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Evaluation of Genetic Diversity through Morphological Characteristics of Olive (*Olea europea* L.) Germplasm in Pakistan

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Abstract: Olive is an evergreen tree of subtropical regions all over the world, hence it is important to evaluate and characterize the genetic variability of olive. This issue has particular importance in areas where a number of cultivars show adaptation to difficult local environmental conditions. Present study was carried out during 2014-2015, to evaluate various morphological characteristics for the identification of 21 olive cultivars available at the Barani Agricultural Research Institute (BARI), Chakwal, Pakistan.

Characterization of olive tree comprised tree vigour, attitude of branches and density of branches, while leaf characterization included the length, width, shape, colour and its angle. It was found that characterization of morphological characteristics is very crucial during parent selection for developing new commercial olive cultivars.

Key words: Evergreen, Germplasm, Genetic diversity, Morphological characterization.

INTRODUCTION

Olive (*Olea europea* L.) belongs to a dicotyledonous family *oleaceae*. It is an ancient tree which has been found in Egyptian tombs from 2000 years BC. Olive tree of Vouves is considered as the oldest olive tree in the world and it is estimated to be over 3,000 years old (Maravelakis *et al*., 2012). It is found in all regions of the world except arctic. However, 98% of the world olive cultivation is carried out in Mediterranean region, and it contributes a major share in olive oil production (Hashmi *et al*., 2015). More than 2,000 olive cultivars are present in Mediterranean basin and these cultivars are characteristically distinguished through tree and fruit morphology (Bartolini *et al*., 1998; Ganino *et al*., 2006).

Botanically, olive is an evergreen tree of subtropical nature. It can attain the height of up to 10 m or more. Leaves are shortly stalked, oblong or lanceolate in their shape. White creamy flowers are produced in leaf axils. Fruit is drupe, ovoid in shape and blackish-violet in colour when ripe (Shu, 1996). It is a monoecious plant and pollination occurs through wind. Genetically, it possesses a diploid set of chromosomes as 2n = 46 (Kumar *et al*., 2011).

Olive thrives well in climates having hot summers with low humidity and cold winters. Winter chilling of at least two months is required for flower bud initiation. However, it cannot withstand freezing temperature, which ultimately leads to death of the plant. It can tolerate drought very well and can be successfully grown in areas with annual rainfall of 900-1000 mm. It can withstand moderate soil conditions but water logging conditions are injurious for plant health (Munir, 2009).

Local production of edible oil in Pakistan provides only 25% of its need and the rest of 75% is imported. Now, Pakistan is the 3rd largest importer of edible oil after China and India (Anonymous, 2017). To lessen the import bill, local production of edible oil should be increased and in this respect olive is a good alternative. Olive has the dominance over other edible oils regarding its chemical composition and health benefits. By promoting olive cultivation import bill of edible oil can be reduced.

In the past, olive has not been cultivated as a commercial fruit tree in Pakistan. Wild olive species, i.e., *Olea cuspidata* and *Olea ferrugenea*, are present in the Western parts of Baluchistan, Potohar region, Dir, Chitral, Swat, Azad Kashmir (at lower altitude), Waziristan and Murree hills (Sheikh,

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1993: Zabihullah *et al*., 2006). The existence of wild olive trees in these regions indicates that environmental conditions are appropriate for its commercial production. Recently, the government is also promoting the olive cultivation in Potohar region to help in meeting the edible oil need.

Chakwal lies in the Potohar region of Pakistan. It is mainly a semi-arid, rainfed area. Olive was introduced to Chakwal in 1991. There are more than 45 cultivars present in BARI, at this time. Current study was conducted to evaluate and characterize the promising olive cultivars available at BARI, Chakwal.

MATERIALS AND METHODS

This study was carried out during 2014-2015, to describe various morphological characteristics of 21 olive (*Olea europea* L.) cultivars, i.e., Manzanilla, Azerbaijan, Sevillano, Ottobractica, Pendolino, Leccino, Carolea, Moraiolo, Nocllare, Gemlik, Hamdi, Nabali, Souri, Uslu, Dan, Dermalali, Khodeiri, Mavi, Sorani, Tefahi and Kaissy, which are available at BARI. Ecological position and meteorological distribution data are given in Table 1.

Table 1: Eco-meteorological distribution data of Chakwal, Punjab (Pakistan).

Location	Chakwal
Altitude (m)	498
Latitude	32.9311 °N
Longitude	72.8551 °E
Max. mean temperature °C	39.45
Min. mean temperature °C	15.87
Annual rainfall (mm)	519

Five trees of each cultivar (10 years old), uniform in size and vigour were selected for the study and observations were recorded on morphological characteristics of each cultivar as mentioned.

1) Tree

Following different tree characteristics were observed and recorded:

a) Vigour: Tree vigour was evaluated on the following standards:

Strong: Trunk and canopy display vigorous growth than normal specimen of this species.

Medium: Trunk and canopy display the average growth.

Weak: Trunk and canopy display weak growth than normal specimen of this species.

b) Attitude of branches: Attitude of branches is basically the natural distribution of framework of branches and shoots. Attitude of branches was assessed by the following standards:

Erect: Branches tend to grow vertical and have strong apical dominance.

Drooping: Plagiotropic branching is that in which branches tend to grow downwards.

Spreading: Orthotropic branching is that in which canopy becomes hemispherical in shape and maximum light is available.

c) Density of branches: Density of branches is basically the interaction among the number and vigour of shoot, size of leaves and length of internodes. It was classified into three categories:

Dense: Short internodes, heavy branching and foliage, compact surface and inner parts are shaded.

Medium: Heavy branching but some light can penetrate in internal parts.

Sparse: Long internodes, light branching with fast growing habit, light can easily penetrate.

2) Leaf

a) Leaf length: Blade length was classified into three categories:

Long: Length of leaf was more than 7 cm.

Medium: Length of leaf blade was between 5 and 7 cm.

Short: Length of leaf blade was less than 5 cm.

b) Leaf width: Leaf width was divided in three different portions:

Broad: Width of leaf was more than 1.5 cm.

Medium: Width of leaf was between 1-1.5 cm.

Narrow: Width of leaf was less than 1 cm.

c) Leaf shape: Leaf shape was determined by length/width ratio of leaf and it was further divided in to three classes:

Elliptic: Length and width ratio was less than 4.

Elliptic-Lanceolate: Length and width ratio was between 4 and 6.

Lanceolate: Length/width ratio was more than 6.

d) Colour of leaf: Lower and upper colour of leaf surface was observed per following standards:

Lower leaf surface: Greenish grey and Greyish green.

Upper leaf surface: Green and dark green.

e) Leaf angle: Angle of leaf blade was observed from basal and acute points as per following standards:

Basal: Flat and concave.

Acute: Acute, very acute and open.

Data analysis:

Dendrogram was constructed through software, Minitab, 2017.

RESULTS AND DISCUSSION

Characterization of olive tree

Tree morphology was studied to assess the diversity among 21 genotypes of olive. Olive germplasm exhibited wide variations in tree characteristics as shown in Table 2.

Table 2: Tree characteristics of 21 olive cultivars.

Genotype	Origin	Tree vigor	Attitude of branches	Density of branches
Manzanilla	Spain	Med	Spread	Dense
Azerbaijan	Azerbaijan	Strong	Erect/spread	Dense
Sevillano	Spain	Strong	Erect	Dense
Ottobratica	Italy	Strong	Erect	Sparse
Pendolino	Italy	Strong	Spread	Sparse
Leccino	Italy	Very strong	Spread	Sparse
Carolea	Italy	Strong	Erect	Sparse
Moraiolo	Italy	Very strong	Spread/Drop	Medium
Nocellare	Italy	Med	Dropping	Dense
Gemlik	Turkey	Med	Erect	Medium
Hamdi	Egypt	Med	Spread	Sparse
Nabali	Palestine	Med	Dropping	Medium
Souri	Syria	Med	Spread	Sparse
Uslu	Turkey	Strong	Erect	Dense
Dan	Syria	Weak	Dropping	Medium
Dermalali	Syria	Weak	Spread	Sparse
Khodeiri	Syria	Strong	Erect	Dense
Mavi	Turkey	Strong	Erect	Dense
Sorani	Syria	Med	Spread	Dense
Tefahi	Egypt	Weak	Erect	Medium
Kaissy	Syria	Weak	Spread	Dense

Tree vigour

Two genotypes (Leccino and Moraiolo) had very strong tree vigour, while eight genotypes (Azerbaijan, Sevillano, Ottobratica, Pendolino, Carolea, Uslu, Khodeiri and Mavi) had strong tree vigour. Genotypes (Manzanilla, Nocellare, Gemlik, Hamdi, Nabali, Souri and Sorani) exhibited medium tree vigour while remaining genotypes (Dan, Dermalali, Tefahi and Kaissy) possessed weak tree vigour among all the studied germplasm.

Attitude of branches

Genotypes (Sevillano, Ottobratica, Carolea, Gemlik, Uslu, Khodeiri and Mavi) had erect attitude of branches, while genotypes (Manzanilla, Pendolino, Leccino, Hamdi, Souri, Dermalali, Sorani and Kaissy) exhibited spread attitude of branches. There was dropping attitude of branches in two genotypes (Nocellare and Nabali). Remaining two genotypes had distinct behavior in attitude of branches. Genotype Azerbaijan had erect or spreading nature while genotype Moraiolo had spread or drop nature.

Density of branches

Olive germplasm showed maximum variation regarding density of branches. It was dense in Manzanilla, Azerbaijan, Sevillano, Nocellare, Uslu, Khodeiri, Mavi, Sorani and Kaissy genotypes, the branching density of seven genotypes (Ottobratica, Pendolino, Leccino and Carolea, Hamdi, Souri and Dermalali) was sparse while five genotypes (Moraiolo, Gemlik, Nabali, Dan and Tefahi) showed medium branching density.

Leaf characterization of olive cultivars

Identification of olive cultivars carried out through leaves and high level of diversity occurred in leaf shape, colour and other morphological characteristics.

Leaf length

Wide variations occurred in leaf length of studied germplasm. Genotypes (Azerbaijan, Carolea, Gemlik, Hamdi and Uslu) had long leaves, while genotypes (Ottobratica, Souri, Dermalali, Khodeiri, Mavi and Kaissy) had short leaves. Further detail regarding variations in leaf length of olive germplasm is presented in Table 3. Leaf length is an important varietal character and is used for cultivar identification (Singh *et al.*, 1999). It is a genetic character which may differ from cultivar to cultivar under similar soil and environmental conditions.

Leaf width

Olive cultivars exhibited a higher level of variation in leaf width of studied germplasm. Broader leaf width was found in different genotypes including Manzanilla, Leccino, Gemlik, Souri and Sorani, while two genotypes Sevillano and Ottobratica had narrow leaves. The remaining genotypes showed medium leaf width as listed in Table 3. Leaf width is an important varietal character and is used for cultivar documentation (Cantini *et al.*, 1999; Singh *et al.*, 1999). Moreover, it is a genetic character that may differ from cultivar to cultivar under similar soil and environmental conditions.

Table 3: Leaf characteristics of 21 olive cultivars.

Genotype	Length	Width	Shape	Colour		Angle	
				Upper	Lower	Basal	Apex
Manzanilla	Medium	Broad	Ellip/lanc	Dark G	Grey G	Flat	Acute
Azerbaijan	Long	Medium	Ellip/lanc	Dark G	Grey G	Flat	Acute
Sevillano	Medium	Narrow	Lanceolate	Dark G	Grey G	Flat	Acute
Ottobratica	Short	Narrow	Ellip/lanc	Dark G	Grey G	Flat	Acute
Pendolino	Medium	Medium	Ellip/lanc	Dark G	Grey G	Flat	Acute
Leccino	Medium	Broad	Elliptical	Green	Grey G	Flat	Open
Carolea	Long	Medium	Ellip/lanc	Dark G	Grey G	Flat	Acute
Moraiolo	Medium	Medium	Ellip/lanc	Dark G	Grey G	Flat	Open
Nocellare	Medium	Medium	Ellip/lanc	Dark G	Grey G	Flat	Open
Gemlik	Long	Broad	Elliptical	Green	Green G	Flat	Acute
Hamdi	Long	Medium	Ellip/lanc	Dark G	Green G	Flat	Acute
Nabali	Medium	Medium	Ellip/lanc	Dark G	Green G	Flat	Open
Souri	Short	Broad	Elliptical	Dark G	Grey G	Flat	Acute
Uslu	Long	Medium	Lanceolate	Dark G	Grey G	Concave	Open
Dan	Medium	Medium	Ellip/lanc	Dark G	Green G	Flat	Open
Dermalali	Short	Medium	Elliptical	Green	Green G	Flat	Open
Khodeiri	Short	Medium	Ellip/lanc	Dark G	Green G	Concave	Open
Mavi	Short	Medium	Ellip/lanc	Dark G	Green G	Concave	Open
Sorani	Medium	Broad	Ellip/lanc	Dark G	Grey G	Flat	Acute
Tefahi	Medium	Medium	Ellip/lanc	Green	Green G	Flat	Acute
Kaissy	Short	Medium	Elliptical	Dark G	Green G	Flat	Open

Dark G = Dark green ; Green G = Green grey.

Leaf shape

Leaf shape may vary from one genotype to other and many different genotypes had same leaf characteristics. Maximum genotypes had either elliptical or lanceolate leaf shape. Leaf shape of genotypes (Leccino, Gemlik, Souri, Dermalali and Kaissy) was elliptical, while other two genotypes (Sevillano and Uslu) had lanceolate leaf shape. Leaf shape is a genetic character and is of great importance in cultivar identification (Cantini *et al*., 1999; Singh *et al*., 1999).

Leaf colour

Leaf colour has been divided into two classes, i.e., colour of upper surface and colour of lower surface. Leaf colour of upper surface of different genotypes (Manzanilla, Azerbaijan, Sevillano, Ottobratica, Pendolino, Carolea, Moraiolo, Nocellare, Khodeiri, Mavi, Sorani and Kaissy) was dark green, while in remaining genotypes (Leccino, Gemlik, Dermalali and Tefahi) was green colour. Colour of lower leaf surface of various genotypes (Manzanilla, Azerbaijan, Sevillano, Ottobratica, Pendolino, Leccino, Carolea, Moraiolo, Nocellare, Souri, Uslu and Sorani) was grey green, while other genotypes (Gemlik, Hamdi, Nabali, Dan, Dermalali, Khodeiri, Mavi, Tefahi and Kaissy) exhibited green grey colour of leaves.

Leaf angle

Leaf angle had been further divided into two segments, i.e., basal leaf angle and apex leaf angle. Basal leaf angle in genotypes (Manzanilla, Azerbaijan, Sevillano, Ottobratica, Pendolino, Leccino, Carolea, Moraiolo, Nocellare, Gemlik, Hamdi, Nabali and Souri) was flat, while in remaining genotypes (Uslu, Khodeiri and Mavi) basal leaf angle was concave. Apex angle in genotypes (Manzanilla, Azerbaijan, Sevillano, Ottobratica, Pendolino, Carolea, Gemlik, Hamdi, Souri, Sorani and Tefahi) was acute, while other genotypes (Leccino, Moraiolo, Nocellare, Nabali, Uslu, Dan, Dermalali, Khodeiri, Mavi and Kaissy) possessed open leaf angle.

Cluster analysis

Dendrogram (Ward linkage, Pearson distance) was built on the basis of morphological characteristics of leaves which allocated the 21 genotypes into four main clusters (A, B, C and D), as shown in Fig. 1. Cluster (A), large cluster contained eight genotypes (Manzanilla, Carolea, Azerbaijan, Sevillano, Ottobratica, Pendolino, Souri and Sorani), showed maximum similarity with each other and was further divided into two sub clusters (A1 and A2). Two genotypes (Pendolino and Ottobratica) showed maximum similarity in sub cluster (A1). So, these two

genotypes were very close to each other, as compared to all other genotypes in cluster (A). Cluster (B) comprised four genotypes (Leccino, Gemlik, Dermalali and Tefahi), which were very close to each other and showed maximum relationship with each other. Cluster (C) contained 6 genotypes (Moraiolo, Nocellare, Hamdi, Nabali, Dan and Kaissy), which were very close to each other. It was further divided into two sub clusters (C1 and C2). Moraiolo and Nocellare showed higher level of similarity in sub cluster (C1), while Dan and Nabali had maximum similarity with each other in sub cluster (C2). Cluster (D) comprised three genotypes (Uslu, Khodeiri and Mavi), which were very close to each other. During current study, dendrogram based on morphological characteristics of tree and leaf differed in total number of clusters and position of cultivars within the clusters.

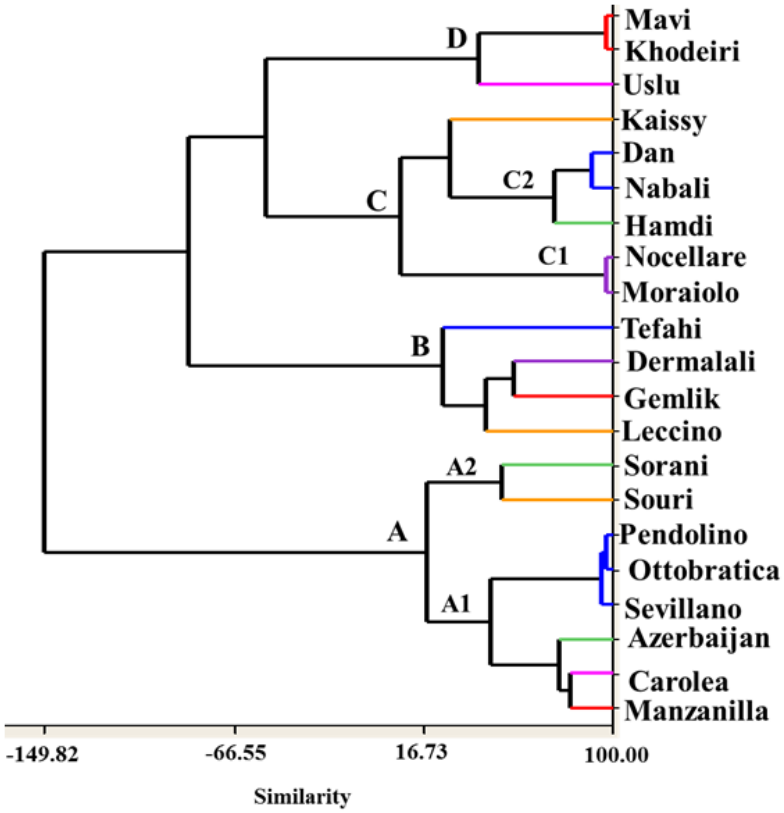

Fig. 1: Dendrogram showing relationship among 21 olive cultivars based on leaf colour, shape and angle.

Genetic make-up, geographical distribution, agronomic practices, plant nutrition and fruit orientation are the possible causes of variations in these morphological characteristics. Similarly, previous studies were carried out to analyse the genetic diversity through morphological characteristics in various horticultural crops (Khurshid *et al*., 2004; Hammadi *et al*., 2009). Morphological descriptors play an important role in germplasm characterization and were frequently used before the advent of molecular markers. Historically, this fact cannot be denied. Now-a-days, morphological markers along with molecular markers are being used for germplasm characterization. Recently, Dridi *et al*. (2018) used morphological and molecular markers to identify the olive germplasm

CONCLUSION

Olive germplasm is being cultivated since long, hence, it is possible to find new cultivars with valuable morphological traits that can be distributed to the farmers and can be utilized for further breeding programs in future. This is true mainly for those attributes that were not taken into consideration during the traditional selection focused for higher oil production. Present study demonstrated that characterization of morphological characteristics is very essential during the parent selection for evolving new commercial olive cultivars.

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