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Genetic Diversity among some Horse Breeds in Pakistan

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ABSTRACT

Genetic diversity among 12 horse breeds present in Pakistan was determined by using RAPD markers. The blood samples of purebred animals of eight exotic breeds (Thoroughbred, Arab, Anglo-Arab, Suffolk, Percheron, Clevland, Noriker and Hanovarian) present in Pakistan were collected from Army Remount Stables while those of four Pakistani indigenous breeds (Morna, Saen, Kakka Bralanwala and Anmol) from local horse breeders of Punjab province. Genomic DNA was extracted from the blood samples and amplified by PCR using 40 RAPD primers. PCR products were separated on an agarose gel electrophoresis and scoring of bands was performed. Phylogenetic tree was constructed by UPGMA method to determine the genetic similarity among the horse breeds. Thoroughbred and Arab horse breeds showed a higher similarity with Pakistani indigenous breeds, suggesting that these two exotic breeds had contributed significantly in the development of indigenous horse breeds of Pakistan. One set of primers OPL-7 revealed a polymorphic band at 450 bp which was present in DNA bulk of the indigenous breeds while absent in that of exotic breeds. High similarity coefficients among breeds implied the loss of genetic diversity and emphasized the need for conservation efforts for the Pakistani indigenous horse genetic resources.

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Authors' Contribution
FH and MSK conceived and planned the study. FH conducted blood sampling and lab work and drafted the manuscript. MSR did statistical analysis of data. MSK, SMRD and MAA revised the manuscript.

Key words
Genetic diversity, RAPD, BSA, Horse breeds, Breed specific marker.

INTRODUCTION

Human civilization has shared an intimate association with horses as companion animal owing to their key roles in agriculture, economics and culture in past and current societies. However, mechanical and industrial revolution in last a few decades has challenged the utility of horses which has been leading to the extinction of many indigenous breeds. The indigenous horses of Pakistan are termed as purebred "desi horses". These are used for riding, tent pegging, polo, dancing, draught and mule production.

In general, the populations of indigenous horses show variety and unevenness because of the continuous import of foreign mares and stallions by various conquerors and invaders, as well as by individual horse breeders today. This event, in combination with the decreasing use of horses, has resulted in the survival of a very small number of individuals that are true for each breed (Zafrakas, 1991).

There are several inconsistencies and doubts in literature regarding evolution of indigenous horse breeds of Pakistan. Most of these horses have had infusion of Arab and Persian blood primarily from Iran. The saints from Iran and Iraq brought elite blooded horses when they migrated to the subcontinent. Muhammad Bin Qasim of Hijaz brought extremely fine graded Arab horses when he conquered Sindh in 712 A.D. Similarly, those migrating to Punjab from Afghanistan like the inhabitants from Gardez brought their own lines. The noble houses maintained purity of these breeds. In other instances natural boundaries, mountains and rivers also helped to reduce genetic admixture among the breeds. Some of the central Asian horses like those from Afghanistan, Turcoman and other republics have also mixed with the local breeds (Amir, 1997).

In the 16th century, the Mughal emperor Akbar imported 500 Arab stallions, which were distributed throughout the subcontinent with a view to upgrade local stock (Amir, 1997). Special emphasis was given on the improvement of local stock by introduction of foreign blood during British regime in the subcontinent (Anonymous, 1998). During this period, English Thoroughbreds were imported from Great Britain to improve local horses to meet the demand of horses for British Army and horse race (Amir, 1997).

Crossbreeding is the most common system of horse breeding being practiced in Pakistan in absence of proper breeding policy for indigenous horses. Moreover, efforts of government departments to improve local horses by introduction of exotic breeds have further deteriorated the situation and the dilution of indigenous breeds is feared.

Besides all this, however, the decision as to whether

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individuals in a breed are the result of crossbreeding or inbreeding cannot be resolved based merely on phenotypic traits since they are hampered by phenotypic diversity, which limits the usefulness of morphological characters in assessing phylogenetic relationships among individuals within and between breeds.

The morphological characteristics have been used for classification of native breeds traditionally but work on phenotypic characterization of breeds is scanty, making it difficult to distinguish among local breeds. Characterization of genetic diversity among these breeds is first step required for conservation of indigenous genetic resources. Furthermore, it also helps to understand the extent and pattern of genetic variation among breeds that ultimately lead to devise suitable breeding programs aimed for improvement and conservation of the breeds (Loftus *et al*., 1994).

Genetic analysis can be very helpful in such an investigation where the phylogenetic relationship and hence the differentiation among the breeds has to be denied. DNA fingerprinting has been extensively used to determine genetic diversity within and among breeds based on polymorphic sites in DNA and hence is a method of choice for predicting the phylogenetic relationship. Several DNA fingerprinting techniques such as restriction fragment length polymorphism (RFLP), amplified fragment length polymorphism (AFLP), simple sequence repeats (SSR) or microsatellites, minisatellites, single nucleotide polymorphism (SNP) and random amplified polymorphic DNA (RAPD) have been extensively used to explore genetic variation and breed specific DNA markers in animal populations.

Studies on population genetic structure and genetic variation using RAPD, produced by the polymerase chain reaction (PCR) (Williams *et al*., 1990), has been used extensively in past. Typically, RAPDs are detected using a single, short (10-mer) oligonucleotide primers and genomic DNA in a PCR with low stringency conditions (Williams *et al*., 1993). This method provides an easy, quick, and inexpensive examination of genomic variation, at many loci especially in case of unknown DNA sequences (Hadrys *et al*., 1992). It has been applied successfully in several organisms such as bacteria, plants, fish and animals (Haig *et al*., 1994). Many studies have been conducted in exploring significant polymorphic regions within a species so that to understand their population structure (Black *et al*., 1992; Dawson *et al*., 1993; Yeh *et al*., 1995; Caccone *et al*., 1997; Ali, 2003). These investigations proved that the polymorphisms revealed by RAPD can be effectively used to determine intra-specific variation. In horses, the RAPD method has been used successfully by Bailey and Lear (1994) to compare Arab and Thoroughbred horse breeds. They found that RAPD markers might be useful to infer relationship and detect undesirable crossbreeding among horse breeds. Recently, RAPD markers have been used to dissect genetic diversity in endangered Himalayan gray langurs in Pakistan (Minhas *et al*., 2018).

Firstly, present study was conducted to explore the utility of RAPD markers in distinguishing the Pakistani indigenous horse breeds. Such markers ideally are those present in one breed but absent in the other breeds. Secondly, we presented a first evaluation on genetic structure and variation among the Pakistani indigenous horse breeds and their relationship with exotic breeds present in Pakistan. Given the limited number of native horses, this study may have important implications for the conservation and perpetuation of these indigenous horse genetic resources.

Table I.- Details of number of samples of each breed used and their respective locations.

S No	Horse breed	No of samples	Location
1	Morna	10	Local Horse Breeders of Okara, Faisalabad, Chiniot, Jhang
2	Shiean	14	Local Horse Breeders of Faisalabad, Chiniot, Jhang
3	Anmol	03	Local Horse Breeders of Sargodha
4	Kaka Bralanwala	02	Local Horse Breeders of Jhang, Faisalabad
5	Percheron	20	Army Remount Stables, Faisalabad, TT Singh, Sargodha
6	Suffolk	15	Army Remount Stables, Faisalabad, TT Singh, Sargodha
7	Thoroughbred	15	Army Remount Stables, Faisalabad, TT Singh, Sargodha
8	Clevland Bay	06	Army Remount Stables, Faisalabad, TT Singh, Sargodha
9	Hanoverian	01	Army Remount Stable, Sargodha
10	Noriker	01	Army Remount Stable, TT Singh
11	Arab	05	Army Remount Stables, Okara, Sargodha
12	Anglo Arab	02	Army Remount Stables, Okara

MATERIALS AND METHODS

Selection of animals and collection of blood samples

Representative individuals from each breed were selected on the basis of their respective breed characteristics and consideration of horse breeders.

The samples of exotic horse breeds *viz*., Thoroughbred, Arab, Anglo Arab, Percheron, Suffolk Punch, Clevland Bay, Noriker and Hanoverian were collected from Army Remount Stables of Chenab Breeding Area while samples of indigenous breeds *viz*., Morna, Siaen (Shiean), Anmol and Kakka Bralanwala from different local horse breeders of Punjab province (Table I). The peripheral whole blood samples were collected from the selected animals of each breed of both sexes and ages into 3 ml EDTA vacutainers. Great care was taken to avoid foreign DNA contamination from dead cells and prokaryotes. Blood samples were kept at -20°C until processed for DNA extraction.

DNA extraction and quantification

Genomic DNA was extracted from whole blood by using the phenol:cholorfarm method as reported earlier (Signer *et al*., 1988; Grimberg *et al*., 1989). Quantification of genomic DNA was performed by DyNA Quant Flourometer. The DNA quality was determined by gel electrophoresis of samples (using 0.8% agarose gel). The DNA samples showing smear were rejected.

Conditions optimization for RAPD analysis

DNA concentration in the working solution was confirmed by the DyNA Quant Flourometer for RAPD analysis (Williams *et al*., 1990). The genomic DNA concentration, 10x PCR buffer with $(NH_4)_2SO_4$, $MgCl_2$, dNTPs, 10 M random primer and *Taq* DNA polymerase were optimized. The DNA bulk of each breed was prepared by mixing equal quantities of DNA from available number of individuals.

A series of 40 arbitrary oligonucleotide primers obtained from Operon Company (Operon Tech. Inc., Alameda, CA, USA.) was used for the amplification of genomic DNA. A detail of primers used along with their sequences is presented in Table II. PCR was carried out in 25 µL reaction mixture through programmable thermal cycler (EPPENDORF, USA). The PCR profile included an initial denaturation for 5 min at 94°C, followed by 1 min denaturation at 94°C, primer annealing for 1 min at 36°C and extension for 2 min at 72°C for 40 cycles, and then a final extension at 72°C for 10 min.

Table II.- Details of primers and amplified fragments scored for each primer.

S No	Primer	Sequence	No. of amplified loci	S No	Primer	Sequence	No. of amplified loci
1	OPC-1	TTCGAGCCAG	5	21	OPG-16	AGCGTCCTCC	4
2	OPC-2	GTGAGGCGTC	4	22	OPI-04	CCGCCTAGTC	8
3	OPC-10	TGTCTGGGTG	6	23	OPI-10	ACAACGCGAG	8
4	OPC-12	TGTCATCCCC	6	24	OPI-13	CTGGGGCTGA	3
5	OPC-13	AAGCCTCGTC	6	25	OPI-15	TCATCCGAGG	9
6	OPC-17	TTCCCCCCAG	5	26	OPI-20	AAAGTGCGGG	11
7	OPE-01	CCCAAGGTCC	8	27	OPL-7	AGGCGGGAAC	6
8	OPE-04	GTGACATGCC	8	28	OPN-8	ACCTCAGCTC	7
9	OPE-05	TCAGGGAGGT	6	29	OPN-15	CAGCGACTGT	4
10	OPE-12	TTATCGCCCC	3	30	OPN-19	GTCCGTACTG	5
11	OPE-15	ACGCACAACC	7	31	OPO-1	GGCACGTAAG	2
12	OPF-01	ACGGATCCTG	7	32	OPO-5	CCCAGTCACT	8
13	OPF-02	GAGGATCCCT	6	33	OPO-8	CCTCCAGTGT	11
14	OPF-04	GGTGATCAGG	5	34	OPO-12	CAGTGCTGTG	5
15	OPF-06	GGGAATTCGG	7	35	OPO-11	GACAGGAGGT	7
16	OPF-13	GGCTGCAGAA	8	36	OPO-15	TGGCGTCCTT	9
17	OPF-15	CCAGTACTCC	5	37	OPO-19	GGTGCACGTT	9
18	OPF-16	GGAGTACTGG	7	38	OPO-20	ACACACGCTG	9
19	OPF-17	AACCCGGGAA	7	39	OPY-02	CATCGCCGCA	6
20	OPG-15	ACTGGGACTC	7	40	OPQ-14	GGACGCTTCA	7

Band scoring and analysis of data

The PCR products were run on 1.2% agarose gel stained with ethidium bromide. The amplified bands were scored on the basis of presence (1) or absence (0) to get total scorable fragments. Amplification profile of 12 horse breeds was compared with each other. Genetic similarity among horse breeds was calculated based on number of shared bands using following statistical equation as described by Nei and Li (1979).

$$F = 2N_{xy} / (N_x + N_y)$$

Where, F is the similarity coefficient in which N_x and N_y are the numbers of fragments in population x and y, respectively; whereas, N_{xy} is the fragment shared by the two populations. Similarity coefficients were utilized to generate a phylogenetic tree (Dendrogram) using Unweighted Pair Group Method of Arithmetic Means (UPGMA) (Sneath and Sokal, 1973).

RESULTS AND DISCUSSION

Condition optimization for RAPD analysis

In RAPD, PCR reaction conditions are crucial to get reproducible patterns. Therefore, in this study we optimized different conditions for PCR like concentrations of $MgCl_2$, *Taq* DNA polymerase and template DNA. The template DNA concentration of 10 ng, 3 mM $MgCl_2$ concentration, one unit of *Taq* DNA polymerase per 25 µL reaction were found to be optimal.

Primer screening and DNA polymorphism

Totally 40 RAPD primers were screened in 12 horse breeds. A total of 297 DNA fragments were generated by these primers with an average of 7.4 bands per primer. Out of 297 bands 57 bands were polymorphic, showing around 19.19% polymorphism. The rest of the bands were monomorphic among the breeds. Size and number of amplified product depended upon the sequences of the primers and template DNA. Reactions were duplicated from time to time depending upon the amount and consistency of amplification. Number of bands amplified by each primer is presented in Table II.

Bulked segregant analysis (BSA)

In the present study, each horse breed was reduced to one aliquot that contained the same quantity of the genomic DNA from available number of animals of that breed. Therefore, all analyses were conducted using one bulk representing each breed. Since bulk samples of DNA were used to produce RAPDs, it was expected to have number of amplified fragments owing to presence of diverse sequences with different degrees of homology with the primer. The studies have reported advantages and disadvantages of BSA earlier (Michelmore *et al*., 1991). BSA is a fast and feasible protocol but unable to detect within breed diversity. In this study, we used available number of samples from each breed making DNA bulks. Larger number of individuals in bulk present problems as it increases chances of addition of a heterogeneous individual to the bulk and makes difficult to identify all but the tightest linkage since one or two recombinant individuals may be found in each bulk (Warburton *et al*., 1996). Thus closer linkages are desirable, which greatly reduces the efficiency of BSA in identifying linkages.

Breed specific markers

One set of primers OPL-7 revealed a polymorphic band at 450 bp which was present in bulk of indigenous breeds but absent in exotic breeds (Fig. 1). To check the specificity of these three primers, they were screened in all individuals of indigenous breeds. Each primer amplified the same fragment in all animals except three individuals belonging to Morna breed. These three individuals were sampled from same location, implying that they may not be true animals of Morna breed as there was no pedigree available for indigenous breeds and animals were selected on the basis of phenotypic characteristics and breeder's opinion. Therefore it is believed that these three markers were specific to four Pakistani indigenous horse breeds. However, screening more number of samples from indigenous breeds can elucidate its implications.

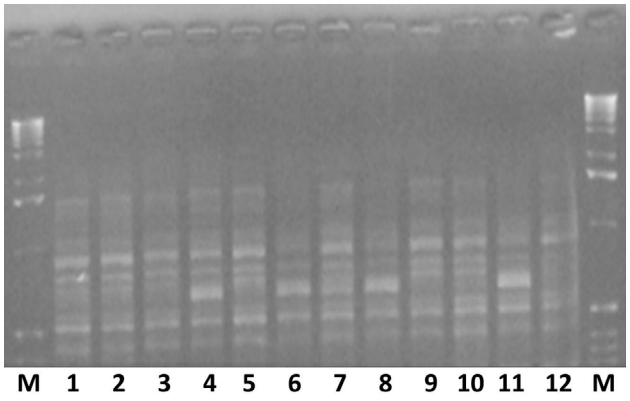

Fig. 1. Profile of PCR amplification of twelve horse breeds with primer OPL-7.

Phylogenetic relationship

Genetic similarity matrix of 12 horse breeds was generated based on RAPD data (Table III). The UPGMA dendrogram (Fig. 2) generated from similarity matrix showed that Suffolk Punch and Percheron were most closely related breeds (0.9846 similarity coefficient). The Percheron horse originated in the area known as

"Le Perche" in the northwest of France in 732 A.D. The Arabian blood was used in the formation of Percheron as Arabian horses abandoned by Moors after their defeat in the Battle of Tours were crossed with the massive Flemish stock and Percheron horses were derived from these crosses (Bongianni, 1988). Suffolk Punch is a breed of Great Britain and dates back to 1506. It was created with the contributions from Norfolk Trotter and the Norfolk Cob, with later contribution from English Thoroughbred (Bongianni, 1988). As Arab is a founder breed in the development of Thoroughbreds, so similarity among Suffolk Punch and Percheron is due to their common genes inherited from Arab breed. Similarly second group of breeds showing a relatively close relationship included Thoroughbred and Anglo Arab (0.983 similarity). This finding may be due to the fact that Anglo Arab is a crossbred of Thoroughbred and Arab breeds.

Both Suffolk Punch and Percheron showed a similarity at 0.9773 with Hanoverian breed. Hanoverian is a breed originated in West Germany (Hanover) and Thoroughbred blood was introduced for the first time into this breed in 1714 when a link was established between Germany and Britain in reign of King George I of England. Toward the end of the 19th century the introduction of Thoroughbred blood was stopped to avoid further modification of the breed that might lessen its suitability for its established use. However, with the advent of mechanization in agriculture and in general transport, the Hanoverian was forced to adapt new requirements, and new blood was once again introduced from English Thoroughbred, Arab and also Trakenhner (Bongianni, 1988).

Cleveland Bay and Anmol showed a similarity of 0.9763. Cleveland Bay was originated in Great Britain and is a light to medium riding horse for draft and farm work. While Anmol is a native horse of Pakistan which descended from horses brought by the Alexander the Great (Amir, 1997). This is almost extinct now due to crossbreeding with other breeds and lack of proper breeding policy for this breed. The close relationship between Cleveland and Anmol may be due to the fact that both have a common founder breed (Thoroughbred) and possible crossbreeding of two breeds during British regime in the subcontinent.

Next closely related group included Suffolk Punch, Percheron and Hanovarian breeds with Thoroughbred and Anglo Arab (0.9753 similarity). The seven breeds Percheron, Suffolk Punch, Hanovarian, AngloArab, Thoroughbred, Cleveland Bay and Anmol grouped together in cluster A. Arab and Noriker breeds showed similarities

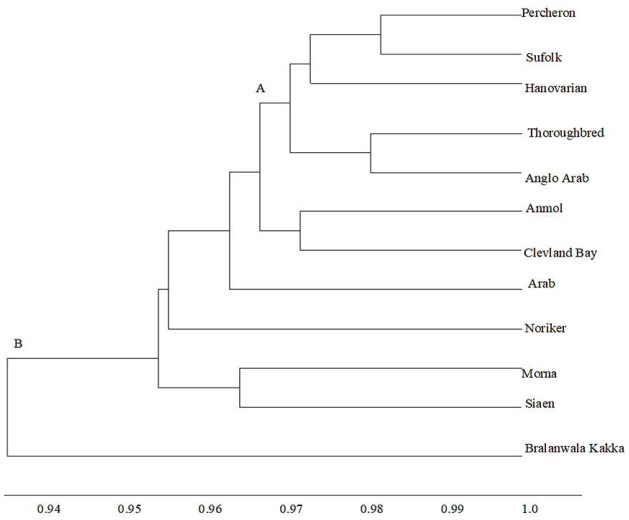

Fig. 2. Dendrogram among twelve horse breeds generated by RAPD data using UPGMA method.

Table III.- Similarity matrix of twelve horse breeds based on RAPD markers.

	Cleveland	Thorough-bred	Suffolk	Anmol	Hanoverian	Morna	Percheron	Siaen	Anglo Arab	Noriker	Kaka	Arab
Cleveland	1											
Thoroughbred	0.9730	1										
Suffolk	0.9754	0.9797	1									
Anmol	0.9763	0.9799	0.9726	1								
Hanoverian	0.9658	0.9702	0.9795	0.9630	1							
Morna	0.9533	0.9620	0.9641	0.9746	0.9556	1						
Percheron	0.9758	0.9845	0.9846	0.974	0.9752	0.9664	1					
Siaen	0.9404	0.95	0.9512	0.9623	0.9644	0.9677	0.9493	1				
Anglo Arab	0.9664	0.9830	0.9768	0.9622	0.9769	0.9550	0.9677	0.9466	1			
Noriker	0.9587	0.9602	0.9618	0.9643	0.9535	0.9581	0.9679	0.946	0.9694	1		
kaka	0.9135	0.9183	0.9181	0.9065	0.8743	0.9404	0.9233	0.9539	0.9181	0.9223	1	
Arab	0.9564	0.9731	0.9701	0.9689	0.9526	0.9609	0.98	0.9551	0.9654	0.9552	0.9438	1

up to 0.9645 and 0.9584 within cluster A, respectively. Noriker is a breed originated in Austria but also distributed in Germany and was modified in 19th century by the introduction of Norman, Cleveland, Holstein, Hungarian, Clydesdale and Oldenburg blood (Bongianni, 1988).

Cluster B revealed Morna and Siaen (Shiean) as most closely related breed with 0.9677 similarity. These are most common and famous indigenous breeds of Punjab province. Both breeds have a nearly common home tract being most commonly found in Jhang, Faisalabad and Okara districts of Punjab province. As there is no proper breeding policy for indigenous horse breeding in Pakistan, so crossbreeding is common and no attention is paid to maintain purity of a breed, as most breeders have no knowledge of selection. The third breed in cluster B is indigenous Bralanwala Kakka horse, which showed a 0.9357 similarity with both Morna and Shiean and rest of the breeds. This relationship of Pakistani indigenous breeds with other exotic breeds especially with Arab and Thoroughbred is in close agreement with earlier reports that most of indigenous horse breeds have had infusion of Arab and Persian blood primarily from Iran (Amir, 1997). Moreover in the past Thoroughbred stallions were extensively used to improve these indigenous breeds (Khan, 1969).

CONCLUSIONS

The genetic variation among all 12 horse breeds was very low as results of clustering analysis showed their very close relationships, in particular for the three most common Pakistani indigenous breeds *i.e.* Morna, Shiean and Bralanwala Kakka due probably to the decline in number of breeding animals and indiscriminate crossbreeding. At the mean time governmental departments have been trying to improve the local horses by the introduction of foreign blood especially Thoroughbreds. The outcome of present study can be helpful for formulating effective conservation strategies of the Pakistani indigenous horse breeds. However, modern genome-wide SNPs analysis based on genotyping array and/or high-throughput sequencing will provide a depth understanding of these important horse genetic resources in Pakistan.

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Statement of conflict of interest

Authors have declared no conflict of interest.

REFERENCES

Ali, B.A., 2003. Genetics similarity among four breeds of sheep in Egypt detected by random amplified polymorphic DNA markers. *Afri. J. Biotechnol*., **2**: 194-197. https://doi.org/10.5897/AJB2003.000-1040

Amir, P., 1997. *Oriental horses of Pakistan*. Asianics Agro-Dev. International, Islamabad, Pakistan.

Anonymous, 1998. *Imperial Gazetteer of India*. Provincial Series, Punjab Vol. 1. Sang-e-Meel Publications, 25 Shahrah-e-Pakistan, Lahore, Pakistan.

Bailey, E. and Lear, T.L., 1994. Comparison of Thoroughbreds and Arabian horses using RAPD markers. *Anim. Genet.,* **25**: 105-108. https://doi.org/10.1111/j.1365-2052.1994.tb00414.x

Black, W.C., DuTeau, N.M., Puterka, G.J., Nechols, J.R. and Pettorini, J.M., 1992. Use of the random amplified polymorphic DNA polymerase chain reaction (RAPD-PCR) to detect DNA polymorphisms in aphids (Homoptera: Aphididae). *Bull. entomol. Res*., **82**: 151-159. https://doi.org/10.1017/S0007485300051671

Bongianni, M., 1988. *The MacDonald encyclopedia of horses*. Macdonald & Co. Ltd., Hampstead Road, London, UK.

Caccone, A., Allegrucci, G., Fortunato, C. and Sbordoni, V., 1997. Genetic differentiation within the European sea bass (*Dicentrarchus labrax*) as revealed by RAPD-PCR assays. *J. Hered*., **88**: 316-324. https://doi.org/10.1093/oxfordjournals.jhered.a023109

Dawson, I.K., Chalmers, K.J., Waugh, R. and Powell, W., 1993. Detection and analysis of genetic variation in *Hordeum spontaneum* populations from Israel using RAPD markers. *Mol. Ecol*., **2**: 151-159. https://doi.org/10.1111/j.1365-294X.1993.tb00104.x

Grimberg, J., Nawoschik, S., Belluscio, L., McKee, R., Turck, A. and Eisenberg, A. 1989. A simple and efficient non-organic procedure for isolation of genomic DNA from blood. *Nucl. Acids Res*., **17**: 8390. https://doi.org/10.1093/nar/17.20.8390

Hadrys, H., Balick, M. and Schierwater, B., 1992. Applications of random amplified polymorphic DNA (RAPD) in molecular ecology. *Mol. Ecol*., **1**: 55-63. https://doi.org/10.1111/j.1365-294X.1992.tb00155.x

Khan, A.W., 1969. *Afzaaish-e-Havanat (in urdu)*. Markaz-e-Urdu Board, 89 Canal Road, Lahore, Pakistan.

Haig, S.M., Rhymer, J.M. and Heckel, D.G., 1994:

Population differentiation in randomly amplified polymorphic DNA of red-cockaded woodpeckers *Picoides borealis*. *Mol. Ecol*., **3**: 581-595. https://doi.org/10.1111/j.1365-294X.1994.tb00089.x

Loftus, R.T., Mchugh, D.E., Bradley, D.G., Sharp, P.M. and Cunningham, P., 1994. Evidence for two independent domestications of cattle. *Proc. natl. Acad. Sci. U.S.A*., **91**: 2757-2761. https://doi.org/10.1073/pnas.91.7.2757

Michelmore, R.W., Paran I. and Kesseli, R.V., 1991. Identification of markers linked to disease resistance genes by bulked segregant analysis: A rapid method to detect markers in specific genomic regions by using segregation populations. *Proc. natl. Acad. Sci. U.S.A*., **88**: 9828-9832. https://doi.org/10.1073/pnas.88.21.9828

Minhas R.A., Khan M.N., Awan M.S., Ahmad B., Bibi S.S., Hanif M. and Mian A., 2018. RAPD based genetic diversity of endangered himalayan gray langur (*Semnopithecus ajax*) populations of Pakistan. *Pakistan J. Zool.,* **50**: 2059-2071.

Nei, N. and Li, W.H., 1979. Mathematical model for studying genetic variation in terms of restriction endonucleases. *Proc. natl. Acad. Sci. U.S.A*., **76**: 5269-5273. https://doi.org/10.1073/pnas.76.10.5269

Signer, E., Kuenzle, C.C., Thomann, P.E. and Hubscher, U., 1988. DNA fingerprinting: Improved DNA extraction from small blood samples. *Nucl. Acids Res*., **16**: 7738. https://doi.org/10.1093/nar/16.15.7738

Warburton, M.L., Becerra-Velasquez, V.L., Goffreda, J.C. and Bliss, F.A., 1996. Utility of RAPD markers in identifying genetic linkages to genes of economic interest in peach. *Theor. appl. Genet*., **93**: 902-925. https://doi.org/10.1007/BF00224094

Sneath, P.H.A. and Sokal, R.R., 1973. *Numerical taxonomy*. Freeman, San Francisco.

Williams, J.G.K., Kubelik, A.R., Livak, K.J., Rafalski, J.A. and Tingey, S.V., 1990. DNA polymorphism amplified by arbitrary primers are useful as genetic markers. *Nucl. Acids Res*., **18**: 6531-6535. https://doi.org/10.1093/nar/18.22.6531

Williams, J.G.K., Hanafey, M.K., Rafalski, J.A. and Tingey, S.V., 1993. Genetic analysis using random amplified polymorphic DNA markers. *Method. Enzymol*., **218**: 704-740. https://doi.org/10.1016/0076-6879(93)18053-F

Yeh, F.C., Chong, D.K.X. and Yang, R.C., 1995. RAPD variation within and among natural populations of trembling aspen *(Populus tremuloides* Michx.) from Alberta. *J. Hered*., **86**: 454-460. https://doi.org/10.1093/oxfordjournals.jhered.a111620

Zafrakas, A., 1991. *The equine and its feeding (in Greek)*. Kyriakidis, Thessaloniki, pp. 200.