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Evaluation by Electron Microscopy of the Stomatal Density in the Leaves of the Sugar Beet (Beta vulgaris L.) Crop Fertilized with Compost Made from Sugarcane By-products

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The aim of this work was to determine the effect of compost made basically from sugarcane by-products on the stomatal density of the leaves of the sugar beet crop. The scanning electronic microscope model Quanta 200+EDS was used to evaluate the stomatal density and other details. This evaluation was contrasted with that resulting from the evaluation of the physical characteristics of the sugar beet crop. Compost was made from bagasse and vinasse that are residues from the sugar industry, complemented with guinea pig guano and dry grass, obtaining an organic fertilizer for the ecological production of sugar beet. The doses of compost were applied to the crop in 5 treatments seven days after transplantation and then the physical, chemical and biological characteristics of the plant were evaluated. The data obtained were subjected to the analysis of variance and Duncan's test. It was determined that in the treatment (T5) the highest values were obtained in the physical characteristics of the crop: plant height (36.67 cm), equatorial diameter (6.57 cm), polar diameter (6.18 cm), weight of the tuber (201.25 g), and commercial yield (20.964 t/ha). However, a greater number of open stomas were found in the treatment (T4).

1. Introduction

Due to the problem of industrial organic waste management, research was done to evaluate the response of the sugar beet crop when using the compost obtained as natural fertilizer under certain normalized conditions of humidity, temperature, and turning frequency for optimal biodegradation of bagasse, vinasse, guinea pig guano, and dry grass; the latter was added to improve the resulting density of the compost and the addition of guinea pig guano is to compensate for the nitrogen deficiency of the first two components mentioned. This is supported by the research of Pérez et al., (2016), who affirm that, from an environmental point of view, the use of waste to make compost is favourable to avoid contaminating the waters and at the same time improving soil fertility.
On the other hand, López Bravo et al. (2017), maintain that compost (based on sugarcane by-products) shows positive indicators of quality, having quantified the humidity of 59%, pH 8.2; the carbon-nitrogen ratio of 12.5; a density of 0.55 g/cm^3, the composition of nitrogen 1%, phosphorus 1.3%, potassium 1.1% and magnesium 1.1%, as well as low salinity, which is beneficial for ecological production.

2. Materials and methods

The research was carried out at the Los Anitos Farm located in the Province of Barranca in the Department of Lima, South latitude 10 ° 45 '48.74'' and West longitude 77 ° 44' 20.864'', with an altitude of 75 meters above sea level, which has a temperature average 19.3 ° C and a sandy loam soil type.

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2.1 Treatment

The application of doses of compost was carried out 7 days after transplanting (when the plant reached 45 days after sowing). For this purpose, the common dose and the control were taken into account; according to Hirzel and Salazar (2016), they indicate that the reference dose of organic amendments is 4 to 8 t/ha of semi-compost and 6 to 12 t/ha of compost. It should also be mentioned that the field work was the same for all the plots, and only the dose of compost was varied. Table 1 below details the amounts of compost for each treatment.

Table 1: Compost dosage for sugar beet cultivation

Treatment	g/plant	kg/ha
T1	00	00
T2	24	4000
T3	36	6000
T4	48	8000
T5	60	10000

2.2 Procedures

Sugarcane by-products were used as a percentage: bagasse and vinasse, as well as guinea pig guano and dry grass, with a percentage composition of 23.5%, 29.4%, 23.5% and 23.5% respectively for a total amount of 85 kg of waste.

Then the compound was turned over with a shovel every week for a period of 112 days. It was dosed according to what is indicated in Table 1. The performance results were compared with the elemental concentration dose of each treatment. The same process was also done with the biological analysis.

2.3 Statistical application

Analysis of variance and F distribution was applied, since it allows to determine whether the treatment is significant or not; that is, whether there was an effect of the doses of compost on the evaluation parameters of the randomized treatments, in an analysis of variance of randomized blocks as shown in Table 2.

Table 2: Analysis of block variance and randomised treatment

Source of Variation	SC 2	Gl	CM	Model I E(CM)	Model II E(CM)	F. cal
Blocks	SC_b	b – 1	$CM_b=SC_b/b-1$	$(\sigma^2_e + \sum\beta^2_j)/(b-1)$	$\sigma^2_e + t\sigma^2_\beta$	CM_b/CM_e
Treatments	SC_{tr}	T – 1	$CM_{tr}=SC_{tr}/t-1$	$(\sigma^2_e + b\sum T^2_i)/(t-1)$	$\sigma^2_e + b\sigma^2_t$	CM_{tr}/CM_e
Error	SC_e	(b-1)(t-1)	$CM_e=SC_e/(b-1)(t-1)$	σ^2_e	σ^2_e	
Total	SC_t	bt – 1				

Source: Núñez & Tusell (2007).

Duncan's Multiple Range Test

To the data obtained from the field and post-harvest evaluation, the Duncan test was applied at 5% error, which made it possible to determine which treatment stood out in relation to the others and if homogeneity is presented. Duncan's Multiple Range Test, Eq(1), is a valid sequential procedure for comparing the contrast of two means. (López and González, 2016).

$$D_P = d_{(p,glee,\propto)} \times \sqrt{\frac{CMee}{r}} \tag{1}$$

Where:
D_P: The Significant Difference or the range value.
d: Duncan's Significant Range Value, with parameters:
p: distance between two compared means
glee: degrees of freedom of experimental error
α: significance level
CMee: mean square of the experimental error
r: number of replicates of the means of the treatments to be compared

3. Results and discussion

The results of the soil analysis are shown in Table 3, which according to the classification of Prialé (2016), the value of electrical conductivity corresponds to a very slightly saline soil, the pH corresponds to a neutral value, organic matter and nitrogen have low values, and phosphorus and potassium show average values. As for the interchangeable cations, according to McKean (1993), we have a high value for Ca, and average values for Mg, Na and K; and for the cationic exchange capacity, we have a low level according to Garrido (1994).

Table 3: Basic soil analysis for sugar beet cultivation

E.C. 1:2.5 mS/cm	pH 1:2.5	O.M. %	N %	P ppm	K ppm	Interchangeable cations (mEq/100 g soil) Ca	Mg	Na	K	CEC
1.19	6.88	1.37	0.07	12	212	16.99	0.68	0.29	0.54	18.50

Source: INIA, 2018.

CEC: Cation exchange capacity
OM: Organic matter
EC: Electrical conductivity
Table 4 shows the laboratory report with the results of the concentrations of the microelements present in the soil where the sugar beet was planted with their respective classification. In these results it can be seen that the iron concentration value has the qualification of normal and copper is in excess, in reference to that established by Toledo (2016), who states that the appropriate values for iron are between 56 and 112 ppm and for copper between 1.7 and 3.4 ppm. Boron levels are found to be low, taking into account McKean (1993), who states that normal boron levels are between 1.5 and 3 ppm.

Table 4: Micro-element concentrations of the experimental area

Micro-elements (ppm)			
Fe	Zn	Cu	B
103.90	7.62	11.04	1.56
Normal	Low	Excess	Low

Source: INIA (2018) National Institute for Agricultural Research. Laboratory 105-108. INIA Huaral**.**

The composition of the compost used is shown in table 5, where we can see that the values of pH, organic matter, nitrogen, potassium and the carbon/nitrogen ratio are within the values recommended by Román et al. (2013), who suggest that ideal ranges of mature compost parameters should be: pH of 6.5 to 8.5, organic matter values greater than 20%, nitrogen of 0.3 to 1.5%, potassium of 0.3 to 1.0% and a carbon/nitrogen ratio in the range of 10:1 to 15:1; however, it can be observed that the humidity and phosphorus parameters are above the values recommended by these authors: humidity of 30 to 40% and phosphorus of 0.1 to 1.0%.

Table 5: Basic analysis of organic fertilizer

E.C. 1:2.5 mS/cm	pH 1:2.5	Humidity %	O.M. %	C %	N %	P_2O_5 %	K_2O %	CaO %	MgO %	C/N %
4.25	7.95	44.96	26.79	15.53	1.14	1,68	0.82	2.85	0.34	13.62

Source: INIA, 2018.

The chemical analysis of the sugar beet leaves after the treatments applied is shown in Table 6. It is observed that in most of the treatments the nutrients are within the normal values compared to the comparison treatment (T_1); which indicates that an adequate amount of nutrients in the compost has a significant influence on the yield. This is supported by Quintero (1995), who mentions that plants require a number of minor elements (micronutrients) in relatively small quantities; which does not mean that they are less important than macronutrients, as they are necessary for enzymatic processes, oxidation-reduction, chlorophyll formation and carbohydrate transport, among others.

Table 6: Complete analysis of leaves according to the doses of compost

Percentage	Treatments				
(%)	T_1	T_2	T_3	T_4	T_5
C	25.1	27.57	32.33	32.7	27.69
O	73.7	71.19	66.36	65.52	70.96
K	0.39	0.45	0.48	0.57	0.5
Ca	0.06	0.09	0.1	0.12	0.05
Mg	0.09	0.14	0.17	0.11	0.18
Na	0.11	0.09	0.06	0.1	0.21
Si	0.12	0.1	0.08	0.25	0
S	0.4	0.5	0.9	1.1	0
Cl	0.2	0.16	0.16	0.25	0.35
parts per million (ppm)					
Fe	700	1100	1300	1400	600
Al	1100	600	400	1300	0

The physical characteristics and commercial yield in tons of the sugar beet (Beta vulgaris L.) crop are shown in Table 7. It can be observed that at a higher dose of compost (T_5), high values were obtained in the parameters of the physical characteristics of the sugar beet, as well as in the yield. Therefore, this dose and the type of raw material used to make the compost favours plant nutrition, as López et al. (2017) conclude that the compost made from sugarcane by-products presents an adequate composition to be used as organic fertiliser in agriculture.

Table 7: Physical characteristics of sugar beet (Beta vulgaris L.)

Treatment	Plant length (cm)	Equatorial diameter (cm)	Polar diameter (cm)	Weight of sugar beet (g)	Yield (t/ha)
T_5	36.67 a	6.568 a	6.178 a	201.25 a	20.964 a
T_4	33.45 ab	6.059 a	5.426 ab	149.27 ab	15.738 a
T_3	29.33 ab	5.339 a	4.943 ab	137.40 ab	14.454 a
T_2	27.33 ab	5.176 a	4.786 ab	108.36 b	13.342 a
T_1	25.72 b	4.765 a	4.684 b	91.95 b	10.242 a
Significance	**	**	**	**	**
CV (%)	16.85	22.32	14.09	29.79	42.72

CV: Coefficient of variation.

Analysis of stomatal density of the leaves by treatment:
The stomata of sugar beet leaves were quantified from the micrographs obtained with the scanning electron microscope, for a degree of magnification of 800X, which is indicated in table 8. It was observed that the highest number of stomata is obtained in the T_2 treatment with 169 stomata per mm^2. However, this number of stomata did not influence the yield, as can be seen in the T_5 treatment with 123 stomata per mm^2. Therefore, it is interpreted that the higher the dose of compost, lower stomatal density and higher yield were obtained. This can be interpreted as taking advantage of the humidity from evapotranspiration, so there is less exchange with the atmosphere, which favours the formation of carbohydrates.

Table 8: Stomatal index, stomatal density and number of open stomata according to treatment

Evaluation	Treatments				
	T_1	T_2	T_3	T_4	T_5
Stomatal index	18.94	21.56	26.66	23.07	19.75
Stomatal density (number of stomata/mm^2)	138	169	153	161	123
Number of open stomata/mm^2	19	21	21	22	15

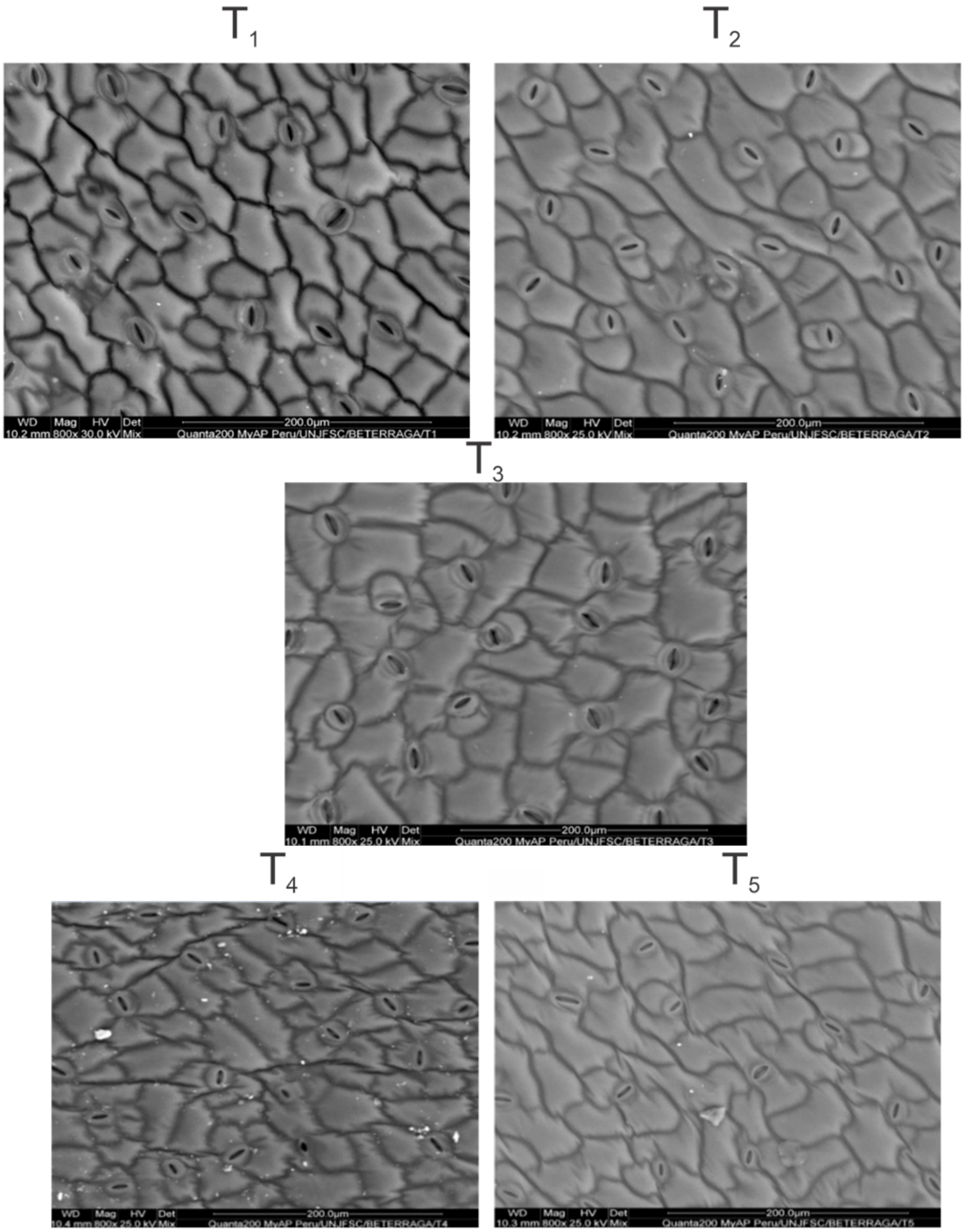

Figure 1: Stomata micrograph of each treatment

The results of the abaxial stomatal density are within that specified by Esau (1976), who states that angiosperms such as Beta vulgaris have between 100-300 stomata/mm^2. This is reaffirmed according to Cañizares et al. (2003), who mention that the decrease in stomatal density increases stomatal resistance, thus increasing the adaptability of plants to stressful conditions in the soil. In this study the T5 treatment presents the best harvest yield, so it can be proposed as a favourable harvest indicator in Beta vulgaris L crops.

4. Conclusions

The variation in the doses of compost per treatment produced variation in the percentage of the stomatal index and in the stomatal density, the highest values being 19.75% for treatment T_3 and 123 stomata/mm^2 of the T_2 treatment respectively. On the other hand, maximum yield was obtained for treatment T5, which corresponds to the highest dose of compost, with a value of 20,964 t / ha, and which is related to values of stomatal indices and low stomatal densities. The gradual increase in the concentration of oxygen, potassium, magnesium, sodium and chlorine was determined until treatment T5, this is favourable since these elements influence the development of the plant, which is why a higher yield per hectare was obtained.

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