



EVALUATION OF MELON (*CUCUMIS MELO. L*) GENOTYPES AIMING EFFECTIVE SELECTION OF PARENTS FOR BREEDING DIRECTED AT HIGH YIELD UNDER DROUGHT STRESS CONDITION

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ABSTRACT

This study examined 36 accessions of melon grown originally in dry and warm zones managed in the gene bank in the Institute of Seed and Plant Improvement, Karaj. To characterize and compare results of the field experiment statistical procedures, hierarchical cluster analysis, principal component analysis (PCA), and multiple linear regressions were applied. The results indicated a high degree of variability for single plant yield, fruit weight, fruit length, and cavity diameter. In addition, the variables of fruit size displayed a high differentiating power. Positive and negative correlation coefficients were identified among the studied traits as well. The highest significant positive correlation was found between the plant yield and fruit weight (0.88). Also, the highest significant negative correlation was observed between relative water content and canopy temperature (−0.58). Melon accessions were classified into three main clusters, indicating that the characterized melon collection has a high potential for particular breeding goals. The usefulness of the identified correlations among traits is valuable for the potential use in breeding projects directed for fruit size and yielding in dry condition. Multiple linear regressions were done and R^2 (Coefficient of determination) and RMSE (Root Mean Squared Error) were 0.97 and 229, respectively.

Key words: Melon, principal component, multiple regression

INTRODUCTION

More than 85000 ha are under the cultivation of melon in Iran (FAO. 2014). Cucurbits (Cucurbitaceae) are among the main plant families supplying people with edible products and useful fibers. Knowledge on genetic variability can help breeders to select parental genotypes for hybridization leading to new cultivars. Studies have been conducted in order to characterize gene bank collections of melon germplasm (Naroui Rad et al. 2015;

Naroui Rad et al. 2014). Using indirect selection criteria to select high yielding genotypes includes the knowledge of component traits (Aparicio et al. 2000). The identification of traits based on phenotypic diversity is also important for the determination of degree of total diversity of a germplasm collection (Mehmood et al. 2014). The multivariate data analysis (De Oliveira et al. 2012) is a powerful statistical technique for analyzing genetic relations between morphological traits. Some useful methods, including the principal components, clusters,

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and factor analyses are currently available for identifying genetic variation and parental diversity, tracing the relationship of traits for more yields in crops, variation and origin, and studying the interaction of genotype and environment (Eivazi et al. 2007). The most popular multivariate procedures for morphological traits and the characterization of genotypes are principal component analysis (PCA) and cluster analysis. PCA describes the pattern of variation of characters among individuals by a descriptive method. It reduces the dimension of data by removing inter-correlations among variables and depicts a multi-dimensional relationship to be plotted on 2 principal axes (Nwangburuka et al. 2011). Cluster analysis and PCA may have relative differences with each other in results. Therefore, before performing cluster analysis, principal components must be avoided. In other words, when the first two principal components account for a high variation percentage, clustering according to these components can certainly be a useful technique to find special groups (Fotokian et al. 2002). Multiple linear regression (MLR) is a useful technique for depicting the linear relationship between dependent and independent variables by fitting a linear equation between the observed and predicted data (Baodi et al. 2008). The aims of this study were to evaluate phenotypic diversity in 36 melon accessions, grown

originally in dry condition, identify specific traits, and detect diversity among the studied genotypes.

MATERIALS AND METHODS

Experimental site and accessions

This study was carried out in 2015–2016 at the Agriculture and Natural Resource Research Station of Zahak, located in Sistan and Baluchistan Province in Iran, at 61°41' South latitude and 30°54' longitude, with a yearly precipitation of 59 mm and average temperature of 23 °C. This area rises to 482 m from the sea level. The basic aim of the study was the selection of traits and accession with a high potential for the improvement of melon productivity under water deficit. The study was conducted on 36 melon accessions, which were chosen from warm and dry zones of Iran for their high adaptability to these regions (Table 1). The seeds were derived from the National Plant Gene bank of Iran and directly sown in field. The field experiment had been assigned to plots in a lattice design with 2 replicates and 6 incomplete blocks. A distance between plants in each row was 2 m × 0.5 m apart. Six plants were assigned to each plot and the plot area was 6 m². Soil water content was measured by a time-domain reflectometer (TDR) to a depth of 50 cm, and irrigation was done after 75% water depletion of soil field capacity.

Table 1. Accessions of melon used in the study, chosen on the base of tolerance to dry growth condition

Number	Accession	Number	Accession	Number	Accession	Number	Accession
1	TN-345	10	KC-257236	19	KC-357020	28	TN-278
2	KC-35700	11	TN-629	20	TN-621	29	TN-272
3	TN-271	12	TN-92334	21	TN-92306	30	KC-357104
4	KC-457250	13	TN-628	22	TN92302	31	KC-357079
5	KC-357238	14	TN92319	23	KC-357044	32	KC-357063
6	TN-441	15	TN-623	24	KC-357154	33	KC-357047
7	TN-92401	16	TN92317	25	KC-357009	34	KC-357062
8	TN-377	17	TN-92312	26	KC-357067	35	Check-Sefidak
9	TN-277	18	TN-622	27	KC-357100	36	Check-Suski

Evaluated Traits

Fourteen traits were evaluated at the ripening of the fruits. They were selected to characterize yield and yield components and some physiological traits that can be important under water deficit condition.

1. Number of fruit per plant (NF).
2. Fruit weight (FW).
3. Fruit length (FL).
4. Fruit width (F.WI.).
5. Total Soluble Solids (TSS), measured by hand refractometer.
6. Single plant yield (SPY).
7. Flesh diameter (FLD).
8. Cavity diameter (CD).
9. Plant length (PL).
10. Chlorophyll content index (CHL), the SPAD-502 measured by portable chlorophyll meter (Minolta Ltd).
11. Canopy temperature (CT), taken with the infrared thermometer, held 0.5–1 m from the edge of the plot and approximately 50 cm above the canopy.
12. Relative water content (%RWC).
 $(\%RWC) = [(W-DW)/(TW-DW)] \times 100$,
 where: W – sample fresh weight, TW – sample turgid weight, DW – sample dry weight.
13. Days to maturity (DM).
14. Root length (RL), measured on the main root after pulling out the soil.

Data scoring and analysis

Data were analyzed via SAS 9.2 and XLSTAT software (version 2015.1). Mean values stands based on three samples and calculated for each studied trait was used to perform the PCA. Due to different units and the variability of range of the measured traits, the data for each raw variable were standardized to obtain the correlation matrix as the variance-covariance matrix before PCA was utilized. Pearson correlation coefficient was employed to calculate the correlation among variables. A dendrogram was constructed from the traits. Euclidean distance was a criterion to estimate phenotypic similarity for the accessions and for the agglomerative hierarchical clustering (AHC), the Ward's method was selected. Multiple linear regression (MLR) is a statistical technique that uses several explanatory variables to predict the outcome of a response variable. The goal of MLR is to model the relationship between explanatory and response variables. MLR

analysis was done to examine the relationship between independent (explanatory) variables and the dependent variable (single plant yield) more closely by fitting a linear equation to the observed data.

RESULTS AND DISCUSSION

Descriptive statistics and correlations

Descriptive parameter results including minimum, maximum, means, variance, standard deviations, and coefficients of variation (CV) of 14 traits are shown in Table 2. These morphological and physiological traits revealed a high variation, and some traits such as single plant yield (0.47) and fruit weight (0.45) displayed a high value for CV. These two traits had more phenotypic variations, indicating that selection can be applied on the traits to select more suitable genotypes. Moreover, moderate positive correlations were observed among all the 14 measured traits (Table 3). Knowledge of the interrelationship between yield and its component may assist the breeder to decide upon the intensity and direction of the selection pressure to be given on related traits for the simultaneous improvement of yield-contributing traits. The highest significant positive correlation was observed between single plant yield and fruit weight (0.88), showing that simultaneous selection for these characters would result in the improvement of yield. Significant positive correlations were also seen between fruit length and width (0.77) as well as root length and relative water content (0.64). This proves that accessions with long roots help to keep more relative water content. Moreover, significant negative correlations were found between traits, for example, relative water content and canopy temperature (–0.58). This valuable information may be useful for any future melon-breeding program to obtain more yield and high-quality cultivars. For example, hybridization can be performed between accessions with large fruits. Based on the relationship between traits, Szamosi et al. (2010) reported that significant results could be important for future melon-breeding programs. The diversity of and relationship between traits are essential information in crop improvement programs, and the success of plant-breeding programs relies heavily on the existence of genetic and phenotypic variability for particular traits. Fruit traits varying in these accessions in size and shape could be observed in a number of accessions (Fig. 1).

Principal component analysis of quantitative variables

By applying PCA, the most significant traits in the datasets were revealed. PCA is a technique that lies within the data framework of multivariate statistical analysis. For determining genetic variation and the relationship among cultivars, PCA has already been employed to identify correlations among traits. The scattered genotypes or accessions based on PC1 and PC2 axes expose the existing phenotypic variation among the accessions and also show how extensively the accessions spread along the axes; eigenvalues of more

than 1 were selected as the threshold (Fig. 2). It is possible to decrease the dimension of the 14 quantitative traits to only six components (Table 4), which could explain 80.87% of the total variation. The first component, which accounted for 26.98% of the total variation, was strongly associated with fruit shape, including fruit length, fruit weight, fruit width, cavity diameter, and single plant yield. Szamosi et al. (2010) reported that the first two components explain 54% of the morphological variation, and the length of fruit, length of seed cavity, hypocotyl length, and width of leaf were the most important variables composing PC1.

Table 2. Descriptive statistics of morphological and physiological traits of 36 melon accessions

Trait	Minimum	Maximum	Mean	Variance	Standard deviation	Variation coefficient
Fruit number	1.0	2.50	1.60	0.17	0.41	0.25
Fruit weight (g)	495	3728	1522	484048	696	0.45
Fruit length (cm)	11	33	20	25	5.0	0.25
Fruit width (cm)	6.5	15.8	12.3	4.0	2.0	0.16
Total Soluble Solids (Brix)	2.25	8.75	5.44	3.75	1.94	0.35
Single plant yield (g)	830	5773	2373	1298292	1139	0.47
Flesh diameter (cm)	5.00	8.75	7.04	0.49	0.7	0.10
Cavity diameter (cm)	2.40	4.65	3.21	0.25	0.5	0.15
Plant length (cm)	95	225	173	1029	32.1	0.18
Chlorophyll content index	31.5	64.5	46.9	52.0	7.2	0.15
Canopy temperature (°C)	26.0	34.5	29.5	3.94	1.99	0.07
Relative water content (%)	0.36	0.65	0.54	0.005	0.07	0.13
Number of days to maturity	79	88	83	5.39	2.3	0.03
Root length (cm)	26	54	35.2	41.6	6.5	0.18



Fig. 1. Diversity in fruits among some accessions of melon in the evaluated collection

Table 3. Correlation coefficients between yield and morphological and physiological parameters

Variables	NF	FW	FL	FWI	TSS	SPY	FLD	CD	PL	Chl	CT	RWC	DM	RL
NF	1													
FW	-0.28	1												
FL	-0.17	0.77	1											
FWI	-0.11	0.63	0.49	1										
TSS	0.18	-0.13	-0.18	-0.22	1									
SPY	0.14	0.88	0.75	0.59	-0.08	1								
F.D	-0.15	0.32	0.05	0.25	-0.19	0.30	1							
CD	-0.34	0.47	0.36	0.46	-0.24	0.31	0.48	1						
PL	0.11	0.18	0.18	0.08	0.02	0.33	0.17	0.03	1					
Chl	-0.05	0.24	0.11	0.04	0.34	0.23	0.05	0.30	-0.09	1				
CT	-0.18	-0.03	0.10	0.04	-0.15	-0.20	-0.34	0.17	-0.28	-0.15	1			
RWC	0.40	-0.06	-0.05	-0.16	0.12	0.19	0.10	-0.07	0.44	0.21	-0.58	1		
DM	-0.30	0.23	0.30	0.03	-0.22	0.13	0.10	0.05	0.11	-0.16	0.16	-0.32	1	
RL	0.10	0.06	0.03	-0.13	0.03	0.11	-0.01	0.02	0.42	0.08	-0.20	0.64	-0.28	1

Values in bold are different from 0 with a significance level $\alpha = 0.05$

NF: number of fruit; FW: fruit weight; FL: fruit length; FWI: fruit width; TSS: total soluble solids; SPY: single plant yield; FLD: flesh diameter; C.D: cavity diameter; PL: plant length; CT: canopy temperature; RWC: relative water content; DM: number days to maturity; RL: root length

Table 4. Squared cosines of the variables, eigenvalues and cumulative variance (%) of the first six principal components related to the studied traits in 36 accessions of melon

	F1	F2	F3	F4	F5	F6
Fruit number	0.08	0.28	0.08	0.14	0.08	0.28
Fruit weight	0.87*	0.03	0.01	0.02	0.07	0.01
Fruit length	0.65	0.01	0.04	0.17	0.05	0.01
Fruit width	0.54	0.01	0.02	0.03	0.01	0.17
Total soluble solids	0.08	0.11	0.28	0.07	0.05	0.09
Single plant yield	0.73	0.10	0.03	0.09	0.01	0.02
Flesh diameter	0.22	0.02	0.02	0.53	0.05	0.02
Cavity diameter	0.42	0.02	0.07	0.19	0.10	0.06
Plant length	0.07	0.31	0.26	0.03	0.08	0.09
Chlorophyll content index	0.04	0.08	0.62	0.02	0.01	0.07
Canopy temperature	0.02	0.50	0.02	0.10	0.16	0.06
Relative water content	0.01	0.72	0.03	0.01	0.07	0.08
Number of days to maturity	0.09	0.19	0.15	0.01	0.28	0.14
Root length	0.02	0.30	0.06	0.02	0.37	0.12
Eigenvalue	3.79	2.66	1.47	1.33	1.05	1.03
Cumulative %	26.98	45.05	56.53	66.04	73.53	80.87

*Bold data are effective in each principal component

The second component, which accounted for 18.93% of the total variation, mainly correlated with the characters of canopy temperature, plant length, and relative water content. The third component, that explained 10.47% of the total variation, was associated with the traits of total soluble solids and SPAD. A PCA plot is presented based on the first two components (Fig. 2). This plot classifies the accessions according to their phenotypic similarity and morphological characteristics. Based on the ordination of melon accessions, materials revealed that accession numbers 35, 31, 32, 26, 33, 12, 6, 23, and 9 were separated for the studied trait. The ordination of the traits on PC1 and PC2 demonstrated that some of the traits were highly inter-correlated as seen in the overlapping pattern of the traits. Variables on the same PC and next to each other indicate positive correlations between them, and increase in one will lead to an increase in the other. Nevertheless, the traits at opposite ends are anti-correlated and increase in one will bring about a decrease in the other and vice versa. Based on the first component, accessions showing high positive component values exhibited relatively high fruit weight, fruit length, fruit width, and cavity diameter.

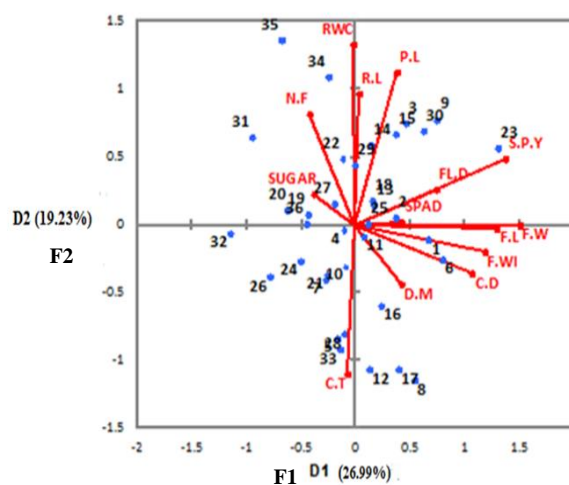


Fig. 2. PCA biplot of the 36 melon accessions in coordinates of the first two principal components (with numbers of accessions and eigenvalue vectors for the traits)

Screen plot explains the percentage of variance associated with each principal component obtained by drawing a graph between eigenvalues and PC. The PC plot shows clearly the highest variation in PC 1 and PC 2 to explain the maximum variation in the data set, so the selection of genotypes from this

PC will be useful. The accession number 1 has the highest fruit width, these results demonstrate the highly positive correlation of fruit diameter, fruit weight and fruit width. Canopy temperature with relative water content has reverse interaction. Also for single plant yield, accession number 23 has the highest yield based on position, next to the single plant yield arrow. Our results are in agreement with the studies, which also confirmed that the fruit size and weight are useful parameters to separate accessions or cultivars (Imen et al. 2013; Naroui Rad et al. 2017). Principal component analysis approved that some traits had the highest influence in the first two components. These traits included the fruit weight, fruit length and fruit width. These results suggested that such traits are suitable both for the estimation of diversity and for the phenotypic characterization of the melon in genetic resources.

Agglomerative hierarchical clustering (AHC) and multiple linear regression

In this experiment, an unsupervised AHC analysis was employed to separate accession data into groups of increasing similarity. The Euclidean dis-

tance was used as a metric to measure this phenotypic similarity among the 36 melon accessions based on the 14-quantitative data. In addition, Ward's method was implemented for agglomeration. The relative dendrogram (Fig. 3) revealed three distinct groups.

C1 comprised 15 accessions, C2 11 accessions, and C3 10 accessions. This result was consistent with the variation that had initially been observed in the data within melon accessions. The highest genetic distance was found between C1 and C3 (1383), followed by the distance between C2 and C3 (1127) and between C1 and C2 (435). According to Tschoeke et al. (2015), dendrograms enable revealing the relationships among accessions basing on various morphological parameters.

Fig. 4 reveals a gap between Cluster 3 and the other clusters regarding fruit weight and single plant yield. In addition, Cluster 1 and Cluster 2 had the greatest values of single plant yield and fruit weight, respectively. Szamosi et al. (2010) found that not all accessions could be separated by their origin and cluster one has a higher cluster mean than others.

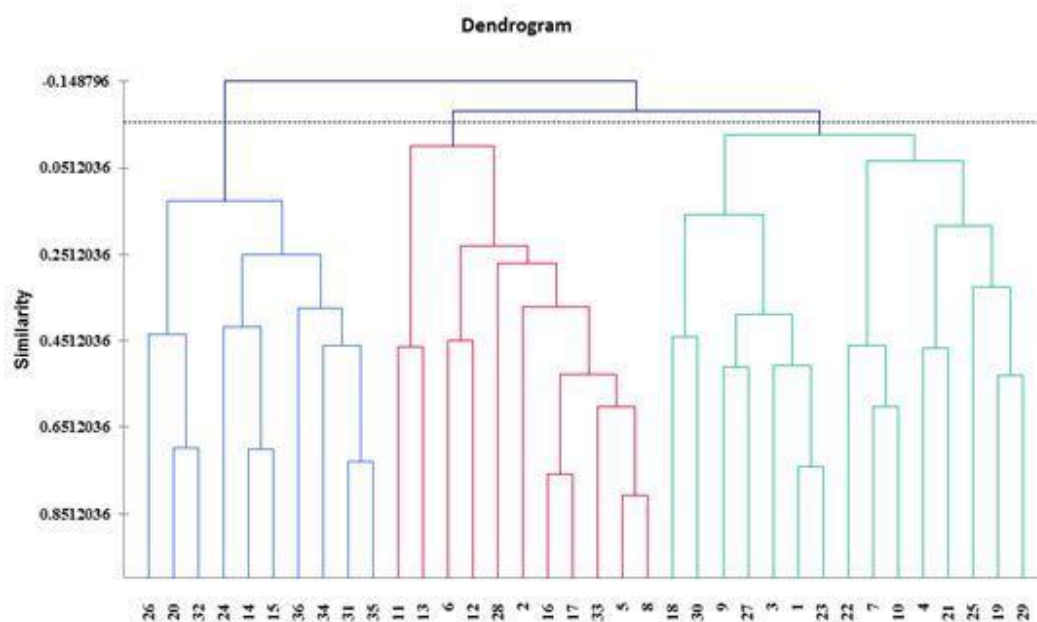


Fig. 3. Dendrogram generated by hierarchical cluster analysis showing the relationships among the melon accessions at using 14 quantitative characteristics

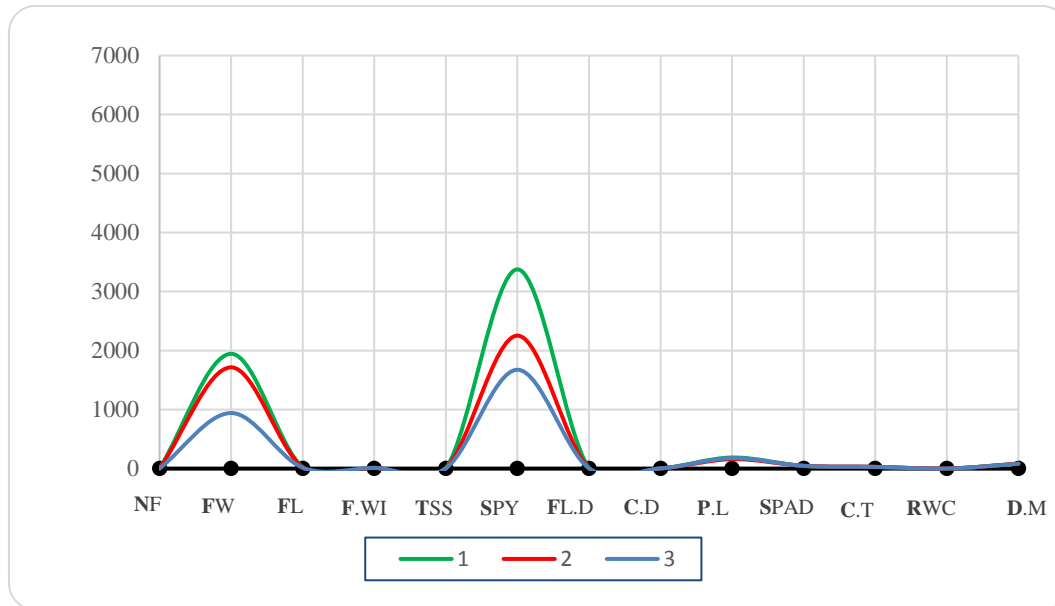


Fig. 4. Profile plot of the clusters, (line with separate color stand for cluster), X axis: traits, Y axis: cluster means

Multiple linear regression (MLR) is the most common form of linear regression analysis. As a predictive analysis, MLR is utilized to explain the relationship between one continuous dependent variable and two or more independent variables that can be continuous or categorical. The purpose of MLR is to predict a single variable from one or more independent variables. Furthermore, the goodness of fit (GOF) of a statistical model describes how well it fits a set of observations. GOF indices summarize the discrepancy between the observed values and the values expected by a statistical model. GOF statistics are GOF indices with known sampling distributions, usually obtained using asymptotic methods, and are used in statistical hypothesis testing. The AIC and BIC are not employed to test the model in the sense of hypothesis testing, but for model selection. Given a data set, a researcher chooses either AIC, BIC, or SBC, and computes it for all the models under consideration. Then, the model with the lowest index is selected. The results of fitting a MLR model are shown in Fig. 5 and Table 5. Standardized MLR analysis strongly correlated single plant yield with the number of fruits per plant, fruit weight, and fruit length. MLR provides a helpful method for analyzing multivariate data. The high coefficient of determination ($R^2 = 0.97$) indicated the high predictability of the model. RMSE is the square root of the variance of residuals, revealing the absolute root of the

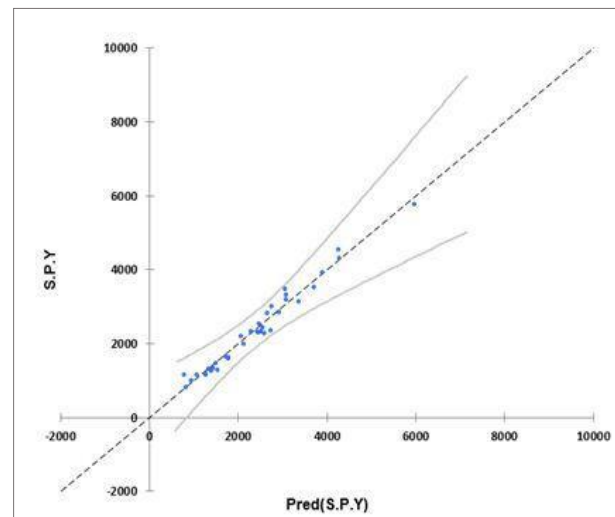


Fig. 5. Single plant yield predicted versus actual observation (95% CI)

variance of residuals, revealing the absolute fit of the model to the data, how the observed data points are close to the model's prediction and for this model, it was 229.19. Zalapa et al. (2008) using path analysis, showed that fruit length and weight have a strong association on yield and suggested them as selection criteria. The MLR equation that optimizes the model for relationship and statistics calculated in this analysis are shown below:

Plant yield = $-0.05 + 0.38$ (number of fruit) + 0.82 (fruit weight) + 0.16 (fruit length) + 0.02 (fruit width) - 0.03 (cavity diameter) - 0.03 (flesh diameter) +

+ 0.09 (plant length) + 0.02 (SPAD) – 0.06 (canopy temperature) + 0.20 (RWC) – 0.01 (days to maturity) – 0.04 (root length). The coefficient number of fruit ($P < 0.01$), fruit weight ($P < 0.01$) and fruit length ($P < 0.01$) were significant, and those for other traits were not ($P > 0.05$). Multiple linear regression is the most effective way in providing information about the relationship of yield and yield

components; this analysis strongly associated fruit yield with the number of fruit per plant and fruit weight. Multiple linear regressions provide a powerful method to analyze multivariate data. However, the goodness of fit was significant and approved by the model based on predicted and observed data into the model, but for better results, need to use more data with repeated data in years.

Table 5. Goodness of fit statistics of the regression and analysis of variance of regression

Observations	Sum of weights	DF	R ²	Adjusted R ²	MSE	RMSE	MAPE	DW	Cp	AIC	SBC	PC
36	36	23	0.97	0.959	52513	229.15	6.87	2.2	13	401.1	421.7	0.056
Source		DF		SS		MS						
Model		13		44232417.548		3686034.796**						
Error		22		1207807.639		52513.376						

** Significant at 1% statistical level

CONCLUSION

In this experiment, significant variation has been revealed based on accessions similarity, regarding the morphological and physiological traits in the evaluated germplasm. This variation allows the effective selection of parents in various breeding programs specially referring to fruit size, besides high yield in drought stress condition. Thus, this experiment emphasizes the importance of some traits, which are preserved in genetic resources for any plant breeding program.

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