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RESEARCH ARTICLE

MORPHOLOGICAL VARIABILITY OF EUSTOMA HYBRIDS OBTAINED FROM INTERSPECIFIC CROSSES BETWEEN *Eustoma grandiflorum* X *Eustoma exaltatum*

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Abstract

The morphological characteristics of hybrid lines obtained from interspecific crosses between *Eustoma grandiflorum* and *Eustoma exaltatum* were analyzed based on the descriptors reported by UPOV for *Eustoma grandiflorum*. In the principal component analysis, a variation of 73.70% was obtained within four main components. The hybrids of the genus *Eustoma* were grown in weather conditions with temperature up to 44.5°C in Yucatan Mexico. The 1512063, 1512096, 1512098, and 1512100 hybrids showed a good development plant height, number of nodal segments, flower number and flower size, so that these hybrids could be cultivated tropical regions for commercial purposes.

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Introduction:-

Eustoma grandiflorum (Lisianthus), is a species whose demand has increased due to its ornamental characteristics, including a long vase life, wide range of colors (from purple to lavender, and from pink to white), as well as high crop profitability. Lisianthus is cultivated as a cut flower and as a potted flower (Anderson, 2006). *E. grandiflorum* of large flowers and restricted distribution conforms to the genus *Eustoma* together with *E. exaltatum*, which has small and more widespread flowers (Turner, 2014). *Eustoma grandiflorum* presents adaptation difficulties at temperatures above 25°C (Roni et al., 2016; Harbaugh et al., 1992; Ohkawa et al., 1991) which has led to studies on genetic improvement programs. Barba-González et al., (2015) that carried out interspecific hybridization of *E. grandiflorum* x *E. exaltatum*, obtaining hybrids that presented different characteristics to parental ones, specifically, hybrids of *E. exaltatum* White showed a wide variation in color flower.

Additionally, Barba-González et al. (2017) performed interspecific hybridization of *E. grandiflorum* and *E. exaltatum*, obtaining F1, which presented important ornamental features such as double and large-sized flowers. One of the most important aspects to consider is the presence of co-dominance of the progeny when crossing *E. grandiflorum* White with pink F1 hybrids.

Variability between hybrids can be analyzed with morphological markers, because these allow showing differences and similarities in the phenotype. It is also possible to estimate and evaluate the genetic distance between different varieties, identifying heterotic groups by grouping methods (Barros et al., 2019).

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Morphological markers are practical, so they have been applied in breeding programs to study genetic diversity (Barros et al., 2019; Mangosongo et al., 2019; Shahid et al., 2019; Piña et al., 2010); selection outstanding hybrids (Khadivi et al., 2019), and interspecific hybrids evaluation (Santos et al., 2012).

The objective of this study was to determine the variability between hybrid lines of *E. exaltatum* x *E. grandiflorum* generated by Barba-González et al. (2017). Plants that were morphologically characterized were grown under *ex vitro* culture conditions in a tropical climate in Yucatan, Mexico.

Materials and Methods:-

ex vitro culture:

The seeds of the hybrids of *Eustoma* were provided by the Center for Research and Assistance in Technology and Design of the State Jalisco (CIATEJ). Hybrid plants were obtained from in vitro germination of hybrid seeds according to the protocol of Falcon Bautista et al., 2018. In vitro plants of four month of age with 5-8 cm in length with four pairs of developed leaves were transferred from in vitro culture to ex vitro conditions, 4 individuals were selected for each of the hybridized lines.

In vitro seedling roots were rinsed with distilled water; subsequently, a rinse was carried out with fungal solution of Binolate® 1g L⁻¹. The seedlings were placed in forks with Agrolite and sterilized organic soil at a ratio of 1:1. During 15 days, the *Eustoma* plants were covered with transparent nylon bags and placed in vitro culture conditions with photoperiod of 16 h light and 8 h dark, at a temperature of 23°C; subsequently, the transparent bags were removed and the seedlings were taken to ex vitro conditions.

During acclimatization process until that fully were developed plants, temperature, relative humidity and luminosity were recorded. ex vitro culture had two flowering periods that were recorded, the first period was from September to December and the second period from March to July. The average temperature recorded was 32.5°C, with a maximum temperature of 44.5°C recorded on May and a minimum temperature of 23.7°C on December. The percentage of maximum humidity was on October with a value of 78.31% and the minimum on April with a value of 31.7%. The maximum luminosity (Lx) is changed during April with a value of 225.25 and a minimum value of 31.5 on December.

Morphological characterization:

Morphological characterization was carried out based on guide TG / 197/1 Guidelines for the execution of the examination of the distinction, homogeneity and stability of *Eustoma* (UPOV, 2002), established by International Union for Protection of New Varieties of Plants (UPOV).

The morphological characteristics measurement was carried out in the period from March to June 2019, during the second flowering. Following what was described by *Eustoma*'s guide TG / 197/1 (UPOV, 2002), the leaf observations were examined on the third sheet from the top; flower and pedicel observations were examined on the second flower that opened and on its inner face; internode length was measured in the fourth internode from the top.

The color observations were made using the HunterLab MiniScan EZ colorimeter, which records data on the CIELab scale (L = luminosity, -a / + a = green to red scale, -b / + b blue to yellow scale), during the anthesis in 3 petals per flower, the results were adapted to the color guide of the Royal Horticultural Society (RHS) as marked by the guide TG / 197/1 (UPOV, 2002) of *Eustoma*.

Statistical analysis:

The statistical parameters (average, minimum, maximum values, standard deviation and coefficient of variation) obtained for 14 quantitative and 10 qualitative morphological characters evaluated in 12 hybrid lines (Table 1).

Analysis of main components was carried out with the objective of representing hierarchical relationships between the hybrids. Subsequently, the PROJ module was used to generate a two-dimensional projection of PCA using the NTSYS PC 2.1 software (Rohlf et al., 2000). A similarity matrix was made using the Euclidean distance coefficient based on the projection of main components to perform a cluster analysis using the UPGMA method (Unweighted Pair Group Method using Arithmetic averages) for the grouping of taxonomic units, using the NTSYS software version 2.1 (Rohlf et al., 2000). The selection of outstanding individuals was made by grouping all the individuals that make up each hybrid line, considering the separate values of the complete siblings, to make the grouping the

FIRST v6 software was used (Clarke, 2006). Statistical analyzes were performed with Statgraphics® statistical software.

Table 1:- Morphological characteristics established by the International Union for the Protection of New Varieties of Plants (UPOV) for the genus *Eustoma*.

Traits	
PL	Plant Length (cm)
SD	Stem Diameter (mm)
NN	Nodes Number
LL	Leaf Length (cm)
LW	Leaf Width (cm)
LSTE	Leaf in relation to Stem
LC	Leaf Color
LS	Leaf Shape
FN	Flowers Number
FT	Flower Type
FL	Flower Length (cm)
FD	Flower Diameter (cm)
FS	Flower Shape
PN	Petals Number
PEL	Petals Length (cm)
FW	Flower Width (cm)
PS	Petal Shape
PU	Petal Undulation
FEP	Fringe of the Edge of the Petal
CN	Colors Number
PC	Petal Color
CHL	Chalice Length (cm)
PELE	Pedicel Length(cm)
SN	Stamens Number

Results and Discussion:-

Assessment of morphological characters in hybrids of the genus *Eustoma* statistically significant differences were found between the hybrids in relation to the morphological traits, except for the variable SD and LSTE, where the data did not present any difference in relation to the hybrid. High values as plant length (PL) and nodal segments number (NN) were observed in 1512063, 1512095, 1512096, 1512098 hybrids contrary the low values were obtained in 1512070, 1512093, 1512029 hybrids (Table 2). As to flowers number (FN) high values were observed in 1512096, 1512098 hybrids and low values in 1512025, 1512070, 1512093 hybrids. Another important trait in cut flowers is pedicel length (PELE), which the high values were observed in 1512063, 1512082, 1512100 hybrids, and the low value in 1512070 hybrid. Associate traits as flower length (FL) and flower diameter (FD), high values were detected in 1512063, 1512098, 1512100 hybrids, and lower values in 1512093, 1512095, 1512105 hybrids. Furthermore, petal length (PEL) trait was the best in 1512100 hybrid, and the worst in 1512070 and 1512105 hybrids. High values in petals width (PW) were observed in 1512093, 1512093 and 1512100, hybrids, and low value in 1512070 value (Table 2).

Higher variation coefficients were observed in FN, flower shape (FS), PEL, and leaf shape (LS) traits (65.99, 49.13, 49.13 and 43.95%, respectively; differently, lower coefficients were observed in stamens number (SN) and leaf color (LC) with 10.89 y 10.69% respectively (Table 2).

Correlation analysis of used variables in characterization of *Eustoma* genus hybrids, 13 significative relationships were obtained, which were considered values with an “r value” greater than 0.7. It is important to mention some significative correlations with shape plant, for example relation of Nodes number (NN)-PL presented a positive r value=0.7321, It means that both variables tend to increase at the same time. The same situation can be observed in floral traits like PN-FT with r value = 0.9996, SN-Flower type (FT) with r value = -1.000 and SN- Petals Number

(PN) with r value = -0.999, this negative value indicate that while one variable tends to increase the other tend to decrease. It means that if a flower presents more petals, the number of stamens is smaller.

Table 2:- Basic statistics and description for qualitative and quantitative traits evaluated in *E. grandiflorum* x *E. exaltatum* hybrids.

Trait	Hybrids												Mean	Min	Max	SD	CV%	Error %
	1512025	1512029	1512063	1512070	1512072	1512082	1512093	1512095	1512096	1512098	1512105	1512100						
PL	22.25	26.53	40.41	13.78	29.68	30.13	21.43	38.65	34.75	42.94	23.37	28.68	29.91	11.89	51.00	11.03	37.77	1.59
SD	4.07	3.48	3.06	3.20	5.27	3.81	4.06	3.49	3.21	4.06	3.09	4.16	3.75	2.12	9.53	1.32	35.38	0.19
NN	7.75	6.25	8.75	6.25	6.75	6.00	5.25	8.75	9.75	10.25	7.00	8.75	7.62	5.00	12.00	1.74	22.89	0.25
LSTE	1.00	1.00	1.25	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.50	1.06	1.00	3.00	0.31	30.11	0.04
LL	3.39	3.68	4.54	3.39	5.09	5.18	4.20	4.33	4.60	4.48	5.27	6.22	4.61	3.19	7.15	0.84	18.30	0.12
LW	1.62	1.73	2.54	2.21	2.81	2.41	2.98	2.22	2.40	2.07	2.22	3.05	2.35	1.54	3.52	0.49	21.12	0.07
LC	41.78	48.70	38.76	42.80	50.46	48.92	44.04	48.75	44.62	48.69	45.56	46.87	45.37	37.89	56.88	4.89	10.69	0.73
LS	1.00	1.00	2.25	3.00	2.00	2.00	3.00	2.00	2.00	1.00	1.00	1.00	1.77	1.00	3.00	0.77	43.95	0.11
FN	1.50	2.75	3.75	1.50	2.50	2.25	1.00	4.00	5.25	6.00	2.50	3.25	3.02	1.00	7.00	1.65	65.59	0.28
FT	1.00	1.00	2.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.08	1.00	2.00	0.27	25.78	0.04
FL	4.02	4.10	4.96	3.79	4.81	4.50	3.26	3.71	4.44	5.06	3.43	5.59	4.30	2.86	5.92	0.76	17.83	0.11
PN	5.00	5.00	13.75	5.00	5.25	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.75	5.00	15.00	2.52	43.86	0.36
FD	4.58	4.78	6.15	3.87	5.21	4.09	3.42	3.46	4.16	5.16	3.65	5.98	4.54	3.17	6.59	0.95	18.37	0.11
FS	1.00	2.00	2.75	1.00	1.00	2.00	1.00	1.00	1.50	3.00	3.00	1.00	1.68	1.00	3.00	0.82	49.13	0.12
PEL	3.55	4.28	5.05	3.31	4.41	4.78	3.51	3.70	4.18	5.02	3.44	5.65	4.24	3.22	5.81	0.77	49.13	0.12
PW	2.41	2.50	3.28	2.37	2.77	3.12	3.48	3.26	2.53	4.13	2.70	3.70	3.02	2.34	4.33	0.57	18.94	0.08
PS	3.00	2.00	3.25	3.00	2.00	2.00	1.00	2.00	3.25	3.00	3.00	3.00	2.54	1.00	4.00	0.71	28.06	0.10
PU	2.00	2.00	1.00	1.00	1.00	1.00	1.00	1.00	1.75	1.25	1.00	1.25	1.27	1.00	2.00	0.44	35.33	0.06
FEP	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.25	1.25	2.00	1.00	1.12	1.00	2.00	0.33	29.70	0.04
CN	2.00	1.75	1.00	1.00	1.00	2.00	1.00	1.00	1.00	1.25	1.00	1.00	1.25	1.00	2.00	0.43	35.00	0.06
CP	91.49	92.14	85.38	77.67	80.79	89.23	53.66	87.84	89.53	88.64	83.95	85.05	84.04	49.16	97.20	11.20	13.33	1.61
CHL	2.12	2.06	2.39	1.33	3.13	2.62	1.94	2.04	2.30	2.48	2.16	2.53	2.26	1.32	4.83	0.56	24.80	0.08
PELE	2.54	3.36	5.37	1.47	4.29	4.75	2.79	3.35	3.28	4.05	3.41	5.10	3.65	1.29	7.10	1.24	34.09	0.17
SN	5.00	5.00	3.25	5.00	5.00	5.00	5.00	5.00	5.25	5.00	5.00	5.00	4.87	3.00	6.00	0.53	10.89	0.07

SD: Standard deviation; CV: Coefficient of variation. SD: Stem diameter (mm); PL: Plant length (cm); NN: Nodes number; LL: Leaf length (cm); LW: Leaf width (cm); LC: Leaf color; LS: Leaf shape; FN: Flowers number; FT: Flower type; FL: Flower length (cm); FD: Flower diameter (cm); FS: Flower shape ; PN: Petals number; PEL: Petal length (cm); PW: Petal width (cm); PS: Petal shape; PU: Petal undulation; FEP: Fringe of the edge of the petal; CN: Colors number; CP: Petal color; CHL: Chalice length; PELE: Pedicel length; SN: Stamens number. LSTE: Leaf in relation to Stem.

Analysis of principal component allowed identifying the best traits to define the phenotypic variation between the hybrid lines. The 73.70% of total variation in 16 traits was explained in the first four principal components (Table 3).

Table 3:- Values and percentage of variation explained in the first four component principals of the analysis of *Eustoma* hybrids.

Principal component	Value	% of total variance	% of accumulate variation
1	7.71	32.12	32.12
2	3.99	16.64	48.76
3	3.66	15.26	64.02
4	2.32	09.68	73.70

The principal component number one explained the 32.12% of total variance, and was conformed to eight traits; two of them are related with shape plant (PL y NN) and the other six with floral traits (FN, FL, FD, LNP, CHL, and PELE). The principal component number two presented the 16.64% of total variance and was conformed with four traits, which, one was foliar trait and three were linked with floral trait (FT, PN, SN) (Table 4).

Table 4:- Vectors of traits which to integrate the first four principal components in *Eustoma* genus hybrids analysis.

Trait	CP1	CP2	CP3	CP4
PL	0.7438	0.1538	0.0648	0.5428
DT	0.2796	-0.7315	0.1709	0.0481
NN	0.6331	0.1634	-0.3343	0.3374
LW	0.3474	-0.4215	0.5136	-0.5552
LS	-0.3624	0.1448	0.7939	-0.196
FN	0.6284	0.124	-0.1992	0.5343
FT	0.5342	0.7183	0.2332	-0.2739
FL	0.8888	-0.1578	-0.1471	-0.0623

PN	0.5389	0.7091	0.2407	-0.2782
FD	0.8392	0.0839	-0.1337	-0.2294
PEL	0.9218	-0.1852	-0.0609	-0.0302
PU	-0.1195	-0.0935	-0.8772	-0.1994
PC	0.1748	-0.1379	0.6832	-0.091
PELE	0.8919	-0.1311	-0.003	-0.1205
SN	-0.5342	-0.7183	-0.2332	0.2739

PL: Plant length; DT: Stem diameter, NN: Nodal number; LW: Leaf width; LS: Leaf shape; FN: Flowers number; FT: Flower type; FL: Flower length; PN: Petals number; FD: Flower diameter; PEL: Petal length; PU: Petal undulation; PC: Petal color; PELE: Pedicel length; SN: Stamens number.

Projection of 12 hybrid lines of *Eustoma* genus from the two first principal components permits to concentrate three groups, while in 1512063, 1512070, 1512093, 1512098 hybrid lines were considered independent groups (Figure 1). Those groups were dispersing in diagram; they showed higher phenotypic variability between materials, similar as Khadivi et al. (2019) reported in walnuts genotypes.

Group I integrated by 1512025, 1512029, 1512105 hybrids lines, these hybrids showed low values in PL: 22.25-26.53 cm. Group II integrated by 1512095 and 1512096 hybrids lines, which showed white flowers and intermediate values in PL: 34.75-38.65 cm. Group III conformed by pink color flowers, and high values in PELE: 4.29-5.10, and PL values of 26.68-30.10 cm.

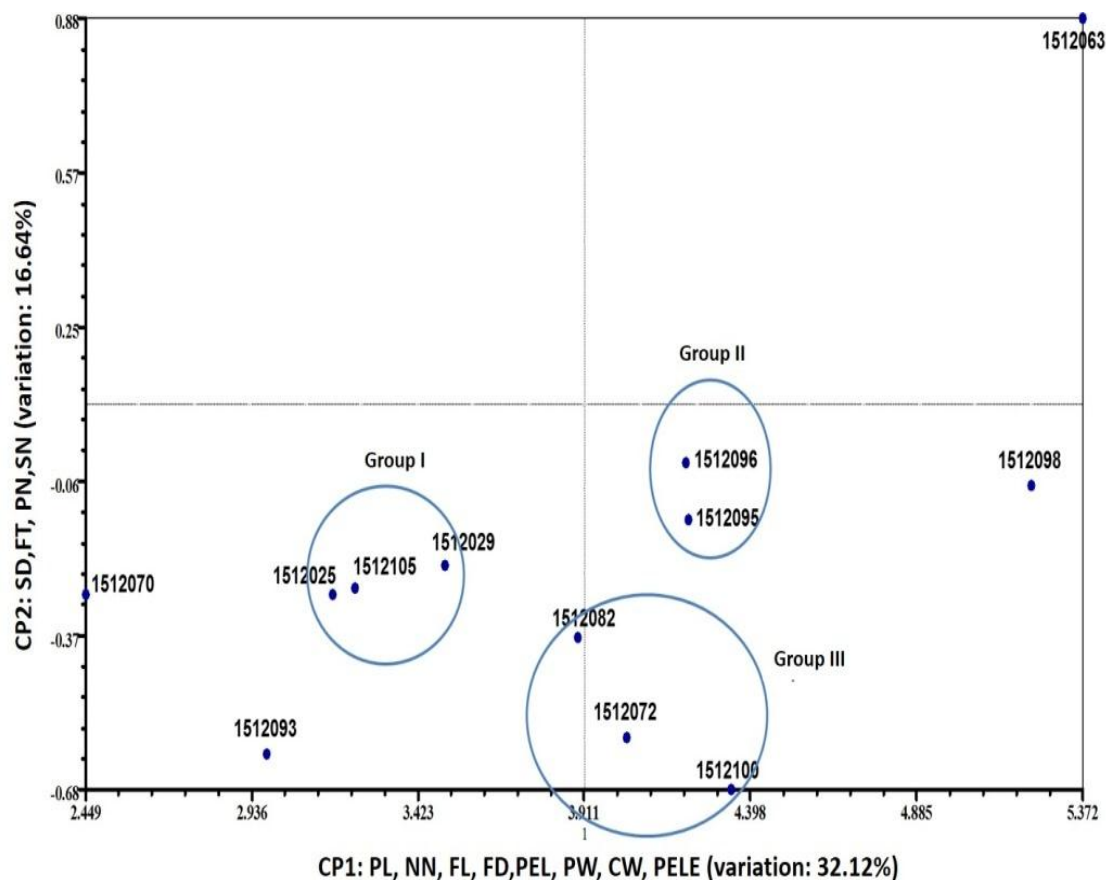


Figure 1:- Analysis saturation graphic of principal components between morphological traits in *Eustoma* genus hybrids.

Twelve *Eustoma* genus hybrids were grouped by UPGMA method using the Euclidean coefficient on the principal components space. The dendrogram showed the same groups observed in Figure 1. Two principal groups and subgroups were formed. Seven groups (from AI to AIV and from BI to BIII) in a distance of 0.71 were observed.

The 1512070, 1512093, 1512063 and 1512098 hybrids formed to the groups AIII, AIV, AV, BI, BIII respectively, which to indicate independence with the others hybrids (Figure 2).

The distribution related to ornamental traits for cut-flower was observed in the UPGMA dendrogram obtained from morphologic data. The group B integrated in its most by hybrids with higher PL, and Higher values in FN and NN. The group B was integrated by hybrids with intermediate values and low characteristics of PL, FN and NN. The flower color trait did not influence in grouping, which to suggest that this morphologic trait is not apt for grouping of *Eustoma* genus, endogamic lines of *Viola wittrockiana* also has been reported by Vemmos (2005).

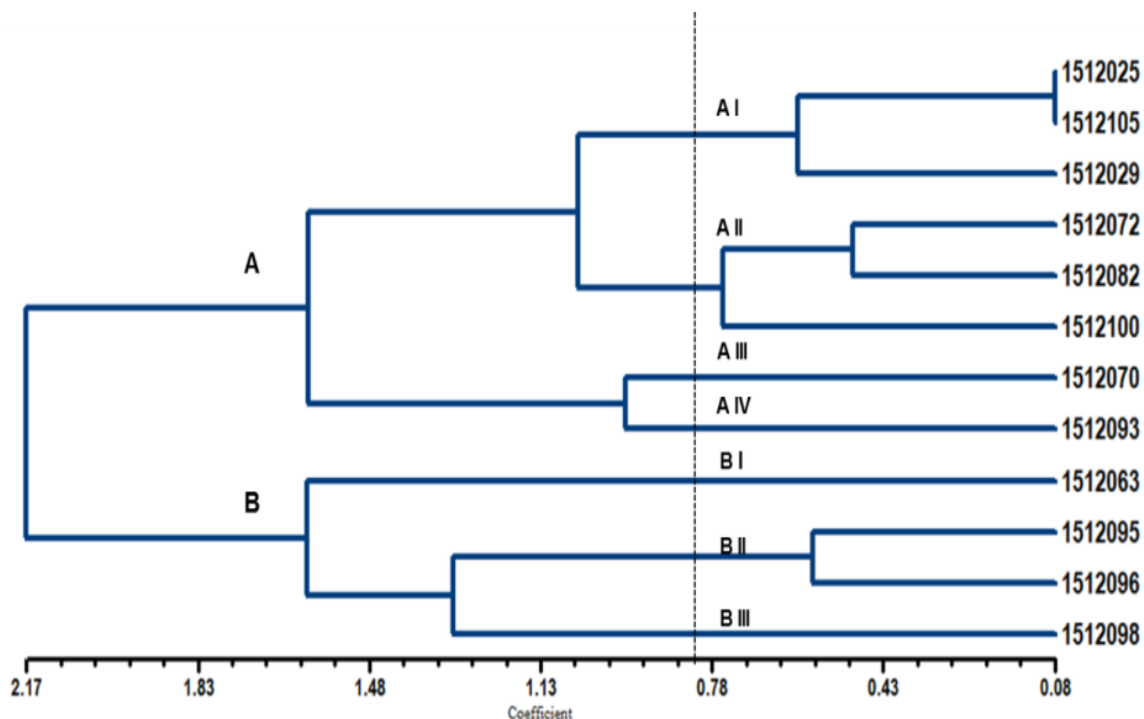
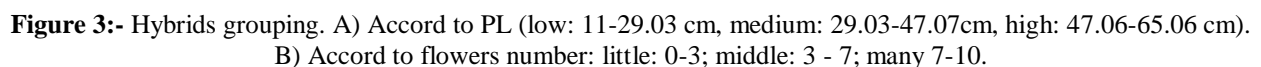


Figure 2:- Eustoma genus hybrids dendrogram based in the first four components by Euclidean distance using the UPGMA grouping method.

Outstanding material selection in Eustoma genus hybrids:

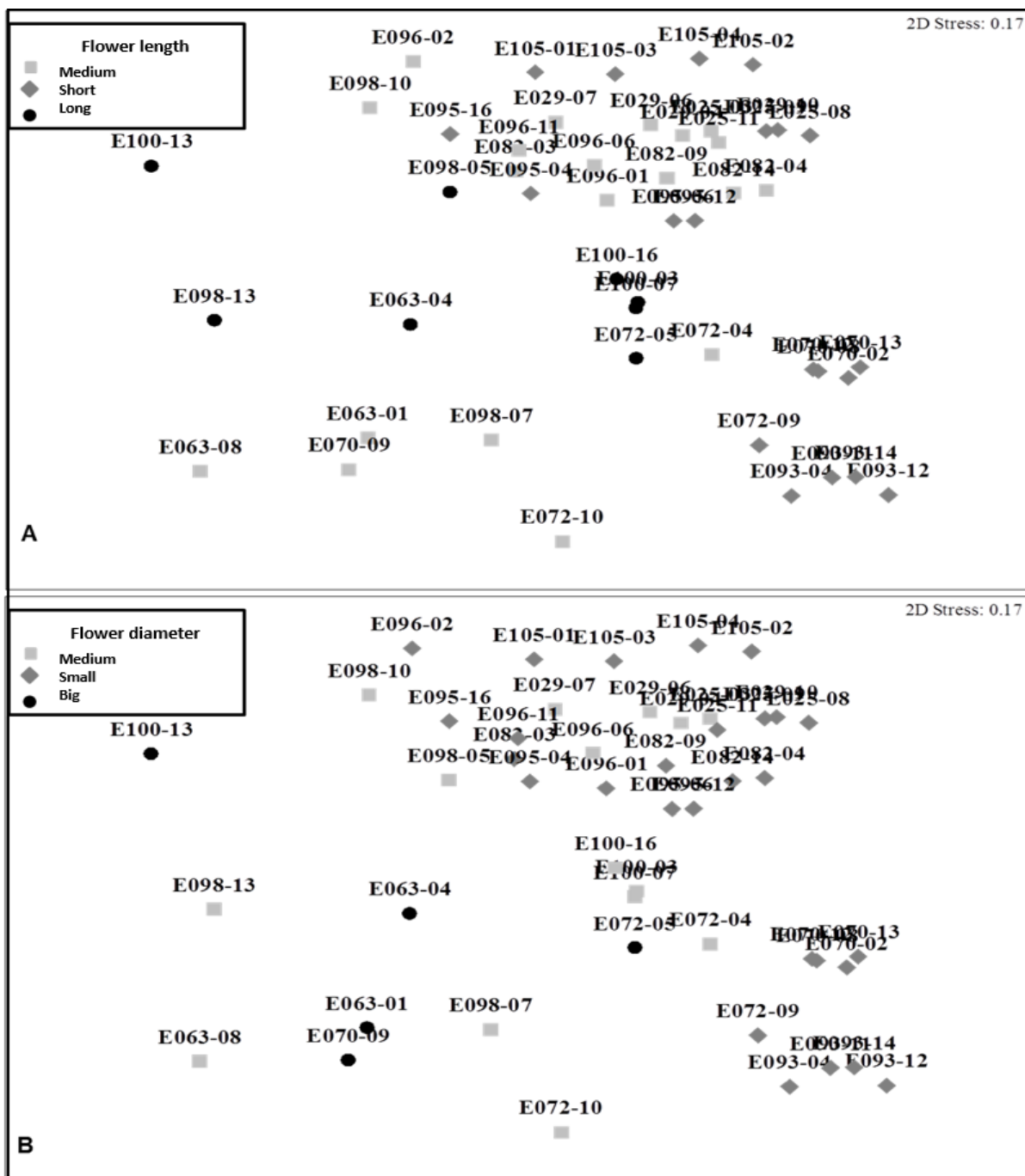
Materials of each Eustoma genus hybrid were grouped accord the important cut-flower trait. The trait considered were PL, floral shoots number, FL, FD, flower color and PELE. Those variables were divided in 3 ranges (excepting the color) to group the materials belonging to each hybrid.

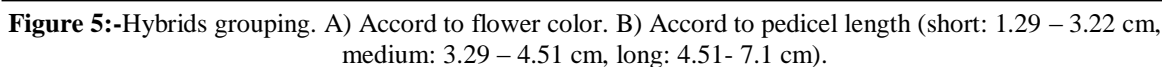
Among the 1512063, 1512098, 1512095 hybrids were individuals with a height of up to 51 cm that are positioned within the High category, despite the fact that the maximum temperature recorded during their culture was 44.5 ° C during May mo (Figure 3A). These results are like the reported by Uddin et al., 2015, who evaluated the behave of 8 *E. grandiflorum* varieties from February to October with a temperature between 28.4- 31.3°C, they reported the higher plant length of 58.9 cm grouped respectively in tall category. Plants with length of 46 cm from November to July cataloged in medium subgroup Uddin et al. (2013). These values differ with Ahmed (2016) that analyzed the yielding of 15 *Lisiantus* lines and mentioned that maximum values in plant length were from 68.8 to 65.9 cm. On the other hand, the 1512095, 1512096 y 1512098 hybrids presented materials with higher flowers number set on range of many value traits with 7 floral shoots per material (Figure 3B), these results are similar with reported by Uddin et al. (2015) with maximum flower number of 8 and Ahmad (2016) with maximum flowers number of 9.



Grouping of Figure 3, 4 and 5, showed materials of a same hybrid in different categories. This variation between complete siblings came from heterozygosity of existing parental that to generate higher diversity inside of progeny.

Figure 4:-Hybrids grouping. A) Accord to flower length (short: 2.86 - 4.00 cm, medium: 4.00 - 5.14 cm, Long: 5.14 - 6.3 cm. B) Accord to flowers diameter (small: 3.2 - 4.6 cm; medium: 4.6 -6.0 cm; big: 6 - 7.4 cm).





Conclusion:-

Evaluation and analysis of morphologic traits helped to confirm the presence of outstanding hybrids, considering important agronomic traits to obtain cut-flower materials. The outstanding hybrids obtained were 1512063, 1512096, 1512098, and 1512100. Our studies revealed that outstanding hybrids obtained by interspecific crosses of *E. grandiflorum* and *E. exaltatum* could be cultured at high temperature.

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