Genetic analysis of Sperata sarwari (Singharee) population fragmentized by Physical barriers in the Indus drainage system of Punjab, Pakistan.

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Abstract

Physical barriers like head works, dams barrages are main cause of fragmentation and declining of freshwater fish population in natural habitat. Present study focused on RAPD marker technique to assess the genetic variability among and between the populations of endangered *sperata sarwari* (Singharee) inhabited in the Indus drainage system of Punjab. Total eight populations (80 speciemen) of *S. sarwari* were collected from the up and downstream of four Rivers (Chenab, Jhelum, Ravi and Indus) of Punjab. Genomic DNA isolated from muscle tissue of adipose side and ten RAPD marker were used, which produced 50 scorable bands with average band ranging from 250–1050 bp which used for further genetic analysis of *S. sarwari*. Downstream Indus population of *S. sarwari* showed highest values of observed alleles (na), effected alleles (ne), Nei's diversity (h), Shannon index (I) and polymorphism which indicated that downstream Indus population was more genetically variant. The genetic variability (0.5124) and genetic flow (0.4758) among the eight population *S. sawari* was observed. The up and downstream population showed the highest genetic distance (0.5738) and lowest genetic similarities (0.5634) which indicated complete isolation of Ravi population from other six population. Dendrogram showed that up and downstream Ravi population was completely isolated from the other six up and down stream population of *S. sarwai.* Overall results indicated that the presence of high fragmentation in River Ravi caused the destruction of habitat and decline in population of *S. sarwai* in the River Ravi.

Introduction

Rivers are complex systems which provides natural habitat to fish species. Fishes show different tolerance level to environmental stressor's that enables fish to survive in hostile condition of the environment (Cooke et al. 2013). Most of the species are considered to be endangered due to the declining of their natural habitat and also reduced in numbers and sizes of populations. The crucial factors responsible for the decline in the number and size of different fish population involve alternation of climate and habitat, pollution, dam construction, fragmentation, eutrophication, replacement of invasive or intentionally introduced species, over-fishing, the aquarium pet trade, river flow modification, and even the consideration of the most optimistic climate change scenario points to the likelihood of *ex-situ* management of many species for their survival (Jeong et al. 2012;Kelly et al. 2013; Reis 2013; Gupta and Homechudhuri 2015; Gallardo et al. 2016, Pauly and Zeller 2016; Medeiros et al., 2016; Gold et al., 2017; Santos et al., 2017; Filho et al. 2018; Martinz et al. 2018; Mahboob et al. 2019; Lopera-Barrero et al. 2019).

Population genetics shows distribution of genetic variability that is affected by natural selection, mutation, migration, population size and genetic drift not only influenced the genetic variations (Hansen 2003; Xia et al. 2014, 2015; Liu et al. 2019) and diversity of the population but was critical to the conservation of species. The genetic variation can be detected by the morphological parameter which was frequently concealed by the environmental factors and infrequency of observable morphological parameters reduced the genetic variability (Hedrick 2005; Mix et al. 2006). The native extinction was high in small fragmentized populations due to loss of genetic diversity and interruption of genetic drift and inbreeding within and among populations (Maskur 2002; Ruzafa et al. 2006; Kahl et al. 2008; Syandri et al. 2013; Coleman et al. 2017). Liu et al. (2019) reported that habitat fragmentation is one of the main cause of reduction in biodiversity of aquatic organism like freshwater mussel and dams had adverse effect on aquatic habitat, population genetics and fish communities and other aquatic animals (Cheng et al, 2013; Cheng et al. 2018; Morita and Yamamoto 2002; Roberts et al. 2013; Terborgh et al., 2001; Wu et al. 2003)

Ferguson et al. (2019) reported the declining of *S.trutta* (sea trout) due to the presences of barriers such as hydroelectric dams which caused the reproductive and genetic isolation of *S.trutta* population. Due to the development of PCR technique (Ferguson et al. 1995), molecular markers such as SNPs (Single Nucleotide Polymorphism), RAPD (Random Amplified Polymorphic DNA) and SSR (Short Sequence repeat or microsatellite) used directly to identify genetic diversity and distribution of the population. (Duran et al. 2009; Pujolar et al. 2009). Genetic information used to determine population structure, size (Luikart et al. 2010; Waples and Do 2010; Ahmad et al. 2012; Mojekwu et al. 2012), stability or declining in population and the relative time of declining in population (Williamson-Natesan 2005). It provides the information about the

patterns of individual migration (DeHaan et al. 2011; Vollestad et al. 2012) and gene flow among populations. Genetic data can also be imported to report the biology and demographic status of a species (Smith et al. 2011; Homola et al. 2012).

RAPD and SSR markers widely used (Liu and Cordes 2004; Muneeret al. 2011) in several different stduies (Ramanadevi and Tharngaraj 2014; Achrem et al. 2015) for different fish population or species RAPD maker used not only for molecular characterization, identification, genetic diversity, genetic verability Genetic technologies have successfully been applied in species identification, studying the phylogenetic structure, conservation, monitoring fisheries and enhancement operations (Muneeret al., 2011; Usman et al. 2013; Asagbra et al. 2014; Vasave et al. 2014; Marimuthu et al. 2015; Kabir et al. 2017; Amit and Preeti 2020; Miah et al. 2020). Furthermore, genetic data have provided useful insight in setting up conservation priorities for many imperiled species (Lal et al. 2006; Muneeret al. 2011; Carison et al. 2015). Genetic variability provides the vital information to evaluate the endangered fish stocks.

In a given environment, the genetic diversity among different populations, either endemic or recent invasion can be detected through phylogenetic reconstruction approach using RAPD and SSR markers. Numerous studies has provided literature regarding the molecular diversity of wild populations of many fish species in the regions (Barman et al. 2003; Islam and Alam 2004; Lal et al. 2006; Sivaraman et al. 2010).

The status of *S. sarwari* is nearly "endangered" due to the declining stock in natural waters due to overfishing, pollution and low migration between population through dams and barrages. Keeping in view of the importance of *S. sarwari*, there is an urgent need to conserve *S. sarwari* in natural water bodies of Pakistan. In Pakistan, limited information is available on the meristic and morphometric characteristics as well as on the genetic diversity of this important fish. The study hypothesized that declining of genetic diversity in different populations of *S. sarwari*due to low level of migration flow, caused the isolation and inbreeding depression. Therefore, the present study was designed to estimate and compare the genetic varitaions between up and downstream population, and also assess the extent of migration flow between riverine population.

Material And Methods

Total 80 specimens of *Sperata sarwari* were collected during the year of 2016-2017 from up and downstream locations of four rivers of Punjab (Chenab, Jhelum, Ravi and Indus) with average body weight 305±0.5 and length 37±0.5 detail showed in Table 1 and fig 1. The Specimens were transferred to Molecular Research Lab of the Department of Zoology, Government college University Faisalabad, Pakistan in ice containing Polythene bag for molecular analysis.

DNA Extraction

Genomic DNA of 54 specimens was extracted by using Genomic DNA isolation kit (Favorgen FATGK-001). Fish samples were weighed up to 25 mg and ground in liquid nitrogen, then transferred into new micro-centrifuge (Sigma, D37520) tube. Added 200 µL FATG 1 buffer and mixed very well by micro pestle or pipette tip. Added 20 µL Proteinase K (10mg/ml) to the sample mixture and mixed through vortex. Incubated the sample at 60^0C until the tissue was lysed completely. Vortex (BV1000) for 10-15 minutes during incubation to break up the tissue sample. Briefly spin the tube to remove drops from the inside of the lid. Added 4µL of 100 mg/ml RNase and incubated for 2 mint at room temperature. Added 200 µL FAGT 2 buffer to the sample mixture. Mixed thoroughly by pluse-vortexing (BV1000) and incubated at 70 ^{0}C for 10 minutes. Briefly spined the tube to remove drops from the lid. Added 200 µL ethanol (96-100%) to the sample and mixed thoroughly by pulse- voterxing (BV 1000). Briefly spined the tube to remove drops from the lid. Placed FATG mini column in collection tube and transfer the mixture to mini column tube. Centrifuged (Sigma, D37520) for 1 minute, then placed FATG mini column tube to new collection tube. Washed the mini column tube with 500 µL with W1 buffer by Centrifuge (Sigma, D37520) for 1minute then discard the follow through. Washed mini column tube with 750 µL wash buffer by centrifuged for 1 minute then discard the follow through. Centrifuged (Sigma, D37520) for an additional 3 mint to dry column. Placed the mini column tube in Elution tube and added 50-200 µL elution buffer or ddH_2O to the center of membrane at mini column and stand FATG mini column tube

for three minutes. Centrifuged (Sigma, D37520) for 2 minutes to elute DNA. Stored the DNA at 4 ^{0}C or -20 ^{0}C for quantification and PCR amplification.

Random Amplified Polymorphic DNA and PCR reaction

To assess the genetic variations in *S. sarwari* population, the condition of polymerase chain reaction (PCR) was optimized by using the Random Amplified Polymorphic DNA (RAPD) markers. Ten RAPD markers were selected from the genomic Library on the basis of GC contents (%) and band reproducibility for the amplification of genomic DNA in *S. sarwari* (NCBI;www.genelink.com; Table 1) and were named as SS Makers.

DNA, 2.5 mm mixed dNTPs, 2.5 uL 10X Taq DNA polymerase buffer, 2.5 uL 25 mm $MgCl_2$, 0.5 ul each primer, 0.5 uL Taq DNA polymerase, that was gently mixed. PCR reaction was as follows: 95 ^{o}C for 3 min, 95^{o}C for 30 sec, 45^{o}C for 30 sec, 72^{o}C for 1min, 35 cycles, followed by a 10 min final extension at the 72 ^{o}C. The detail of concentration of reagents that were applied for the optimization of genetic markers is given in Table 4. PCR profile for amplification of DNA sample was optimized by up and down range of annealing temperature. The PCR optimization conditions of denaturation occurred in two steps, primer annealing, primer extension, final extension and final amplified product of RAPD markers. The PCR optimized pattern is given in Table 3. 1.5% agarose gel was used for resolution of resolution of different band patterns. Amplified fragments of each marker were observed under the UV transilluminator and the photograph was taken through the gel documentation system (Syngene, 2000) for further genetic analyses.

Genetic Analysis

RAPD markers amplified different patterns of genome in *S. sarwari*, examined under the UV transluminator and photographed using Gel documentation (Syngene, 2000). Molecular analysis program of gel documentation system was applied for scoring the amplified locus size of individual genome. These loci patterns of different individuals of *S. sarwari* population were analyzed by molecular software program POPGENE ver16/32and Gen AlEx 6.4 for estimation of genetic diversity in *S. sarwari.*

Results

Genomic Amplification

Total ten RAPD (SS) primer were used to analyze the genetic diversity of endangered species *S. sarwari* and 50 scroable band generated by these primers. The band size ranged from 250-1050bp and showed 37 polymorphic amplified band with 74.0% polymorphism among the eight different populations of *S. sarwari.*The amplification of RAPD primers of different size of loci in all populations of *S. sarwari* collected from various up and downstream of different river of Punjab indicated the variation in allelic frequencies (Table 3).

Genomic diversity among population

The values of genetic indices observed (na) effected (ne) alleles, Nei's diversity, Shannon Index and polymorphism of 80 individuals of eight up and downstream populations of *S. sarwari* collected from different rivers (i.e., River Chenab, Jhelum, Ravi and Indus) of the Punjab analyzed by the 10 RAPD (SS) Primers which showed the lowest polymorphism (2%), na (1.02), ne (1.02), H (0.010,0.008) and I (0.032, 0.001) in up and down stream Ravi populations while the other up and down stream population of Chenab, Jhelum and Indus populations of *S. sarwari* (Table 4). The polymorphism of up and downstream populations of Chenab, Jhelum, Ravi and upstream population of Indus was less than 60% which showed that the level of intra population genetic variation was very low and level of inbreeding was high within populations. Every population of up and downstream formed its own isolated population, which decrease the genetic flow. The downstream population of Indus with highest polymorphism (62%) indicated the high genetic variations (Table 4).

The genetic diversity content such as heterozygosity (Ht), diversity (Hs), Genetic variation (Gst) and genetic flow (Nm) between the populations was used in the present study to detect population structure. The heterozygosity (Ht, 0.3574), intra population diversity (Hs, 0.1743), inter population genetic variations (Gst, 0.5124) and genetic flow (Nm, 0.4758)in eight populations of *S. sarwari*. The genetic variability higher and low genetic flow observed between eight populations of *S. sarwari*(Table 5).

Genetic Distance and Genetic Identity

The genetic distance found between the up and the downstream population of *S. sarwari* ranged from 0.0013 to 0.5738 and genetic similarities between up and downstream populations of *S. sarwari* ranged from 0.5634 to 0.9987. Both up and downstream Ravi populations were completely isolated due to large genetic distance (0.5738) and the lowest genetic similarities (0.5634) from the other six up and down stream populations of *S. sarwari*.

Dendrogram

Cluster analysis was done on the basis of RAPD genetic distances through POPGENE 32 among and between the various up and downstream populations of *S. sarwari*. The 1st group consists of the upstream and the downstream population of the River Ravi (5,6) which showed the complete isolation of these two populations due to large genetic distance from the other populations, 2nd group composed of the upstream and the downstream population of the River Chenab (1, 2), whereas 3rd group formed by the upstream and the downstream population of the River Jhelum and the River Indus. The 3rd group was subdivided into 3 subgroups, the 1st sub group was the combination of the up and downstream population of the River Jhelum and the River Indus (3,8) and 2nd and 3rd subgroups were developed by the downstream of the River Jhelum (4) and upstream of the River Indus (7) (Fig. 1).

Discussion

Genome amplification is necessary for the genetic analysis, such as genetic diversity, heterozygosity, gene flow between populations and genetic diversity among and between the populations. Ten RAPD primers were used to amplify eight populations of *S. sarwari* collected from the various up and downstream areas of the four rivers (i.e., River Chenab, Jhelum, Ravi and Indus). Ten RAPD primers generated 50 scorble bands with an average range from 250–1050 bp. The spacious range of the band size was comparable with previous studies, where 5 RAPD markers produced 45 amplicon, (250–2000 bp) in *Eutropiichthys vacha*population (Chandra et al. 2010). The six RAPD primer produced 48 and 44 scorable bands in tilapia (Mahboob et al. 2019; Khafaji et al. 2019), 16 RAPD markers generate 197 bands ranged from 114–2000 bp in six golden mahseer populations (Shafi et al. 2016) and 12 RAPD primers produced 87 bands, amplified band ranged from 400–1250 bp in four Rohu populations (Kabir et al. 2017). Moreover the present studies was corelated with the previous studies Vasave et al. 2014 (rainbow trout and snow trout), Hasan and Goswami 2015 (cat fish (*Mystus vittatus)*), and six RAPD prime produced 89 and 60 amplified bands with 50-1500 bp in genetic analysis of *Capoeta copeta gracilis* and *piracanjuba* fish population (; Lopera-Barrero et al. 2019) respectively. The result revealed that the genetic measures (polymorphism, observed alleles, effected alleles, gene diversity and Shannon index) of downstream Indus population (na 1.60, ne 1.44, h 0.250, I 0,367) was high as compared to other up and downstream population of *S. sarwari.* The lowest value of na (1.02), ne (1.02), h (0.010, 0.008) and I (0.032, 0.001) was observed in both up and downstream Ravi populations of *S. sarwari.* These results are in line with the isolated up and downstream populations of *Capoeta copeta gracilis*,in which the genetic measure reliably higher in downstream population of *Capoeta copeta gracilis* (Hossein et al. 2013). The result showed that the average polymorphism (47%) of eight populations of *S. sarwari* was less than 60%. The pervious results of polymorphism were also related to the present study. The average 46.81% polymorphism was observed in walking catfish (*C. batrachus*) (Miah et al. 2020). The average 48.38% polymorphism was observed in four populations of Rohu collected from different geographical areas (Kabir et al. 2017), polymorphism in Rohu population of 47.89% (Fayyaz et al. 2014), 45% (Barman et al. 2003), and 46.5% (Islam & Alam, 2004). The polymorphism ranged from 2–62% in all populations of *S. sarwari* collected from the up and downstream of selected rivers, i.e., River Chenab, Jhelum, Ravi and Indus. The average polymorphism of eight populations of *S.sarwari*

was less than 60%, which indicated the low genetic variability among eight populations and the present study is similar to the previous study of Kabir et al. (2017) in four populations of Rohu sampled from different geographical regions.

The total mean values of na, ne, h and I was 1.42, 1.31, 0.174 and 0.255 which was moer or less similar to pervious study of Shafi et al. (2016) in six population of golden mahseer inhabited in different geographical region. The total mean value of Nei's gene diversity and Shannon Index in present research work was less than 0.5 which indicated that there was less genetic variability and inbreeding coefficient was high (Shafi et al. 2016). The present study was in agreement with the previous study on golden mahseer, where the average values of Nei's gene diversity and Shannon Index was very low (Shafi et al. 2016). The heterozygosity (Ht), diversity (Hs), genetic variation (Gst) and genetic flow (Nm) ranged from 0.101–0.478, 0.052–0.273, 0.282–0.743 and 0.178–1.670, respectively for eight populations of *S. sarwari* (Table 4). The total heterozygosity (Ht 0.3574), gene diversity (Hs 0.1743), genetic variation (Gst 0.5124) and gene flow (Nm 0.4758) was observed in eight population of *S. sarwari.* Vasave et al. (2014) found relatively similar results in rainbow trout and snow trout. He observed high value of genetic variation (Gst 0.6835) with low gene flow (0.2316) among populations of rainbow trout and snow trout. The present study was in line with the previous studies for genetic variation that was found in tilapia species (Gst = 0.583) (Heba et al.2013), *Capoeta capoeta gracilis* (Hossein et al. 2013), *Elops machnata* (Ramandaevi and Thangaraj 2014) and in walking catfish (*C. bartrachus*) (Gst = 0.19823) (Miah et al. 2020). Due to natural barriers the freshwater fish showed low migration rate and higher genetic variation (Rodriguez et al. 2007; Kusmini et al. 2011).

The genetic distance and similarity was observed among and between the eight populations ranged from 0.0496 to 0.5378 and 0.5634 to 0.9987, respectively (Table 5). The above findings are comparable with the previous studies on other fish species. Hossein et al. (2013) also reported that the genetic distance between two up and downstream populations of *Capoeta capoeta gracilis*ranged from 0.1455 to 0.7382 and observed that the two populations were isolated from each other. In the present study, eight populations of *S. sarwari* collected from the up and downstream of the different rivers of the Punjab also showed the segregation among and between the populations. The isolation of populations was reflected by the genetic distances and complicated mechanism of genetic flow (Rana et al. 2004). Low migration of fish significantly affected the fish population of the up and downstream region and causes the reduction in genetic exchange among two populations (McAllister et al., 2001). Furthermore, the dams and barrages extend to striking changes in aquatic environment which directly affect fish populations (Craig 2000). The genetic variations found in present study were also studied and observed in the up and downstream populations of *C. c. gracilis*, which were also the cause of barriers established by dams and barrages in rivers. These barriers were geographically segregated from each other because there was no chance for gene flow among and between populations of *C. c. gracilis* (Hossein et al. 2013). Barriers effect on the gene flow and caused the isolation of up and downstream population with high genetic variation and fragmentation interacted with genetic diversity of freshwater fish populations (Van Leeuwen et al. 2017). Our study investigated the lowest level of genetic flow (0.178–1.670) between the up and downstream of dams and barrages populations of *S. sawari*, ultimately causing the increase of genetic variation (population divergence) towards the highest level of inbreeding depression. However, the populations of *S. sarwari* above and below dams/barrages showed a significant higher level of genetic difference, which is the indication of isolation in populations. This study also reported that the higher inbreeding coefficient caused the declining of *S. sarwari.*

Conclusion

In this study it was concluded that the genetic analysis of *Sperata sarwari* sampled from different up and downstream populations of four rivers (i-e Chenab, Jhelum, Ravi and Indus) is main source to observed genetic difference between the populations. The physical barriers cause the isolation of *S.sawari* and it also showed that Ravi population was completely isolated due to presence of high genetic variability and low gene flow between populations. The highest genetic distance and lowest genetic similarity was assess between both up and downstream Ravi population and other up and downstream riverine populations.

Declarations

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Authors contribution

Concept and design of study was carried out by Dr. Farhat Jabeen and Dr. Inayat Ullah Malik, data acquisition and data analysis by Dr. Asma Aziz, Dr. Farhat Jabeen, and Dr. Inayat Ullah Malik. Fish Sampling was done with the help of Zahid Sahrif Mirza, Muhammad Ahmad Raza, Sami Ullah Bhati and Noor Afshan. The final draft was prepared Dr. Asma Aziz and reviewed by Dr Farhat Jabeen, Mr. Muhammad Nafees and Adiba Khan Sehrish.

Declaration

We declare that this manuscript is an original research, has been written by us and has not been submitted in any other journal for publication. The experimental work is almost entirely our own work; the collaborative contributions have been clearly indicated. It has been declared that all authors are agreed to submit in this journal.

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Tables

Table 1. Different populations of *S. sarwari* collected from the upstream and downstream of four rivers (River Chenab, Ravi, Jhelum and Indus).

Sr. No	Location	Sites	Sample size
1	River Chenab	Tarimue Head Upstream	10
		Downstream	10
2	River Ravi	Head Balloki Upstream	10
		Downstream	10
3	River Jhelum	Head Rasool Upstream	10
		Downstream	10
4	Indus river	Chashma Barrage Upstream	10
		Downstream	10
Total			80

Table 2. List of RAPD primers used for the amplification of genome pattern in RAPD markers.

Sr. No	Primer Sequence	% of GC contents
1	5\ CTGCTGGGAC 3\	70%
2	5\ CATCCCCCTG 3\	70%
3	5\ TGGACCGGTG3\	70%
4	5\ TCTGGTCAGG 3\	60%
5	5\ GTCGCCGTCA 3\	70%
6	5\ GGGTAACGCC 3\	70%
7	5\ AGCCAGCGAA3\	60%
8	5\ TGCCGAGCTG 3\	70%
9	5\ GGTGACGCAG 3\	70%
10	5\ GGTCCCTGAC 3\	70%

Table 3. Description of amplicons in genomic DNA with polymorphism, gene pool frequency and range size of 10 primers on different population of *S. sarwari* collected from different rivers of the Punjab, Pakistan.

RAPD Markers	No. of Amplicon	No. of Polymorphic bands	Polymorphism (%)	Gene pool (%)	Loci Range bp
SS1	5	4	80	10	300-850
SS2	4	4	100	8	300-850
SS3	5	3	60	10	300-850
SS4	4	3	75	8	300-850
SS5	3	2	67	6	300-650
SS6	7	5	71	14	250-1050
SS7	6	4	67	12	300-1050
SS8	5	4	80	10	300-800
SS9	7	5	71	14	250-1050
SS10	4	3	75	8	300-1050
Total	50	37	74	100	250-1050

Table 4. Description of genetic indices; observed alleles (na), effected alleles (ne), Nei's diversity (h) and Shannon Index (I) of the upstream (US) and the downstream (DS) population of *S. sarwari* from four different rivers of the Punjab, Pakistan.

Riverine Population	Polymorphism	na	ne	h	I
US Chenab	58	1.56	1.44	0.240	0.346
DS Chenab	56	1.56	1.44	0.240	0.346
US Jhelum	54	1.54	1.37	0.214	0.315
DS Jhelum	52	1.52	1.42	0.231	0.331
US Ravi	2	1.02	1.02	0.010	0.032
DS Ravi	2	1.02	1.02	0.008	0.001
US Indus	56	1.56	1.33	0.202	0.303
DS Indus	62	1.60	1.44	0.250	0.367
Mean		1.42	1.31	0.174	0.255

Table 5. Estimation of Genetic variations (Hetrozygosity among population= Ht; Diversity within population= Hs, Genetic variations among populations= Gst and Genetic flow= Nm) among and between the eight populations of *S. Sarwari* collected from different upstream and downstream of the four rivers of the Punjab, Pakistan.

RAPD Primer	Ht	Hs	Gst	Nm*
SS1	0.226	0.097	0.485	0.960
SS2	0.101	0.052	0.432	0.822
SS3	0.401	0.177	0.542	0.706
SS4	0.469	0.127	0.7364	0.247
SS5	0.422	0.088	0.743	0.178
SS6	0.478	0.269	0.434	0.771
SS7	0.241	0.114	0.467	0.968
SS8	0.275	0.193	0.282	1.670
SS9	0.467	0.273	0.427	0.904
SS10	0.439	0.236	0.488	1.069
Mean	0.3574	0.1743	0.5124	0.4758

Table 6. Genetic distance (d) and genetic similarity (S) among eight populations of *S. sarwari* collected from the upstream (US) and the downstream (DS) of the River Chenab (C), Jhelum (J), Ravi (R) and Indus (I) based on the construction of Phylogenetic relationships by using POPGENE 32.

	Population ID	1 (USC)	2 (DSC)	3 (USJ)	4 (DSJ)	5 (USR)	6 (DSR)	7 (USI)	8 (DSI)	
Genetic Distance (d)= 0.0013-0.5738	1 (USC)	****	0.9516	0.8211	0.7744	0.6202	0.6137	0.7949	0.8432	Genetic Similarity (S)=0.5634-0.9987
	2 (DSC)	0.0496	****	0.794	0.7849	0.6202	0.6137	0.8463	0.8348	
	3 (USJ)	0.1971	0.2307	****	0.9575	0.5698	0.5634	0.9274	0.9670	
	4 (DSJ)	0.2556	0.2422	0.0434	****	0.5769	0.5705	0.9343	0.9303	
	5 (USR)	0.4777	0.4777	0.5625	0.5501	****	0.9987	0.5736	0.5963	
	6 (DSR)	0.4883	0.4883	0.5738	0.5613	0.0013	****	0.5673	0.5898	
	7 (USI)	0.2295	0.1669	0.0754	0.068	0.5557	0.5669	****	0.9302	
	8 (DSI)	0.1705	0.1806	0.0335	0.0722	0.5169	0.528	0.0724	****	

Figures

Figure 1

Specimen of Sperata sarwari collected from different up and downstream regions of four Rivers (Chenab, Jhelum, Ravi and Indus) of Punjab.

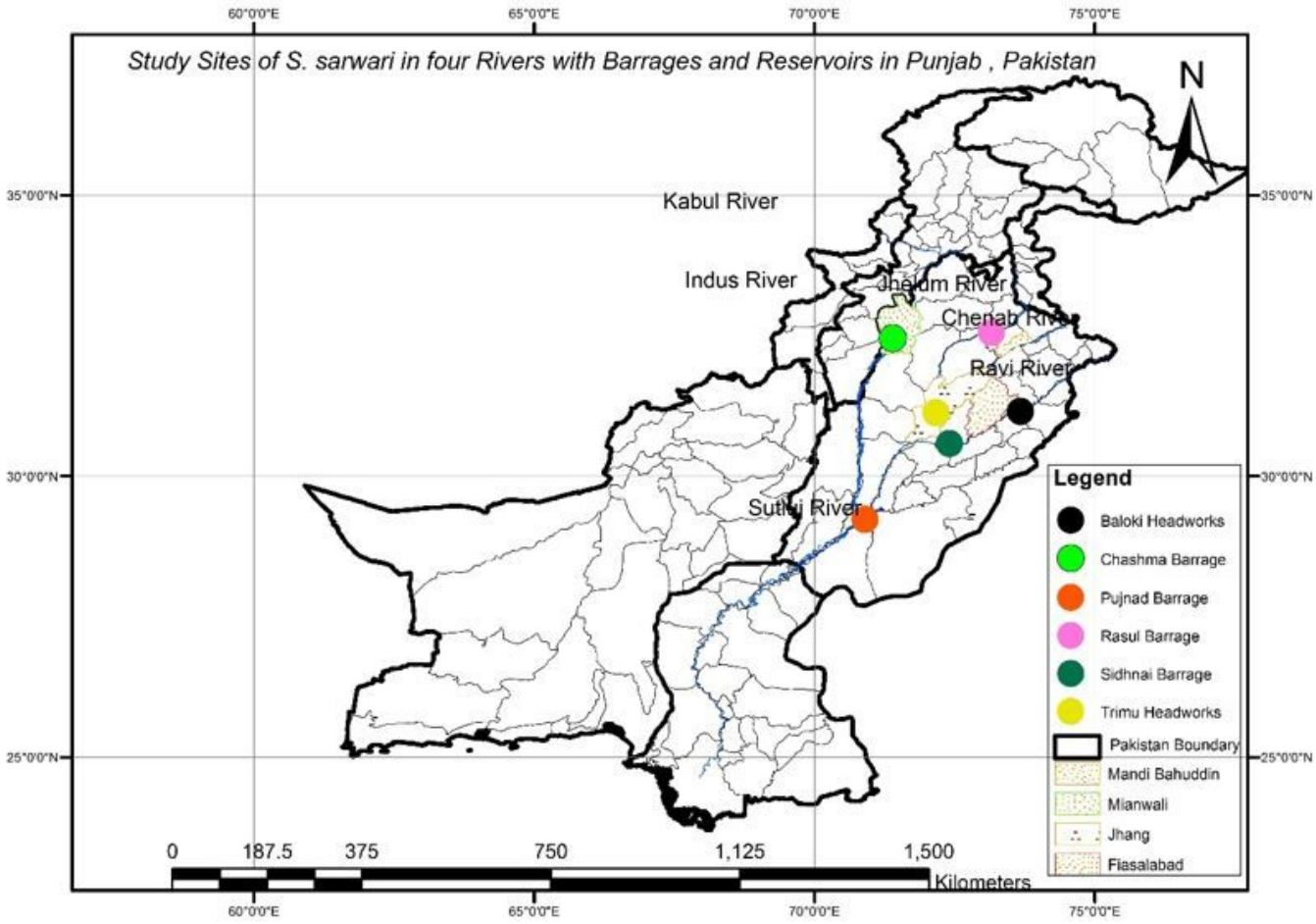

Figure 2

The map of Sample collection. Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.

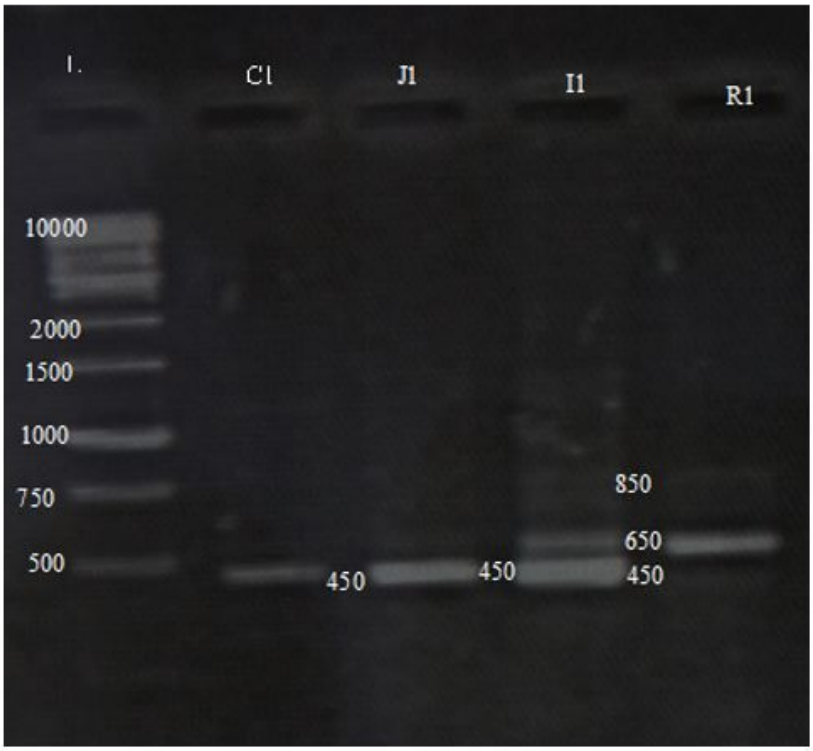

Figure 3

The amplification of 1SS (RAPD) Primer in four different riverine population, 1 Ladder; 2 River Chenab specimen; 3 River Jhelum; 4 River Indus and 5 River Ravi

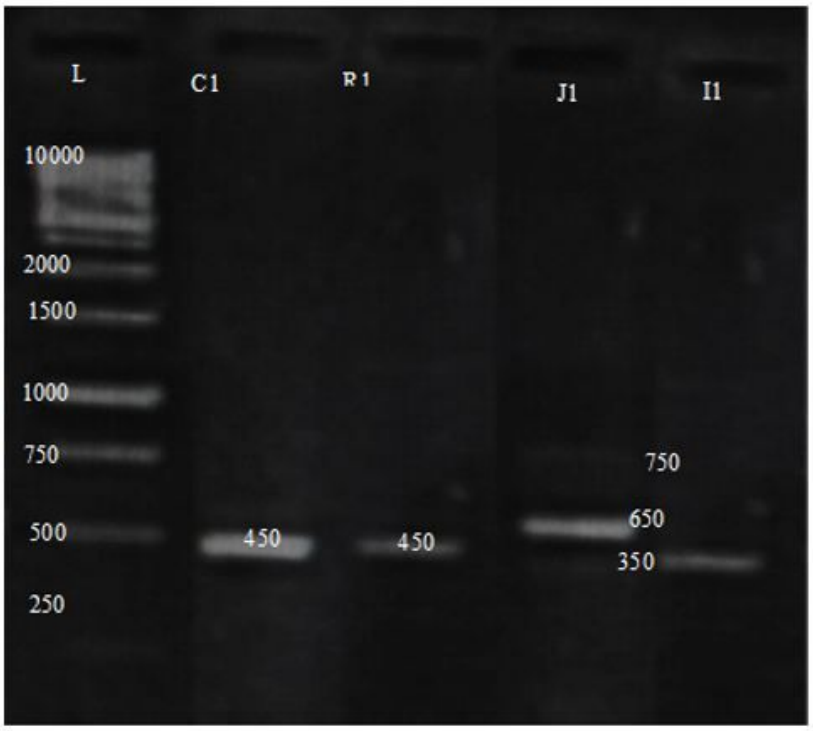

Figure 4

The amplification of 2SS (RAPD) primer in four different riverine population 1 marker; 2 River Chenab specimen; 3 River Ravi; 4 River Jhelum and 5 River Indus.

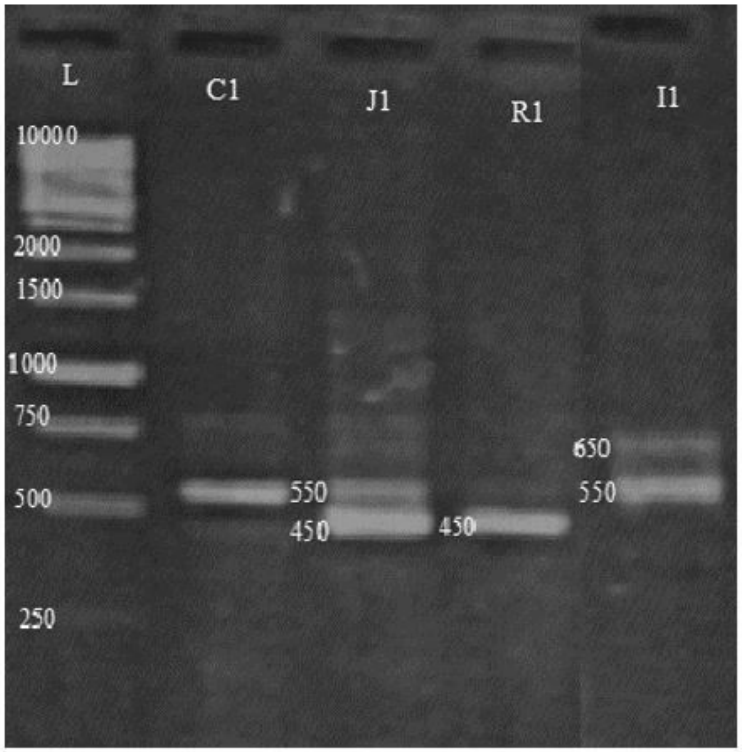

Figure 5

The amplification of 3SS (RAPD) primer in four different riverine populations 1 Ladder; 2 River Chenab specimen; 3 River Jhelum; 4 River Ravi and 5 River Indus.

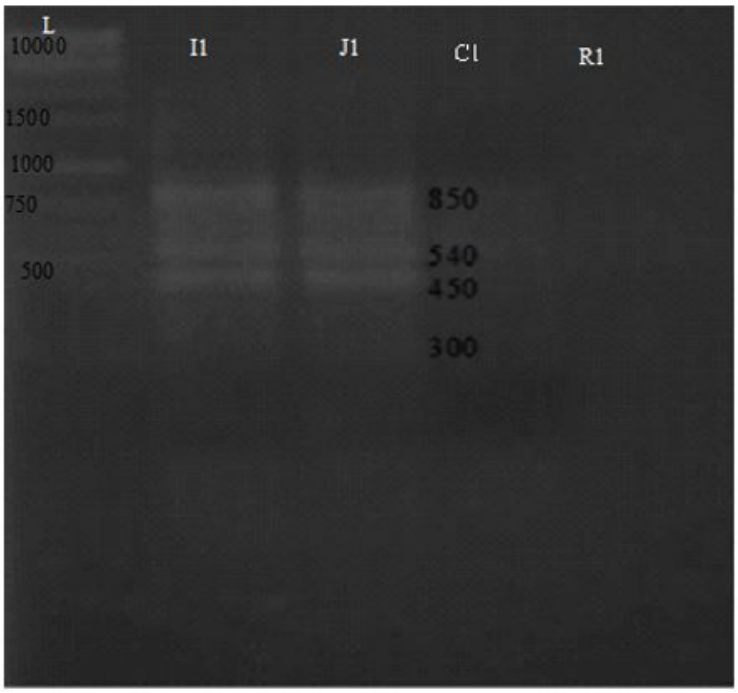

Figure 6

The amplification of 4SS (RAPD) primer in four different riverine population 1 Ladder; 2 River Chenab specimen; 3 River Ravi; 4 River Jhelum and 5 River Indus.

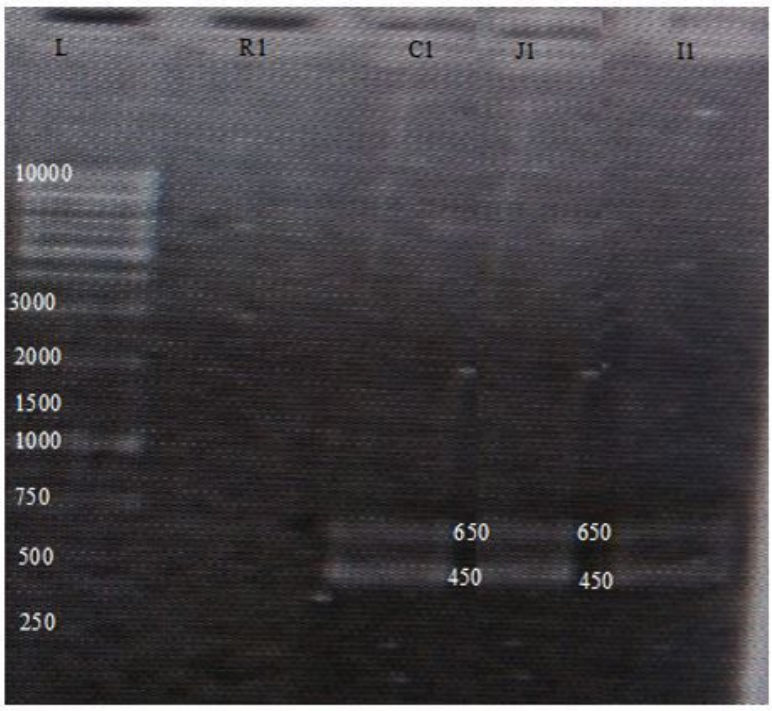

Figure 7

The amplification of 5 SS (RAPD) primer in four different riverine populations 1 marker; 2 River Ravi specimen; 3 River Chenab; 4 River Jhelum and 5 River Indus.

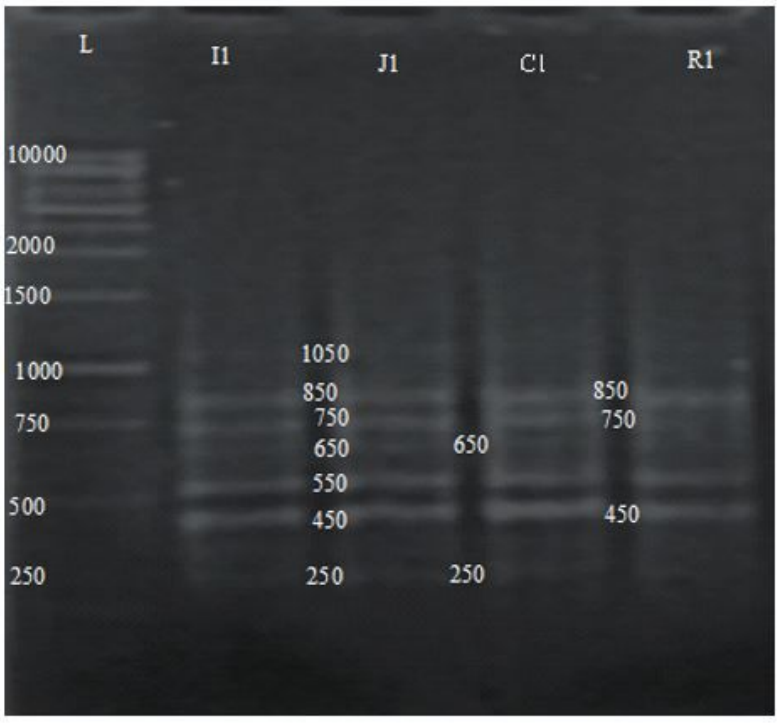

Figure 8

The amplification of 6SS (RAPD) primer in 4 different riverine population 1 Ladder; 2 River Ravi specimen; 3 River Jhelum; 4 River Indus and 5 River Ravi.

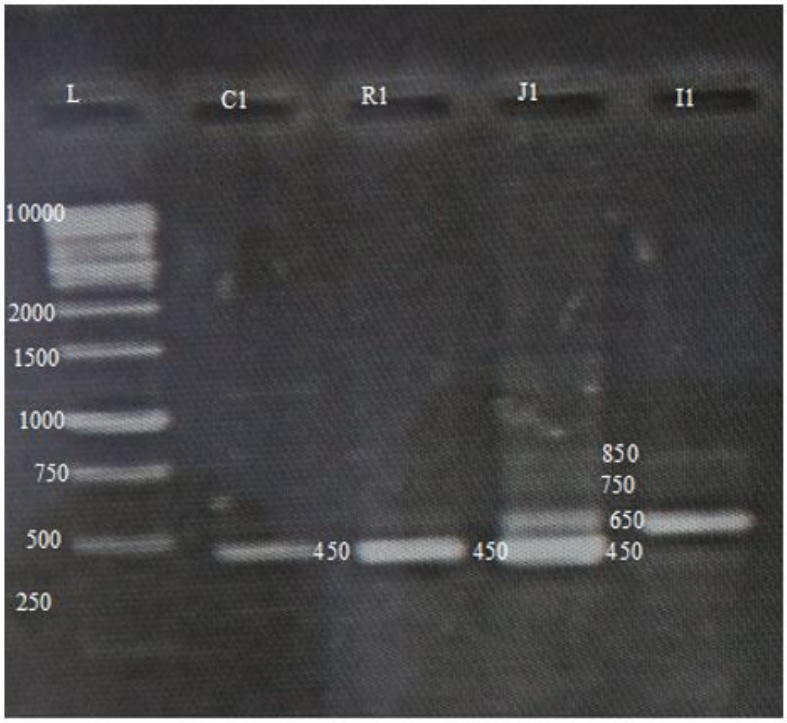

Figure 9

The amplification of 7 SS RAPD primer in 4 different riverine population 1 marker; 2 River Chenab specimen; 3 River Ravi; 4 River Jhelum and 5 River Indus.

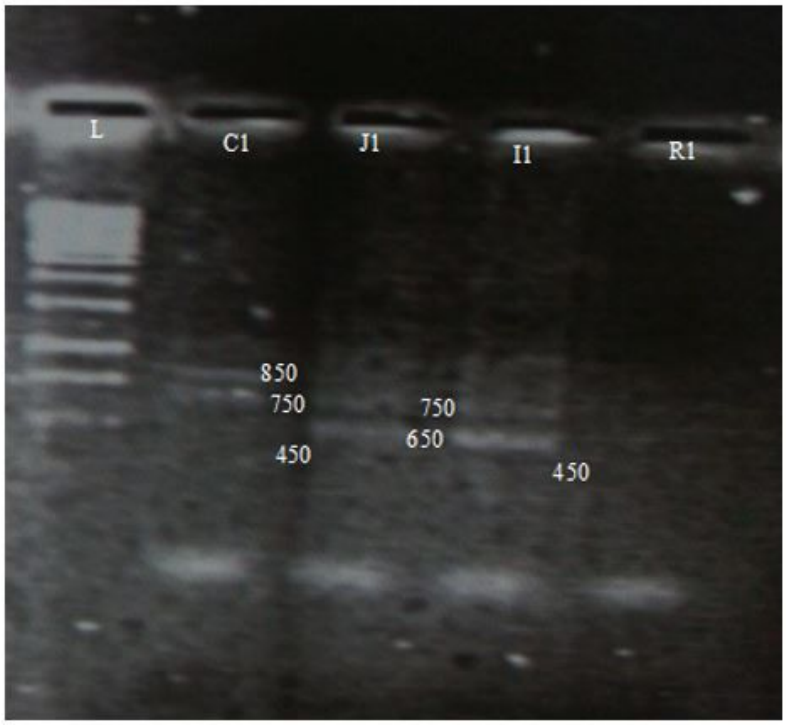

Figure 10

The amplification of 8SS RAPD Primer in 4 different riverine population 1 marker; 2 River Chenab specimen; 3 River Jhelum; 4 River Indus and 5 River Ravi.

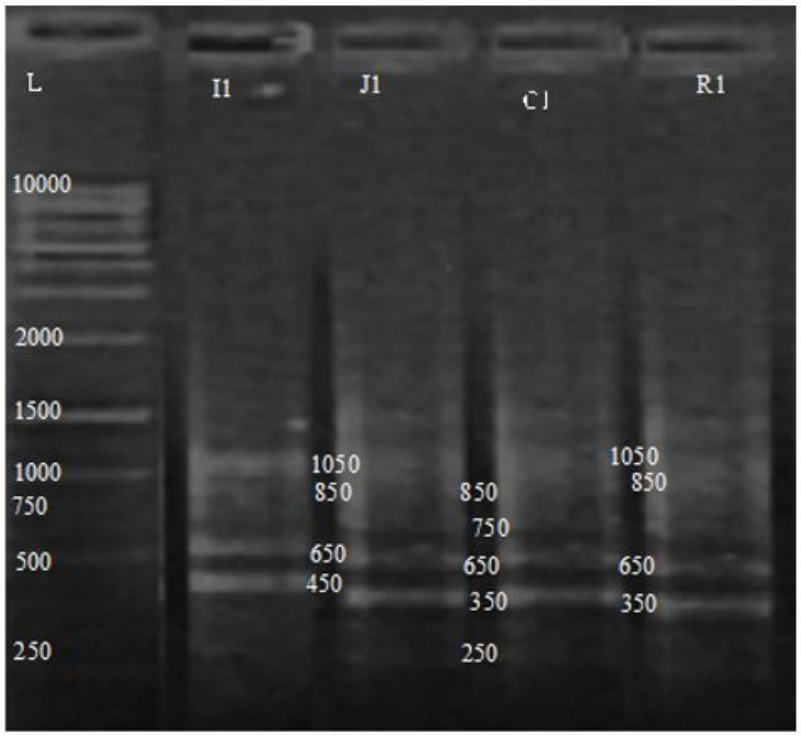

Figure 11

The amplification of 9SS RAPD Primer in 4 different riverine population 1 Ladder; 2 River Chenab specimen; 3 Jhelum; 4 River Ravi and 5 River Indus

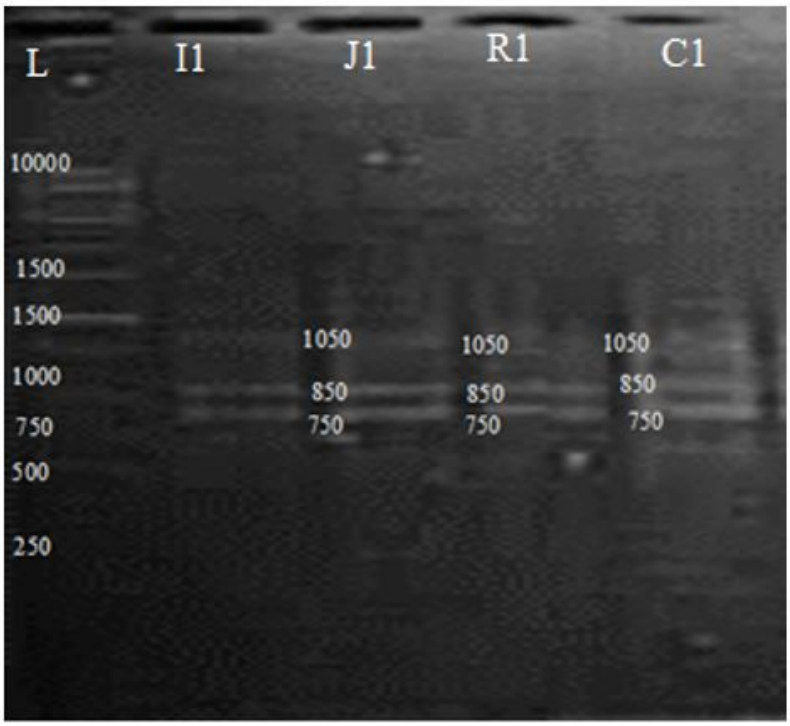

Figure 12

The amplification of 10SS (RAPD) primer in 4 different riverine population 1 Ladder; 2 River Indus specimen; 3 Jhelum; 4 River Ravi and 5 River Chenab.

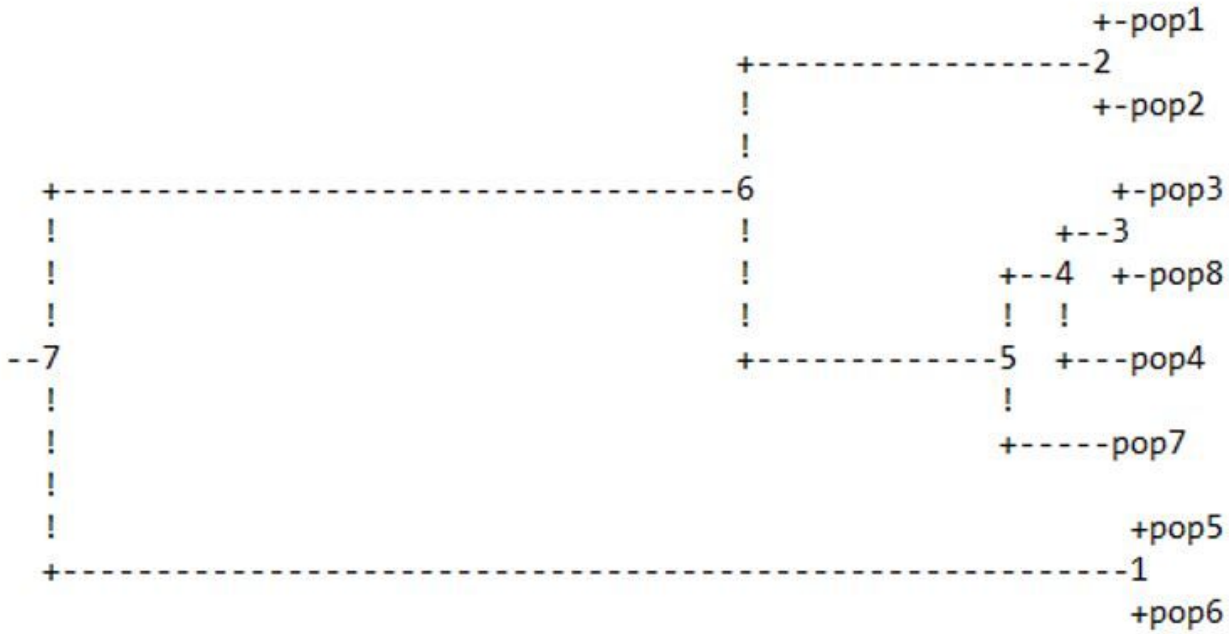

Figure 13

UPMGA dendrogram based on genetic distances by RAPD markers among eight different populations of S. sarwari of the four rivers (Chenab=1, 2, Jhelum=3, 4, Ravi=5, 6 and Indus=7, 8) of the Punjab, Pakistan.