GENETIC DIVERSITY OF TWO PHENOTYPES OF EGYPTIAN DOMESTIC GEESE *Anser anser* (AVES: ANATIDAE) BASED ON SSR MARKERS

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The geese of the genus *Anser* Linnaeus 1758 belongs to the subfamily *Anserinae* including true geese and swan (Livezey, 1986; Carboneras, 1992). Currently, 10 species of *Anser* are recognized, which span nearly the whole range of true goose shapes and sizes (Carboneras, 1992). Two only of these species have been domesticated (Crawford, 1990) and one of them is *Anser anser* which is a European species and is represented by many breeds (Wójcik and Smalec, 2007). Greylag goose *Anser anser anser* is common breed in Egypt. According to the publication of the Egyptian Environmental Affairs Agency (EEAA, 1997), these geese are characterized by having brownish grey or whitish grey or totally pure white feather. The tail is grey with a white tip and the tail coverts are white. The beak, legs and feet color ranges from orange to pinkish. There is high similarity in appearance of the two sexes.

Taxonomic studies of avian species typically dealt with analysis of morphology, plumage, behavior and cytological approaches (Johnsgard, 1961; Livezey, 1986; Ata *et al*., 2005; 2007 and 2017). Nevertheless, molecular analyses are recognized as being of value to avian taxonomy, beginning with Sibley's electrophoretic evaluation of avian egg-white proteins (Sibley, 1960). As Sibley's avian studies (Sibley *et al.*, 1969; Sibley, 1970; Sibley and Ahlquist, 1972) are focused at the generic and familial levels, later studies largely extended to identify avian species and their relationships. In addition, taxonomic controversies have been clarified in many taxa by using molecular techniques that help to study the genetic variation by using DNA markers and create new possibilities for the selection and genetic improvement of livestock (Salem *et al*., 2005).

One of the efficient DNA molecular markers is Microsatellites markers (SSR). They are suited to distinguish between closely related genotypes and to characterize the genetic variations of animal species based on DNA polymorphism (Simianer, 2006). Microsatellites; Simple Sequence Repeats (SSR) are tandem repeated of DNA section. They are arranged throughout the genome of eukaryotes and these repeats are mono or di, tri, tetra or penta units (Powell *et al*., 1996). These markers are polymorphic, co-dominant and highly reproducible. They have been

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extensively used in forensics, genetic mapping, population genetics and evolutionary studies (Vignal *et al*., 2002; Sasazaki *et al.*, 2004; Yoon *et al.*, 2005). A large number of microsatellite markers have been mapped for various waterfowl species (Fields and Scribner 1997; Maak *et al*., 2000; Sruoga *et al.*, 2005; Baublys *et al.*, 2006; Ahmadi *et al.*, 2007; Basha *et al*., 2016; Seo *et al*., 2015 and 2016).

Currently there are known some microsatellite markers isolated and evaluated in the wild forms of geese as Greylag goose *Anser anser* (Weiß *et al*., 2008), Canada goose *Branta canadensis* L. (Cathey *et al.*, 1998), Swan goose *Anser cygnoides* L. (Tu *et al*., 2006; Li *et al*., 2007), White-fronted goose *Anser albifrons* (Fields *et al*., 1997), Pink-footed Goose *Anser brachyrhynchus* (Noreikiene, 2012). Moreover, microsatellite markers were used to study genetic diversity of anatidae in Chinese (Tu *et al*., 2006; Liu *et al.*, 2008), Hungarian, Embden (Aliczki, 2007), Zatorska (Andres and Kapkowska, 2011; Mindek *et al*., 2014) and Alaska′s Emperor Goose population (Gravley *et al*., 2017).

As regards, the Egyptian domestic geese *Anser anser* population is still poorly studied in views of molecular aspects that must be subjected to further investigation to throw light on the taxonomy and genetic information of this genus in Egypt. So, this study was carried out to assess the genetic make-up of domestic geese *Anser anser* populations of different phenotype in Egypt, based on 10 microsatellite loci analysis.

MATERIALS AND METHODS

1. Sample collection and DNA preparation

Venous blood of geese samples were taken from the Minia governorate localities as illustrated in Fig. (1) and kept in heparinized tubes at -20°C. DNA samples were extracted from 100 to 200 µl of blood samples using Cornell extraction buffer (Sambrook *et al*., 1989). Subsequently, the DNA extracts were purified using the phenol-chloroform-isoamyl alcohol extraction method and precipitated by ice cold absolute ethanol. The DNA quantity and quality was determined using UV/V spectrophotometer based on absorbency at 260 and 280 nm, respectively. The sample size, sex and feather color patterns are indicated in Table (1).

2. PCR condition

The microsatellite DNA analysis was carried out using 10 SSR microsatellite primers. Sequence and annealing temperatures of the used primers are listed in Table (2). The amplified PCR products were in a final volume of 25 µl. Each PCR reaction contains (5 µl of DNA template, 2 µl of primer and 10 µl of master mix taq. DNA polymerase, Sigma Scientific Services Co., Egypt). The reaction volume was completed to 25 µl with sterilized deionized water. PCR amplifications were carried out in an initial denaturation at 95°C for 5 min followed by 40 cycles

of 30 s at 94°C, 60 s at 55-60°C, 120 s at 72°C, and a final extension at 72°C for 10 min using Biometra Uno-Thermoblock Thermal Cycler. The amplified products were resolved by gel electrophoresis 2% agarose gel for one hour**.**

3. Statistical analysis

Gel images detected via PCR-based methods were analyzed using Gel Analyzer version three, 2007. Molecular sizes of the amplified fragments, presence (1) or absence (0) of DNA fragments, frequencies through samples, and polymorphism type (either monomorphic or polymorphic) as well as the polymorphism percentage for each primer were determined.

Dice's (1945) genetic similarity coefficient values (*S*) within geese populations expressed as band sharing frequency (BS) were calculated for all possible pairs or operational taxonomic units (OUT) by using the software SPSS (version 12.0.1, 2004) based upon coding of the amplified bands numbers related to their presence or absence; (1) for their presence and (0) for absence. Hierarchical cluster analysis was conducted with the PAST software version 1.88 (Hammer *et al*., 2009) based on Dice's (1945) similarity coefficient matrix within geese populations.

RESULTS AND DISCUSSION

Ten SSR microsatellite primers were screened to identify genetic variation in both males and females of two phenotype of domestic geese (whitish grey and white) occurring in Minia province, Egypt. Only eight of the used ten SSR microsatellite primers produced scorable amplified bands, while the other two primers (TTUCG-1 and APH17) generated no amplicons in any of the studied geese populations examined (Fig. 2 and Table 3). These scorable amplified bands displayed variable polymorphism amongst the whitish grey and white populations (Tables 3 and 4). The total number of amplified bands produced by the eight primers was 29 bands with an overall mean (3.63 ± 0.89) and ranged from 1 band for both TTUCG-2 and TTUCG-4 primers to 8 bands in SFIMU-1 primer) (Table 3). One monomorphic band separately produced by each of the two primers TTUCG-2 and TTUCG-4 with molecular size 135 and 96 bp, respectively in all investigated geese population (Table 4). In comparison with the results revealed by Baublys *et al*. (2006) who used the same primers (SFIMU-1, TTUCG-1, TTUCG-2 and TTUCG-4) for studying genetic variability of Lithuanian native goose breeds, their results were contradict with the present study. SFIMU-1 primer was monomorphic in all investigated species of Baublys *et al.* (2006) with DNA fragment number (4). Here in, SFIMU-1 primer result was polymorphic and revealed 8 amplified bands with molecular size ranged from (67 to 743 bp) as shown in (Tables 3 and 4). Moreover, the largest number of DNA fragments (9 bands) was detected in the Vistines breed geese obtained with the TTUCG-4 primer with molecular size ranged from 35 to 360 bp,

and the smallest number (1 band with 30 bp) was obtained in Vistines geese with the TTUCG-1 primer (Baublys *et al.*, 2006).

SSR amplicons produced by primers APH11, APH21 and SFIMU-1 exhibited 100% polymorphism among the studied geese samples, while those of APH18 and CAUD019 primers showed 66.7% polymorphism (Table 3). Moreover, the six SSR primers; APH11, APH17, APH21, APH18, CAUD019 and CAUD024 were used in parallel with Fandy (2017). According to results of the parallel studies (Fandy, 2017) on Egyptian ducks populations, these six microsatellite (SSR) primers generated amplified bands in all duck breeds. The number of amplified bands per primer was variable among the five studied Egyptian duck breeds. Moreover, some studies used the six SSR primers APH11, APH17, APH21, APH18, CAUD019 and CAUD024 (Huang *et al.*, 2005; Hui-Fang *et al.*, 2010; Ismoyowati and Purwantini, 2011; Weimann *et al.*, 2016). They scored 39 diagnostic bands and 37 (94.87%) was recognized as polymorphic and the other 2 (5.13%) was recognized as monomorphic bands.

Here in, the same six SSR primers generated amplified bands in all geese samples except APH17 primer. The amplified products and polymorphism percentage were different. APH18 SSR primer revealed 3 amplified bands (one monomorphic with molecular size 65 bp and two polymorphic with 91 and 218 bp) within and among domestic geese samples (Table 3 and Fig. 2) while, it generate one fixed band with 292 bp molecular size among duck breeds (Fandy, 2017).

In the present study, six of the eight SSR primers (SFIMU-1, APH11, APH18, APH21, CAUD019 and CAUD024) revealed polymorphism within the tested geese samples and the polymorphism was not color or sex dependent. These data were in agreement with those reported by Ata *et al.* (2012) and Shahin *et al.* (2014) and suggests the relatedness of whitish grey and white domestic geese.

In order to determine the genetic variability within the two phenotypes of geese populations, matrix of Dice's (1945) similarity coefficient (S) was calculated based upon band sharing frequency (BS) as shown in Table (5). The average mean of similarity was found between whitish grey geese individuals S = (0.710 ± 0.0129) and ranged from 0.467 to 0.889. The average mean of similarity was S = (0.702 ± 0.0202) within white geese population and ranged from 0.476 to 0.963. However, average of similarity between females in both studied populations (whitish gray and white) was S = (0.698 ± 0.0138) while that of males was S = (0.694 ± 0.0185). As clearly observed from matrix, there was great affinity and relatedness of the two geese phenotypes together and the two sexes.

The dendrogram was constructed using the hierarchical cluster analysis method with the average linkage between pairs from the matrix of Dice's (1945)

and similarity coefficient values (S) within geese individuals (Fig. 3). Within populations, the dendrogram showed two main clusters, the first of which clustering of some males whitish grey individuals. The second cluster included whitish grey individuals with some white samples. However, there were many sub clusters group the white individuals together (Fig. 3). In addition to matrix, dendrogram revealed the great similarity and relationship between the two geese phenotypes (whitish grey and white). These results suggested the relatedness degree to each other and white geese involved with the genus *Anser* (Ata *et al.*, 2012; Shahin *et al*., 2014).

The relationship between the whitish grey and white geese populations indicated that the whitish grey and white geese truly belong to the genus *Anser*. This finding supports the hypothesis assumed by Cramp (1977); Madge and Burn (1988); Carboneras (1992) and Dudley *et al.* (2006) who included the white geese (genus *Chen*) within the genus *Anser* comprising basically the whitish grey geese. However, it contradicts the assumption of AOU (1998) and IUCN (2007) that treat them as separate.

In conclusion, the SSR microsatellite primers APH11, APH21, APH18, CAUD019, CAUD024 and SFIMU-1 displayed obvious polymorphism within the domestic geese population examined in Egypt and thus suggest their potentiality for usage as intra- and inter-population specific markers. There was polymorphism between the different phenotypes of geese and this polymorphism is not dependent on sex or color. Moreover, the Egyptian strain is not pure strain and need further cytological, molecular studies based on pure lines to emphasize the genetic make-up of this strain in Egypt.

SUMMARY

Genetic variation within and among domestic whitish grey and white Greylag geese populations collected from Minia, Upper Egypt were examined using ten SSR microsatellite primers. Of these primers, only eight primers produced scorable amplified bands, while TTUCG-1 and APH17 primers generated no amplification products in any of studied geese population. These scorable amplified bands displayed variable polymorphism amongst the whitish grey and white populations. The total number of amplified bands produced by eight primers was 29 bands with an overall mean (3.63 ± 0.89) and ranged from one band with TTUCG-2 and TTUCG-4 primers to eight bands with SFIMU-1 primer. Primers APH11, APH21 and SFIMU-1 revealed 100% polymorphism between geese populations. Cluster analysis of similarity within populations indicated: 1) the existence of two distinct evolutionary clusters corresponding to whitish grey and white geese groups and 2) clustering of white populations with the whitish grey population. The average similarity among the two geese phenotypes was nearly similar; this suggests their relatedness to each other. This relationship between the two geese

phenotypes strongly supports the previous hypothesis of including the white geese within the genus *Anser* basically containing the Greylag geese. It is the first time to use SSR markers for identification of Egyptian domestic geese breed.

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Table (1): Colour and sex variation of the collecting samples of domestic geese *Anser anser* populations.

Number of collected samples based on colour pattern				Total number of collected samples
Whitish grey		White		
Males	Females	Males	Females	
6	6	2	6	20

Table (2): Microsatellite SSR-primer names, sequences, melting and annealing temperatures used for amplification in *Anser anser* geese populations.

No	Name	Sequence (5′-3′)	Tm C°	Ann. T. C	References
1	SFIMU-1	F: 5′- CAC AAG GAA GCA TGA CCT CAG AA-3′ R: 5′- CTC ATG CCT CCT GTT AGT CAT CT-3′	58.3 58.0	55	Tu *et al*., 2006 Baublys *et al*., 2006
2	TTUCG-1	F: 5′- CCC TGC TGG TAT ACC TGA-3′ R: 5′-GTG TCT ACA CAA CAG C-3′	51.5 44.1	55	Tu *et al*., 2006 Baublys *et al*., 2006
3	TTUCG-2	F: 5- GAG AGC GTT ACT CAG CAA A -3 R: 5- TCA CTC TGA GCT GCT ACA ACA-3	53.6 57.1	55	Tu *et al*. 2006 Baublys *et al*., 2006
4	TTUCG-4	F: 5′- GGT GTA CTC TGC TGA GTG TC-3′ R: 5′- TTA GAA CTA GTG GAT CTC TC-3′	54.0 41.1	55	Tu *et al*., 2006 Baublys *et al*., 2006
5	APH11	F: 5′- GGA CCT CAG GAA AAT CAG TGT-3′ R: 5′- GCA GGC AGA GCA GGA AAT A-3′	45-57	51	Ahmadi *et al*., 2007
6	APH17	F:5′- GGA CAT TTT CAA CCA TAA ACT C-3′ R: 5′- CAT CCA TGA CAG ACA GAA GA -3′	60.0	60	Maak *et al*., 2003
7	APH18	F: 5′- TTC TGG CCT GAT AGG TAT GAG-3′ R: 5′- GAA TTG GGT GGT TCA TAC TGT-3′	56.0	56	Maak *et al*., 2003
8	APH21	F: 5′- CTT AAA GCA AAG CGC ACG TC-3′ R: 5′- AGA TGC CCA AAG TCT GTG CT -3′	59.0	59	Maak *et al*., 2003
9	CAUD019	F: 5′- CTT AGC CCA GTG AAG CAT G-3′ R: 5′- GCA GAC TTT TAC TTA TGA CTC-3′	58.1	58	Huang *et al*., 2005
10	CAUD024	F: 5′- TCG CAT TAA GCT CTG ATC T-3′ R: 5′- ATC AAC AGA ATC CAA AAT ATG-3′	55.5	55	Huang *et al*., 2005

Table (3): Total number of unique bands and polymorphic bands obtained by using SSR primers for studied geese populations.

Primers	Monomorphic bands	Unique bands	Polymorphic bands		Total number of bands	Polymorphism (%)
			without Unique	with Unique		
SFIMU-1	0	4	4	8	8	100.0
TTUCG-2	1	0	0	0	1	0.0
TTUCG-4	1	0	0	0	1	0.0
APH11	0	2	0	2	2	100.0
APH18	1	0	2	2	3	66.7
APH21	0	1	5	6	6	100.0
CAUD019	1	2	0	2	3	66.7
CAUD024	1	2	2	4	5	80.0
Mean ± SE	0.63± 0.18	1.38±0.73	1.63±0.46	3.0±1.0	3.63±0.89	64.18±14.9

Table (4)**:** A summary of monomorphic, polymorphic and unique bands molecular weight (size) amplified by SSR microsatellite primers for the whitish grey and white Greylag geese populations.

Primers	Molecular size		
	Monomorphic bands	Polymorphic bands	Unique bands
TTUCG-4	0.096 **(1)**	**(0)**	**(0)**
CAUD024	0.219 **(1)**	0.073, 0.183 **(2)**	0.131, 0.108 **(2)**
APH21	**(0)**	0.070, 0.050, 0.117, 0.094, 0.038 **(5)**	0.202 **(1)**
SFIMU-1	**(0)**	0.201, 0.176, 0.076, 0.067 **(4)**	0.331, 0.454, 0.573, 0.743 **(4)**
APH18	0.065 **(1)**	0.091, 0.218 **(2)**	**(0)**
TTUCG-2	0.135 **(1)**	**(0)**	**(0)**
CAUD019	0.108 **(1)**	0.293, 0.997 **(2)**	**(0)**
APH11	**(0)**	0.091, 0.218 **(2)**	**(0)**

Table (5): Dice's similarity coefficient matrix within the whitish grey and white greylag geese populations examined based on bands polymorphism of SSR primers.

Population (sample's no. on the gel)	Population																		
	Wg♂1	Wg♂2	Wg♂3	Wg♀4	Wg♀5	Wg♀6	Wg♂7	Wg♂8	Wg♂9	Wg♀10	Wg♀11	Wg♀12	W♂13	W♂14	W♀15	W♀16	W♀17	W♀18	W♀19
Wg ♂2	0.516	-																	
Wg ♂3	0.645	0.714	-																
Wg ♀4	0.741	0.684	0.737	-															
Wg ♀5	0.737	0.467	0.600	0.615	-														
Wg ♀6	0.769	0.757	0.703	0.848	0.640	-													
Wg ♂7	0.600	0.581	0.581	0.667	0.737	0.615	-												
Wg ♂8	0.696	0.588	0.706	0.800	0.818	0.759	0.87	-											
Wg ♂9	0.714	0.769	0.769	0.857	0.667	0.882	0.714	0.839	-										
Wg ♀10	0.696	0.529	0.647	0.733	0.727	0.621	0.783	0.846	0.710	-									
Wg ♀11	0.762	0.688	0.562	0.643	0.500	0.815	0.571	0.583	0.759	0.500	-								
Wg ♀12	0.833	0.686	0.800	0.839	0.783	0.867	0.750	0.889	0.875	0.741	0.720	-							
W♂13	0.800	0.516	0.645	0.741	0.842	0.769	0.600	0.783	0.714	0.609	0.667	0.833	-						
W♂14	0.762	0.562	0.688	0.643	0.900	0.667	0.762	0.833	0.690	0.833	0.545	0.800	0.762	-					
W♀ 15	0.706	0.429	0.429	0.583	0.625	0.522	0.824	0.700	0.560	0.700	0.667	0.571	0.588	0.667	-				
W♀ 16	0.700	0.581	0.581	0.741	0.526	0.692	0.700	0.696	0.714	0.609	0.762	0.750	0.700	0.476	0.706	-			
W♀ 17	0.778	0.414	0.552	0.64	0.824	0.667	0.556	0.762	0.615	0.667	0.632	0.727	0.889	0.737	0.667	0.667	-		
W♀ 18	0.870	0.647	0.647	0.733	0.636	0.897	0.522	0.692	0.839	0.615	0.917	0.815	0.783	0.667	0.600	0.696	0.762	-	
W♀ 19	0.833	0.686	0.686	0.774	0.696	0.933	0.583	0.741	0.875	0.593	0.880	0.857	0.833	0.720	0.571	0.667	0.727	0.963	-
W♀ 20	0.750	0.444	0.444	0.522	0.667	0.545	0.750	0.632	0.500	0.632	0.706	0.600	0.625	0.706	0.923	0.625	0.714	0.632	0.600

Wg = whitish grey and W= white

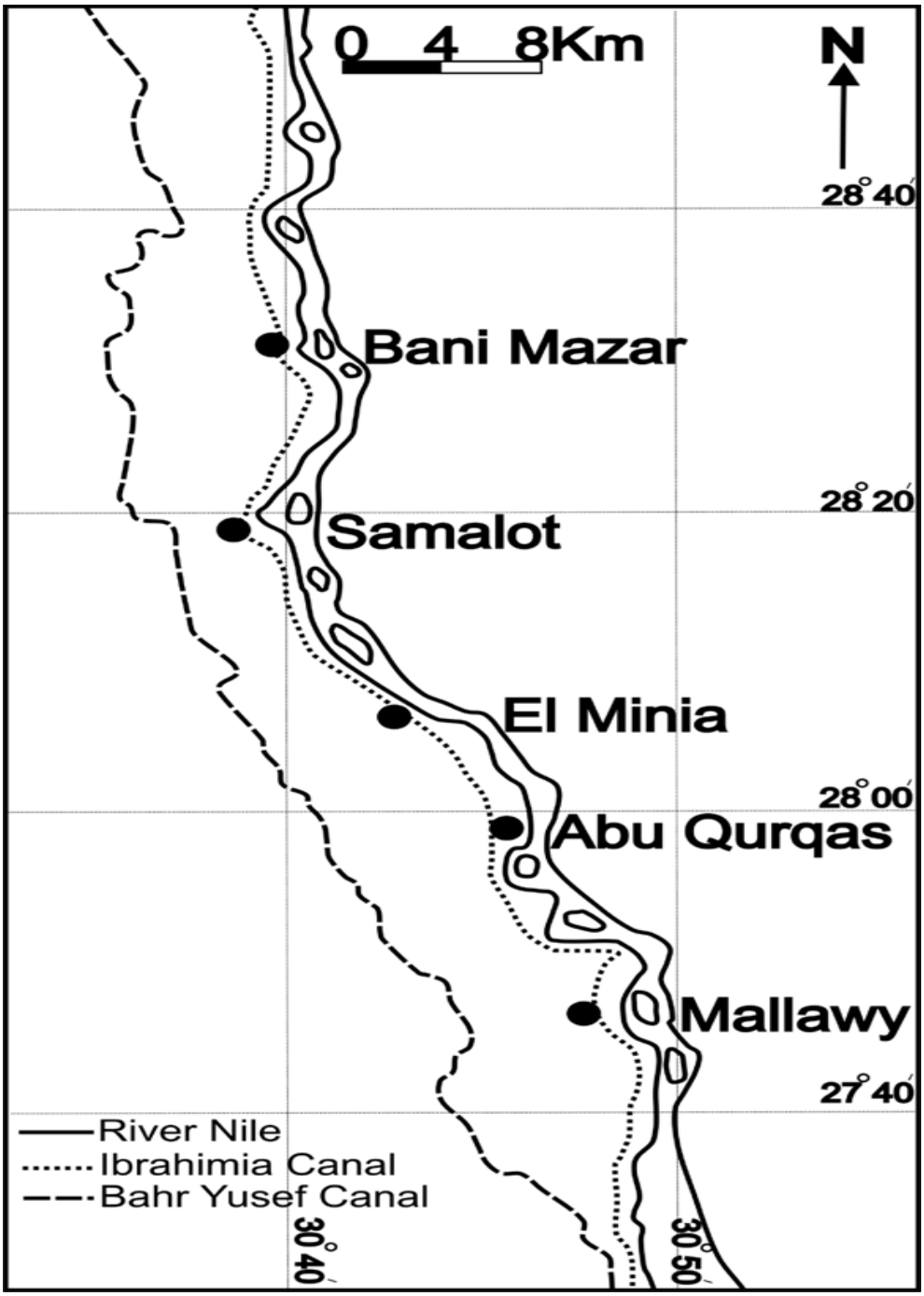

Fig. (1): The localities from which samples were collected in El Minia province (Upper Egypt).

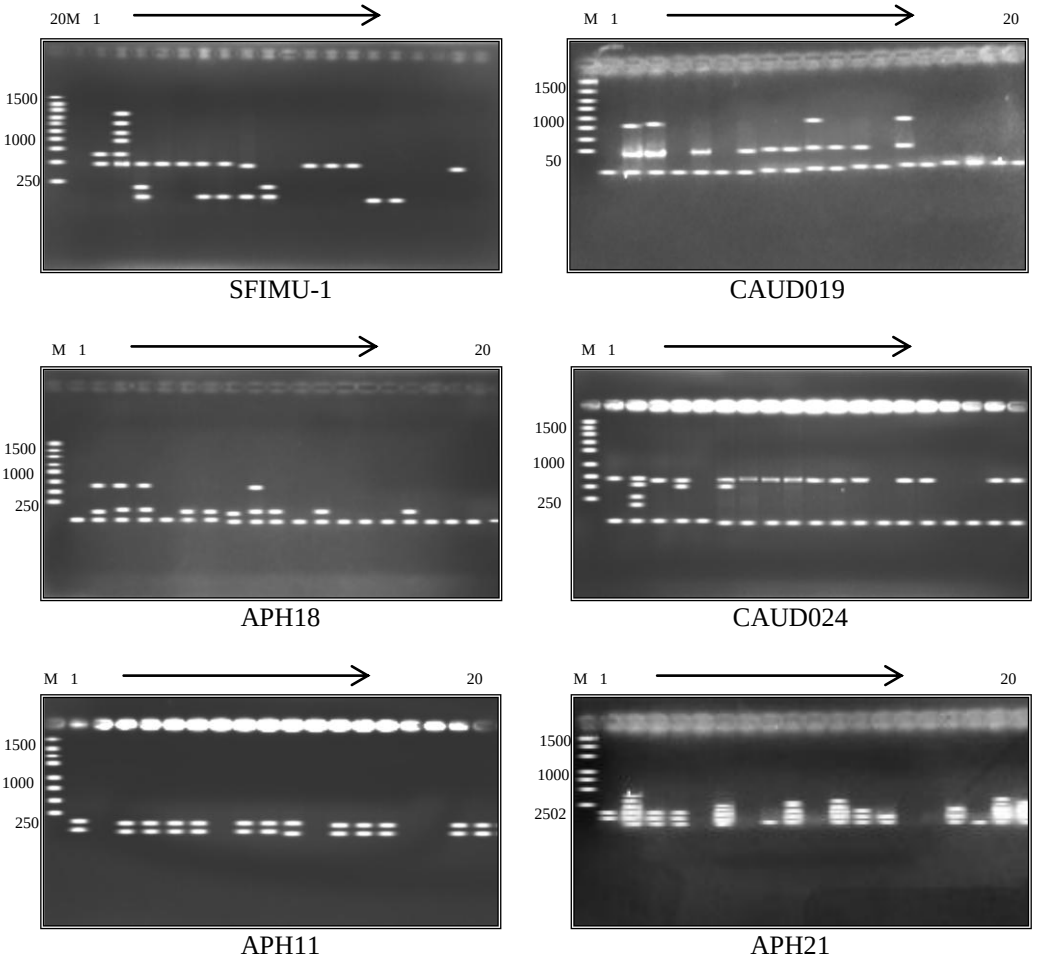

Fig. (2): Electrophoretic gel patterns of SSR DNA products of SFIMU-1, APH18, APH11, APH21, CAUD024 and CAUD019 primers. Lanes (1-3) whitish grey males, lanes (4-6) whitish grey females, lanes (7-9) whitish grey males, lanes (10-12) whitish grey females, Lanes (13-14) white males and lanes (15-20) white females.

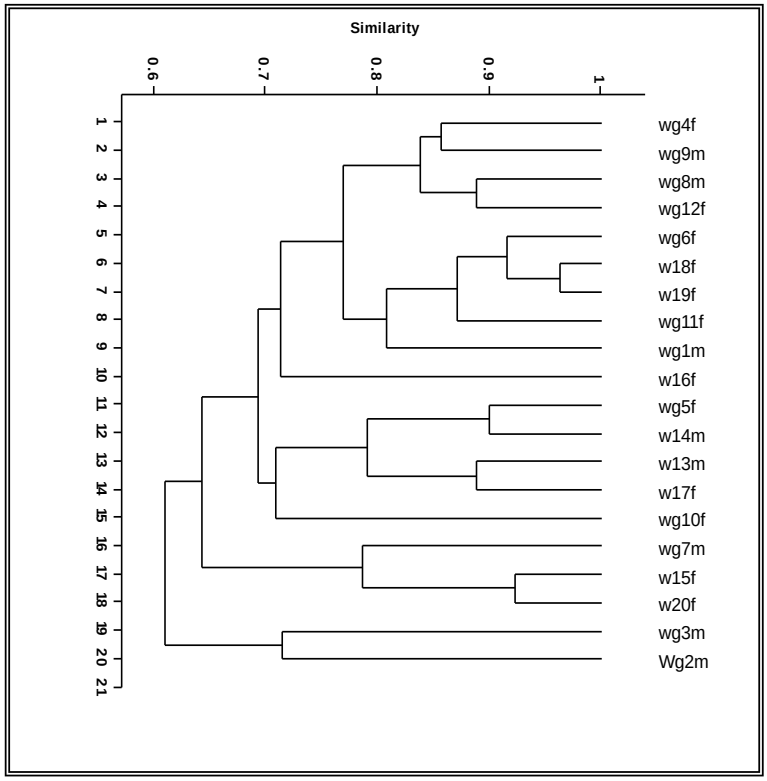

Fig. (3): Dendrogram constructed from the matrix of Dice′s (1945) similarity coefficient values (S) between all pair-wise individual comparisons within populations using hierarchical cluster analysis method with the average linkage between groups. Numbers refer to individuals in gel as in Fig. (2).