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Molecular characterization of tyrosinase gene (exon 1) in camels of Saudi Arabia

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ABSTRACT

We detected genetic variations and single nucleotide polymorphisms (SNPs) of tyrosinase gene (*Tyr*) among seven camel populations in Saudi Arabia. Exon 1 of *TYR* was amplified from 166 DNA samples representing six indigenous camel populations and one exotic population (Alsumalia) that yielded a 474-bp fragment. Two SNPs (T/C) were detected at coding positions 200 and 523 bp. Significant differences in genotypic frequencies were observed at coding position 200. Three different genotypes (CC, TT, CT) were detected at position 200 in each studied population, except Alsumalia, which only had CT. At position 523, the CC genotype was detected in Majaheem and TT was detected in the other populations. The C allele dominated over T allele suggesting that coat color might be associated with it. The populations Majaheem, Maghateer, Hamra, Sofr, and Sawahli had higher C allele frequency than Shaul and the exotic Alsumalia. The cluster analysis of genotypic frequencies at positions 200 and 523 indicated that Majaheem was not closely related to other Saudi populations. The detection of polymorphism at position 523 in Majaheem and in wild Bactrian camel led us to conclude that wild Bactrian camel could be the immediate ancestor of Majaheem populations and other Saudi Arabian populations, as well.

Key words: Camels, Genetic variation, SNPs, Tyrosinase gene.

INTRODUCTION

Arabian camels belong to the genus *Camelus* (the old world camels), which includes only two species, the one-humped (*Camelus dromdarius*) and two-humped camels (*Camelus bacterianus*). Camels are unique animals in many aspects and cannot be compared with other farm animals in their physiological responses or in their adaptation to arid environment (Sweet, 1965; Schwartz, 1992). They are used in transportation, trade, agricultural work, tourism, race and beauty contests and as a source of economically important products, such as meat, milk and wool (Groeneveld *et al*., 2010).

Mehaia *et al*. (1995) reported that indigenous camels in Saudi Arabia can be classified into different ecotypes: Majaheim or Malha, Wadah, Hamra, Safrah and Omani (Wardeh, 1989; Wardeh and Al-Mustafa, 1990; Elamin and Wilcox, 1992). The Majaheim ecotype is restricted to central Arabia, with some spread in the east as well. It is large, blackish-brown in color, and the breeders consider it the best local variety available for milk production. The Wadah ecotype is the camel of the western part of Arabia and is also found in the central and northern part of the country. It is small, white in color and produces small amounts of milk. The Hamra ecotype, fawn in color, is found in small numbers in the central and northern parts of the country. The Safrah ecotype is restricted to the northern part of the country. It is brownish-yellow with a small head and a large abdomen. The Omani ecotype is light in weight and is used mainly for riding and racing.

In the last decades, the population of dromedary camel have declined at a very high rate because of the continuous development of modern transportation, the improvements in agricultural mechanization, and the development of camel products (Ming *et al*., 2016). To avoid a decline in the population of dromedary camel, its genetic resources should be preserved for sustainable utilization and conservation through assessment of genetic variability using genetic markers.

Single nucleotide polymorphism (SNP) in tyrosinase gene (*TYR*) is now widely used as a genetic marker for analyzing genetic variations and mammalian coat color phenotypes (Everts *et al.,* 2000). *TYR* was identified and characterized by Giebel *et al*. (1991) who showed that TYR consists of five exons and four introns. Exon1 of *TYR* is of interest in the investigation of genetic variation and in the analysis of coat phenotypes in *C. dromedarius*. The TYR gene is expressed into its product tyrosinase, which is located in melanocytes. The cells of melanocytes are specialized in

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producing melanin, a pigment that confers color to skin, hair, and eyes (Shah *et al*., 2005). Mutations in *TYR* were responsible for the albino phenotype in mammals and chicken, and influence quantitative traits in mice (Schmidtz *et al.*, 2001).

Shah *et al.* (2005) designed a primer pair based on the partial sequences of mouse and human that can be used for the amplification of an 820-bp fragment in exon 1 of the camel tyrosinase gene. By sequencing the exon 1 of *TYR* from different Pakistani camel breeds, they detected a single nucleotide polymorphism (C>T) at coding position 200 after the ATG, causing an amino acid substitution (Pro>Leu). Shah *et al*. (2008) investigated the genetic variation between the breeds of *C. dromedarius* in Pakistan by genotyping of *TYR* using PCR-RFLP, recording significant differences in the genotype frequency between the breeds. The allelic variants of *TYR* in Sudanese camel breeds were studied using PCR-RFLP analysis (Ishag *et al*., 2013) revealing that there are no significant differences in the allele frequency between the breeds and there is no significant association between the camel coat color and *TYR* genotypes (TT, CC, TC). In Egyptian populations, genotyping of *TYR* was done using PCR-RFLP technique (Alam *et al*., 2015), and only one SNP (C/T) was detected in exon1 among the five tested camel breeds and this nucleotide substitution was recommended as a marker for assessing the genetic biodiversity among camel breeds reared in Egypt.

Previous studies on genetic variability among *C. dromedarius* populations based on polymorphisms in sequences of exon 1 of *TYR* showed considerable discrepancies in the genetic variation data in *C. dromedarius* populations from different countries. No strong evidence on the relation between polymorphisms in the *TYR* exon 1 and coat color was reported as in other mammals, and limited data are available on genetic variation in Saudi Arabian populations. The objective of this study, therefore, was to evaluate the genetic variability in *C. dromedarius* populations of Saudi Arabia, and to determine the relationship between the polymorphism in *TYR* exon 1 and coat color phenotypes.

MATERIALS AND METHODS

Animal Resources: Hair samples were collected from 166 camel individuals representing six indigenous breeds (28 Magaheem, 29 Maghateer, 18 Shaul, 28 Hamra, 19 Sofr, 24 Sawahli) and one exotic breed (20 Alsumalia). The collected samples were kept at -20°C for further use. The samples taken from Alsumalia population were collected from individuals immediately released from quarantine.

DNA Extraction: Genomic DNA was extracted from hair roots using the QIAgen DNeasy blood and tissue kit (Hildane, Germany). The concentration and quality of DNA was measured with a NanoDrop 2000 spectrophotometer (Thermo Scientific, Wilmington, Delaware, USA). All the extracted DNA samples were stored immediately at -80°C for further use.

Amplification of Exon 1: The exon 1 of *TYR* was amplified from camel DNA samples using two PCR primers, forward 52 -AGC CTG TGC CTC CTC CAA GAA-32 and reverse 52 -TGC ATC CAT ACA AAG AAG TCA TAA-3′, which yielded a 474-bp product. Polymerase chain reaction (PCR) amplifications were carried out in a 25-µL reaction volume containing 100 ng of template DNA and 2 µL of each of the 10 µM primers. To reduce the possibility of cross contamination and variation in the amplification reactions, master mixes containing all PCR reagents including the Kapa Taq polymerase enzyme (KAPA Biosystems, Boston, MA, USA), except DNA template and primers, were used. The amplification was performed using Gene Amp PCR system 9700 thermocycler (Applied Biosystems, Warrington, UK). The amplification protocol included denaturation at 94°C for 2 min, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 60°C for 40 s and extension at 72°C for 1 min, and final extension at 72°C for 5 min. PCR products were electrophoresed on 1% agarose gel, which was subsequently stained with ethidium bromide and the amplified bands were detected under UV light using a gel documentation system (Amersham Biosciences, Uppsala, Sweden).

DNA sequencing and sequence analysis: PCR products were extracted from the gel and sequenced at the Advanced Genetic Technologies Center (http://www.uky. edu /Centers/ AGTC/). The DNA sequences were edited and aligned using BioEdit software (Hall; http://www.mbio. ncsu edu Bioedit/ bioedit.html). The BLAST algorithm was used to search the NCBI GenBank database (http www.ncbi.nlm.nih.gov) for homologous sequences. The BioEdit software was also used to detect SNPs and indel mutations.

Data analysis: A dendrogram was constructed based on Nei's genetic distance employing UPGMA (Nei, 1978) using SYSTAT 13.1 software.

RESULTS AND DISCUSSION

Tyrosinase has a role in determining the coat color and significantly effects some phenotypic characteristics in farm animals (Oetting and King, 1999; Aigner *et al*., 2000; Beermann *et al*., 2004; Schmutz *et al*., 2004; Ishag *et al*., 2013). This gene codes for the TYR enzyme that is a key enzyme in the melanogenic metabolic pathway leading to coat color pigmentation in mammals. Mutations in *TYR* are responsible for the albino phenotype in these organisms. The albinism is due to the loss of expression of TYR, which prevents melanin synthesis.

In this study, a 474-bp fragment from exon 1 of *TYR* was amplified and sequenced from camels representing seven populations (Majaheem, Maghateer, Shaul, Hamra, Sofr, Sawahli, and Alsumalia) raised in Saudi Arabia (Fig 1). Two

nonsynonymous SNPs were detected. The first one (C>T) was at coding position 200, which led to a Leu>Pro substitution at amino acid position 67 of the TYR protein (Fig 1) and the second (C>T) was at coding position 523, which led to a Leu>Phe substitution at amino acid position 175.

There were significant differences in the genotypic frequencies among the seven populations (Table 1). The genotypic frequencies at position 200 were 0.48, 0.084 and 0.43 for CC, TT, and CT genotypes, respectively. All the populations possessed the three detected genotypes in different frequencies with the exception of the HA population that lacked the TT genotype and SU that lacked the CC and TT genotypes and only had the CT genotype (Table 2). The detection of the CT genotype only in Alsumalia population contradicted the findings of Alam *et al*. (2015) who reported the three genotypes in Alsumalia breed. This discrepancy can be interpreted on the basis that samples in their study might have been taken from individuals introduced to Egypt and there was an exchange of genes with other Egyptian breeds through breeding. The highest CC, TT and CT genotype frequencies were found in SO (0.79), SH (0.28), and SU (1.00) camels, respectively (Table 1). The highest genotype frequency was for CC followed by those for CT and TT and the frequency of allele C was dominant over that of allele T (Table 1). The dominance of allele C over allele T was also reported by Shah *et al.* (2008, 2012) and Alam *et al*. (2015) in their study on Pakistani and Egyptians camel breeds. The similar frequency of C allele in MG and MJ breeds could be due to the fact that these two breeds are often raised together in mixed herds (Ishag *et al*., 2013). The low frequency of TT genotypes was also reported by Shah *et al*. (2005, 2008) and Alam *et al.* (2015) in their studies on Pakistani camel breeds. They attributed their results to the active selection against TT genotype resulting

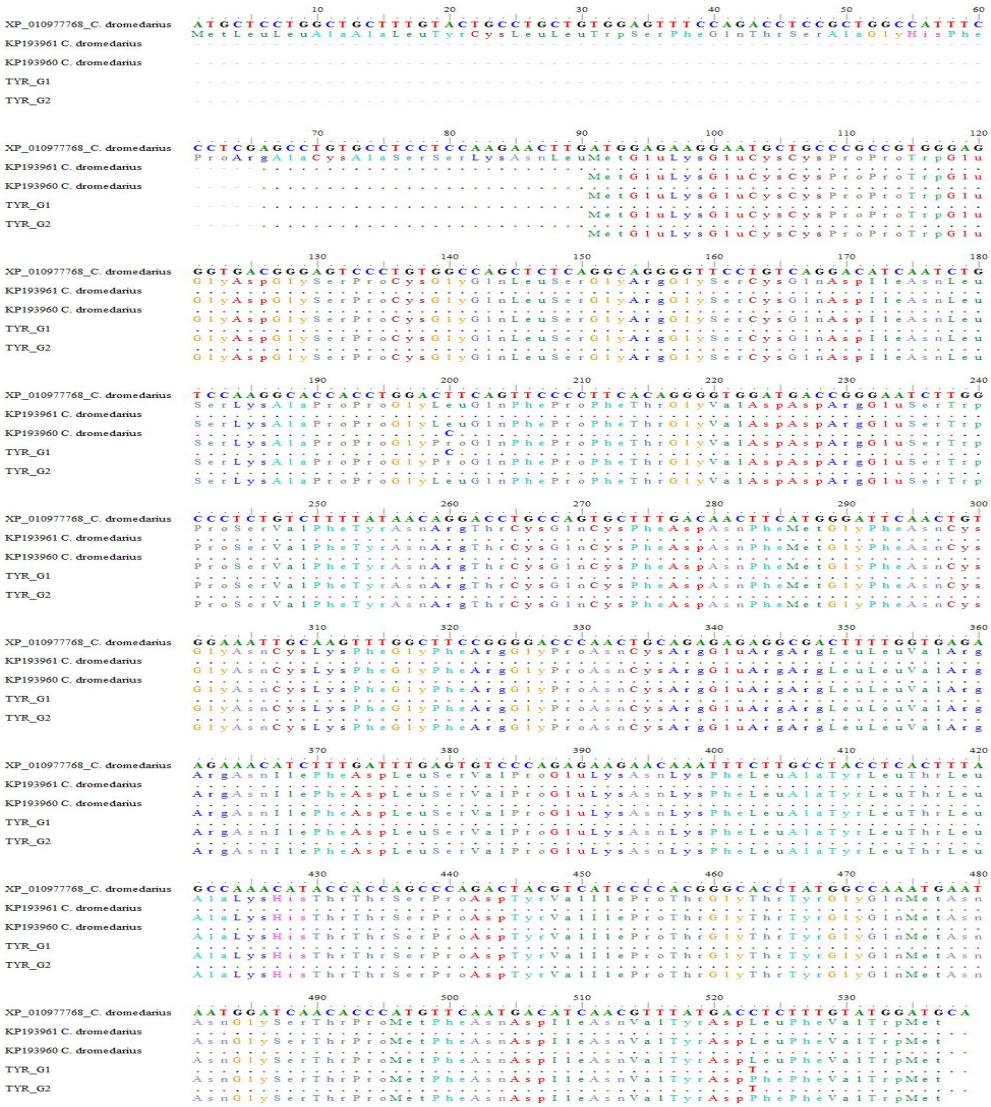

Fig 1: Aligned DNA sequences of the 474-bp fragments of *tyrosinase* (*TYR*) exon 1. TYR-G1 and TYR-G2 genotypes were generated in this study from Saudi camels, whereas XP_010977768, KP193960, and KP193961 accession numbers were retrieved from NCBI-GenBank.

Table 1: Genotype and allele frequencies of C>T at coding position 200 and of C>T at position 523 in tyrosinase gene in Saudi Arabian camel populations.

Breed[a]	Coat color	Genotype frequency at position 200			Allele Frequency at position 200		Genotype frequency at position 523			Allele Frequency at position 523	
		CC	TT	CT	C	T	CC	TT	CT	C	T
MG	Black	0.54	0.11	0.35	0.71	0.29	1.00	0.00	00.0	1.00	00.0
MJ	White	0.48	0.07	0.45	0.71	0.29	0.00	1.00	00.0	00.0	1.00
SH	Brown	0.39	0.28	0.33	0.56	0.44	0.00	1.00	00.0	00.0	1.00
HA	Clear Brown	0.64	0.00	0.36	0.82	0.18	0.00	1.00	00.0	00.0	1.00
SO	Dark brown	0.79	0.05	0.16	0.87	0.13	0.00	1.00	00.0	00.0	1.00
SL	Red	0.54	0.08	0.38	0.73	0.27	0.00	1.00	00.0	00.0	1.00
SU	Brown	0.00	0.00	1.00	0.50	0.50	0.00	1.00	00.0	00.0	1.00
Overall		0.48	0.084	0.43	0.70	0.30	0.14	0.86	00.0	1	0.86

[a] The breed designations are as follows: MG: Majaheem, MJ: Maghateer, SH: Shaul, HA: Hamra, SO: Sofr, SL: Sawahli, SU: Alsumalia

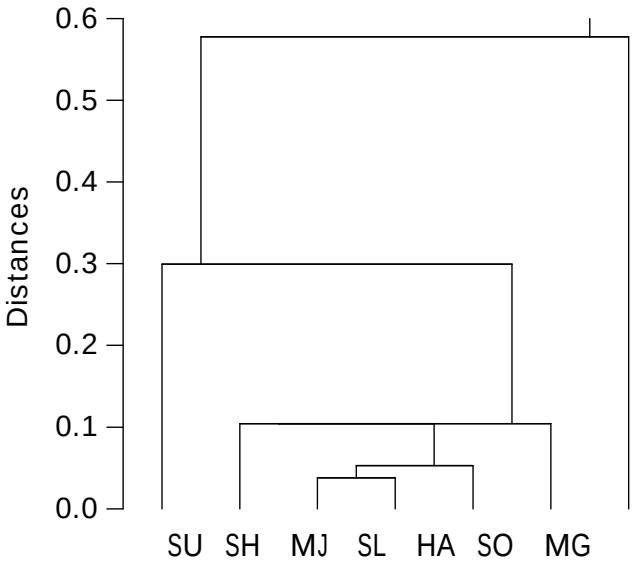

Fig 2: UPGMA dendrogram showing the relationships among seven camel populations based on the data of genotypic frequencies at coding positions 200 and 523 in *tyrosinase* exon1.

from local preference. However, because active selection against TT genotype was not restricted to Pakistani camel breeds but was extended to Egyptian and Saudi Arabian camel breeds, it can be suggested that the active selection against TT genotypes could be due to global environmental preferences.

The frequency of genotypes CC, CT, and TT matched their frequencies in the study of Ishag *et al*. (2013) and Alam *et al*. (2015) on Sudanese and Egyptian camel breeds, respectively. Although, Ishag *et al*. (2013) reported that there was a significant effect of SNPs in *TYR* on shoulder height and there was no significant influence on other phenotypic measurements or on the coat color in Sudanese camel; our results suggest a possible association between *TYR* allele C and the darkness of camel coat color, where the highest frequencies of allele C were present in MG (0.54), SO (0.79) and SL (0.54) and the camels of these breeds possess dark colors ranging from black to red (Table 2). In addition, MG, which has black color, was found to have genotype CC at position 523 where the other breeds had TT. The association of C allele with coat color contradicted the suggestion of Alam *et al*. (2015) and Shah *et al*. (2008 and 2012) that the coat color is association with T allele. The active selections of Pakistani, Egyptian and Saud Arabian camel breeds against the TT genotype and the lack of TT genotype in HA breed supports our suggestion.

The cluster analysis of the data for genotypic frequencies showed the presence of two clusters, one included the MG population and the other contained the other populations (Fig 2). The second cluster was divided into two sub-clusters: one included the SU population and the other contained SH, MJ, SL, HA, and SO populations. Based on these data, it can be suggested that the SH, MJ, SL, HA, and SO breeds were derived from SU breeds. Moreover, the genetic distance between MG and other breeds led us to conclude that the Saudi camel populations were derived from MG. In addition, the genetic distance between MG and other populations and the presence of polymorphism at position 523 in both MG and wild Bactrian camel (the combination of mutations in *TYR* deposited in GenBank under the accession number 010973375.1) suggests that wild Bactrian camel might be the immediate ancestor of Saudi populations.

CONCLUSION AND RECOMMENDATIONS

It is concluded that there is (1) a moderate but significant difference in the allele frequencies of *TYR* exon 1 among the Saudi Arabian camel breeds, (2) a significant association between coat color and CC genotypes, (3) a new mutation at position 523 in exon 1 of *TYR* in MG breed (genotype CC) and (4) active selection against the TT genotype. It is also concluded that wild Bactrian camel might be the immediate ancestor of Saudi Arabian breeds. Further studies with larger number of camel breeds, collected from a wide geographical area, are required to investigate and verify the associations between *TYR* exon1 and coat color.

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REFERENCES

Aigner, B., Besenfelder, U., Müller, M., Brem G. (2000). Tyrosinase gene variants in different rabbit strains. *Mammalian Genome*. **11**: 700-702.

Alam, S.S., Abd El-Kader, H.A.M., Abd El-Aziem, S.H., Othman, O.E. (2015). Genetic variation and SNP of tyrosinase gene among some camel breeds reared in Egypt. *Biosciences Biotechnology Research Asia*. **12:** 23-28.

Beermann, F., Orlow, S.J., M., Lamoreux, M.L. (2004). The Tyr (albino) locus of the laboratory mouse. *Mammalian Genome*, **15:** 749-758.

Elamin, F.M, Wilcox, C.J. (1992). Milk composition of Majaheim camels. *Journal of Dairy Science*. **75:** 3155-3157.

Everts, R.E, Rothuizen, J., van Oost, B.A. (2000). Identification of a premature stop codon in the melanocyte-stimulating hormone receptor gene (MC1 R) in Labrador and Golden retrievers with yellow coat color. *Animal Genetics*. **31:** 194-199.

Giebel, L.B, Strunk, K.M, Spritz, R.A, (1991). Organization and nucleotide sequences of the human tyrosinase gene and a truncated tyrosinase-related segment. *Genomics*. **9:** 435-445.

Groeneveld, L.F. Lenstra, J.A., Eding, H., Toro, M.A., Scherf, B., Pilling, B., Negrini, R., Finalay, E.K., *et al*. (2010). The GLOBALDIV consortium genetic diversity in farm animals–a review. *Animal Genetics*. **41**: (Suppl 1) S6-S31.

Ishag, I.A., Reissmann, M., Eltaher, H.A., Ahmed, M.K.A. (2013). Polymorphisms of tyrosinase gene (Exon 1) and its impact on coat color and phenotypic measurements of Sudanese camel breeds. *Scientific Journal of Animal Science*. **2:** 109-115.

Oetting, W.S., King, R.A. (1999). Molecular basis of albinism: mutations and polymorphisms of pigment genes associated with albinism. *Human Mutation*. **13**: 99-115.

Mehaia, M.A., Hablas, M.A., Abdel-Rahman, K.M., El-Mougy, S.A., (1995). Milk composition of Majaheim, Wadah and Hamra camel in Saudi Arabia. *Food Chemistry*. **52:** 115-122

Ming, L., Yi, L., Sa, R., Ji, R., Ha, S. (2016). Polymorphisms of the tyrosinase (TYR) gene in Bactrian camel (*Camelus bactrianus*) with different coat colour. *Journal of Camel Practice and Research*. **23:** 47-51.

Nei, M. (1978). Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics*, **89**: 583-590.

Shah, M.G, Reissmann, M., Qureshi, A.S, Schwartz, H.J. (2005). Sequencing and mutation screening in exon 1 of camel tyrosinase gene. The global food and product chain-dynamics, innovations, conflicts, strategies. *Deutscher Tropentag*, 13-15.

Shah, M.G., Reissmann, M., Qureshi, A.S., Schwartz, H.J. (2008). Evaluation of six camel breeds for heterozygocity through restriction fragment length polymorphism. *Pakistan Veterinary Journal*. **28:** 13-16.

Shah, M.G., Reissmann, M., Qureshi, A.S., Schwartz, H.J., Khan, M.S., Zaman, T.A., *et al*. (2012). Determination of genotype differences through restriction endonuclease in camels (*Camelus dromedarius*). *African Journal of Biotechnology*. **11:** 11543-11547.

Schwartz, H.J., (1992). The camel (*Camelus dromedarius*) in Eastern Africa. The one-humped camel (*Camelus dromedarius*) in Estern Africa: a pictorial guide to diseases, health care and management. Verlag Josef, Scientific Books D- 6992 Weikersheim Federal Republic of Germany. pp. 1-7.

Schmidtz, B.H., Buchanan, F.C., Plante, Y., Schmutz, S.M. (2001). Linkage mapping of tyrosinase gene to bovine chromosome 26. *Animal Genetics*. **32:** 119-120.

Schmutz, S.M., Berryere, T.G., Ciobanu, D.C., Mileham, A.J., Schmidtz, B.H., Fredholm, M. (2004). A form of albinism in cattle is caused by a tyrosinase frame shift mutation. *Mammalian Genome*. **15:** 62-67.

Sweet, L.E., (1965). Camel pastoralism in North Arabic. In: Man, culture and animals. (Edited by A. Leeds and A.P. Vayda). AAAS Washington, D.C. pp. 129-152.

Wardeh, M.F. (1989). Arabian camels: Origin, Breeds and Husbandry. Ist. Eds. Arabic Version. Al-Mallah Publishers. Damascus. Syria. pp. 500.

Wardeh, M.F. and Al-Mustafa. M. (1990). Camel breed types in the Arab countries North and West Africa. In Arab Symp. Camel Husbandary and Diseases and Methods of Their Control, Marsh 24-26. 1990. Alger, Algeria. ACSAD/AS/p.105/1990. Damascus.