

Agronomic and morphological evaluation of six genotypes and two hybrids of Poblano peppers in field conditions

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ABSTRACT

Objective: to evaluate agronomic and morphological traits of six Poblano-type pepper genotypes, compared to a commercial hybrid and an experimental hybrid, in order to select genotypes with potential to continue with a plant breeding program.

Design/Methodology/Approach: treatments and statistical model were arranged in randomized complete blocks, with eight treatments (six F_2 genotypes, and two hybrids Carranza (commercial F_1) and F402 (experimental F_1). Treatments were analyzed with analysis of variance ($p \leq 0.05$); then tested with Multiple Mean Comparison (Tukey, $p \leq 0.05$) and Pearson Correlation Analysis.

Results: no statistical differences were found in yield per plant, number of fruits per plant, average weight per fruit, plant height, stem thickness, leaf length and leaf width. Regarding width at the base of the fruit the hybrids were superior; in average width of the fruit the genotypes G4, G6 and the hybrids were better; in fruit length G2, G5 and G6 stood out; in calyx depth G1, G3 and G4; in length of the peduncle G4 was different from the others; and in thickness of the mesocarp the genotypes G2, G4, G6 and hybrids were superior. According to Pearson's correlation, the yield depended on NFP (0.66), MT (0.46), FBW (0.38), as it is shown by their coefficients.

Limitations/Implications of the study: F_1 hybrids do include market features preferred by consumers and producers, since those hybrids were created to favor those traits; for this reason, those hybrids were compared to second generation (F_2) genotypes obtained by directed manual pollination.

Findings/Conclusions: The evaluated G2, G4 and G6 second generation (F_2) genotypes are highlighted for their agronomic potential compared even to the tested hybrids. So, their genetic potential is inferred and could be useful to continue selecting them within a plant breeding program.

Keywords: *Capsicum annuum*, F_2 , agronomic behavior, correlation analysis.

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INTRODUCTION

The cultivation of chili peppers in Mexico is of great importance due to its origin and domestication. There is archaeological evidence which suggests that peppers were

cultivated from 7000 to 2555 B.C. in regions as the Coaxtlán Cave, Tehuacán Valley, Puebla, Ocampo, Tamaulipas and Mitla, Oaxaca (Perry & Flannery, 2007; Kraft *et al.*, 2014). Within the genus *Capsicum* 43 species and five varieties are recognized; among them there are five taxa of economic importance which are *Capsicum annuum* L. var. *annuum*, *C. baccatum* L. var. *pendulum* (Willd.) Eshbaugh, *C. baccatum* L. var. *umbilicatum* (Vell.) Hunz. & Barboza, *C. chinense* Jacq. and *C. frutescens* L. (Purkayastha *et al.*, 2012; Barboza *et al.*, 2022).

Mexico ranks second worldwide in green production with 3 113 244 tons and fourteenth place in dry or dehydrated with a production of 60 987 tons (FAOSTAT, 2022). Those varieties with the highest production are Jalapeño, Poblano and Serrano peppers. In the case of Poblano pepper, it is grown in 16 696.88 hectares, with a production of 414 656 tons, with an average yield of 25 tons per hectare. The five states that lead production are Zacatecas, Guanajuato, Jalisco, Baja California Sur and Sinaloa. For this type of pepper, but dried, that after dehydration is known as “Ancho pepper”, 15 247 hectares are used with a production of 151 270 tons and yield of 9.9 tons per hectare. There are five states of the Mexican republic that lead the production of this Poblano pepper dehydrated, which are Zacatecas, San Luis Potosí, Durango, Puebla and Oaxaca (SIAP, 2022).

Poblano-type pepper is appreciated for its gastronomic, economic, social, and cultural importance (Rodríguez *et al.*, 2007; Toledo *et al.*, 2011). This crop is adapted to diverse environmental conditions in various producing regions of the country; where there are native varieties with morphological diversity of interest to be integrated into conservation and genetic improvement projects (Rodríguez *et al.*, 2007; Toledo-Aguilar *et al.*, 2011; Tripodi & Kumar, 2019). However, these native varieties are highly susceptible to pests and diseases; environmental consequences derived of climate change further limit their productivity (Rodríguez *et al.*, 2007; Herrera-Fuentes *et al.*, 2023). In addition, the seed for pepper production is generally imported by foreign companies, which generates high costs for small producers before starting cultivation (Toledo-Aguilar *et al.*, 2011).

Hence the importance of genetic improvement of crops, since it allows the creation of new variants from plant materials with genetic diversity, promoting the combination of traits conferred by the original selections, inducing mutations or retro-crossing wild varieties with commercial ones (Duvick, 2007). In the improvement of chili pepper, several objectives are pursued such as high yield, resistance to pests and diseases or to abiotic stress, to create ornamental plants, and even to increased pungency, meeting market demands (Padilha & Barbieri, 2016). Therefore, several techniques have been applied in chili peppers to improve the crop, such as mass selection, pedigree or genealogical technique, single-seed offspring, recurrent selection, retro-crossing and hybridization (Srivastava & Mangal, 2019; Karim *et al.*, 2021).

For these reasons, a genetic improvement program using native or creole genotypes will increase yield, productivity, tolerance to adverse abiotic and biotic agents, increasing the quality and range of adaptation in the different producing areas in medium and long terms (Bailey-Serres *et al.*, 2019). However, in any plant breeding program, it is necessary

to evaluate the genotypes for their agronomic and morphological traits, to identify their actual genetic potential. Therefore, the objective of this study was to evaluate genotypes of second generation of selection (F_2) Poblano peppers, compared to two hybrids for selecting the best genotypes. Once selected, those shall continue in the breeding program, which would eventually allow generating hybrids or outstanding varieties in medium and long terms.

MATERIALS AND METHODS

Location

This research was established in field conditions at the experimental area of the Department of Horticulture at the Universidad Autónoma Agraria Antonio Narro, Saltillo (Coahuila) Mexico, located at coordinates 25° 21' 23.44" N and 101° 2' 5.18" W, with an average annual temperature of 24 °C, and average annual rainfall of 400 mm; However, during the cultivation period 22 °C was the average temperature, 6.7 °C as minimum and 36.93 °C as maximum temperatures, according to the local University Network of Atmospheric Observatories.

Genetic material

Six genotypes generated from directed manual pollination were used between a genotype that served as a female parent, with the pollen bulk of three genotypes that served as a male. As a result of this recombination, from plants that were cultured in 2022, F_1 was obtained out of which six genotypes were selected for their distinctive and outstanding phenotypic characteristics. This research was the subsequent evaluation of those F_2 genotypes, the stage that was evaluated in 2023. Likewise, it was compared with two hybrids, one of them was the commercial breed from SEMINIS, the Carranza® (F_1) Ancho pepper that ripens from green to red, suitable for fresh and dry markets, with high yield potential and excellent quality of green fruit, good fresh/dried fruit ratio. The other was the experimental hybrid F402 (F_1) generated at the Center for Training and Development in Seed Technology of the "Antonio Narro" Autonomous Agrarian University (Table 1). Both types of genotypes were considered as treatments.

Table 1. Identification of the genetic material used to evaluate agronomic and morphological behavior in field conditions in Saltillo (Coahuila), Mexico.

Key	Description
G1	Genotype 1
G2	Genotype 2
G3	Genotype 3
G4	Genotype 4
G5	Genotype 5
G6	Genotype 6
F402	UAAAN Experimental Hybrid F_1
CARR	Carranza F_1 -Seminis

Plant production

The genetic material was sown on February 18, 2023, in 200-cavity polystyrene germination trays, with substrate for germination of peat-moss and perlite at a 70:30 (%) ratio. Seeds were sown at a 0.5 cm depth, covered with a thin layer of the same substrate mixture. To induce germination, they were covered with black plastic for 72 h, then left in the greenhouse for the emergence and growth of the plants. In order to have good quality plants, fertilization was supplied after six days of emergence with soluble Triple 20 (20-20-20), added with microelements at a dose 1 g L^{-1} .

Establishing the crop

Transplant was done 60 days after planting, the genotypes were distributed in four culture beds raised to 30 cm in height, by 40 cm in width and 15 m in length, plastic mulch was used to prevent weed abundance. Distance between beds was 1.8 m; distance between plants was 30 cm in double rows, with a separation of 25 cm between rows. Planting density calculated under that configuration was 37 000 plants per hectare.

Crop nutrition and crop management

Crop nutrition was supplied by irrigated fertilization (fertigation). After 5 days from transplantation, a modified Steiner-type solution was used in ascending concentrations, according to the stage in crop phenology, 50% (at transplanting), 75% (during vegetative stage) and 100% (at fruiting). As it is shown in Table 2, these occurred at 5, 21 and 50 days after transplantation respectively. It was intended to maintain pH ranging 5.9 to 6.1, electrical conductivity from 1.5 to 2.7 dS m^{-1} depending on the stage.

Weed elimination between beds and in the holes of the mulch was made every 15 days to prevent their spread. Tutors for plants were placed according to plant growth by the trellis method; it began when the plants had an average height of 25 cm, in order to keep the plant upright throughout the crop cycle. To prevent the proliferation of pests such as whiteflies, thrips (order Thysanoptera) and spider mites, Spirotetramat at 15.3%, Spiromesifen at 23.1% and Imidacloprid 17% + betacylfutrin 12% at a rate of 1 mL L^{-1} were applied. And

Table 2. Nutrient chemical composition of the nutrient solution used in the nutritional management of Poblano-type peppers.

Macroelements (mEq L^{-1})										
SN (%)	Cl^{-}	NO_3^{-}	$\text{H}_2\text{PO}_4^{-}$	SO_4^{2-}	HCO_3^{-} & CO_3^{2-}	K^{+}	Mg^{+2}	Ca^{+2}	NH_4^{+}	Na^{+}
50	3.26	6	0.5	3.5	1	3.5	2	5.5	1	3
75	3.26	8.6	0.75	5.25	1	5.25	3	8.25	1.5	3
100	3.26	12	1	7	1	7	4	11	2	3
Microelements (ppm)										
SN (%)	Fe^{+3}	Mn^{+2}	H_3BO_3	Zn^{+2}	Cu^{+2}	MoO_4^{2-}	EC(dS/m)	pH		
50	1.5	0.74	0.14	0.12	0.06	0.04	1.5	5.9-6.1		
75	2.25	1.1	0.21	0.18	0.09	0.06	2.1	5.9-6.2		
100	3	1.48	0.28	0.24	0.12	0.08	12.7	5.9-6.3		

for diseases such as Damping-off and mildew (*Laveillula taurica*), Metalaxil-M 45.28%, and Azoxistrobin 50% were used, both in a dose of 0.5 mL L⁻¹.

Harvest

The harvest was made when the fruits presented the external coloration characteristic of the genotype; this occurred 90 days after transplantation. Bags were separated and marked for the subsequent evaluation of each genotype. Two harvests were accomplished during the experiment.

Determination of yield variables and Agronomic behavior

To determine total yield in grams per plant, the yields obtained from both harvests per plant of each genotype were added. This sum was made using a digital scale OHAUS[®] Scout-Pro[®]. On each time, the number of fruits per plant (NFP) also was recorded. To calculate the average fruit weight (AFW), the total yield per plant was divided by the total number of fruits collected from that plant. The fruit length (FL) in centimeters was determined using a Vernier caliper (Steren[®] Her-411, Mexico). This same instrument was used to measure the width at the base, the mean width, the thickness of the mesocarp and the depth of the calyx, all expressed in millimeters. The number of locules was also counted when sectioning the fruits in half.

To quantify the total soluble solids in the fruit, four fruits were randomly selected by replicate, which presented harvest maturity and green color. This measurement was made using a digital refractometer (SOONDA[®] TD6010, China) and was expressed in °Brix.

With a measuring tape in centimeters (Truper[®] FH-5M, Mexico), morphological and agronomic behavior variables were measured. Total plant height was measured from the ground to the apical part, as well as leaf length, leaf width and peduncle length. In addition, stem thickness was measured using a Vernier caliper (Steren[®], Her-411, Mexico).

Data analysis

The design of treatments was in complete randomized blocks with four replicates. An analysis of variance was performed ($p \leq 0.05$) with the general linear model for a factorial arrangement with six treatments and four replicates. Significant variables were tested with the multiple means comparison of Tukey ($p \leq 0.05$). To analyze interaction and relationship among variables, in order to guide genotype selection, a Pearson correlation analysis was performed (Shumbulo *et al.*, 2017). All these analyses were run in R-Studio (2023 version).

RESULTS AND DISCUSSION

Agronomic variables

The analysis of variance performed for genotypes and hybrids in Poblano-type peppers, in the variables of yield (YPP; kg per plant), number of fruits per plant (NFP), average fruit weight (AFW) did not detect significant differences as observed in Table 3. However, at comparing percentages, hybrids F402, CARR, and genotypes G3 and G6 outperformed G1 and G2 by more than 20%. This statistically insignificant response is probably due to the genotypes are of a second filial generation (F₂); therefore, it is an expected response in

individuals. In terms of number of fruits per plant, G6 was 24% higher than the Carranza hybrid (CARR), which means that G2 shows genetic potential in this trait; a similar response was observed in the average fruit weight (AFW). Number of fruits per plant is an attribute directly related to yield; it is also a determining variable for recurrent selection in breeding programs (Monge-Pérez *et al.*, 2022).

In the case of the variable number of locules (NL), there was a significant difference; G3, G4, G5 and G6 were statistically equal to hybrids with two locules on average (Table 3), whereas G2 and G1 had 2.5 and 2.2 locules. In Poblano-type peppers, it is known they have two locules. Since most consumers prefer Poblano peppers with fewer veins and seeds, this can make them easier to clean and prepare for cooking. However, some farmers reported that for dried Poblano peppers (then called “Ancho” peppers) they prefer three veins.

Although the variable of total soluble solids ($^{\circ}$ Brix) did not show significant differences between genotypes and hybrids as observed in Table 3, in a selection and breeding program it is important to consider the total soluble solids content of the Poblano pepper genotypes, especially if the aim is to increase their sweetness, when dried. Therefore, even if there were no statistical differences between genotypes and hybrids, their inclusion in the analysis is still important to assess the sweetness potential of Poblano peppers, for selecting those which best align with market preferences.

Regarding the fruit quality variables, as observed in Figure 1A, there was a significant difference between genotypes and hybrids in the variable of fruit base width, the hybrids CARR and F402 were statistically equals, but superior to the genotypes. A similar trend was observed in average fruit width (Figure 1B), although in this variable G4 and G6 were also similar to hybrids.

Table 3. Analysis of variance and comparison of means for yield and its components; number of locules and total soluble solids of six genotypes and two hybrids of Poblano peppers, evaluated in field conditions, in Saltillo (Coahuila), Mexico.

Genotype	YPP (Kg plant ⁻¹)	NFP	AFW (g)	NL (mm)	TSS ($^{\circ}$ Brix)
CARR	0.80 a	14.86 a	53.62 a	2.11 b*	8.71 a
F402	0.69 a	15.16 a	67.87 a	2.03 b	8.89 a
G1	0.55 a	11.91 a	49.59 a	2.28 ab	10.43 a
G2	0.53 a	9.93 a	54.09 a	2.53 a	8.60 a
G3	0.69 a	15.81 a	44.61 a	2.22 b	8.70 a
G4	0.61 a	12.71 a	49.15 a	2.17 b	8.75 a
G5	0.66 a	13.74 a	47.11 a	2.17 b	8.68 a
G6	0.78 a	15.11 a	55.82 a	2.08 b	8.95 a
ANVA P \leq	0.0515	0.3361	0.7911	0.0008	0.6674
LSD	0.29	8.94	48.84	0.28	3.58
CV %	15.61	22.73	32.15	4.48	13.85

*Different letters in the same column indicate statistically significant differences (Tukey $p \leq 0.05$). LSD=Least Significant Difference, CV=Coefficient of Variation, YPP=Yield per plant, NFP=Number of fruits per plant, AFW=Weight per fruit, NL=Number of locules, TSS=Total soluble solids.

In fruit length at Figure 1C, it is observed that G2, G5 and G6 were statistically equal to the CARR hybrid, whereas the rest of the genotypes and the F402 hybrid showed lower results. Regarding the calyx depth variable (Figure 1D), genotypes G1, G3 and G4 were statistically equal to the CARR hybrid, whereas the rest of the genotypes and F402 showed minor calyx depth.

In the variable of peduncle length (Figure 1E), it was observed that, with the exception of G4, the rest of the genotypes showed a statistical response similar to each other. In terms of mesocarp thickness, the best genotypes were G2, G4, G6 since they showed a statistical response similar to the hybrids (Figure 1F). These variables are of great importance because they are indicators of fruit quality, according to the Mexican Standard (NMX-FF-025-SCFI-2014).

Morphological variables

In the plant height variable, no statistically significant difference was found between the genotypes and hybrids evaluated, as observed in Table 4. However, the height of the evaluated genotypes ranged from 110.48 to 121.46 cm, which is higher than that found

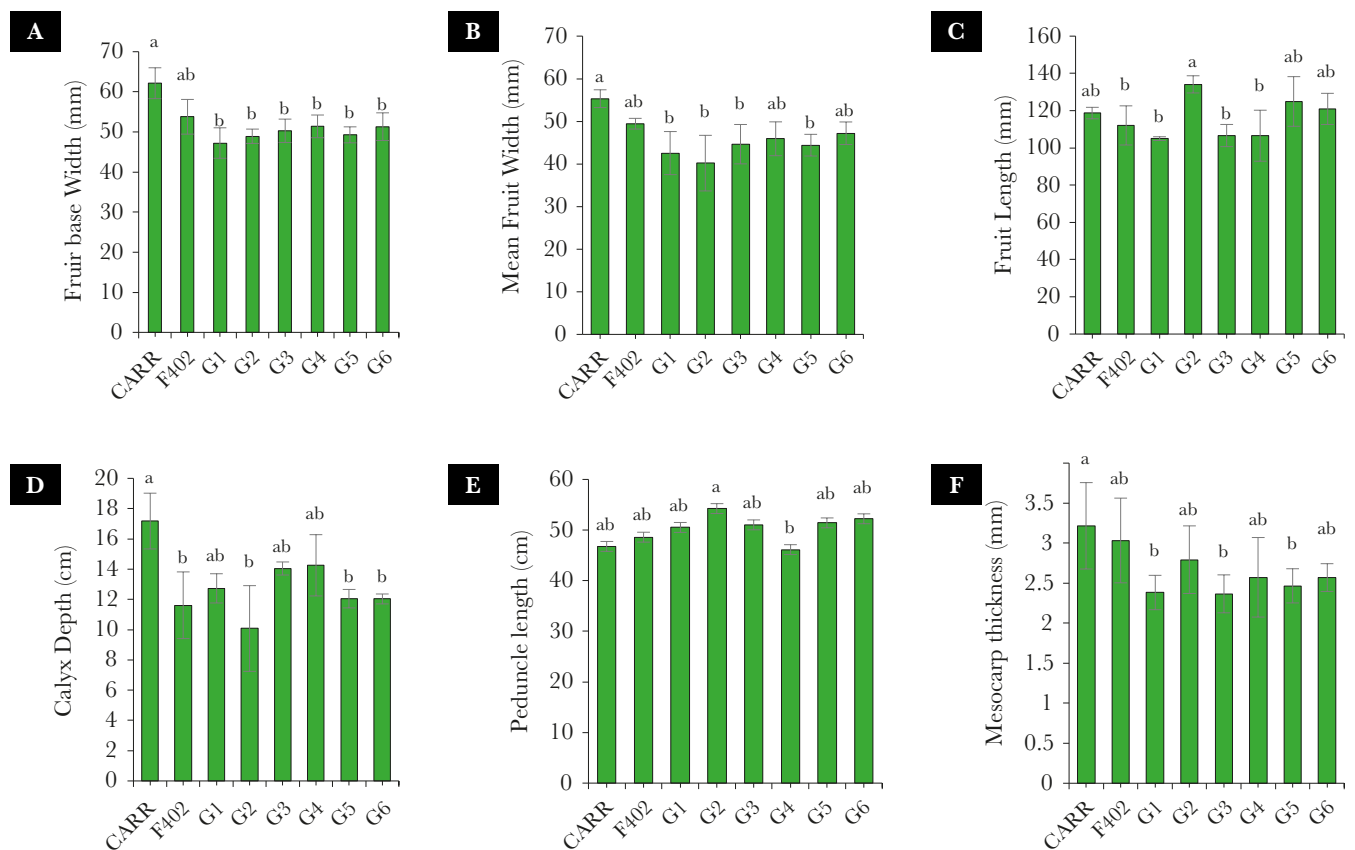


Figure 1. Multiple means comparison (Tukey, $p \leq 0.05$). A: fruit base width; B: mean fruit width; C: fruit length; D: calyx depth; E: peduncle length; and F: mesocarp thickness of six genotypes and two hybrids of Poblano pepper evaluated in field conditions in Saltillo (Coahuila) Mexico. Vertical bars correspond to the standard deviation.

Table 4. Analysis of variance and multiple comparison of means (Tukey, $p \leq 0.05$) of morphological variables of six genotypes and two hybrids of Poblano peppers evaluated in field conditions, in Saltillo (Coahuila), México.

Genotype	TPH(cm)	SD (mm)	LL (mm)	PL (mm)	LW (mm)
CARR	110.49 a	16.26 a	94.60 a	75.07 a*	42.03 a
F402	99.79 a	15.17 a	96.37 a	69.78 ab	42.36 a
G1	121.46 a	15.23 a	91.93 a	64.93 ab	44.33 a
G2	132.15 a	16.03 a	93.38 a	59.62 b	46.43 a
G3	120.63 a	14.94 a	90.16 a	66.59 ab	42.47 a
G4	127.98 a	14.85 a	89.23 a	67.25 ab	43.48 a
G5	122.40 a	15.38 a	92.09 a	64.98 ab	39.03 a
G6	110.48 a	15.53 a	83.40 a	59.89 a	39.41 a
ANVA $P \leq 0.05$	0.07160	0.36015	0.40200	0.01423	0.09446
LSD	33.62	2.27	18.65	12.73	8.12
CV %	9.87	5.11	7.08	6.70	6.64

Different letters in the same column indicate statistical differences (Tukey $p \leq 0.05$), LSD=Least Significant Difference, CV=Coefficient of Variation, TPH=Total plant height, SD=Stem diameter, LL=Leaf length, PL=Petiole length, LW=Leaf width.

by Toledo-Aguilar *et al.* (2011) who reported 37.9 to 56.8 cm. Morphological variables are indicative of crop production, since they represent vigor linked to physiological processes. Stem thickness is a clear example of this, where no statistical difference was detected and measurements ranged 14.9-16.3 mm; stem thickness is one of the morpho-physiological parameters used to evaluate the genetic diversity and heritability of chili pepper genotypes (Pandiyaraj *et al.*, 2017). It is a trait that can contribute to the selection of genetically superior parents and inbred lines for reproduction due to vigor (Vinodhini *et al.*, 2019). In addition, it is a horticultural trait that can be associated with other important characteristics, such as plant height, number of branches, and yield (Karim & Nesa, 2021).

The length of the leaf petiole can be used as a parameter to evaluate the health and plant growth, since it is related to the response of the plant to light intensity (Tsukaya *et al.*, 2002). The results obtained in this variable indicate that except for G2, all obtained the same statistical result with values 59.89-75.07 mm. This indicates that there is potential of the genetic material evaluated, compared with the commercial one, and it is feasible to continue with the plant breeding program.

Correlation coefficients

Pearson correlation coefficients of the evaluated variables were determined (Figure 2), such correlations constitute a measure of the magnitude of the linear association between two variables, without considering cause and effect between them, regardless of the units of measurement. Among the variables of the yield components, the highest correlation corresponded to the YPP with NFP ($r=0.88^{**}$), followed by YPP with MT (0.46^{**}) and with FBW (0.38^*) in Figure 2. The results are similar to those reported by Monge-Pérez *et al.* (2022), where they obtained a positive and highly significant correlation between

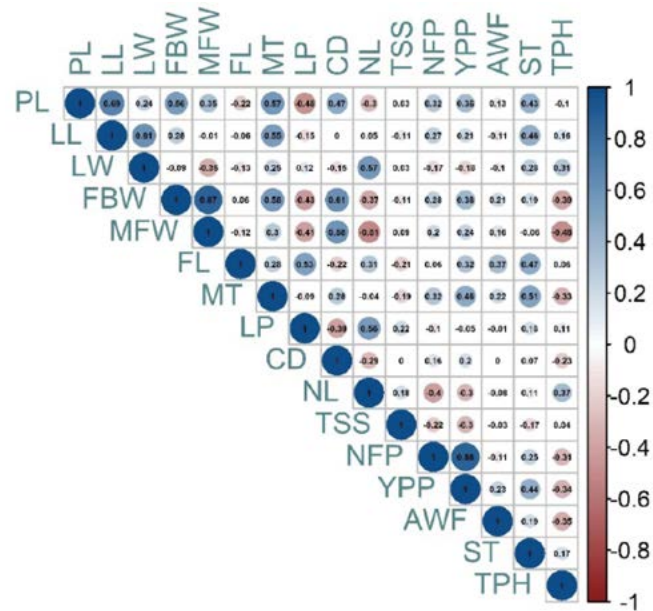


Figure 2. Pearson correlation diagram. PL: petiole length, LL: leaf length, LW: leaf width, FBW: fruit base width, MFW: mean fruit width, FL: fruit length, MT: mesocarp thickness, LP: peduncle length, CD: calyx depth, NL: number of locules, TSS: total soluble solids, NFP: number of fruits per plant, YPP: yield per plant, AWF: average weight per fruit, ST: stem thickness, and TPH: total plant height.

commercial yield and number of fruits per plant ($r=0.51^{**}$), which means that yield is correlated in the selection of these variables.

Genetic improvement of chili peppers also involves using correlation analysis to understand the relationships between different traits for effective selection and generational advancement. Several studies have shown positive correlations between traits such as yield, fruit length, and the number of seeds per fruit (Islam *et al.*, 2023; Karim & Nesa, 2021). In addition, genotypic and phenotypic correlations have been observed between traits such as plant height, fruit weight, and various biochemical contents such as ascorbic acid and capsaicin (Rahevar *et al.*, 2019). By analyzing the interrelationships between different phenotypic traits, plant breeders can make informed decisions to improve attributes, especially yields and fruit quality.

CONCLUSIONS

The agronomic response of the genotypes and hybrids was similar in some of the response variables, but different in others. In the morphological variables they also showed a similar response; therefore, it is inferred that the G2, G4 and G6 genotypes have genetic potential that may be useful to continue with the plant breeding program. The similarity in agronomic and morphological response between the commercial and experimental hybrid is similar, indicating the genetic potential of the experimental hybrid.

The correlation between the variables evaluated revealed that the number of fruits per plant, fruit length, mesocarp thickness, and width of the fruit base are variables that contribute to a greater extent to crop yield. These findings provide valuable information

for the breeding program, highlighting those promising genotypes and key variables to consider in future evaluations in order to clearly understand their behavior.

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