Pak. J. Agri. Sci., Vol. 52(4), 1065-1071; 2015
ISSN (Print) 0552-9034, ISSN (Online) 2076-0906
http://www.pakjas.com.pk

GENETIC VARIABILITY IN DENGUE MOSQUITO *Aedesaegypti* (DIPTERA: CULICIDAE) FROM PUNJAB, PAKISTAN USING RAPD MARKERS

Zonash Arshad[1], Waseem Akram[2], Muhammad Kashif Zahoor[1], Naureen Aziz Qureshi[3], and Shabab Nasir[1,*]

[1]Department of Zoology, Government College University, Faisalabad, Pakistan;
[2]Department of Entomology, University of Agriculture, Faisalabad, Pakistan;
[3]Government College Women University, Faisalabad;
[*]Corresponding author's e-mail: flourenceshabab@yahoo.com

The mosquito *Aedesaegypti*is the main vector of dengue virus. Although, the epidemiology of dengue fever has been widely described; however, the genetics of the vector mosquito remained poorly understood in Pakistan. Hence, this study was performed to estimate the genetic variability in *Ae. aegypti*populations from different areas of the Punjab, *Ae. aegypti m*osquitoes were collected by using standard techniques (dippers) from Faisalabad, Nankana Sahib and Lahore districts. The collected populations were reared separately in the laboratory and then DNA was extracted from single individual. Ten decamer random primers (GL Decamer A-01 to A-10) were selected for Polymerase Chain Reaction (PCR). The PCR products were analyzed using 1.5% Agarose gel electrophoresis. The amplification profile data showed monomorphism and polymorphism between different districts as well as between samples of even same district. The primer A-04 showed a clear polymorphic banding pattern in different samples from Nankana Sahib, Faisalabad and Lahore. Maximum number of bands were produced by A-010 primer (75%) followed by A-02 (67%). The allele and genotype frequencies were measured at each locus. The Nm value showed a migration between Nankana Sahib and Faisalabad (4.65). While, *Ae. aegypti*were freely moving within district. However, the populations were also slightly genetically different (heterozygosity; Ht: 029). The dendrogram based on genetic similarities revealed three clusters which showed that *Ae. aegypti*populations from three districts were slightly different from each other. The genetic similarity ranged from maximum 3.16 to minimum 1.00. Based on the inter- and intra-populations polymorphic data from all three districts, it was concluded that *Ae. aegypti* populations were slightly genetically different in selected areas.

Keywords: Mosquito, genetic diversity, RAPD-PCR, dengue fever

INTRODUCTION

Mosquito and mosquito borne diseases are often discussed, as a major concern for climate change resulting due to global warming and rise in sea level (Scott *et al*., 2000). The greatest concern is that when temperature increases then the geographic range of the pest and vector species will increase automatically and with it the risk of human diseases also raises particularly for tropical diseases like Malaria, West Nile Virus, Yellow Fever, Filariasis and Dengue fever (Silva *et al*., 2008). However, there are many factors besides temperature including the availability of suitable habitats and short term changes in rainfall and tidal heights as well as urbanization and mosquito control programs (Nasir *et al.,* 2015) that influence the distribution and abundance of mosquitoes as well as the incidence of human disease. Two mosquito species *Aedesalbopictus* and *Aedesaegypti*play very important role in spreading different diseases like Yellow fever, West Nile virus and is a primary vector of four serotypes of dengue virus and yellow-fever virus (Herrera *et al*., 2006).

Ae. *aegypti* was originally known from Africa later spreading to Southeast Asia where it became major vector of dengue fever. The epidemiological importance and ease of laboratory handling of *Ae. agypti*greatly facilitated the studies on its population genetics (Fraga*et al*., 2003). Information about the genetic structure of this vector may help in the control programs. As a result of progress in molecular biology a list of useful DNA markers have been used for identification and recognition of genetic polymorphism (Santos *et al*., 2011).

The genetic diversity in *Ae. aegypti*populations had been widely reported. The RAPD method has been used as an efficient tool to detect differentiation of geographically and genetically isolated populations around the world (Ballinger-Crabtree *et al*., 1992; Apostol *et al*., 1994; Gorrochotegui-Escalante *et al*., 2000; Souza *et al*., 2001; Ayres *et al*., 2002; Franco *et al*., 2002; Ayres *et al*., 2003; Paduan*et al*., 2006). The genetic basis of mosquito species and genetic analysis of *Ae. aegypti* has been neglected in Southeast Asia despite its damaging aspects in outbreaks of dengue fever in Pakistan and Sri Lanka. There were many similarities among all the populations from different locations and significant

Fst(fixation index) values showed that *Ae. aegypti* migrated from one location to other locations due to road infrastructure development (Jain *et al*., 2010). For reasons it became really crucial to go further to know more about *Ae. aegypti* and its prevalence on genetic basis using modern techniques to find out control methods to avoid its expansion (Santos *et al*., 2011).

In Pakistan dengue fever was reported firstly during 1994 followed by outbreak in 2007 mainly from September to November which caused mortality and morbidity on high scale (Siddiqui, 2009). Since then outbreak was also seen in 2011 specifically severe in Lahore. High prevalence of dengue fever was observed in Lahore and handful number of confirmed cases was reported from district Faisalabad. In addition, a very few cases were reported in Nankana Sahib. This record enhances the efforts towards genetic and molecular study of dengue fever (DF) vector *Ae. aegypti* in aforementioned districts on regional bases. There is dire need of time to know maximum about dengue vector to combat the dengue disease. Because *Ae. aegypti*was detected in Pakistan shortly after its introduction, it may provide a rare opportunity to study several aspects of the population genetics of the species and the genetic events which initially induced colonization. So, the present study focussed on the genetic variability in *Ae. aegypti* populations from three different districts of the province Punjab, Pakistan using random amplified polymorphic DNA technique (RAPD-PCR).

MATERIALS AND METHODS

***Sample collection*:** *Aedesaegypti* mosquitoes were collected from three different districts of the province Punjab, Pakistan, i.e., Faisalabad, Nankana Sahib and Lahore. Faisalabad is located in northeast of Punjab, between longitude 73°74 East, latitude 30°31.5 North, with an elevation of 604 feet above sea level and Lahore covers more than 6,200 square mile,lying between 31°15′-31°45′ N and 74°01′-74°39′ E while the district of Nankana Sahib is located about 75 km west ofLahoreand about 55 km east ofFaisalabad (Fig. 1).

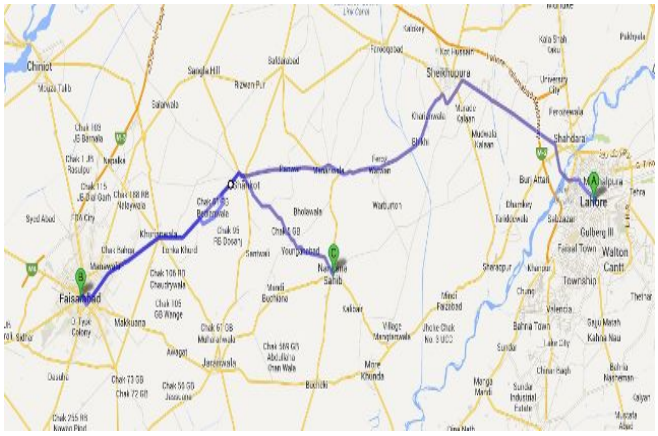

Figure 1. Map of Punjab showing the districts for sampling.

A total of fifteen populations were collected from three districts. From each district five to six locations situated at far off (>20 km) distances were chosen for sample collection (Table 1). The adult mosquitoes were collected with the help of battery-operated aspirator (Herrel*et al*., 2001; Shortall*et al*., 2009; Florencio *et al*., 2014). The larvae and pupae of mosquitoes were collected by using standard dipper (375ml) from each collection site (Naeem-Ullah*et al*., 2010). The collected samples were placed in 250 ml plastic vials. These vials were placed separately in the rearing cages for the rearing process. After emergence, the adults were preserved in 70% alcohol in labelled vials and stored at 4 °C for DNA extraction.

Table 1. Specimen detail of *Aedesaegypti*used during the study.

Sr.	District	Area/village	Type of collection	Date of Collection	Code
1	Nankana sahib	Shahkot	Net/Aspirator	Sep. 2012	NS1
2	Nankana sahib	Iqbal pura	Net/Aspirator	Sep. 2012	NS2
3	Nankana sahib	Anandpura	Net/Aspirator	Aug./Sep. 2012	NS3
4	Nankana sahib	Chak 560 GB	Net/Aspirator	Aug./Sep. 2012	NS4
5	Nankana sahib	Kot hari singh	Net/Aspirator	Sep. 2012	NS5
6	Lahore	Krishan nagar	Net/Aspirator	Aug./Sep. 2012	LH1
7	Lahore	Ichra	Net/Aspirator	Sep. 2012	LH2
8	Lahore	Iqbal town	Net/Aspirator	Aug./Sep. 2012	LH3
9	Lahore	Mazang	Net/Aspirator	Aug./Sep. 2012	LH4
10	Lahore	Mughal Pura	Net/Aspirator	Aug./Sep. 2012	LH5
11	Faisalabad	Malik pur	Net/Aspirator	Sep. 2012	FS1
12	Faisalabad	NIAB	Net/Aspirator	Sep. 2012	FS2
13	Faisalabad	Raza abad	Net/Aspirator	Sep. 2012	FS3
14	Faisalabad	D-type Colony	Net/Aspirator	Aug./Sep. 2012	FS4
15	Faisalabad	Gulfishan Colony	Net/Aspirator	Sep. 2012	FS5

Table 2. RAPD Primers used for PCR and their amplified bands.

Sr.	Primers	Nucleotide Sequence	Size (bp)	Number of amplified bands
1	GL-Decamer A-01	5'-CAG GCC CTT C-3'	250-2000	8
2	GL-Decamer A-02	5'-TGC CGA GCT G-3'	250-1600	9
3	GL-Decamer A-03	5'-AGT CAG CCA C-3'	240-1800	3
4	GL-Decamer A-04	5'-AAT CGG GCT G-3'	210-1100	5
5	GL-Decamer A-05	5'-AGG GGT CTT G-3'	250-1800	8
6	GL-Decamer A-06	5'-GGT CCC TGA C-3'	200-1200	9
7	GL-Decamer A-07	5'-GAA ACG GGT G-3'	240-1890	0
8	GL-Decamer A-08	5'-GAA ACG GGT G-3'	250-2000	7
9	GL-Decamer A-09	5'-GAA ACG GGT G-3'	210-1600	9
10	GL-Decamer A-10	5'-GGG TAA CGC C-3'	250-2000	8

***DNA extraction*:** Single female mosquito from each population was homogenized in 400 µl of TNE buffer containing 100 µl of Proteinase K (20 mg/µl) and 40 µl of 20% sodium dodecyl sulphate (SDS). The homogenates were put in incubator at 55°C for one hour. The contents were centrifuged at 12,000 rpm for 12 min after adding 300 µl of 5M NaCl and gently vortexing for at least 30 sec. The supernatant was shifted to new appendorf tube and 300-400 µl isopropanol or ice-cold 100% ethanol was added and kept at -21°C for 1 hour. DNA was precipitated by centrifugation at 12,000 rpm for 12 min, and pellets were washed with 70% ethanol, air dried and re-suspended in 50µl of sterile water (d_3H_2O). At the end, spectrophotometer at 260 nm was used to estimate the quantity of DNA (Bibi *et al*., 2015; Shakir*et al.,* 2015).

***RAPD-PCR analysis*:** Ten primers were chosen on the basis of their reproducibility and efficiency for PCR amplification (Table 2). Each PCR reaction was carried out in a final volume of 25 µl, containing 2.5 µl of genomic DNA, 3 mM of $MgCl_2$, 20 pmol of each primer, 2.5 µl buffer, 1.0 units of *Taq* DNA polymerase and 0.3 mM of each dNTPs with 11.8 µl water. PCR amplification was performed in a thermo cycler. PCR protocol followed included 35 cycles hot start at 94°C for 3-5 min, denaturation at 94°C for 30 sec, primer annealing at 36°C for 45 sec, extension at 72°C for 45 sec, final extension at 72°C for 5 min, then hold at 4C until tubes were removed. PCR products were analyzed on 1% Agarose gel at 80 volts for 2 hours. The gels were observed on transluminator and digital photographs were recorded (Bibi *et al*., 2015).

***Data analysis*:** Bands were scored and numbered as 1 or 0 on the base of presence and absence of bands. Ambiguous bands were ignored and only clear intense bands were scored. A dendrogram summarizing the genetic relationships among all populations was generated using un weighted pair-group mean analysis (UPGMA) algorithm. Effective migration rates (Nm) were estimated based on inbreeding indices (G_{ST}) according to McDermott and McDonald (1993), where $Nm = 0.5 (1 - G_{ST})/G_{ST}$. Calculations were performed with the help of the POPGENE (version 1.32) population genetics package (Ayres *et al*., 2002).

RESULTS

In the present study, *Ae. aegypti*mosquitoes were molecularly characterized to reveal their genetic variations in natural populations using RAPD-PCR analysis with 10 oligonucleotide primers (Genelink; Supplementary Table) from different areas of a district and among districts. The results were formulated on the basis of data collected from UV images recorded as shown in Fig. 2. Intense bands were seen between 300 to 2000 base pairs when compared with 1 Kb ladder. Names, sequence of Primers used in RAPD and number of fragments obtained after amplification of DNA samples (Table 2).

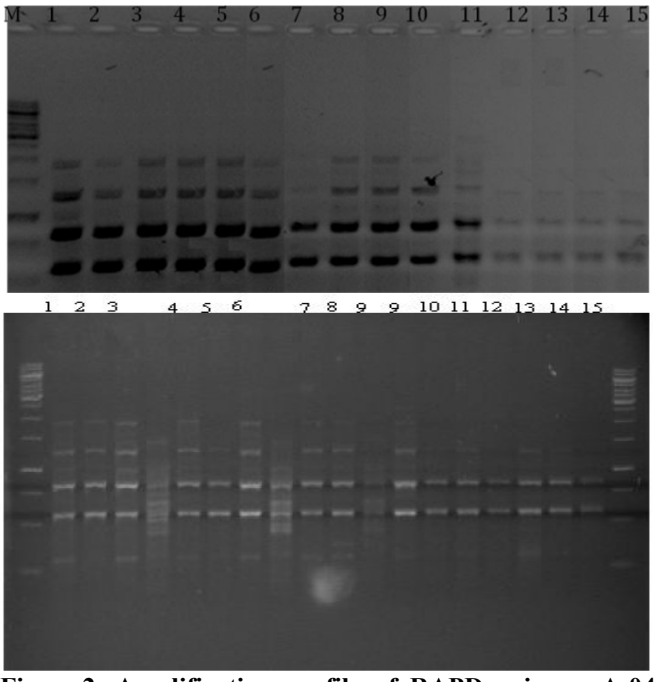

Figure 2. Amplification profile of RAPD primers A-04 and A-06.

M=1Kb DNA ladder, 1= NS1 (Shahkot), 2= NS2 (Iqbal pura), 3=

NS3 (Anandpura), 4= NS4 (Chak 560 GB), 5= NS5 (Kotharisingh), 6= LH1 (Krishan nagar), 7= LH2 (Ichra), 8= LH3 (Iqbal town), 9= LH4 (Mazang), 10= LH5 (Mughal pura), 11= FS1 (Malikpur), 12= FS2 (NIAB), 13= FS3 (Raza Abad), 14= FS4 (D-Type Colony) and 15= FS5 (Gulfishan Colony).

A total of 66 DNA fragments were generated by 10 primers (Table 3) with an average of about 6 bands per primer (Fig. 3). Bands that a primer yielded per PCR were three to seven. Generally, the size and number of the fragments produced strictly depended upon the nucleotide sequence of the primer used and upon the source of the template DNA. The amplified fragments were in the range of 200 to 2000 bp as defined.

Table 3. Number of amplified loci by each RAPD marker.

Primer	Monomorphic Loci	Polymorphic Loci	Total No. of Loci	%age of Polymorphism
A-01	4	4	8	50
A-02	3	6	9	67
A-03	2	1	3	34
A-04	2	3	5	60
A-05	3	5	8	63
A-06	5	4	9	45
A-07	0	0	0	0
A-08	3	4	7	58
A-09	4	5	9	56
A-10	2	6	8	75
Total	28	38	66	58
Mean	2.8	3.8	6.6	5.8

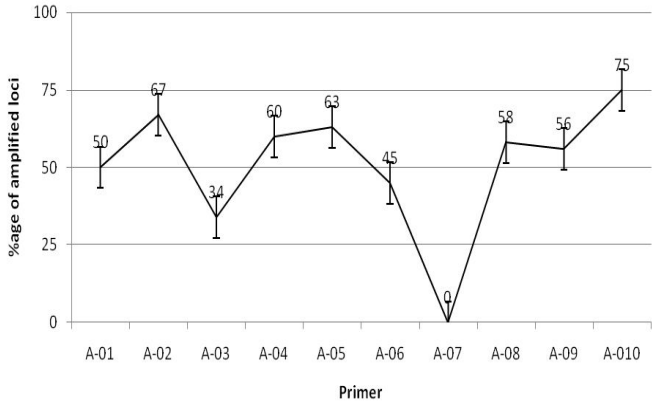

Figure 3. Percentage of polymorphic loci amplified by each RAPD marker.

Two markers were exclusive for FS (Faisalabad) population and remaining for LH (Lahore), NS (Nankana Sahib) and FS together. A dim band shown beside the fragment 2000 of molecular marker showing variations with other populations which was not shown in amplified products. Another variation illustrated (Fig. 2) is a dim band little below 1500 bp fragment showing diversity within the district Lahore and ensures emigration of few species from other regions showing a low degree of divergence.

The gene diversity (Ht) ranged from 0.298 in Faisalabad (FS) populations to 0.311 in the Nankana Sahib (NS) populations. The total average genetic diversity in all populations of three districts was 0.375 and the genetic distances were between 0.00 and 3.32 (Table 5 & 6). The overall differentiation among the fifteen populations presented Gst = 0.136 and Nm = 3.176. The Gst value for the five populations within the district of Lahore was 0.122 (Nm = 3.598), the value for the five populations within the district of Nankana Sahib was 0.127 (Nm = 3.437) while for Faisalabad populations was Gst = 0.129 and Nm = 3.375. The Gst value between districts of NS and FS was 0.097 while the highest value of Gst between districts (LH and NS) was 0.134 and the highest number of migrants was 4.654 between NS and FS while the lowest number of migrants was within the three districts (Table 4).

Table 4. Diversity (Ht), genetic differentiation (Gst) and gene flow (Nm) among *Aedesaegypti* populations, pooled by district based on RAPD-PCR analysis.

Populations	Gst	Nm	Ht
LH	0.122	3.598	0.304
NS	0.127	3.437	0.311
FS	0.129	3.375	0.298
LH, NS	0.134	3.231	0.326
LH, FS	0.130	3.346	0.375
NS, FS	0.097	4.654	0.314
LH, NS, FS	0.136	3.176	0.375

LH=Lahore, NS=Nankana Sahib, FS=Faisalabad

The cluster analysis was used to explore the homogeneous groups of mosquitoes on the basis of presence of different bands. The variables (bands and some abiotic factors) were used to identify the clusters of mosquitoes with similar genetic makeup. Ward's Heirarchical clustering method was considered and the resulting dendrogram is shown in Fig. 4. Figure 4 showed that mosquitoes from four localities (Shahkot, Iqbal pura, Anandpura and Kot Hari Singh) of the district Nankana Sahib formed one cluster while the six other localities, one from district Nankana sahib and all five from district Faisalabad (Chak 560 GB, Malik pur, NIAB, Raza abad, D-Type Colony and Gulfishan Colony) had the mosquitoes with similar genetics. The third cluster represented that mosquitoes from all localities (Krishan nagar, Ichra, Iqbal town, Mazang and Mughal pura) of district Lahore were similar in their genetics. There are two subgroups in group A, the subgroup A_1 represented mosquitoes from two closely related sites (Shahkot and Anandpura) of district Nankana Sahib while subgroup A_2 also represented the mosquitoes from district Nankana Sahib. The second group B comprised of five sub-groups, the subgroup B_1 represented only one locality from district

Table 5. Distance matrix between fifteen *Aedesaegypti* populations based on random amplified polymorphic DNA analysis and abiotic factors.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	0.00	1.41	1.00	2.45	1.73	2.45	2.65	2.45	2.24	2.24	3.32	2.83	2.83	3.00	2.65
2	1.41	0.00	1.00	2.00	1.00	2.00	2.65	2.45	2.24	2.24	3.00	2.45	2.45	2.65	2.65
3	1.00	1.00	0.00	2.24	1.41	2.24	2.45	2.24	2.00	2.00	3.16	2.65	2.65	2.83	2.45
4	2.45	2.00	2.24	0.00	1.73	2.00	2.65	2.00	2.24	2.24	2.65	2.00	2.00	2.24	2.24
5	1.73	1.00	1.41	1.73	0.00	1.73	2.45	2.24	2.00	2.00	2.83	2.24	2.24	2.45	2.45
6	2.45	2.00	2.24	2.00	1.73	0.00	1.73	1.41	1.00	1.00	2.65	2.00	2.00	2.24	2.24
7	2.65	2.65	2.45	2.65	2.45	1.73	0.00	1.73	1.41	1.41	3.16	2.24	2.24	2.45	2.00
8	2.45	2.45	2.24	2.00	2.24	1.41	1.73	0.00	1.00	1.00	2.65	2.45	2.00	2.24	1.73
9	2.24	2.24	2.00	2.24	2.00	1.00	1.41	1.00	0.00	0.00	2.83	2.24	2.24	2.45	2.00
10	2.24	2.24	2.00	2.24	2.00	1.00	1.41	1.00	0.00	0.00	2.83	2.24	2.24	2.45	2.00
11	3.32	3.00	3.16	2.65	2.83	2.65	3.16	2.65	2.83	2.83	0.00	2.65	2.24	2.00	2.45
12	2.83	2.45	2.65	2.00	2.24	2.00	2.24	2.45	2.24	2.24	2.65	0.00	1.41	1.73	1.73
13	2.83	2.45	2.65	2.00	2.24	2.00	2.24	2.00	2.24	2.24	2.24	1.41	0.00	1.00	1.00
14	3.00	2.65	2.83	2.24	2.45	2.24	2.45	2.24	2.45	2.45	2.00	1.73	1.00	0.00	1.41
15	2.65	2.65	2.45	2.24	2.45	2.24	2.00	1.73	2.00	2.00	2.45	1.73	1.00	1.41	0.00

Nankana Sahib (Chak 560 GB) and remaining subgroups represented the district Faisalabad. The third group C comprised of three subgroups and all from the district Lahore. The clustering suggested that mosquitoes from each of the three districts are similar in genetics but genetic variability existed among the mosquitoes of three districts i.e., Faisalabad, Lahore and Nankana Sahib.

Table 6. Distances between Cluster Centroids.

	Cluster1	Cluster2	Cluster3
Cluster1	0.00	2.14	1.96
Cluster2	2.14	0.00	1.76
Cluster3	1.96	1.76	0.00

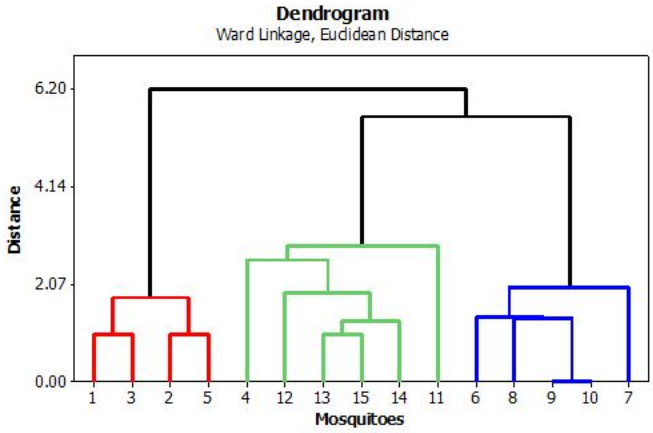

Figure 4. Dendrogram resulting from grouping among the fifteen populations of *Aedesaegypti*, using RAPD, on the basis of genetic distance.

DISCUSSION

Genetic diversity depicts variations present among populations of a species located in different regional boundaries or members of particular species located in a single distinct region. Diversity enables to find out the inheritance pattern of a species in a particular area. Comparisons based on genetic diversity helps to relate populations of a species on molecular bases with reference to genetic structures present in them.

The World health Organization (WHO) declares dengue and dengue hemorrhagic fever to be endemic in South Asia and Pakistan is at high risk of epidemics. In 2011, 0.6 million suspects of dengue fever were reported out of which 22000 were declared as confirmed (Jahan, 2011). High prevalence of dengue fever was observed in Lahore 2011, and handful number of confirmed cases was reported in district Faisalabad as well. This (Jahan, 2011) record enhances the efforts towards genetic and molecular study of DF vector *Ae. aegypti* on regional bases. Our results or RAPDs showed 66 DNA bands, with the percentage of polymorphic loci varying from 45 to 75. Similarly, RAPD markers revealed genetic variability of *Ae. aegypti* populations all over the world (Garcia-Franco *et al*., 2002). de Lourdes Munoz *et al*. (2013) described the patterns of gene flow among *Ae. aegypti* populations in Mexico. A moderate gene flow was recorded among nine *Ae. aegypti* populations from 6 cities in Oaxaca, Mexico. Genetic variation was observed in ND4 mitochondrial gene using single-strand conformation polymorphism analysis, and they detected 3 haplotypes. Cluster analysis using genetic distances showed that *Ae. aegypti* populations were significantly diverse across 800 km range. Another pattern of geographical distribution of the mosquitoes showed a genetic drift among 9 *Ae. aegypti* populations in Mexico. Most importantly, the molecular analysis of variance revealed that there was much

genetic variation among populations 4 km apart. In addition, it was suggested that efficient mosquito dispersion was most likely due to commercial transportation.

Gorrochotegui-Escalante *et al*. (2000) described the polymorphism in mosquito populations from 10 cities of the Mexico through RAPD markers. The genetic variation was observed among populations separated by 90-250 km. Similarly, the heterozygosity observed in current study among *Ae. aegypti*populations from all three districts (Lahore, Nankana Sahib and Faisalabad) and from two districts (Faisalabad and Lahore) (Ht. 0.375) was found to be consistent to the results described in 15 Brazilian populations of *Ae. aegypti*using 27 RAPD *loci* (Ht = 0.390) (Ayres *et al.*, 2003). In addition, the average heterozygosity of 57 *loci* in Puerto Rico (HT = 0.354) and of 21 *loci* (HT = 0.388) in Brazil revealed through RAPD methodologies (Apostol *et al.*, 1996) are consistent to the present results.

Franco *et al*. (2002) also studied the genetic diversity in 131 *loci* of 20 *Ae. aegypti* populations from Mexico using RAPD markers. The genetic variations were also observed due to distance existed among mosquito populations. The distance between Lahore and Faisalabad is ~120 km. Genetic variations existed among *Ae. aegypti* populations collected from these cities. Thus, similar to Franco *et al*. (2002), the polymorphic amplification in our findings showed genetic variations by distance.

The genetic diversity in *Ae. aegypti*populations from three districts (Lahore, Nankana Sahib and Faisalabad) showed that these populations were genetically differentiated (GST = 0.136). In contrast, Souza *et al*. (2001) studied these species in Argentina with values (GST = 0.249; Nm = 0.75) and Ayers *et al*., (2003) in Brazil with values (GST = 0.317; Nm = 0.54). Santos *et al*. (2011) found high level of polymorphism and distinguished 52 markers in the four populations through RAPD analysis, ranging in size from 300 to 2072 bp. Their percentage of polymorphic *loci* varied from 82.69 to 94.23. In contrast; we amplified *loci* ranged from 200 to 2000 bp with a percentage of polymorphism varied from 34 to 75 (Fig. 2).

Hiragi*et al*. (2009) found genetic diversity and demonstrated 50% similarity in *Ae. aegypti* populations through RAPD markers. Paduan*et al*. (2006) described a low population differentiation (GST = 0.208) with a higher degree of gene flow (Nm = 1.90). Similarly, the current study showed a population differentiation (GST = 0.129) from Faisalabad with gene moderate flow (Nm = 3.375); among Lahore populations (GST = 0.122) with a higher degree of gene flow (Nm = 3.598); among Nankana Sahib populations (GST = 0.127) with high degree of gene flow (Nm = 3.437) indicating both intra- and inter-population genetic variability in *Ae. aegypti* populations existed in three districts (Faisalabad, Nankana Sahib and Lahore). The polymorphic amplification could also be attributed to the human activity to mosquito spread (Table 2).

***Conclusion*:** Based on the existence of genetic variation exists in *Ae. aegypti*populations in different cities, further investigations are needed on large scale using gene specific markers in order to devise a dengue control program.

REFERENCES

Apostol, B.L., W.C. Black 4th, P. Reiter and B.R. Miller. 1996. Population genetics with RAPD-PCR markers: the breeding structure of *Aedesaegypti* in Puerto Rico. Heredity 76:325-334.

Ayres, C.F.J., M.A.V. Melo-Santos, A.M. Sole-Cava and A.F. Furtado. 2003. Genetic differentiation of *Aedesaegypti*(Diptera: Culicidae), the major dengue vector in Brazil. J. Med. Entomol.40:430-435.

Ayres, C.F.J., T.P.A. Romao, M.A.V. Melo-Santos and A.F. Furtado. 2002. Genetic diversity in Brazilian populations of *Aedesalbopictus*. Mem.Inst. *Oswaldo. Cruz*. 97:871-875.

Ballinger-Crabtree, M.E., W.C. Black and B.R. Miller. 1992. Use of genetic polymorphisms detected by the random-amplified polymorphic DNA polymerase chain reaction (RAPD-PCR) for differentiation and identification of *Aedesaegypti*subspecies and populations. Am. J. Trop. Med. Hyg.47:893-901.

Bibi, M., M.K. Zahoor, M.A. Zahoor, H.M. Ashraf, H.N. Majeed, S. Nasirand B. Rasool. 2015. Genetic Analysis of Mosquitoes from Rural and Urban Areas of Sialkot, Pakistan. Int. J. Agric. Biol. 17:809-814.

de Lourdes Munoz, M., R.F. Mercado-Curiel, A. Diaz-Badillo, G.P. Ramirez and W.C. Black IV. 2013. Gene Flow Pattern Among *Aedesaegypti* Populations in Mexico.J. Am. Mos. Cont. Assoc. 29:1-18.

Florencio, M., C. Diaz-Paniagua, C. Gomez-Rodriguez and L. Serrano. 2014. Biodiversity patterns in a macro invertebrate community of a temporary pond network. Insect Conserv.Divers. 7:4–21.

Fraga, E.C, J.M.M. Santos and J.F. Maia. 2003. Enzymatic variability in *Aedesaegypti* (Diptera: Culicidae) populations from Manaus- AM, Brazil. Genet. Mol. Biol. 26:181–187.

Franco, F.G., M.D.L. Munoz, S.L. Fuentes, L.F. Salas, J.G. Rejon, B.J. Beaty and W.C. Black. 2002. Large genetic distances among *Aedesaegypti*populations along the south pacific coast of Mexico. Am. J. Trop. Med. Hyg. 6:594-598.

Gorrochotegui-Escalant, N., M.L. Munoz, I. Fernandez-Salas, B.J. Beaty and W.C. Black. 2000. Genetic isolation by distance among *Aedesaegypti*populations along the northeastern coast of Mexico.Am. J. Trop. Med. Hyg. 62:200-209.

Herrel, N., F.P. Amerasinghe, J. Ensink, M. Mukhtar, W. Van Der Hoek and F. Konradsen. 2001. Breeding of

Anopheles mosquitoes in irrigated areas of South Punjab, Pakistan. Med. Vet. Entomol. 15:236-248.

Herrera, F., L. Urdaneta, J. Rivero, N. Zoghbi, J. Ruiz, G. Carrasquel, J.A. Martinez, M. Pernalete, P. Villegas, A. Montoya, Y. Rubio-Pais and E. Rojas. 2006. Population genetic structure of the dengue mosquito *Aedesaegypti* in Venezuela. Mem.Inst. Oswaldo. Cruz. 101:625–633.

Hiragi, C., K. Simoes, E. Martins, P. Queiroz, L. Lima and R. Monnerat. 2009. Genetic variability in *Aedesaegypti*(L.) (Diptera: Culicidae) populations using RAPD markers.Neotrop.Entomol.38:542-547.

Jahan, F. 2011. Dengue Fever (DF) in Pakistan. Asia Pac. Fam. Med. 10:1.

Jain, K.S., B. Neekhra, D. Pandey and K. Jain. 2010. RAPD marker system in insect study: A review. Ind. J. Biotech. 9:7-12.

McDermott, J.M. and B.A. McDonald. 1993. Gene flow in plant pathosystems. Ann. Rev. Phytopathol. 31:353-373.

Naeem-Ullah, U., W. Akram, A. Suhail and S.A. Rana. 2010. Grouping of different mosquito species on the bases of larval habitats. Pak. J. Agri. Sci. 47:124-131.

Nasir, S., I. Nasir, I. Yousaf and I. Zaib. 2015. Larvicidal and pupicidal action of kitchen used plant extracts against *Culex* mosquitoes (Diptera: Culicidae). J. Glob. Innov. Agric. Soc. Sci. 3:68-71.

Paduanet, K.S., J.P. Araujo-Junior and P.E.M. Ribolla. 2006. Genetic variability in geographical populations of *Aedesaegypti* (Diptera, Culicidae) in Brazil elucidated by molecular markers. Genet. Mol. Biol. 29:391-395.

Santos, J.M.M., E.C. Fraga, J.F. Maia and W.P. Tadei. 2011. Genetic diversity in dengue mosquito, *Aedesaegypti*(Diptera: Culicidae) from Amazon region: Comparative analysis with isozymes and RAPD loci. The Open Trop. Med. J. 4:11-20.

Shakir, H.U., N.A. Anjum, Q. Ali, S. Saleem and T. Anwar. 2015. Molecular systematic of *Chrysoperlacarnea* group (Neuroptera: Chrysopidae) in Punjab, Pakistan. J. Glob. Innov. Agric. Soc. Sci. 3: 12-15.

Scott, T.W., A.C. Morrison, L.H. Lorenz, G.G. Clark, D. Strickman, P. Kittayapong, H. Zhou and J.D. Edman. 2000. Longitudinal studies of *Aedesaegypti* (Diptera: Culicidae) in Thailand and Puerto Rico: Population dynamics. J. Med. Entomol. 37:77-88.

Shortall, C.R., A. Moore, E. Smith, M.J. Hall, I.P. Woiwod and R. Harrington. 2009. Long-term changes in the abundance of flying insects.Insect Conserv. Divers.2:251-260.

Siddiqui, F.J., S. Haider and Z.A. Bhutta. 2009. Endemic dengue fever: a seldom recognized hazard for Pakistani children. J. Infect. Dev. Ctrie. 3:306-312.

Silva, W.J., G.A.A. Doria, R.T., Maia, R.S. Nunes, G.A. Carvalho, A.F. Blank, P.B. Alves, R.M. Marcal and S.C.H. Cavalcanti. 2008. Effects of essential oils on *aedesaegypti* larvae: alternatives to environmentally safe insecticides. Biores. Technol. 99:3251-3255.

Souza, G.B., A. Blanco and C.N. Gardenal. 2001. Genetic relationships among *Aedesaegypti*(Diptera: Culicidae) populations from Argentina using random amplified polymorphic DNA polymerase chain reaction markers. J. Med. Entomol.38:371–375.