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Full Length Article

Diversity and Divergence in Cultivated and Wild Olive Germplasm Collected from Northern Pakistan

Safia Naz[1], Muhammad R. Khan[1], Azmat A. Awan[2], Manzoor Hussain[3] and Sajid Ali[1*]
[1]*Institute of Biotechnology & Genetic Engineering, the University of Agriculture, Peshawar, Pakistan*
[2]*Pakistan Oilseed Development Board, Peshawar, Pakistan*
[3]*Department of Botany, Hazara University, Mansehra, Pakistan*
For correspondence: bioscientist122@yahoo.com

Abstract

Assessment and exploitation of indigenous genetic diversity is important for crop genetic improvement. Little is known about the diversity and divergence in cultivated and indigenous wild olives in Pakistan. We aimed to estimate the diversity and divergence between cultivated and wild olive collection from Buner, Bajaur, Malakand and Upper-Dir regions of Khyber-Pakhtunkhwa, based on 30 olive genotypes using eight Randomly Amplified Polymorphic DNA (RAPD) primers. Polymorphic single bands were considered as a single allele/locus for all genetic analysis of these olive genotypes. A total of 36 loci were amplified, scored as dominant markers (present or absent). When considering 30 genotypes, all the markers were polymorphic. A minimum number of loci was recorded for OPA1B1, OPB2B1 and H20B1 while the maximum was recorded for OPA1B7 and OPR3B7. Maximum gene diversity 0.515 was recorded for loci OPA1B3 and OPR3B2, while the minimum gene diversity (0.067) was recorded for loci OPR3B7, OPB2B5, H07B4, H20B1, H20B2 and A14B2. An overall high diversity was observed within 30 olive individuals, each individual was a distinct multilocus genotype. The RAPD based FCA analyses revealed a clear divergence between the cultivated and wild genotypes collected from various locations of Khyber Pakhtunkhwa. Individuals sampled in Buner, Malakand and Bajaur were relatively closer to each other, than those sampled from Upper Dir. The divergence and diversity observed in the present study could be useful for exploitation of local and exotic olive genetic resources. © 2019 Friends Science Publishers

Keywords: *Olea europaea; O. cuspidate*; Himalayan region; exotic vs. indigenous germplasm

Introduction

Olive is an important crop grown worldwide in semi tropical climate (Neal, 1965). Olive (*Olea europaea* L. subsp. *europaea* var. *europaea*) belongs to family Oleaceae, which includes about 30 genera and six hundred species (Cronquist, 1981). It is an ever-green plant with long life span and adapts very easily in different climatic conditions. The plant has a shrubby appearance and flowers have hermaphrodite nature (Fontanazza *et al.*, 1990). It is grown for its oil and fruit which are used for baking and cooking food items, as lubricants, pharrmaceuticals, perfumes and lighting purposes (Crossman, 2002; Campus *et al.*, 2018). Although most of the world's olive is produced in the Mediterranean region, a significant increase has been reported in olive oil production and consumption in last 30 years in many parts of the world, where olive was not indigenous (Spennemann and Allen, 2000; Kaniewski *et al.*, 2012).

The olive plant is known as an image of the Mediterranean basin (Besnard *et al.*, 2018). In the Mediterranean region, cultivated olive is the most important tree crop species and it contributes ~90% to both olive production and olive groves. Though Spain, Greece and Italy alone contribute 75% of global olive oil production, it is also cultivated eastward in Georgia, Azerbaijan and Iran, which are known as a standout amongst the most eastern olive-cultivating nations. There is a long history of olive development documentation in the Middle East, incorporating references in religious texts and description by archaeo-botanists (Kaniewski *et al.*, 2012; Mousavi *et al.*, 2017). In the north of Iran, mainly the old commercial olive orchards are present, which produce more than 85 % of olive production of the eastern Middle East region (Noormohammadi *et al.*, 2007; Mousavi *et al.*, 2014). However, further east to the Middle-East i.e., in Pakistan, the olive production is still very limited (Jan *et al.*, 2017), though wild olive plants are widely distributed in different regions of the country, with climatic conditions suitable for olive cultivation.

Cultivation of olive is feasible to a wide range of climates, even on marginal lands. Moderate cold winters

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and prolonged hot summers having less humidity are the best conditions for growing olives. It is recommended to grow on soil without a lot of fertility requirements with adaptation to soils with acidic to fairly basic nature, with some tolerance to salt stress (Awan *et al.*, 2015; Khan *et al.*, 2015). However, it cannot withstand poorly drained soil and quickly dies if water remains around its root for longer times. In many parts of the world, like Greece, Spain, France and Italy, it is widely present under limited rainfed condition in hilly areas, where other crops would require more efforts for irrigation and crop management.

In many hilly parts of Pakistan with limited rainfall, olive could be successfully grown, considering the growth behavior of olive plant, especially the regions where wild olive trees are already growing. It can be grown in different ecological zones ranging from Swat, Malakand and Dir in the north to FATA, Khuzdar, Loralai and Quetta in the south, where various morphological and ecological types of wild olive is widely present, particularly considering the potential grafting of wild olive germplasm (Anwar *et al.*, 2013).

Wild olive could be differentiated from cultivated olive based on morphological differences i.e., wild olive has smaller fruit size with low quantity of oil and seed. However, both species can be grown in areas with similar climatic conditions (Terral and Simard, 1996; Besnard *et al.*, 2018). This suggests that the cultivated olive can be grown in areas where wild olive is naturally growing after suitable acclimatization efforts. Similarly, crossing between wild and cultivated olive may allow the introduction of genes from the wild relatives into cultivated olives to increase diversity for further selection (Mousavi *et al.*, 2017). In addition, transformation of wild olive into cultivated olive could also be exploited in future. This, however, would require genetic characterization of both available and introduced olive germplasm.

Genetic characterization could be carried out using both morphological and molecular markers. Morphological markers are limited in number and could be affected by environment. Genetic diversity studies using protein based markers and DNA based markers have proven useful in a large number of organisms ranging from plants to microbes (Gladieux *et al.*, 2011; Ali *et al.*, 2014a; 2016). A wide range of DNA based markers could be exploited ranging from random amplified polymorphic DNA (RAPDs), restriction fragment length polymorphism (RFLPs), amplified fragment length polymorphism (AFLPs), short sequence repeats (SSRs) to Single Nucleotide Polymorphism (SNP) and full genome sequencing. These molecular markers have been widely exploited to characterize diversity in olive germplasm in various international research groups (Angiolillo *et al.*, 1999; Sanz-Cortés *et al.*, 2003). However, the genetic relationship among wild and cultivated olive genotypes from Pakistan is poorly known.

The present study was designed to assess diversity and divergence in olive germplasm in Pakistan. Characterization of wild olive germplasm in relation to cultivated germplasm may enable to better exploit olive germplasm for their commercial exploitation in Pakistan. The study was aimed at i) molecular characterization of selected exotic commercial and indigenous olive genotypes introduced/growing in Khyber Pakhtunkhwa and iii) describing relationship between exotic commercial and indigenous wild genotypes.

Materials and Methods

The present research was planned to conduct molecular characterization of indigenous and introduced olive germplasm in Khyber Pakhtunkhwa, Pakistan. The research work involved sample collection of indigenous and commercial olive genotypes from distant areas of Khyber Pakhtunkhwa with subsequent genotyping and population genetics analyses. The research work was conducted at Institute of Biotechnology & Genetic Engineering, the University of Agriculture Peshawar, Pakistan. A total of 30 genotypes of both indigenous and exotic origins were characterized molecularly in the present study (Table 1).

Sample Collection from Indigenous and Commercial Genotypes

Attempts were made to collect at least five genotypes of indigenous wild olive from each region (Table 1). The commercial genotypes included Arbequina, Koroneika, Frantoio, Leccino, Coratina, Chetoui, Pendollino, Kalamata and Megaton. At least 10 leaves were collected from each genotype for DNA extraction and molecular genotyping.

DNA Extraction

Genomic DNA was extracted from young leaves using the CTAB (cetyl trimethtyl ammonium bromide) method from the leaves crushed in liquid nitrogen and CTAB buffer as described earlier (Ali *et al.*, 2017) The quality of DNA extracted was checked through gel electrophoresis. Additionally, the nanodrop was used to quantify extracted DNA for the Polymerase Chain Reaction (PCR). The DNA extracted was store at -20°C for further use in PCR amplifications and molecular genotyping.

Selection of Molecular Markers and PCR Amplification

Thermo scientific PCR kit was used for performing PCR reactions (Table 2). A set of eight RAPD markers was selected for molecular characterization of olive germplasm (Table 3). Reaction volume for each PCR reaction was 20 µL containing, 0.7 µL dNTPs, 1.6 µL $MgCl_2$, 2 µL taq buffer, 0.5 µL Taq polymerase, 1 µL of each primers (RAPD markers), 1 µL of template DNA and at last ddH_2O

Table 1: Details of 30 olive genotypes selected from both cultivated and wild olive plantation

Species	Location	Genotype	Cultivation status	Source
Olea europa	Exotic germplasm	Koroneiki	Cultivated	PODB, Tarnab
		Arbiquina	Cultivated	PODB, Tarnab
		Kalamata	Cultivated	PODB, Tarnab
		Coratina	Cultivated	PODB, Tarnab
		Megaron	Cultivated	PODB, Tarnab
		Pendolino	Cultivated	PODB, Tarnab
		Lacino	Cultivated	PODB, Tarnab
		Chetoui	Cultivated	PODB, Tarnab
		Arbosana	Cultivated	PODB, Tarnab
		Frantino	Cultivated	PODB, Tarnab
O. cuspidata	Upper Dir	UD_1	Wild	Collected for this study
		UD_2	Wild	Collected for this study
		UD_3	Wild	Collected for this study
		UD_4	Wild	Collected for this study
		UD_5	Wild	Collected for this study
	Buner	Bun_1	Wild	Collected for this study
		Bun_2	Wild	Collected for this study
		Bun_3	Wild	Collected for this study
		Bun_4	Wild	Collected for this study
		Bun_5	Wild	Collected for this study
		Bun_6	Wild	Collected for this study
	Malakand	Mala_1	Wild	Collected for this study
		Malak_2	Wild	Collected for this study
		Mala_3	Wild	Collected for this study
		Mala_4	Wild	Collected for this study
		Mala_5	Wild	Collected for this study
	Bajaur	BAJ_1	Wild	Collected for this study
		BAJ2	Wild	Collected for this study
		BAJ3	Wild	Collected for this study
		BAJ4	Wild	Collected for this study
		BAJ_5	Wild	Collected for this study
		BAJ_6	Wild	Collected for this study

was added to make the final volume of 20 µL. PCR conditions were optimized and applied to all RAPD primers. The PCR amplification of DNA was done by incubating the DNA samples for 5 min at 95°C for initial denaturation followed by 40 cycles comprising denaturation at 95°C for 30s, annealing of primers for 45 s at 42°C and extension at 72°C for 3 min, with a final extension step at 72°C for 5 min, using BioRad T100^{TM} Thermal Cycler. The PCR products were run on a 2.5% agarose gel to reveal the amplification products. Data in the form of 1 (for presence of band) - 0 (for absence of band) was scored and stored in MS Excel for further population genetics analyses.

Data Analyses

Scored alleles were formatted in MS excel sheet to make input files for different population genetics softwares. Divergence and diversity analyses were done following Ali *et al.* (2014b). The RAPD markers was checked by assessing the *psex* using GENCLONE (Arnaud-Haond and Belkhir, 2007) and the number of MLGs detected was plotted versus the number of loci (Stenberg *et al.*, 2003) and by estimating the linkage disequilibrium across loci in R software.

Table 2: Details of optimized conditions for PCR reaction as adopted for RAPD primers amplified in olive genotypes

Primers	PCR step	Temperature checked	Min/sec	Optimized condition
OPB6	Denaturation	95°C,95°C	5min/30sec	
	Annealing	30°C,33°C,35°C,37°C, 39°C, 41°C	45sec	41°C
	Extension	72°C,72°C	3min/5min	
OPA8	Denaturation	95°C,95°C	5min/30sec	
	Annealing	30°C,32°C,35°C,37°C, 39°C, 41°C	45sec	41°C
	Extension	72°C,72°C	3min/30sec	
OPA1	Denaturation	95°C,95°C	5min/30sec	
	Annealing	37°C,39°C , 41°C,42°C,44°C	45sec	41°C
	Extension	72°C,72°C	5min/10min	
OPR3	Denaturation	95°C,95°C	5min/30sec	
	Annealing	30°C,354°C,36°C,38°C ,40,42°C	45sec	42°C
	Extension	72°C,72°C	3min/5min	
OPB2	Denaturation	95°C,95°C	5min/30sec	
	Annealing	33°C,35°C,37°C,39°C, 40°C,42°C	45sec	42°C
	Extension	72°C,72°C	3min/5min	
HO7	Denaturation	95°C,94°C	5min/30sec	
	Annealing	33°C	30sec	33°C
	Extension	72°C,72°C	30sec/10min	
A 14	Denaturation	95°C,94°C	5min/30sec	
	Annealing	33°C	30sec	33°C
	Extension	72°C,72°C	30sec/10min	
H 20	Denaturation	95°C,95°C	5min/30sec	
	Annealing	33°C,35°C, 37°C	45sec	37°C
	Extension	72°C,72°C	30min/5min	

The population subdivision was tested across locations by carrying out factorial correspondence analysis using GENETIX program. This was further confirmed through estimation of F_{ST}. Neighbor-joining (NJ) tree was constructed with POPULATION software (Langella, 2008) to represent the relationship among germplasm from wild (*O. cuspidata*) and cultivated olive (*O. europea*) from the RAPD dataset.

Results

RAPD Genotyping of Olive Species

The sampled 30 olive genotypes were characterized with a set of 8 RAPD markers. Varying level of polymorphism was observed for the amplified 36 loci, while all of the markers were polymorphic when all 30 genotypes were considered (Table 4). Minimum number of loci (1) was recorded in OPA1B1, OPB2B1 and H20B1 while the maximum number of loci (7) was found to be in OPA1B7 and OPR3B7.

The maximum gene diversity was found to be 0.515 in loci OPA1B3 and OPR3B2, while the minimum gene diversity was (0.067) in loci OPR3B7, OPB2B5, H07B4, H20B1, H20B2 and A14B2. The maximum Simpsons diversity index (0.498) was recorded for loci OPA1B3 and OPR3B2, while the minimum Simpson diversity index (0.064) was recorded in loci OPR3B7, OPB2B5, H20B1,

Table 3: The Random Amplified Polymorphic DNA markers (RAPDs) employed for diversity and divergence analysis

Name of primers	Sequences
OPA1	(CAGGCCCTTC)
OPA8	(GTGACGTAGG)
OPB2	(TGATCCCTGG)
OPR3	(ACACAGAGG)
OPB6	(TGCTCTGCCC)
H- 20	(GGGAGACATA)
A- 14	(TCTGTGCTGG)
H-07	(CTGCATCGTG)

Table 4: Gene diversity, Simpson's diversity and Evenness indices detected for RAPD markers in olive germplasm from wild (*O. cuspidata*) and cultivated olive (*O. europea*)

RAPD primer	Loci	Gene diversity	Simpsons diversity index	Evenness
OBP6X	OBP6X5	0.508	0.491	0.983
	OBP6X6	0.186	0.180	0.571
OBP6Y	OBP6Y4	0.129	0.124	0.512
	OBP6Y5	0.480	0.464	0.933
OPA8	OPA8B5	0.370	0.358	0.772
	OPA8B6	0.370	0.358	0.772
OPA1	OPA1B1	0.186	0.180	0.571
	OPA1B2	0.497	0.480	0.961
	OPA1B3	0.515	0.498	0.996
	OPA1B4	0.508	0.491	0.983
	OPA1B5	0.497	0.480	0.961
	OPA1B6	0.460	0.444	0.899
	OPA1B7	0.460	0.444	0.899
OPR3	OPR3B2	0.515	0.498	0.996
	OPR3B3	0.460	0.444	0.899
	OPR3B4	0.370	0.358	0.772
	OPR3B5	0.508	0.491	0.983
	OPR3B6	0.331	0.320	0.725
	OPR3B7	0.067	0.064	0.438
OPB2	OPB2B1	0.370	0.358	0.772
	OPB2B2	0.287	0.278	0.676
	OPB2B3	0.287	0.278	0.676
	OPB2B4	0.370	0.358	0.772
	OPB2B5	0.067	0.064	0.438
H07	H07B4	0.067	0.064	0.438
	H07B5	0.434	0.420	0.860
	H07B6	0.129	0.124	0.512
H20	H20B1	0.067	0.064	0.438
	H20B2	0.067	0.064	0.438
	H20B4	0.370	0.358	0.772
	H20B5	0.480	0.464	0.933
	H20B6	0.497	0.480	0.961
A14	A14B2	0.067	0.064	0.438
	A14B3	0.434	0.420	0.860
	A14B4	0.239	0.231	0.625
	A14B5	0.287	0.278	0.676

H20B2, H07B4, A14B2. The maximum Evenness index (0.996) was recorded for OPA1B3and OPR3B2, while the minimum Evenness index (0.438) was recorded for OPR3B7, OPB2B5, HO7B4, H20B1, H20B2 and A14B2.

The detection of maximum number of multilocus genotypes (MLGs) under panmixia and their plotting against the number of loci re-sampled confirmed the suitability of RAPD markers to detect the genotypic variability in the studied populations (Fig. 2A). The lack of strong linkage between most of the loci also reflected on the utility of RAPD markers for the study (Fig. 2B).

Fig. 1: Photographs of olive plants: A. *O. europaea*, cultivated olive plant; B. *O. cuspidata*, wild olive plant

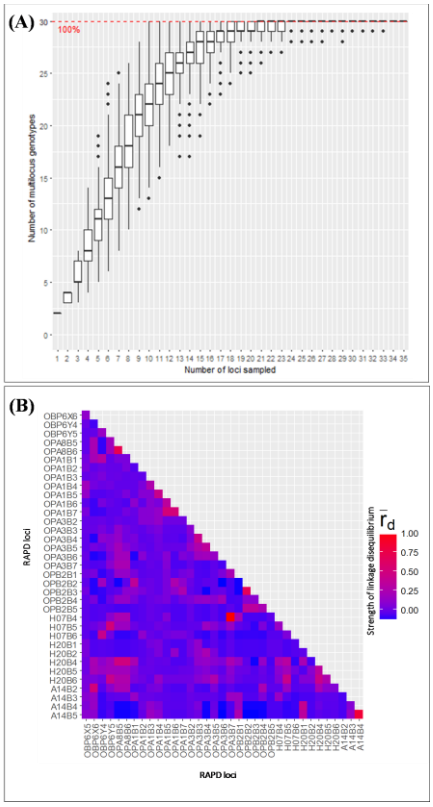

Fig. 2: Multilocus genotypes detected against re-sampling of loci (A) and linkage among RAPD loci (B) in olive germplasm from wild (*O. cuspidata*) from Khyber Pakhtunkhwa in relationship with cultivated olive (*O. europea*)

Diversity in Wild vs. Cultivated Olives

An overall high diversity was observed in both cultivated and wild olive germplasm (Table 5). Each individual represented a distinct multilocus genotype i.e., 30 MLGs detected out of 30 individuals genotyped. The Simpson

Table 5: Diversity parameters in olive germplasm from wild olive (*O. cuspidata*) from Khyber Pakhtunkhwa in relationship with cultivated olive (*O. europea*)

Diversity parameter	*O. cuspidate*				*O. europea*	Overall population
	Bajaur	Buner	Malakand	Upper_Dir	Exotic	
Sample size	5	6	5	5	9	30
No. of different MLGs	5	6	5	5	9	30
Simpson's Index	0.800	0.833	0.800	0.800	0.889	0.967
Evenness index	1.000	1.000	1.000	1.000	1.000	1.000
Gene diversity	0.244	0.296	0.194	0.256	0.343	0.332
Index of association (rDbar)	0.110	0.046	0.024	0.017	0.009	0.030

Table 6: Divergence in terms of F_{ST} (upper diagonal) and its significance (lower diagonal) in olive germplasm from wild olive (*O. cuspidata*) collected from various locations of Khyber Pakhtunkhwa and from the cultivated olive lines (*O. europea*)

Species	Locations	*O. europa*	*O. cuspidate*			
		Exotic	Upper Dir	Buner	Malakand	Bajaur
O. europa	Exotic	-	0.118	0.196	0.303	0.214
O. cuspidata	Upper Dir		-	0.146	0.266	0.272
	Buner	0.00	0.00	-	0.052	0.061
	Malakand	0.00	0.00	0.200*	-	0.160
	Bajaur	0.00	0.00	0.00	0.750*	-

*non-significant F_{ST} values are marked as bold

diversity index was more than 0.800 across all locations, while the Simpson index observed for overall population was 0.967. The cultivated olive though had relatively higher genotypic diversity (0.889) and gene diversity (0.343), but had smaller value for Index of association (0.009).

Diversity in wild relative across geographical regions also varied, with the maximum genotypic (Simpson's) diversity index (0.833) and gene diversity (0.296) observed at Buner, whereas the genotypic diversity remained the same across Bajaur, Malakand and Upper Dir (0.800). The gene diversity among these three locations ranged from 0.194 (observed at Malakand) to 0.256 (observed at Upper Dir), while it was 0.244 at Bajaur.

Divergence and Population Subdivision

Distribution of individuals based on factorial correspondence analyses revealed a clear divergence between cultivated and wild olive, though the subdivision due to location could not be fully explained as some of the individuals from one region were re-sampled across different region (Fig. 3A). When all the RAPD based data was considered, the three axes cumulatively captured 93% of the total genetic information. A maximum genetic variance of 53% was represented by the 1st axis followed by the 2nd that contributes 25% while the 3rd axis represented the minimum genetic variance of 15%. This was confirmed by the Principal Coordinate Analyses, reflecting on a clear divergence of cultivated and wild olive germplasm (Fig. 3B).

The divergence was further assessed through estimation of F_{ST} values, wild olive from all locations, which exhibited significant differentiation from cultivated olive. Within wild olive, all of the observed F_{ST} among locations were significant, except for Malakand with Buner and Bajaur (Table 6). The maximum F_{ST} value (0.272) was

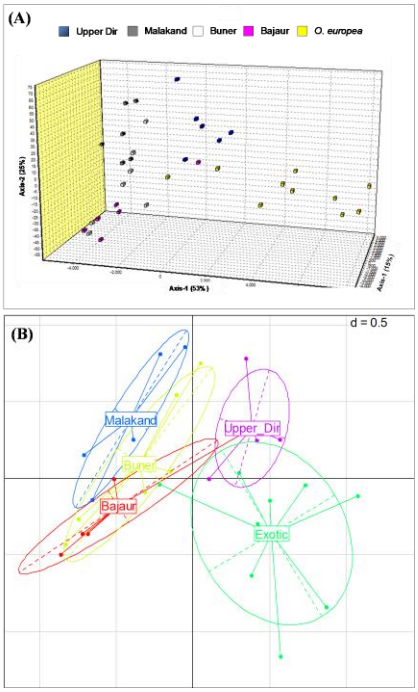

Fig. 3: Distribution of olive individuals from wild (*O. cuspidata*) and cultivated olive (*O. europea*) as evidenced through factorial correspondence analysis (A) and Principal Coordinate Analyses (B). Exotic refers to *O. europea*

recorded between Upper Dir and Bajaur while the minimum value (0.052) was observed between Buner and Malakand.

The Neighbor Joining tree further confirmed the overall divergence between wild and cultivated olives (Fig. 4). Most of the cultivated olive individuals were divergent from the wild olive, except Koroneiki and Arbiquina, along with which the Upper Dir individuals were grouped.

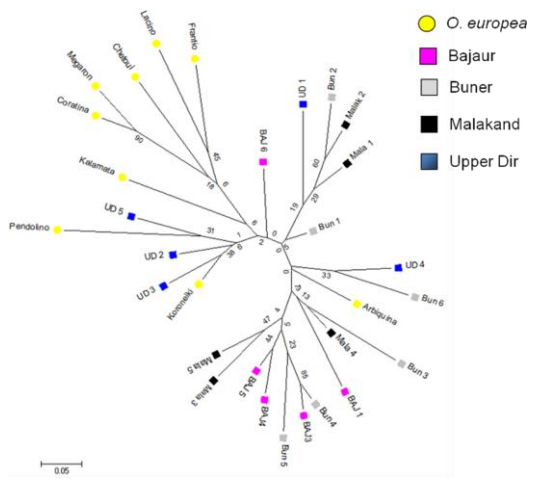

Fig. 4: Neighbor-joining (NJ) tree representing relationship between olive germplasm from wild (*Olive cuspidata*) and cultivated olive (*O. europea*)

Discussion

The identification of duplications within and between olive genotypes is very important for better management of olive genetic resources in the collection (Belaj *et al.*, 2001; Ben Abdeljelil *et al.*, 2017; Besnard *et al.*, 2018). Olive cultivars that are mainly propagated asexually or by grafting, and whose genotype variation seems to be low, comparison of RAPD profiles of several olive genotypes would enable to distinguish between olive germplasm (Sanz-Cortés *et al.*, 2001). Identification of the olive cultivars by using different RAPD primers suggests the high discrimination capacity of these markers, even in vegetative propagated crops. This capacity is particularly useful for management of a germplasm bank, as it provides an inexpensive method for identification of a large number of cultivars. Considering the observed variability of RAPD markers, these could be used for preliminary fingerprinting of olive germplasm. Identification of some of the most important Mediterranean olive cultivars by cultivar-specific RAPD markers and banding patterns, have provided useful tool to certify their plant material (Belaj *et al.*, 2001). Moreover, the number of RAPD markers observed in this study suggested these to be very useful tool for certification of plant material in the nursery industry to identify true-to-type individuals. It would also be useful to identify distinct genotypes for further genetic improvement as well as for asexual propagation (Rehman *et al.*, 2013). These results however, must be further explored with molecular sequencing and other useful markers, considering the fast growing next generation sequencing technologies, which have more precision.

Olive-tree is an old crop in Asia, including in Pakistan, however, very little information is available at the molecular level about the different olive plantation in the region. We have utilized RAPD markers based genotyping of olive germplasm, previously confirmed to be useful for olive cultivar identification (Belaj *et al.*, 2001; Sanz-Cortés *et al.*, 2003). The present study identified a high diversity in the Pakistani olive germplasm represented by 21 wild genotypes from four locations (Malakand, Buner, Bajaur and Upper Dir) and nine genotypes from the cultivated olive germplasm. Varying level of polymorphism was observed for the amplified 36 loci, while all of the markers were polymorphic when all 30 isolates were considered (Table 4). In our study a total of eight RAPD markers were successfully amplified for 30 olive genotypes, represented by 2 (marker OPA1B1, OPB2B1 and H20B1) to 7 (marker OPA1B7 and OPR3B7) loci and were useful to provide information on diversity.

The RAPD loci identified a high diversity in the studied olive germplasm. Each individual represented a distinct multilocus genotype. The cultivated olive though had relatively higher genotypic diversity and gene diversity and a smaller value for Index of association, compared to the wild olive germplasm. Information on olive diversity is essential for breeding programs, development of olive cultivars and transformation of wild olive into cultivated plantation through horticultural practices (Awan *et al.*, 2015; Khan *et al.*, 2015). This high level of polymorphism observed agrees with results of previous studies carried out in olive germplasm (Belaj *et al.*, 2001; Sanz-Cortés *et al.*, 2001; 2003; Mousavi *et al.*, 2017). This high degree of variability could be explained by large diversity in natural plantation of olive and the complexity of the olive genome; i.e., the olive has 23 pairs of chromosomes and is believed to have been originated by allopolyploidy (Bronzini *et al.*, 2002; Ipek *et al.*, 2012). This information of diversity must thus be complemented in future with more thorough sampling and high-resolution markers to identify and exploit traits of interest in future breeding programs.

Our results revealed a clear divergence across the species and locations in the olive germplasm collected from different locations of Khyber Pakhtunkhwa. A clear divergence was evident between *O. europea* (cultivated) and *O. cuspidata* (wild) samples. The F_{ST} values confirmed this divergence. Among the wild olive genotypes from various locations Malakand individuals were closer to Buner and Bajaur, while Upper Dir individuals were the most divergent. The use of molecular markers for deciphering divergences could be coupled with morphological descriptors both within and across species variation in olive (Ipek *et al.*, 2012; Besnard *et al.*, 2018).

Within wild olive divergence among locations was also observed as revealed through FCA, F_{ST} and phylogenetic tree. The divergence within wild olive and across the two species must be helpful for breeding programs, development of olive cultivars and transformation of wild olive into cultivated plantation through horticultural practices.

Conclusion

The wild olive clearly diverged from the cultivated olive genotypes. Among the wild olive genotypes, Malakand individuals were closer to Buner and Bajaur, while Upper Dir individuals were the most divergent. Overall, there was a high diversity in the germplasm, which could be exploited in further olive cultivation and improvement, may be helpful for breeding programs, development of olive cultivars and transformation of wild olive into cultivated plantation through horticultural practices. Finally, we recommend a more extensive sampling and more powerful genotyping tools to further elucidate the wild olive population structure in Pakistan in comparison with worldwide collections.

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References

Ali, S., M.R. Khan, A. Gautier, Z.A. Swati and S. Walter, 2017. Microsatellite genotyping of the wheat yellow rust pathogen *Puccinia striiformis. In: Wheat Rust Disease-Methods in Molecular Biology*, p: 445. Periyannan, S. (ed.). Humana Press, New York, USA

Ali, S., P. Gladieux, H. Rahman, M.S. Saqib, M. Fiaz, H. Ahmad, M. Leconte, A. Gautier, A.F. Justesen and M.S. Hovmøller, 2014a. Inferring the contribution of sexual reproduction, migration and off-season survival to the temporal maintenance of microbial populations: a case study on the wheat fungal pathogen Puccinia striiformis f. sp. tritici. *Mol. Ecol.*, 23: 603–617

Ali, S., S. Soubeyrand, P. Gladieux, T. Giraud, M. Leconte, A. Gautier, M. Mboup, W. Chen, C. Vallavieille-Pope and J. Enjalbert, 2016. Cloncase: Estimation of sex frequency and effective population size by clonemate resampling in partially clonal organisms. *Mol. Ecol. Resour.*, 16: 845–861

Ali, S., P. Gladieux, H. Rahman, M.S. Saqib, M. Fiaz, H. Ahmed, M. Leconte, A. Gautier, A.F. Justesen, M.S. Hovmøller, J. Enjalbert and C. de Vallavieille-Pope, 2014b. Inferring the contribution of sexual reproduction, migration and off-season survival to the temporal maintenance of microbial populations: a case study on the wheat fungal pathogen *Puccinia striiformis* f.sp. *tritici*. *Mol. Ecol.*, 23: 603–617

Angiolillo, A., M. Mencuccini and L. Baldoni, 1999. Olive genetic diversity assessed using amplified fragment length polymorphisms. *Theor. Appl. Genet.*, 98: 411–421

Anwar, P., A. Bendini, M. Gulfraz, R. Qureshi, E. Valli, G.D. Lecce, S.M. Naqvi and T.G. Toschi, 2013. Characterization of olive oils obtained from wild olive trees (*Olea ferruginea Royle*) in Pakistan. *Food Res. Intl.*, 54: 1965–1971

Awan, A.A., M. Zubair, S. Ali, I. Jan, A. Iqbal and A. Ahmad, 2015. The effect of different climatic zones on total phenolics and fatty acid profile of various olive cultivars. *Pak. J. Agric. Sci.*, 52: 359–364

Belaj, A., I. Trujillo, D.L. Rosa, L. Rallo and M.J. Gimenez, 2001. Polymorphism and discriminating capacity of randomly amplified polymorphic markers in an olive germplasm bank. *J. Amer. Soc. Hortic. Sci.*, 126: 64–71

Ben Abdeljelil, Z., M. Tekaya, B. Mechri, G. Flamini and M. Hammami, 2017. Changes in volatiles of olive tree *Olea europaea* according to season and foliar fertilization. *Intl. J. Agric. Biol*., 19: 1633–1639

Besnard, G., J.F. Terraland and A. Cornille, 2018. On the origins and domestication of the olive: A review and perspectives. *Ann. Bot.* 121: 385–403

Bronzini, D.C.V., J. Maury, C. Gambotti, C. Breton, A. Bervillé and J. Giannettini, 2002. Mitochondrial DNA variation and RAPD mark oleasters, olive and feral olive from Western and Eastern Mediterranean. *Theor. Appl. Genet.*, 104: 1209–1216

Campus, M., N. Değirmencioğlu and R. Comunia, 2018. Technologies and Trends to Improve Table Olive Quality and Safety. *Front. Microbiol*., 9: 617

Cronquist, A., 1981. *An Integrated System of Classification of Flowering Plants.* Columbia University Press, New York, USA

Crossman, N.D., 2002. The impact of the European olive (*Olea europaea* L. subsp. *europaea*) on grey box *(Eucalyptus microcarpa* Maiden) woodland in South Australia. *Plant Prot. Quat.*, 17: 140–146

Fontanazza, U., L. Baldoni and C. Corona, 1990. *Osservazioni Preliminari Sul Valor Agronomico di una nuova Cultivar da Olio:"Fs-17",* pp: 69–75. Proc Problematiche qualitative dell'olio d'oliva. Accademia Nazionale dell'Olivo, Spoleto, Italy

Gladieux, P., E. Byrnese, M.C. Fisher, G. Aguleta, J. Heitman and T. Giraud, 2011. *Epidemiology and Evolution of Fungal Pathogens in Plants and Animals*, pp: 1-59-106. Elsevier Inc. USA

Ipek, A., E. Barut, H. Gulen and M. Ipek, 2012. Assessment of inter-and intra-cultivar variations in olive using SSR markers. *J. Sci. Agric.*, 69: 327–335

Jan, S., M. Ilyas, I.K. Samar, N. Ali, M.M. Anjum, A. Ullah, R.U. Zahir and M.N. Shuaeb, 2017. Response of rooting of various olive cultivars to Iba (Indol Butaric Acid). *Agric. Res. Technol.*, 9: 555–757

Kaniewski, D., E. Van Campo, T. Boiy, J.F. Terral, B. Khadari and G. Besnard, 2012. Primary domestication and early uses of the emblematic olive tree: palaeobotanical, historical and molecular evidence from the Middle East. *Biol. Rev.*, 87: 885–899

Khan, M.N., R. Soherwardy and A. Sarwar, 2015. Nutritional assessment of olive fruit (*Olea europaea*) available at local market. *Intl. Food Res. J.*, 22: 26–531

Mousavi, S., M.H. Mazinani, K. Arzani, A. Ydollahi, S. Pandolfi, L. Baldoni and R. Mariotti, 2014. Molecular and morphological characterization of Golestan (Iran) olive ecotypes provides evidence for the presence of promising genotypes. *Genet. Resour. Crop Evol.* 61: 775–785

Mousavi, S., R. Mariotti, L. Regni, M. Bufacchi, S. Pandolfi, L. Baldoni and P. Proietti, 2017. The first molecular identification of an olive collection applying standard simple sequence repeats and novel expressed sequence tag markers. *Front. Plant Sci.*, 8: 1283

Neal, M.C., 1965. *In Gardens of Hawaiâ€™i. Bernice P. Bishop Museum Specia l Publication 40.* Bishop Museum Press, Honolulu, Hawaii, USA

Noormohammadi, Z., M. Hosseini-Mazinani, I. Trujillo, L. Rallo, A. Belaj and M. Sadeghizadeh, 2007. Identification and classification of main Iranian olive cultivars using microsatellite markers. *Hort Sci.*, 42: 1545–1550

Rehman, M., A.A. Awan, O. Khan and I. Haq, 2013. Response of olive cultivars to air - layering at various timings. *Pak. J. Agric. Sci.*, 50: 555–558

Sanz-Cortés, F., M. Badenes, S. Paz, A. Iniguez and G. Llacer, 2001. Molecular characterization of olive cultivars using RAPD markers. *J. Amer. Soc. Hort. Sci.*, 126: 7–12

Sanz-Cortés, F., D. Parfitt, C. Romero, D. Struss, G. Llacer and M. Badenes, 2003. Intraspecific olive diversity assessed with AFLP. *Plant Breed.*, 122: 173–177

Spennemann, D. and L. Allen, 2000. Feral olives (*Olea europaea*) as future woody weeds in Australia: A review. *Anim. Prod. Sci.*, 40: 889–901

Terral, J.F. and G.A. Simard, 1996. Beginnings of olive cultivation in eastern Spain in relation to Holocene bioclimatic changes. *Quat. Res.*, 46: 176–185

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