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# IDENTIFICATION OF SEVEN OLIVE VARIETIES USING RAPD MOLECULAR MARKERS

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## ABSTRACT

Randomly amplified polymorphic DNA (RAPDs) markers system was used for development of DNA fingerprints and assessment of genetic diversity among seven olive varieties at Genetically Modified Organism Testing Lab, Agricultural Biotechnology Research Institute, AARI, Faisalabad, Pakistan during the year 2015. A total of 62 RAPD markers were used out of which 59 were found highly polymorphic (95.2% polymorphism) and three RAPD markers were monomorphic. Out of total 583 alleles were amplified by 59 markers and 85 were monomorphic and 498 were polymorphic). Number of alleles varied from 3 (for I-4, I-18 and R-10) to 19 (for G-16), with an average of 9.85 alleles per marker. A combination of three RAPD primers (A-13, Q-1 & R-15 or I-3, R-13, R-15 or I-2, A-13, I-17 ) can distinguish all seven olive varieties under study. RAPD primers Q-1 can individually identify four olive varieties among seven under study whereas locus I-1, I-14, I-2, I-5, R-13, G-13, I-7, R-15, G-18 and A-13 each alone can distinguish three olive genotypes individually. Variety specific alleles were not found for Bulkasar selection and Arbequina. Further statistical analysis i.e. estimation of confusion probability (Cj) and primer efficiency [discriminating power (DL), Discriminating power (Dj)] also endorsed these findings. Clustering analysis at 70% similarity as base line produced seven different clusters indicating different genetic background of these varieties. Genetic similarity varied from 0.548 to 0.728.

KEYWORDS: Discriminating powers, Molecular markers, Olive, RAPD, Pakistan.

## INTRODUCTION

Olive is an ancient fruit tree species being cultivated in Mediterranean basin which later on spread to other world as well. It is largely consumed as oil and also for table purpose. People outside Mediterranean region are showing interest in olive cultivation because of its balanced fatty acid composition and presence of other functional food supplements. Local production of olive is significantly lower as compared to its demand. Olive cultivation needs special focus in two aspects i.e. improvement in oil quality and its yield. Pakistan is heavily deficient in edible oil and gap between domestic consumption and production is expanding year by year. For filling of this gap government spends handsome amount every year on imports of oilseeds and edible oil. Geographical climate of different areas of Punjab (Gujrat, Narowal, Jehlum, Khushab, Sialkot, Attock, Rawalpindi and Chakwal), KPK (Loralai, Swat, Malakand, Dir and Peshawar) and Baluchistan (Zhob) is ideal for olive cultivation. Olive cultivation is possible on these marginal lands which can significantly reduce the countries import bill.

Cultivated olive varieties belong to *Oleaeuropae* species which is diploid and grown in subtropical areas. There are many subspecies depending on different geographical origins and variation in morphological traits (Green, 2002). It is an *Allogamous* species, having high outcrossing rate and high heterozygosity. There is great variation in genetic architecture of varieties and high degree of DNA polymorphism among varieties (Angiolillo *et al.,* 1999; Rallo *et al.* 2000). Both hermaphrodite and staminate flowers are found in olive (Ateyyeh *et al.,* 2000) on panicles (Griggs *et al.,* 1975). Olive is naturally cross pollinated via wind (Cuevas *et al.,* 2001). Growers should be aware of cross-compatibility between olive cultivars when planning orchard for maximum fruit setting and high yield. Since domestication, olive was propagated through vegetative propagation to fix gene combination which develops either through mutations or random crosses (Carriero *et al.,* 2002). So, great variation is present in all countries cultivating olive and there are several issues for identification of varieties and management of germplasm.

Characterization and genetic diversity assessment of olive is a pre-requisite as it is difficult to identify large number of varietal synonyms (cultivars genetically similar but having different names) and homonyms (cultivars genetically different but having same names). It requires comprehensive discrimination method for classification and cultivar identification. Certified olive nursery production also requires reliable and fast techniques for cultivar identification because olive being trees differ from other horticultural plants as they

are long lived trees. Previously cultivars and rootstocks were identified on phenotypic trait basis; which was very long and tedious process because of long juvenile periods of olive trees.

Previously agronomical, biological and morphological characterizations were used for genetic diversity assessment (Barranco and Rallo, 2002) and cultivar identification (Cantini *et al.,* 1999). Lately, isozymes (Grati *et al.,* 2002; Lumaret *et al.* 2004 and Trujillo I and Rallo, 1995) were also used as a tool for variety identification. Isozymes were effective for olive variety identification but lack of specificity among isozymes and their sensitivity to developmental factors and environmental changes limited their use for varietal identification (Mousavi *et al.,* 2017). Different DNA markers types i.e. SSRs (Bracci *et al.,* 2009; Ercisli *et al.,* 2011), AFLPs (Bandelj *et al.,* 2004; Grati *et al.,* 2007), RFLPs (Cavallotti *et al.,* 2003) were also introduced along with phenotypic traits and allozymes for better characterization of cultivars. RAPD markers are a type of DNA markers extensively used in cultivar identification of different crops (Belaj *et al.,* 2001; Belaj *et al.,* 2004; Durgac *et al.,* 2010; Lobato *et al.,* 2012; Sesli *et al.,* 2009; Sesli *et al.,* 2010; Zitoun *et al.,* 2008; Zhang *et al.,* 1999).

RAPD markers are suitable due to efficiency and low cost which can be performed in a moderate laboratory, needing limited quantities of DNA. Moreover, these are simplified version of PCR and require design of only single primer. Present study was conducted to generate DNA fingerprints/Barcodes for rapid and easy identification of seven olive varieties using RAPD markers.

## MATERIALS AND METHODS

Seven olive varieties were introduced from Syria, Iran and Iraq at Barani Agricultural Research Institute (BARI), Chakwal, Punjab, Pakistan. Their profiling related to different agronomic traits i.e. fruit shape, size, quality, bearing was already collected by BARI. The step to generate DNA fingerprints/barcodes was initiated to promote propagation and cultivation of purely true to type olive varieties at the farmer's field by testing at early stage so farmer may not suffer at lateral stages.

***DNA extraction and Plant Material:*** Seven olive varieties viz. Sohawa selection, Manzanilla, Bulkasar selection, Chugtai selection, Arbequina, Arbosana and Koroneikai were studied using 62 RAPD primers. Total genomic DNA of seven varieties was isolated from succulent leaves (Guerin et al., 2002). EDTA was used as a chelating agent for $Mg^{+2}$ (Co-factor for nucleases). Olive DNA contains heavy contamination of polysaccharides, pigments, tannins, RNA and proteins. Proteins were removed using chloroform: Isoamyl alcohol and was subjected to further centrifugation. RNase (RNA degrading enzyme) was added for removal of RNA. NaCl along with CTAB removed polysaccharides. Nanodrop spectrophotometer (ND 2000, Thermo Scientific, USA) was used for quantification of DNA which measure absorbance at 260nm. DNA quality was determined by loading 50ng DNA on 0.8% agarose gel prepared in 0.5X TE buffer and stained with ethidium bromide.

***Primers, reaction mixture and PCR program:*** Sixty two RAPD primers belonging to A, G, H, I, J, Q and R series reported to be polymorphic were selected from previous studies (Belaj *et al.,* 2001; Belaj *et al.,* 2004; Durgac *et al.,* 2010; Lobato *et al.,* 2012; Sesli *et al.,* 2009; Sesli *et al.,* 2010; Zitoun *et al.,* 2008; Zhang *et al.,* 1999) and got synthesized from Gene Link, USA. PCR was assembled using reaction mixture (20 µL) comprising 2.0µL of 10X PCR buffer {Tris (pH 8.3) 50mM KCl}, 0.3mM each of dATP, dCTP, dGTP and dTTP, 30ng genomic DNA, 3mM of $MgCl_2$, one unit of Taq DNA polymerase and 0.6µM of each forward and reverse primers. The thermal cycler was set at 94°C for 5 minutes followed by 35 cycles of different temperature ranges i.e. 94°C for 45 seconds for denaturation, annealing temperature (variable for each primer), 72°C for 1 minutes for extension, 72°C for 7 as final extension and 4°C as holding temperature for infinite time. 10 micro liters of the PCR mixture was examined by 2% agarose gel on horizontal gel electrophoresis system and stained with ethidium bromide.

***Data analysis:*** Only unambiguous DNA fragments were scored. Scoring was done as presence (1) and absence (0) of DNA bands among genotypes. 100 bp DNA ladder was used as reference for estimation of band size. For confirmation of observed DNA bands, DNA was omitted from control reaction and one negative control was also used in each PCR for checking of validity of master/reaction mixture. Binary data were analyzed in NTSyspc 2.0 software to construct dendrogram using Unweighted Pair Group Method of Arithmetic Means (UPGMA). The Resolving power (Rp) was worked for each RAPD marker using the function described by Lobato *et al.* (2012). Discrimination power (Dj) of markers was also worked out to check their efficiency in varietal identification using formula described by Zhang *et al.* (1999). The polymorphism information content was computed on Microsoft Excel 2010 according to formula described by Powell et al. (1996).

## RESULTS AND DISCUSSION

***Polymorphism features:*** Sixty two RAPD primers were amplified among which 59 were polymorphic and 03 were monomorphic with a percentage polymorphism of 95.2% for markers. The 59 polymorphic markers generated 583 alleles (85 were monomorphic and 498 were polymorphic) with amplicon length ranging from 220bp to 2020bp. Alleles per polymorphic RAPD marker ranged from 3 (for I-4, I-18 and R-10) to 19 (for G-16), averaging 9.85 alleles per primer. Earlier studies also reported that RAPD markers gives maximum variation for alleles per locus (Belaj *et al.,* 2001; Belaj *et al.,* 2004; Durgac *et al.,* 2010; Lobato *et al.,* 2012; Sesli *et al.,* 2009; Sesli *et al.,* 2010; Zitoun *et al.,* 2008; Zhang *et al.,* 1999).

***Allele sizing and cultivar identification:*** RAPD markers have potential for providing unique alleles/DNA fingerprints for effective genotyping of different alleles i.e. olive. Unique banding pattern of each marker and their different sizes in each variety of olive is shown in Table 1, which identify each individual genotype on the base of specific alleles/bands. It may be possible that one marker is insufficient to distinguish all genotypes in question than some other marker may be taken into consideration and sometime a combination of two markers or may be three markers is used to distinguish all the genotypes.

Forty five RAPD primers can identify one, two, three, four or five olive varieties under study. All seven olive varieties can be distinguished successfully with the combination of three RAPD markers i.e. A-13, Q-1 and R-15. Similarly three RAPD markers I-3, R-13, R-15 and I-2, A-13, I-17 can also identify all seven varieties under study (Table 1). RAPD marker Q-1 can distinguish/identify four olive varieties among the seven under study. Similarly, markers I-1, I-14, I-2, I-5, R-13, G-13, I-7, R-15, G-18 and A-13 each alone can distinguish three olive genotypes individually. The A-3, I-13, J-9, J-18, Q-3, Q-4, G-8, I-6, G-16, G-19 and H-3 RAPD marker distinguished two olive varieties under study. Arbosana and Manzanilla can be recognized by 12 RAPD markers. So these genotypes can be distinguished at early stage during their multiplication in nursery, using one or may be more than one RAPD markers.

Nineteen RAPD markers identified Bulkasar selection and eight different RAPD markers can be used to identify Chugtai selection. Koroneiki and Arbequina varieties can be identified using 18 and 4 different RAPD markers, respectively. Sohawa selection showed a specific allele at locus I-4 (825 bp) and Manzanilla at Q-13 (825bp) for its identification (Fig. 1). Manzanilla can also be identified by 1000bp allele produced by Q-6 and 995bp allele yielded by H-12. The RAPD primer I-16, R-1 and G-3 gave unique bands of 625, 400 and 1050bp, respectively for the documentation of Bulkasar selection. Chugtai selection can be recognized by unique allele of 1200, 1470 and 1210 bp yielded by J-13, G-14 and G-17 respectively. The RAPD primer G-5, G-16 and H15 produced 880, 490 and 1500 bp bands respectively for identification of Arbosana variety. Koroneiki can be recognized by unique allele of 410, 750, 890, 1050 and 950bp yielded by J-19, Q-18 (Fig. 1), Q-12, R-2 and H-8 RAPD primers respectively (Table1). DNA fingerprinting/ barcoding key was developed olive genotypes under study which is given in Table 1 and Fig. 2. Lobato *et al.* (2012) also characterized the olive genotypes using RAPD markers and reported that all cultivars could be identified by a combination of three primers (OPF-6, OPA-8, and OPK-16); four cultivar-specific markers were detected. Similar results were also reported by Sesli and Yegenoglu (2009; 2010).

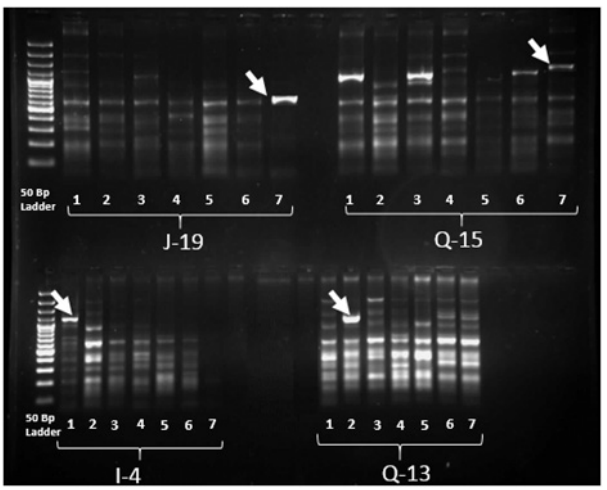



**Fig. 1. Specific Banding Pattern of 04 RAPD Markers i.e. J-19, Q-15, I-4 and Q-13.**

Arrows are indicating the Specific band which is an identification of that genotypes whereas 1-7 are genotypes Sohawa Selection, Manzanilla, Bulkasar Selection, Chugtai Selection, Arbequina, Arbosana and Koroneiki respectively.

**Table 1. Identification of olive varieties through banding pattern with RAPD primers and their respective base pair.**

| Primers | Sohawa Selection | Manzanilla | Bulkasar Selection | Chugtai Selection | Arbequina | Arbosana | Koroneiki |
|---|---|---|---|---|---|---|---|
| Q-1 | 1450 | 620 | 650 | | | | 1170 |
| I-1 | 1900 | | 1480 | | | | 1225 |
| I-14 | 900 | 950,500 | | 1300 | | | |
| I-3 | 1050,780 | 1325,730 | 900 | | | | |
| I-2 | 1300,360 | 1175 | | | | | 1275,680 |
| Q-5 | 1560 | 890,380 | | 540 | | | |
| R-13 | 750 | | | | | 615 | 1250 |
| G-13 | 605 | 685 | | | | 1400 | |
| I-7 | - | 1000 | | | | 1100,530 | 820 |
| R-15 | - | | 1550,580 | 680 | 370 | | |
| G-10 | - | | 615 | | 730 | | 300 |
| G-18 | - | | 810 | 1320 | | | 895 |
| A-13 | - | | | 1650 | | 1250 | 1225 |
| A-3 | 730 | 375 | | | | | |
| I-13 | 1175 | | | | 340 | | |
| J-9 | 900 | | | | | 950 | |
| J-18 | 1220,1130,1000,890,500,335 | | | | | | 585 |
| Q-3 | 300 | | | | 1100 | | |
| Q-4 | 830 | 900 | | | | | |
| G-8 | 1250 | | | | | 830 | |
| I-6 | - | 1125 | | | | 1300 | |
| G-16 | - | | 780 | | | | 1500,440 |
| G-19 | - | | | | | 1280,710 | 690 |
| H-3 | - | | | | | 880 | 1250 |
| I-4 | 825 | | | | | | |
| Q-13 | | 825 | | | | | |
| G-4 | 720 | | | | | | |
| H-18 | 830 | | | | | | |
| Q-6 | | 1000 | | | | | |
| H-12 | | 995 | | | | | |
| I-16 | | | 625 | | | | |
| R-1 | | | 400 | | | | |
| G-3 | | | 1050 | | | | |
| J-13 | | | | 1200 | | | |
| G-14 | | | | 1470 | | | |
| G-17 | | | | 1210 | | | |
| G-5 | | | | | | 880 | |
| G-16 | | | | | | 490 | |
| H-15 | | | | | | 1500 | |
| J-19 | | | | | | | 410 |
| Q-15 | | | | | | | 750 |
| Q-18 | | | | | | | 750 |
| Q-12 | | | | | | | 890 |
| R-2 | | | | | | | 1050 |
| H-8 | | | | | | | 950 |

**Statistical approach for primer efficiency**

For comparing the efficiency of different RAPD markers for varietal identification, Discriminating power (DL), Discriminating power (Dj), Resolving power (Rp) and Confusion probability (Cj) were computed (Table 2). Lower Cj alongwith high Dj and DL indicates that marker is more useful for generation of DNA fingerprints/Barcodes. The Cj value varied from 0 (I-1 & Q-12) to 0.5079 (I-12) with an average of 0.2405. Dj value ranged from 0.4921 (I-12) to 1.0000 (I-1 & Q-12) with an average of 0.7590 per primer. The DL values oscillated from 0.4626 (I-12) to 0.9796 (Q-12 and I-1) with an average of 0.7245 per primer. There was highly negative correlation between DL and Confusion probability (Cj) whereas highly positive correlation was observed between DL and discrimination power (Dj).

The Rp of markers ranged from 0.2857 (Q-12 & H-20) to 18.8571 (J-9) with an average of 7.6949 and a total Rp of 368.6. The relative high polymorphism and Rp value of primers J-9 and J-20 rendered these primers as the most useful for genetic diversity studies (Table 2). Belaj *et al.* (2001) studied polymorphism and discrimination capacity of randomly amplified polymorphic markers in an Olive Germplasm Bank. It was reported that a set of four markers OPA-01, OPK-08, OPX-01, and OPX-03 efficiently discriminated all 51 genotypes under study. Similarly, Belaj *et al.* (2004) used RAPD markers for varietal identification in olive and reported that a combination of three primers OPK-16, OPK-19 and OPZ-11 effectively distinguished 103 cultivars with a very low cumulative confusion probability.

***Clustering analysis and genetic relationships:*** The dendrogram was drawn using UPGMA algorithm to show genetic distances (Fig. 3). The dendrogram produced seven different clusters when drawn using 70% similarity as base line (Figure 2). It indicated that all seven varieties varied from each and are genetically distant at 70% similarity level. Genetic similarity values varied from 0.548 to 0.728. The RAPD primer indicated that Bulkasar Selection and Chugtai Selection were the most diverse genotypes with each other in this study. Bandelj *et al.* (2004) also studied the genetic relationship among olive cultivars using AFLP markers and grouped olive cultivars into different classes based on their evolution. Rallo *et al.* (2000) also studied the genetic relationship among olive cultivars using SSR markers and grouped cultivars on the basis of their banding pattern.

## CONCLUSION

The RAPD molecular markers were found useful for the identification of olive varieties and propagation of certified nursery. The seven varieties under study are genetically different from each other and can be distinguished with three or more RAPD markers. The variety specific markers were also available for all the seven varieties under study except Arbequina.

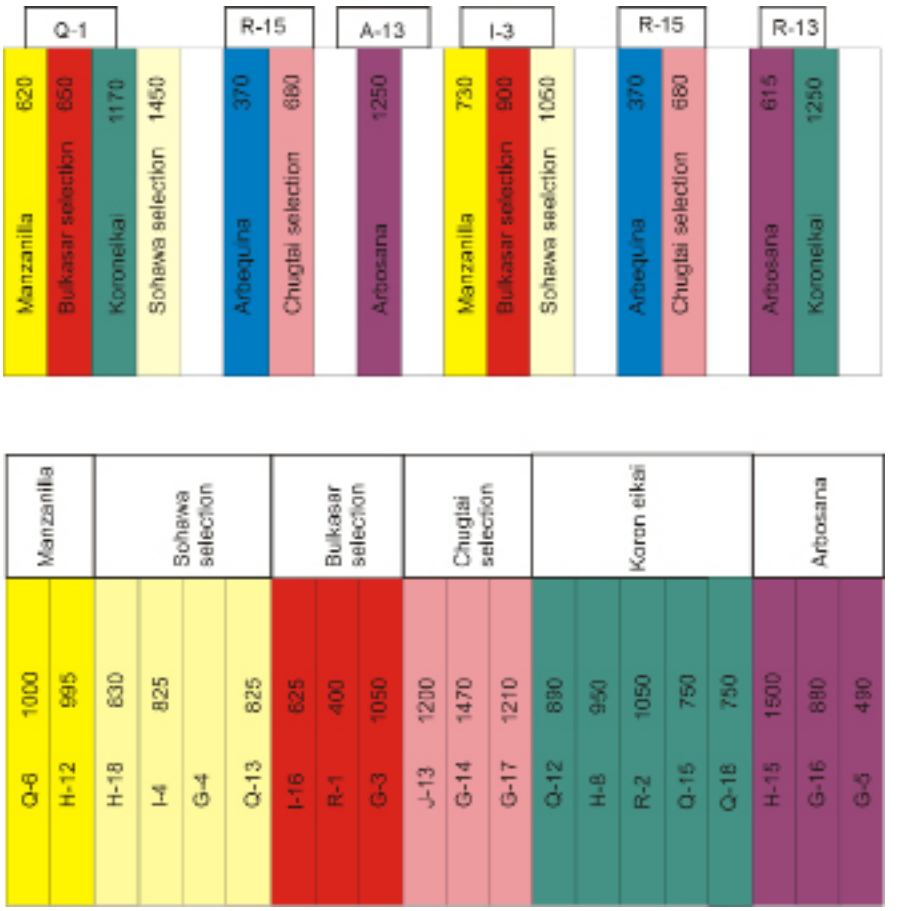



**Fig. 2. Identification key of seven olive cultivars and varieties specific allele based on RAPD primers**

**Table 2. Number of allele, gene frequency (pi), Confusion probability (Cj), Discriminating power (Dj), Discriminating power when N tends to infinity (DI) and Resolving power (Rp) of RAPD primer used in study.**

| S. No. | Primer No. | No. of Allele | Gene frequency | Cj | Dj | DL | Rp |
|---|---|---|---|---|---|---|---|
| 1. | A-3 | 13 | 0.4524 | 0.2063 | 0.7937 | 0.7585 | 10.8571 |
| 2. | A-13 | 15 | 0.3956 | 0.1722 | 0.8278 | 0.7959 | 10.2857 |
| 3. | I-1 | 4 | 0.1429 | 0.0000 | 1.0000 | 0.9796 | 0.8571 |
| 4. | I-2 | 15 | 0.3407 | 0.1282 | 0.8718 | 0.8414 | 8.8571 |
| 5. | I-3 | 12 | 0.3714 | 0.1619 | 0.8381 | 0.8082 | 7.4286 |
| 6. | I-4 | 3 | 0.4286 | 0.2063 | 0.7937 | 0.7619 | 2.5714 |
| 7. | I-6 | 6 | 0.3095 | 0.0794 | 0.9206 | 0.8878 | 3.7143 |
| 8. | I-7 | 10 | 0.3750 | 0.1905 | 0.8095 | 0.7832 | 6.0000 |
| 9. | I-12 | 7 | 0.7143 | 0.5079 | 0.4921 | 0.4626 | 8.5714 |
| 10. | I-13 | 13 | 0.4945 | 0.2894 | 0.7106 | 0.6813 | 12.8571 |
| 11. | I-14 | 14 | 0.3469 | 0.1190 | 0.8810 | 0.8484 | 9.7143 |
| 12. | I-16 | 9 | 0.3968 | 0.1640 | 0.8360 | 0.8027 | 7.1429 |
| 13. | I-17 | 11 | 0.4762 | 0.2328 | 0.7672 | 0.7324 | 8.5714 |
| 14. | I-18 | 3 | 0.5714 | 0.3095 | 0.6905 | 0.6531 | 4.5714 |
| 15. | J-9 | 16 | 0.6286 | 0.4286 | 0.5714 | 0.5429 | 18.8571 |
| 16. | J-12 | 5 | 0.5714 | 0.3524 | 0.6476 | 0.6163 | 5.7143 |
| 17. | J-13 | 7 | 0.6122 | 0.4218 | 0.5782 | 0.5510 | 8.5714 |
| 18. | J-16 | 4 | 0.5714 | 0.3333 | 0.6667 | 0.6327 | 3.4286 |
| 19. | J-17 | 4 | 0.6429 | 0.4286 | 0.5714 | 0.5408 | 5.1429 |
| 20. | J-18 | 11 | 0.2078 | 0.0260 | 0.9740 | 0.9481 | 4.5714 |
| 21. | J-19 | 8 | 0.4898 | 0.2517 | 0.7483 | 0.7143 | 6.8571 |
| 22. | J-20 | 13 | 0.5844 | 0.3463 | 0.6537 | 0.6197 | 12.8571 |
| 23. | Q-1 | 13 | 0.4026 | 0.1905 | 0.8095 | 0.7792 | 8.8571 |
| 24. | Q-3 | 13 | 0.4405 | 0.1984 | 0.8016 | 0.7670 | 10.5714 |
| 25. | Q-4 | 11 | 0.4000 | 0.1524 | 0.8476 | 0.8122 | 8.0000 |
| 26. | Q-5 | 7 | 0.4490 | 0.2313 | 0.7687 | 0.7376 | 6.2857 |
| 27. | Q-6 | 12 | 0.4881 | 0.2738 | 0.7262 | 0.6956 | 11.7143 |
| 28. | Q-13 | 9 | 0.4643 | 0.2321 | 0.7679 | 0.7347 | 7.4286 |
| 29. | Q-15 | 8 | 0.5000 | 0.2778 | 0.7222 | 0.6905 | 6.0000 |
| 30. | Q-16 | 8 | 0.6000 | 0.3714 | 0.6286 | 0.5959 | 6.0000 |
| 31. | Q-18 | 9 | 0.4898 | 0.2721 | 0.7278 | 0.6968 | 6.8571 |
| 32. | Q-12 | 6 | 0.1429 | 0.0000 | 1.0000 | 0.9796 | 0.2857 |
| 33. | R-1 | 12 | 0.5079 | 0.2963 | 0.7037 | 0.6735 | 9.1429 |
| 34. | R-2 | 6 | 0.5429 | 0.3238 | 0.6762 | 0.6449 | 5.4286 |
| 35. | R-10 | 3 | 0.6190 | 0.3810 | 0.6190 | 0.5850 | 3.7143 |
| 36. | R-13 | 12 | 0.4000 | 0.1762 | 0.8238 | 0.7918 | 8.0000 |
| 37. | R-15 | 11 | 0.3506 | 0.1255 | 0.8745 | 0.8423 | 7.7143 |
| 38. | R-19 | 6 | 0.6667 | 0.4286 | 0.5714 | 0.5374 | 8.0000 |
| 39. | G-2 | 12 | 0.3714 | 0.1333 | 0.8667 | 0.8327 | 7.4286 |
| 40. | G-3 | 13 | 0.5397 | 0.3175 | 0.6825 | 0.6508 | 9.7143 |
| 41. | G-4 | 7 | 0.4286 | 0.1973 | 0.8027 | 0.7697 | 6.0000 |
| 42. | G-5 | 11 | 0.3714 | 0.1524 | 0.8476 | 0.8163 | 7.4286 |
| 43. | G-8 | 13 | 0.4405 | 0.2222 | 0.7778 | 0.7466 | 10.5714 |
| 44. | G-9 | 8 | 0.5714 | 0.3265 | 0.6735 | 0.6385 | 8.0000 |
| 45. | G-10 | 14 | 0.5000 | 0.2738 | 0.7262 | 0.6939 | 12.0000 |
| 46. | G-13 | 8 | 0.3878 | 0.1837 | 0.8163 | 0.6472 | 5.4286 |
| 47. | G-14 | 10 | 0.3429 | 0.1048 | 0.8952 | 0.8612 | 6.8571 |
| 48. | G-16 | 19 | 0.4082 | 0.2007 | 0.7993 | 0.7697 | 11.4286 |
| 49. | G-17 | 12 | 0.6349 | 0.4286 | 0.5714 | 0.5420 | 11.4286 |
| 50. | G-18 | 16 | 0.3776 | 0.1463 | 0.8537 | 0.8207 | 10.5714 |
| 51. | G-19 | 13 | 0.4396 | 0.2125 | 0.7875 | 0.7551 | 11.4286 |
| 52. | H-3 | 10 | 0.5357 | 0.3214 | 0.6786 | 0.6480 | 8.5714 |
| 53. | H-8 | 6 | 0.5143 | 0.3048 | 0.6952 | 0.6122 | 5.1429 |
| 54. | H-12 | 11 | 0.4286 | 0.1799 | 0.8201 | 0.7846 | 7.7143 |
| 55. | H-13 | 9 | 0.5714 | 0.3197 | 0.6803 | 0.6443 | 8.0000 |
| 56. | H-15 | 9 | 0.3673 | 0.1224 | 0.8776 | 0.8426 | 5.1429 |
| 57. | H-18 | 13 | 0.4127 | 0.2116 | 0.7884 | 0.7596 | 7.4286 |
| 58. | H-19 | 14 | 0.4714 | 0.2333 | 0.7381 | 0.7327 | 9.4286 |
| 59. | H-20 | 4 | 0.5714 | 0.3095 | 0.6905 | 0.6531 | 2.2857 |
| | Average | 9.8475 | 0.4623 | 0.2405 | 0.7590 | 0.7245 | 7.6949 |

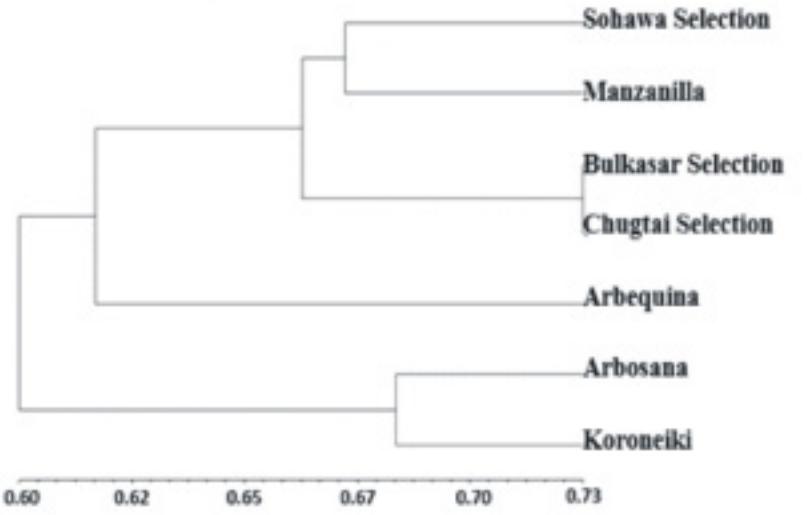



**Fig. 3. Dendrogram of seven olive varieties revealed by cluster analysis of genetic similarity estimate for 583RAPD Fragment.**

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| 1. | Muhammad Zafar Iqbal | Analysed data and prepared writeup |  |
| 2. | Shakra Jamil | Conducted research work and analysed data |  |
| 3. | Abid Mehmood | Planned expeirment and reviewed manuscript |  |
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