

African Journal of Biological Sciences

Research Paper

Open Access

Investigation of Several Caprifig Genotypes in Northwestern Syria

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Article Info

Volume 6, Issue 8, April 2024 Received: 02 Feb 2024 Accepted: 13 March 2024 Published: 07 April 2024

Abstract

The fig tree is one of the most important trees spread in the Mediterranean region. In Syria, there is a great genetic diversity of this tree, both in edible fig varieties and Caprifig. Caprifigs are very important for pollinating edible figs, so this research studied six types of Caprifigs spread in northwestern Syria (Idlib Governorate, which is the main area for fig cultivation in Syria) between 2021 and 2022. The results of the study showed the diversity of the types studied in terms of leaf characteristics, especially the number of lobes (3, 5, 7), as well as the shape of these lobes and the shape of the leaf base. Fruits also varied in the length of the inflorescences (4.14-6.83 cm), their width (4.25-7.02 cm), and their number on the branch (6.00-7.02). Concerning pollen vitality, the highest value of pollen vitality was in the small Pangani variety (99.67%), superior to the rest of the models. Bunduqi variety was early when the wasp pollinating fig emerged, and the amount of pollen in it was high, making it ideal for pollinating early female figs (which is the dominant characteristic of the female fig varieties widespread in Syria). The results of the cluster analysis of the characteristics of the studied models also showed that the studied genotypes were grouped into two main clusters. The first cluster included five genotypes, while the second group included one genotype, the big Pangani, and the highest degree of relatedness was between the Azrak and big Pangani genotypes. PCA analysis indicated that three factors explained 93.65% of the total variance. Inflorescence length was also positively associated with its width because of studying the correlation between traits. The results of this research indicate the great diversity among the types of Caprifigs spread in northwestern Syria, and therefore the necessity of choosing the appropriate pollinator in terms of the quantity and vitality of pollen grains and the timing of the appearance of Blastophaga insects.

Key words: Caprifug, Syconia, Leaves, Pollen Vitality, Morphological Characterization.

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Introduction

The fig tree is one of the most important trees spread in Mediterranean countries, and it has adapted to the climatic conditions prevailing in this region (Ighbareyeh, *et al.*, 2018). It is a drought-tolerant tree that survives and produces even in difficult conditions (Mars, 2003). It has great nutritional and medicinal importance (Hajam and Saleem., 2022; Kiralan, *et al.*, 2023). The fig Ficus carica L. belongs to the family Moraceae (Falistocco, 2009). The Ficus genus includes 750 species of trees and shrubs (Condit, 1969). Figs have two sexual forms: the male fig (Caprifig) and

the female (edible) fig. The male fig produces pollen, so it is practically male, but at the same time, it contains female flowers with the male flowers, so they are functionally hermaphrodite trees. As for the female fig (edible), it contains only long-styled, unisexual female flowers (Stover, *et al.*, 2007). The flowers are unisexual, either male, female, or gall flowers (short-styled female), and fig pollen is carried by the fig wasp (*Blastophaga psenes* L.), which develops with the fig tree (Kjellberg, *et al.*, 1987).

Figs are generally divided into four main groups. The first group is the male fig (Caprifig), which is spread in many regions of the world, including Syria, and its species are very similar to each other. It is believed that they are the origins of the cultivated figs, through continuous hybridization processes (Condit, 1947). The fruits of this type are inedible and contain two types of flowers: male, which produces pollen grains (near the ostiole), and female, short-styled or gall flowers (in the lower two-thirds of the cavity). The fig wasp develops inside the tuberous flowers, and this type produces three crops during the year. (Stover, et al., 2007; Fleishman, et al., 2008). The male fig produces three growth cycles during the year, to temporarily house the eggs, larvae, and wasp of the fig wasp, which are the Profichi crop (matures in early summer and gives only pollen), the Mammoni crop (ripens in the fall), and the Mamme crop (during the winter) (Oukabli, et al., 2001; Anjam, et al., 2017). Adult insects (females) emerge after insect pollination in the wild fig crop, searching for a place to lay eggs. They enter the female fig fruit and scatter the pollen that is attached to its body, and pollination of the female flowers occurs (Galil and Neeman, 1977). The second group is the female fig, which produces two crops during the year. This type contains longpenciled female flowers that need to be pollinated by a fig wasp carrying pollen from the Profichi crop of the male fig to produce fruits (Çalişkan and Polat, 2012; Ferrara, et al., 2016; Núñez-Gómez, et al., 2021). The third group is the common (virgin) fig, which contains a gene that enables it to bear fruit without the need for pollination (Dickson and Dickson, 1996). The fourth group is the San Pedro fig, which produces two crops, the first is virgin without pollination, while the second needs pollination (Chai, et al., 2017).

(Aytürk, 2019) found that the inflorescences of the two types of figs (male and female) bear 3 types of flowers, which are tuberous flowers (in which the fig wasp develops), male flowers (which are found in male figs), and female flowers that are found in female (edible) figs. Just. The process of pollination in figs is called caprification and Profichi crop is used for this process. The pollen crop produces pollen in much greater quantities than the other two crops (Mammoni and Mamme), and for the pollen crop to be highly effective and effective, it is better that the pollen germination rate be high, and the amount of pollen produced is high (Balci, *et al.*, 2001).

On the other hand, a phenomenon known as xenia is seen in fruit trees and other plants, which is the effect of genes from the male father on the development of fruits or seeds. Therefore, the zinnia phenomenon can be used to determine the best pollinating parents to reduce the fruit growth period and increase production in farms of different varieties (Wallace and Lee, 1999; Koşar, *et al.*, 2022; Liu, *et al.*, 2022). As for the phenomenon of metaxenia, it is the effect of pollen on the shape of the fruit and its other characteristics, and it may be possible to use the phenomenon of metaxenia to determine the best pollinating parents for each variety (Olfati, *et al.*, 2010; Barakat and Draie., 2023c). Pollen sources significantly affect fruit yield, early fruiting rate, fruit size, bone width, skin, flesh thickness, pH, soluble solids content, and fruit weight (Koşar, *et al.*, 2022). Also, selecting pollen of good quality and quantity is important in fig orchards, as several studies show that the Caprifig variety used affects skin color, Syconium size, and shape, total dissolved solids, maturity time, and seed germination (Rahemi and Jafari, 2005; Gaaliche, *et al.*, 20011).

Pollen vitality and germination rate, as well as the amount of pollen grains, affect the yield of pollinated fruit trees. Various testing procedures have been used to determine pollen viability in fruit trees (Heslop-Harrison, *et al.*, 1984; Parfitt and Ganeshan, 1989). The amount of pollen per flower is also important for better pollination (Stanley and Linsken, 2012). Pollen vitality to identify the best pollinator has been studied in many researches (Awamura, *et al.*, 1995; Zeybekoglu, *et al.*, 1997; Ilgin, *et al.*, 2007; Vego and Miljković, 2012; Gaaliche, *et al.*, 2013). A study (Khadivi-Khub and Anjam, 2014;) indicated great morphological diversity among accessions of Caprifig genotypes, based on morphological characters. (Aljane and Ferchichi, 2007; Bilgin, *et al.*, 2020) studied Caprifig based on the quantitative and qualitative characteristics of the fruit. It has been noted that there are differences between Caprifig varieties, in terms of fruit characteristics

and pollen (Yaman and Çalışkan, 2016; Çalışkan, *et al.,* 2021; Caliskan, *et al.,* 2023; Barakat and Draie., 2023c).

The fig tree has great genetic diversity (Giraldo, *et al.*, 2010; Barakat and Draie., 2023a), and this diversity must be exploited in the process of breeding and genetic improvement (Falistocco, 2020; Barakat and Draie., 2023b). The study of the Caprifig is also important, because of its major role in the pollination process of edible figs. The effect of pollination on the growth of fig fruits on the tree and in stores has been studied. At harvest, the inoculated fruits showed superiority in the commercially desirable physical and taste characteristics (larger diameter and weight, and better hardness compared to virgin fruits). During storage, senescence and deterioration of inoculated fruits were slower than in virgin fruits, as internal texture, weight, size, wilting, and rot (Rosianski, *et al.*, 2016). On the other hand, the selection of Caprifig is important in breeding and genetic improvement processes, due to the clear effects of pollen source on fruit quality and chemical properties of edible figs (Zare, 2005; Rahemi and Jafari, 2008; Gaaliche, *et al.*, 2011; Pourghayoumi, *et al.*, 2012; Mirheidari, *et al.*, 2020; Barakat and Draie., 2023c). This research was carried out to study the Caprifig types widespread in northwestern Syria, characterize them, preserve them in a genetic bank to protect them from extinction and use them in genetic improvement programs for the tree.

Materials and Methods

Location:

The samples were collected from different areas within Idlib Governorate, which is the first governorate in Syria to grow and produce figs and has great genetic diversity in both male and female fig types.

Plant Material:

The plant material included six genotypes of male figs (which were collected during the research), trees planted at 7-10 years old were studied, and readings were taken on 5 trees of each type and from each site studied during the years 2021 and 2022.

Descriptive Study:

A comprehensive morphological characterization of five trees of each genotype was conducted at the study site following the fig characterization guide approved by the International Plant Genetic Resources Institute (IPGRI and CIHEAM, 2003), and the Profichi crop was mainly studied as it is the pollinator crop of female figs. The following biological characteristics were studied:

- Leaf shape: the number of lobes, leaf color, shape of the lobes, and the shape of the leaf base were studied.
- Fruit length: The measurement was taken with a graduated ruler from four inflorescences from each tree, then the average of the five trees was calculated.
- Fruit width: The measurement was taken with a graduated ruler from four inflorescences from each tree, then the average of the five trees was calculated.
- Number of fruits on the branch: Fruits were counted from four branches in the four directions, then the average of each tree and the general average of the trees were calculated.
- Fig wasp emergence time: early, medium, late.
- Quantity of pollen: Pollen grains were collected from inflorescences, and their quantity was calculated using a graduated cylinder.
- Fruit color at maturity.
- Pollen vitality study: Caprifig wild fig fruits were harvested from the studied male types, during the emergence of the pollen crop in June. 100 fruits of each variety were collected. The fruits were cut crosswise and placed on paper sheets in the laboratory (a cool, dry place) to collect pollen grains. Pollen viability was tested using methyl blue dye. After adding methyl blue dye, the colored pollen grains were considered alive, while the uncolored pollen grains were considered dead, so 100 pollen grains were tested, the

percentage of colored grains in them was calculated, and the process was repeated three times for each sample of the types studied, and the average was taken.

Statistical Analysis:

The data were analyzed statistically using the GenStat-12 statistical analysis program, and the averages were compared using the least significant difference (L.S.D) test at a significance level (0.05 for field studies, and 0.01 for testing pollen vitality), and the coefficient of variation (C.V) %. The relatedness between the types was studied using SPSS, a cluster analysis tree diagram, and a tree cluster analysis diagram to determine the degree of relatedness between the studied types. Principal components analysis (PCA) was also performed using SPSS to determine the factors that most contribute to the morphological diversity present, and the correlation between the studied traits was studied using the same program.

Results and Discussion

Morphological Specifications of Caprifig

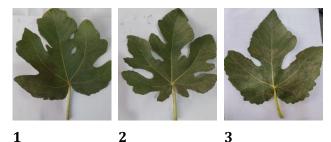
3-1-1-Specifications of Leaves:

Table (1) shows the most important leaf characteristics (number of lobes, leaf color, shape of the lobes, leaf base) in the studied Caprifig genotypes.

Table (1): Leaf characteristics (number of lobes, leaf color, shape of the lobes, shape of the
leaf base)

N	Genotype	Number of Lobes	Leaf Color	Lobular Shape	Leaf Base Shape
1	Azrak	5	light green	wide	Harpsichord
2	Bunduqi	7	light green	Long spoonful	Extended downward
3	Big Panjani	3	green	Wide, serrated edges	Harpsichord
4	Small Panjani	7	green	Long spoonful	Extended downward
5	Merry	7	light green	Long spoonful	Extended downward
6	Merry Armanaz	5	green	Severely serrated	Harpsichord

We note from Table (1) the diversity of the studied Caprifig types in terms of the number of leaf lobes, as the number of lobes was 3 in the big Pangani genotype, 5 in the two genotypes, Merry and Azrak, and 7 in the Bunduqi, small Pangani, and Merry Armanaz genotypes. As for the color of the leaves, it was green in the large and small Panjani, and light green in the Azrak, Bunduqi, and bitter genotypes. The shape of the lobes also varied depending on the type. It was wide in the Azrak genotype and spoon-serrated in the Bunduqi genotype, broad with a serrated edge in the big Panjani genotype, and long, spoon-shaped in the small and bitter Panjani genotype. The shape of the lobes was strongly serrated in the Merry Armanaz genotype. As for the base of the leaf, it was lyre in Azraq, Panjani Kabir, and Merry Armanaz, while the base of the leaf was extended downward in Nudhaqi, Panjani Saghir, and Merry. Figure (1) shows the leaf shape in the studied male fig genotypes.



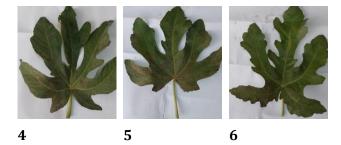


Figure (1): Leaf shape in the studied Caprifig genotypes

3-1-2-Fruit Specifications of Wild Figs:

In this research, the characteristics of male fig fruits were studied (fruit color, length and width, number of fruits on the branch, percentage of pollen vitality, quantity of pollen, date, and duration of insect emergence). As shown in Table (2).

N	Genotype	Fruit Color	Frui t Leng th	Fru it wid th	Numb er of Fruits	Polle n Vital	Quan tity of Polle n	When Insects Emerge	Duratio n of Insect
			cm	cm	/ Branc h	ity			Emerge nce
1	Azrak	Greenish purple	4.14 ^e	4.5 8 ^d	6.00 ^c	98.6 7 ^{ab}	Few	Medium (08-06)	Medium
2	Bunduqi	Reddish green	5.88 ^b	4.8 4 ^c	6.20 ^{bc}	97.3 3 ^b	too many	Early (01- 06)	short
3	Big Panjani	purple	6.83ª	7.0 7ª	6.03 ^{bc}	98.0 0 ^b	a lot	Medium (08-06)	Medium
4	Small Panjani	purple	5.12°	5.4 0 ^b	6.50 ^{abc}	99.6 7ª	a lot	Medium (08-06)	Medium
5	Merry	Yellowish green	4.96 ^c	4.6 9 ^{cd}	7.02ª	98.0 0 ^b	a lot	late (14-06)	Medium
6	Merry Armanaz	light green	4.52 ^d	4.2 5 ^e	6.60 ^{ab}	97.3 3 ^b	a lot	Medium (08-06)	Medium
CV%	0		-	4.3	9.9	0.8		-	-
L.S.I)		-	0.2	0.57	1.95		-	-
Pr			-	<.0 01	0.006	0.02 6		-	-
F									

Table (2): Characteristics of fruits in Caprifigs

It is noted from Table (2) that the genotypes of Caprifigs differed in the color of the fruit. The color was greenish-violet in the Azrak genotype, reddish green in the Bunduqi genotype, purple in the big Panjani and small Panjani genotypes, yellowish green in the Merry genotype, and light green in the genotypes. Genetic model: Merry Armanaz. Figure (2) shows the external color of the fruit in the studied male fig varieties Caprifig. The external color of the fruit is important in characterizing wild figs (Caliskan, *et al.*, 2023).

Table (2) also shows the variation of the studied types in fruit length. The highest value was for the big Panjani genotype, 6.83 cm, which outperformed the rest of the genotypes with highly significant differences, followed by the Bunduqi genotype, followed by the small Pangani and bitter genotypes, followed by the bitter genotypes. Armanaz, the lowest value was in the Azrak genotype, with a value of 4.14 cm. As for the fruit display characteristics, the genotypes differed greatly among themselves. The highest value was in the big Panjani genotype, reaching 7.02 cm, which outperformed the rest of the studied genotypes with highly significant differences, and the lowest value was in the Mery Armanaz genotype, with a value of 4.25 cm. As can be seen from

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Table (2) the diversity of the genotypes in the number of fruits on the branch. The highest value was in the Merry genotype and amounted to 7.02, outperforming the rest of the genotypes with clear significant differences. In contrast, the lowest value was in the Azrak genotype and amounted to 6.00. In a study conducted by (Yaman and Çalışkan, 2016), the fruit length of the studied genotypes of Caprifigs ranged between 37.54 and 50.25 mm, and the number of fruits on each branch ranged between 3.0 and 8.2. In another study (Caliskan, *et al.*, 2015), the length of the fruit in the Caprifigs genotypes studied ranged between 27.70 and 67.68 mm, and the width of the fruit ranged between 30.15 and 60.43 mm.



Figure (2): Fruit color and shape in the studied Caprifig genotypes

As for the vitality of pollen grains, the highest value was in the small Pangani genotype, reaching 99.67%, superior to the rest of the genotypes except for Azrak, with highly significant differences, while there were no significant differences between the rest of the genotypes studied. Pollen characteristics are important for characterizing Caprifigs (Caliskan, *et al.*, 2023), and pollen vitality is an important characteristic for pollination efficiency, as selecting pollen of high quality and a good quantity is important in fig orchards (Rahemi and Jafari, 2005; Gaaliche, *et al.*, 2011). Figure (3) shows the result of testing the vitality of male fig pollen using methyl blue dye. The Caprifig types also differed in the quantity of pollen, as it was very abundant in the Bunduqi genotype, and abundant in the big Pangani, small Pangani, Merry, and Merry Armanaz genotypes, and few in the Azrak genotype.

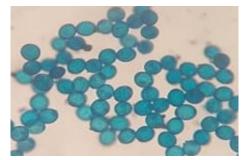


Figure (3): Pollen vitality test using methyl blue dye

From Table 2, we find that the Bunduqi genotype was one week earlier in the time of insect emergence than the Azrak, big Pangani, small Pangani, and Mery Armanaz genotypes, and by two weeks later than the Mery genotype, which was later than all genotypes. This gives great importance to the Bunduqi genetic pattern as an early pollinator for early edible figs, which represent most of the edible figs spread in the region. On the other hand, the duration of insect emergence was short in the Bunduqi genotype, while it was medium in the rest of the genotypes. The date of emergence of the fig Blastophaga insect from the fruit, and the duration of the insect's emergence, are important criteria for determining the efficiency of Caprifig genotypes in pollinating feminized fig models (Caliskan, *et al.*, 2015). Figures (4 and 5) show male and female fig dragonflies and galls and male flowers in the genotypes of Caprifig studied.



Figure (4): Male and female of Blastophaga insects in the studied Caprifig genotypes

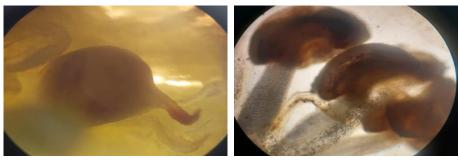
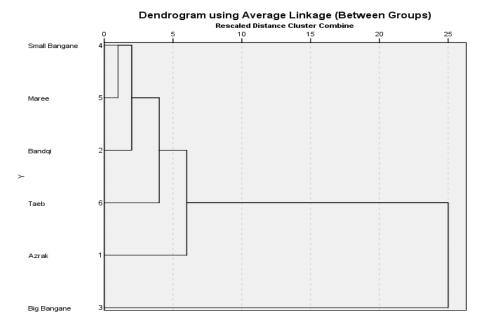


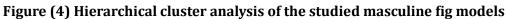
Figure (5): galls and male flowers in the studied Caprifig genotypes

In a study (Khadivi-Khub and Anjam, 2016), traits including the number of fig wasps, fruit weight, pit diameter, fruit flesh diameter, and harvested fruit diameter were evaluated for 36 Caprifig genotypes in Iran. A large discrepancy was found in the genotypes that were evaluated, and significant differences were found between them in all fruit characteristics. In a study (Yaman and Çalışkan, 2016) to study the pollination indicators of some Caprifig genotypes selected from the city of Hatay, Turkey, the rates of emergence of fig dragonflies from Profichi fruits, the number of fruits per branch, the weight of the fruit (g), and the diameter of the fruit (mm), fruit length (mm), fruit neck length (mm), and fruit skin color. The research (Caliskan, *et al.*, 2015) studied 77 genotypes of male figs grown in Turkey on the characteristics of the fruit, and a large phenotypic diversity was discovered between the genotypes of Caprifig based on the characteristics of the fruits

3-2- Cluster Analysis:

The kinship between the studied genotypes was studied based on hierarchical cluster analysis, and Figure (4) indicates the distribution of the Caprifig genotypes into two main groups. The first group (A) included 5 of the six studied types, while the second group (B) included a single type, which is big Pangani. The first group (A), in turn, was divided into two subgroups. The first subgroup was divided into two groups. The first subgroup included the Bunduqi model, which is the most distant in the group from the rest of the varieties and genotypes, Mery, small Pangani, and Azrak, as the highest degree of similarity was between Azrak and small Pangani. As for the second subgroup of A, the genotype included only Mery Armanaz.



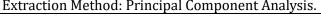


3-3-Principal Components Analysis:

Tables (3 and 4) and Figure (4) show the results of the analysis of the principal components responsible for the variance between the studied fig models.

Total Va	Total Variance Explained								
Commo	Initial Eigenvalues				raction S quared I	Sums Loadings	Rotation Sums of Squared Loadings		
Compo nent	Total	% of Varian ce	Cumula tive %	To tal	% of Varia nce	Cumula tive %	To tal	% of Varia nce	Cumula tive %
1	5.155	46.860	46.860	5. 15 5	46.86 0	46.860	3. 78 0	34.36 5	34.365
2	3.160	28.723	75.583	3. 16 0	28.72 3	75.583	3. 66 0	33.27 6	67.641
3	1.987	18.064	93.647	1. 98 7	18.06 4	93.647	2. 86 1	26.00 6	93.647
4	.699	6.353	100.000						
5	1.164E -15	1.058E -14	100.000						
6	5.782E -16	5.257E -15	100.000						
7	1.006E -16	9.149E -16	100.000						
8	- 4.303E -17	- 3.911E -16	100.000						
9	- 1.225E -16	- 1.113E -15	100.000						
10	- 3.091E -16	- 2.810E -15	100.000						

11	- 5.356E -16	- 4.869E -15	100.000						
Extraction Method: Principal Component Analysis.									



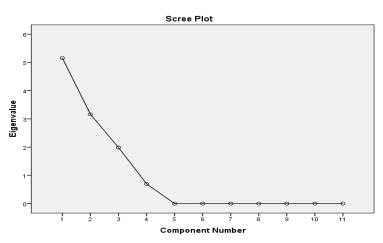


Figure 4: Factors composing variance

Table 4: Principal components of the factors responsible for the variance

Component Matrix ^a						
	Component					
	1	2	3			
The Shape of the Leaf Base	.907	.029	381			
Number of Lobes	905	308	.106			
Fruit Color	.887	249	.185			
Lobular Shape	.740	.313	595			
Number of Fruits/Branches	669	377	.473			
Fruit Width	.658	.562	.485			
Fruit Length	.195	.904	.357			
Pollen Vitality	.502	802	.244			
The Amount of Pollen	620	.743	.205			
Duration of Blastophaga Exit	.568	629	.396			
Plant Leaf Color	.550	.196	.773			

Principal component analysis (PCA) indicates the presence of three main factors that explain 93.647% of the total variance. The first factor, PCA1, accounts for 46.860% and is given by the following factors: the shape of the leaf base, color of the fruit, shape of the lobes, width of the fruit, length of the fruit, vitality of the pollen, and duration of Blastophaga emergence. The second factor, PCA2, explains 28.723% of the total variance and consists of fruit length, pollen quantity, inflorescence width, leaf base shape, leaf color, and lobe shape. The third factor, PCA3, explains 18.064%, and the most important characteristics related to this factor are leaf color, number of fruits/branches, fruit width, pollen grain vitality, pollen grain quantity, duration of Blastophaga emergence, number of lobes, and leaf color, Tables (3 and 4) and Figure (4).

3-4-Correlation Analysis:

The linear correlation between the quantitative morphological traits of the studied Caprifig genotypes was studied. The correlation analysis table (Table 4) shows that there is a positive linear correlation between the length and width of the fruit.

	Numb er of Lobes	Fruit Leng th	Frui t Wid th	Number of Fruits/Bran ches	Pollen Vitality	Duration of Blastophaga Exit
Number of Lobes	1					
Fruit Length	-0.340	1				
Fruit Width	-0.581	.851*	1			
Number of		-	-			
Fruits/Branches	0.566	0.31	0.43	1		
		6	1			
Pollen Vitality	0.049	- 0.13 8	0.27 3	-0.185	1	
Duration of Blastophaga Exit	-0.400	- 0.32 1	0.14 3	0.239	0.442	1

Table (5): Correlation matrix between the morphological characters of the studied
Caprifig types

* Correlation is significant at the 0.05 level (2-tailed)

Conclusion

The genetic patterns of the male figs studied varied in different characteristics. In leaf characteristics, the number of lobes was (3-5-7), the leaf shape was varied, the length of the fruits varied between (4.14 and 6.83) cm, and the width ranged between (4.25 and 7.07) cm. The types varied between early, medium-early, and late, with the Bunduqi genotype being the earliest of the Caprifig types in terms of fruit maturity, pollen abundance, and time of emergence of wasps. Therefore, it is recommended for use in pollinating early female fig varieties. Pollen vitality was also very high in all genotypes studied, and there were no significant differences between the genotypes in this trait. Analysis of genetic kinship based on the morphological characteristics studied showed the distribution of the studied male genotypes into two main groups. The first included five genotypes (Azrak, small Pangani, Bunduqi, Mery, and Merry Armanaz), and the second group included the big Pangani type. The principal components analysis (PCA) showed that three main factors explained 93.647% of the total variance. The first factor accounted for 46.860%, the second factor accounted for 28.723%, and the third factor accounted for 18.064% of the total variance. The leaf's specifications had the greatest share in the distribution of these factors. Finally, the correlation analysis between the studied morphological traits indicated the presence of a positive linear correlation between the length and width of the fruit.

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