately 30 cm from the lamps for 4 days.

2.8 Establishment of greenhouse cultivation

Seeds inoculated with cells suspensions of the 6 strains selected for the two varieties were used and germinated in plastic Petri dishes in a germination chamber set at 28-30^{o}C for 3 days. The seedlings obtained were transplanted into polyethylene bags and their growth was observed during the first 65 days after sowing (das). Irrigation was carried out every third day, using a half-liter container so it was homogeneous in all the pots.

2.9 Variables tested

The variables were: total germination percentage (TGP), which was calculated by adding the daily germination values up to the third day; germination speed (GS) was calculated with the formula of Maguire, (1962); root and stem length were

measured using a sheet of millimeter paper (Fig 1); To get the dry root biomass and the dry stem biomass, these were separated and preserved in an oven set at a temperature of 45°C for 5 days and then in another oven set at a temperature of 70°C, for 4 days; the chlorophyll content was obtained using the Konica Minolta SPAD 502 PLUS meter, considering the average of three readings per seedling; stem length of the plant (SL) in the greenhouse was measured with a ruler at 3, 45 and 65 das.

2.10 Statistical analysis

The analysis was divided into three parts. In the first, 10 treatments with 3 repetitions were considered, the variables TGP, GS, RL, SL, DRB and DSB were analyzed and the variation factors were varieties and strains; In the second, 8 treatments with 3 repetitions were considered (the repetitions represented the type of lighting used), the variables TGP, GS, RL, SL, DRB, DSB and CC were analyzed and the variation factors were varieties, strains and type of lighting; in the third, 8 treatments with 3 repetitions were considered, only the variable SL was analyzed at 3, 45 and 65 das and the variation factors were strains and varieties. The treatments were distributed under a completely random design. For the analysis of variance and comparison of means (Tukey, p = 0.05), the ANOVA procedures of the statistical software package SAS (Statistical Analysis System) version 8.0 (SAS, 1999) were used.

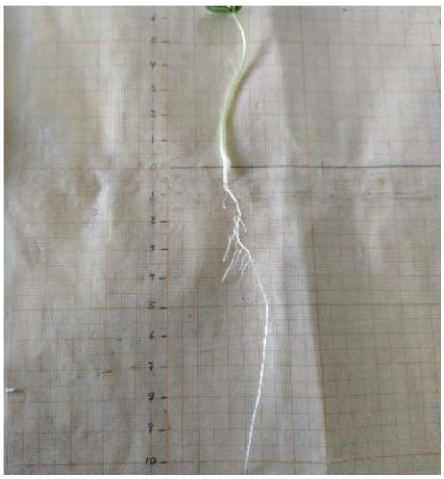

FIGURE 1: Appearance of the Roselle seedlings developed in Petri dishes from which the root and stem lengths were obtained

FIGURE 2: Appearance of the Roselle seedlings developed in a greenhouse from which the stem lengths of Roselle plants were obtained

III. RESULTS AND DISCUSSION

A total of 271 isolates derived from washing and macerated root preparation of the two Roselle varieties were obtained, of which 7.7% corresponded to isolates of fluorescent strains from the root washing and 14.6% from the root maceration preparations (Table 1).

When the variables TGP, GS, DSB, DRB, SL and RL were tested, it was found that the type of variety had a significant effect ($p \leq 0.05$) on the germination speed, the dry biomass of the stem and root and the stem and root length (Table 2), getting higher values for the Spider variety in all the tested criteria. However, when the strains were tested, a significant difference was observed between strains on all the tested criteria (Table 3). However, no strain showed a significant effect when compared with its respective control (M67, L168, M82_C, M3 and M61 were tested with the Creole variety and M83, M88 and M82_A with the Spider variety) on total germination, germination speed, stem dry weight, root dry weight and root length.

The only significant positive effect of strain M88 was obtained on stem length when compared to the Spider Control. There was a significant effect of the strains on most of the criteria tested, but without considering the effect of the variety (Table 3). Smith and Goodman (1999) pointed out that the genotype of the organisms involved play an important role in the association between microorganisms and plants, determining the biological result of said association. Total germination percentage was not influenced by the type of variety or by inoculation with strains used in the same variety, which is under what was reported by Méndez and Campos (2007), who got unsatisfactory results when testing germination percentage, stem length, radicle length and fresh stem biomass in the laboratory.

TABLE 1
BACTERIAL ISOLATES GOT FROM THE ROOT TIPS OF TWO VARIETIES OF ROSELLE

Process	Number of bacterial isolates	Number of fluorescent bacterial isolates	Percentage of fluorescent bacterial isolates
Root washing	169	13	7.7
Root macerate	103	15	14.6

A significant effect ($p \leq 0.05$) of the type of variety was obtained in the germination speed, dry biomass of the stem and the root, root length and chlorophyll content (Table 4). Getting higher values for the Spider variety in all the tested criteria. No differences were observed between varieties when testing the total germination percentage and the stem length. Light is a vital environmental factor that affects the growth and development of plants by acting not only as the only source of energy for photosynthesis but also as a type of external signal (Ding et al., 2010; Liu, 2012). The type of lighting did not have a significant effect on the Roselle seedlings (Table 4). Only differences in root length were observed (Table 5), achieving greater root growth with the lamp composed of 15% white light, 27% blue light and 58% red light. Xiaoying et al., (2012) mention that the combination of red-blue and red-blue-green LEDs were shown to be beneficial factors in the growth and photosynthesis of cherry tomato (*Solanum esculentum*var. *cerasiforme*) seedlings.

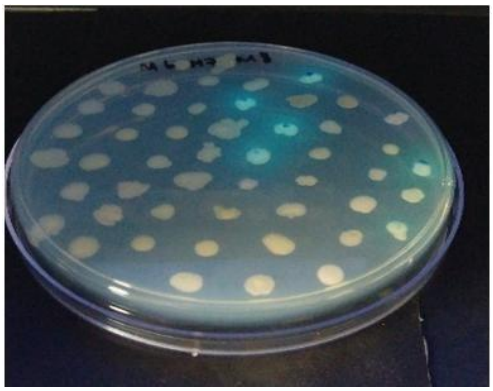

FIGURE 3: Fluorescent bacterial isolates

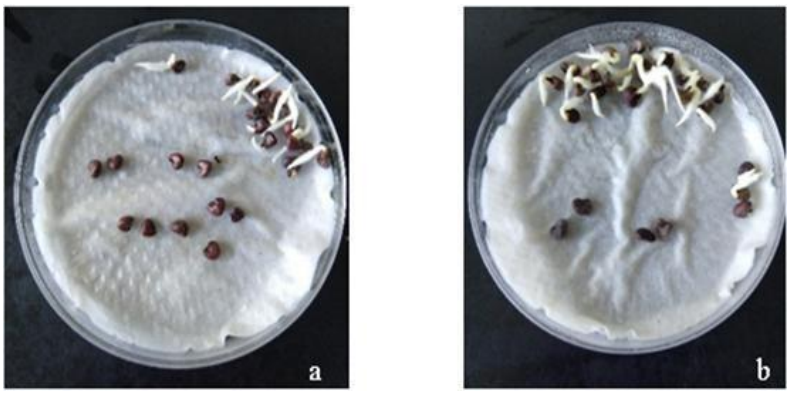

FIGURE 4: Aspect of the germinated seeds of the two varieties of Roselle
(a: Creole variety; b: Spider variety)

Table 6 shows that there was no positive effect of the strains on the total germination percentage and the stem length, however, on the germination speed positive effects were observed when inoculating the Creole variety with the M67 strain, which speed up germination with regarding the Creole Control. In contrast, strains M83, M88 and M82_C strains compared to the Spider Control did not show significant effects. The dry stem biomass, dry root biomass and chlorophyll content showed significant differences between the type of cells of the strains used but not between those used for the same variety. So, the variety influences the cells of the strains and for this reason, this difference is obtained. The cells of the M83 strain had a positive effect on the root of the seedlings, increasing their length considerably regarding the Control Spider. In contrast, there was no strain that promoted root growth in the Creole variety.

To confirm the effect of the strains on the stem length of the Roselle seedlings, the stem lengths of the plants were measured in the two varieties established in the greenhouse during their first days of development (Fig 2). No significant differences were obtained on the stem lengths of both varieties (Table 7). However, when testing the effect of inoculation with cells of bacterial strains, if there were contrasts between the strains M67, M83 and M82_A at 3 das (Table 8), but when comparing them with the controls, no significant differences were obtained. At 45 das and 65 das, in the same way, no differences were perceived. It reflects the above shows that the effect of inoculation with cells of bacterial strains only in the first days of plant development. Méndez and Campos (2007) got similar results when testing the growth of Roselle plants at the field level, where the greatest difference in plant height was obtained at 8 das.

TABLE 2
DETERMINATION OF THE PARAMETERS SHOWED ON THE VARIETIES OF ROSELLE CREOLE AND SPIDER

Variety	Total Germination (%) **		Germination speed		Drystem biomass (mg)		Dryroot biomass (mg)		Stem length (mm)		Root length (mm)	
Creole	4.02	a*	9.76	b	39.50	b	6.72	b	36.59	b	24.48	b
Spider	4.47	a	16.11	a	72.12	a	12.29	a	44.55	a	44.63	a
MSD	1.06		2.94		2.55		3.87		1.78		4.18	

**** Means with the same letter in a column are not significantly different (Tukey, p = 0.05). MSD = Minimum significant difference. ** TGP was transformed with the formula ROOT (100- (TGP / 25 * 100)). * Means with the same letter in a column are not significantly different (Tukey, p = 0.05). MSD = Minimum significant difference. ** TGP was transformed with the formula SQRT (100- (TGP / 25 * 100)).***

TABLE 3
TESTED CHARACTERISTICS OF THE SEED INOCULATED WITH CELLS OF THE SHOWED STRAINS

Strain	Total germination (%) **		Germination speed		Dry stem biomass (mg)		Dry root biomass (mg)		Stem length (mm)		Root length (mm)	
M67	5.40	a*	8.70	c	37.50	b	9.17	bcd	36.33	c	19.80	d
L168	4.31	ab	9.33	c	38.67	b	3.33	d	36.33	c	32.80	bcd
M82_C	4.28	ab	9.87	c	38.17	b	3.50	d	34.27	c	23.67	cd
M3	4.12	ab	9.93	c	42.33	b	7.83	cd	37.67	c	24.33	cd
M61	1.82	b	11.93	bc	40.33	b	7.50	cd	39.53	bc	22.80	cd
Creole Witness	4.20	ab	8.80	c	40.00	b	9.00	bcd	34.50	c	23.47	cd
M83	3.64	ab	17.93	a	76.00	a	15.17	abc	41.00	bc	52.33	a
M88	5.29	a	14.87	ab	70.83	a	22.16	a	51.33	a	42.33	ba
M82_A	4.28	ab	16.33	ab	68.00	a	15.16	abc	45.00	ba	46.20	ba
Spider Witness	4.76	a	15.30	ab	73.67	a	16.67	ab	40.87	bc	37.67	abc
MSD	2.57		4.63		9.20		7.92		6.83		16.02	

**** Means with the same letter in a column are not significantly different (Tukey, p = 0.05). MSD = Minimum significant difference. ** TGP was transformed with the formula ROOT (100- (TGP / 25 * 100)). * Means with the same letter in a column are not significantly different (Tukey, p = 0.05). MSD = Minimum significant difference. ** TGP was transformed with the formula SQRT (100- (TGP / 25 * 100)).***

TABLE 4
CRITERIA TESTED IN THE SEEDS OF TWO VARIETIES OF ROSELLE

Variety	Total germination (%) **		Germination speed		Dry stem biomass (mg)		Dryroot biomass (mg)		Stem length (mm)		Root length (mm)		Chlorophyll Content (SPAD Units)	
Creole	3.93	a*	13.27	b	78.83	b	14.33	b	53.62	a	32.71	b	36.33	b
Spider	4.49	a	17.22	a	139.04	a	30.21	a	55.46	a	69.22	a	40.76	a
MSD	0.80		1.57		29.32		8.64		2.33		5.66		2.18	

**** Means with the same letter in a column are not significantly different (Tukey, p = 0.05). MSD = Minimum significant difference. ** TGP was transformed with the formula ROOT (100- (TGP / 25 * 100)). * Means with the same letter in a column are not significantly different (Tukey, p = 0.05). MSD = Minimum significant difference. ** TGP was transformed with the formula SQRT (100- (TGP / 25 * 100)).***

TABLE 5
TESTED CHARACTERISTICS OF THE DEVELOPMENT OF THE SEEDLINGS OF TWO VARIETIES OF ROSELLE ILLUMINATED WITH THE TYPE OF LIGHT SHOWED

Lighting type	Dry stem biomass (mg)		Dryroot biomass (mg)		Stem length (mm)		Root length (mm)		Chlorophyll Content (SPAD Units)	
100% White	110.31	a*	19.69	a	52.94	a	40.24	b	37.22	a
15% White, 27% Blue, 58% Red	108.12	a	23.12	a	58.37	a	54.92	a	39.18	a
29% Blue, 71% Red	108.37	a	24.00	a	57.30	a	43.97	a	39.24	a
MSD	43.63		12.85		3.43		8.33		3.20	

**** Means with the same letter in a column are not significantly different (Tukey, p = 0.05). MSD = Minimum significant difference. ** TGP was transformed with the formula ROOT (100- (TGP / 25 * 100)). * Means with the same letter in a column are not significantly different (Tukey, p = 0.05). MSD = Minimum significant difference. ** TGP was transformed with the formula SQRT (100- (TGP / 25 * 100)).***

TABLE 6
CRITERIA TESTED IN THE SEEDS THAT HAD BEEN PREVIOUSLY INOCULATED WITH CELLS OF THE SHOWED STRAINS

Strain	Total germination (%) **		Germination speed		Dry stem biomass (mg)		Dry root biomass (mg)		Stem length (mm)		Root length (mm)		Chlorophyll Content (SPAD Units)	
M67	3.04	a*	16.83	ab	77.83	b	14.83	b	54.87	a	28.90	e	37.84	ab
L168	3.43	a	14.10	abc	78.50	b	15.00	b	53.03	a	33.63	de	32.74	b
M82_C	4.85	a	12.93	bc	80.67	b	12.83	b	54.63	a	33.07	de	36.70	ab
Creole Witness	4.38	a	9.20	c	78.33	b	14.67	b	51.93	a	35.23	cde	38.06	ab
M83	5.01	a	15.83	ab	144.67	a	30.83	a	56.60	a	89.10	a	39.60	a
M88	4.43	a	17.83	ab	136.33	a	29.00	a	55.57	a	63.50	abc	42.13	a
M82_A	4.21	a	16.8	ab	133.33	a	30.33	a	53.80	a	66.26	ab	40.89	a
Spider Witness	4.31	a	18.43	a	141.83	a	30.67	a	55.87	a	58.00	bcd	40.41	a
DMS	2.82		5.19		81.27		20.96		8.12		28.64		6.09	

**** Means with the same letter in a column are not significantly different (Tukey, p = 0.05). MSD = Minimum significant difference. ** TGP was transformed with the formula ROOT (100- (TGP / 25 * 100)). * Means with the same letter in a column are not significantly different (Tukey, p = 0.05). MSD = Minimum significant difference. ** TGP was transformed with the formula SQRT (100- (TGP / 25 * 100)).***

TABLE 7
STEM LENGTHS OF PLANTS OF TWO VARIETIES OF ROSELLE ESTABLISHED IN THE GREENHOUSE AT 3, 45 AND 65 DAS

	Stem length (cm)					
Variety	**3 das**		**45 das**		**65 das**	
Creole	2.2083	a*	5.275	a	8.358	a
Spider	2.167	a	4.95	a	8.558	a
MSD	0.624		1.147		1.781	

****Means with the same letter in a column are not significantly different (Tukey, p = 0.05). MSD = Minimum significant difference. ** TGP was transformed with the formula ROOT (100- (TGP / 25 * 100)). * Means with the same letter in a column are not significantly different (Tukey, p = 0.05). MSD = Minimum significant difference. ** TGP was transformed with the formula SQRT (100- (TGP / 25 * 100)).***

TABLE 8
STEM LENGTHS OF PLANTS OF TWO ROSELLE VARIETIES ESTABLISHED IN THE GREENHOUSE AT 3,45 AND 65 DAS INOCULATED WITH CELLS OF THE SHOWED STRAINS

	Stem length (cm)					
Strain	**3 das**		**45 das**		**65 das**	
M67	1.33	b*	5.167	a	8.667	a
L168	2.33	ab	5.5	a	7.667	a
M82_C	3	ab	5.77	a	9.167	a
Creole witness	2	ab	4.67	a	7.93	a
M83	1.33	b	4.5	a	8.667	a
M88	2.167	ab	4.9	a	7.73	a
M82_A	3.5	a	5.067	a	8	a
Spider Witness	1.83	ab	5.33	a	9.83	a
MSD	2.04		3.746		5.818	

**** Means with the same letter in a column are not significantly different (Tukey, p = 0.05). MSD = Minimum significant difference. ** TGP was transformed with the formula ROOT (100- (TGP / 25 * 100)). * Means with the same letter in a column are not significantly different (Tukey, p = 0.05). MSD = Minimum significant difference. ** TGP was transformed with the formula SQRT (100- (TGP / 25 * 100)).***

IV. CONCLUSION

The criteria of total germination percentage, dry stem biomass and dry root biomass are not safe criteria for the selection of Plant Growth-Promoting Strains, since the seedlings from Roselle seeds inoculated with cells of the bacterial strains did not show significant differences with respect to the controls corresponding to each variety. It must be taken into account that the benefit that the plant obtains from bacteria must occur after they colonize its roots more than just during the germination process. The germination speed and the stem length of the seedlings showed significant differences when inoculating the seeds, therefore, selection criteria could be considered, however to consider them safe or not, the important thing is the relationship of what happens at the moment of the germination and development of the seedlings in the laboratory and what happens in the development of the seedlings in the greenhouse, because, when measuring the stem length of the plants in the greenhouse, it was concluded that differences between strains can be noticed, but not regarding the control and that it is also only observed in the first days of development (3 days after sowing). Subsequently, no significant differences are shown. The same happened with the root length, they only showed favorable results in the experiments carried out in the laboratory, but in the same way it would have to be verified if the same effect had in the greenhouse. Chlorophyll content also did not show to be a safe selection criterion, since there were no significant differences between strains applied to the same variety. The type of lighting did not have a significant effect on the Roselle seedlings. Only, the lamp composed of 15% white light, 27% blue light and 58% red light was the one that most promoted root growth. The mean values of most of the criteria tested were higher for the Spider variety. Therefore, it is important to consider the type of variety used when making the selection of strains.

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