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GENETIC DIVERSITY OF GUAR (*CYAMOPSIS TETRAGONOLOBA* L.) LANDRACES FROM PAKISTAN BASED ON RAPD MARKERS

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Abstract

Genetic diversity is very important for developing new crop varieties with high yield and other desirable traits. Consequently it assists in increasing food production and improving the level of human nutrition. Genetic diversity among 30 guar (*Cyamopsis tetragonoloba* L.) accessions was observed at DNA level using random amplified polymorphic DNA (RAPD) markers. High quality genomic DNA was extracted and PCR conditions were optimized for amplification using 12 random primers. Twelve decamers generated a total of 76 amplification products, of which average proportion of polymorphic loci was 73%. The number of amplified products produced by each primer ranged from 3 to 10 with an average of 6.3 bands per primer. Dice similarity coefficients ranged from 0.49 to 0.93. The highest similarity index of 93% was observed between accessions '27350' and '27351', whereas '24293' and '24302' were the most varied accessions with similarity index of 49%. On the basis of similarity matrix analysis via unweighted pair group method with arithmetic averages (UPGMA), accessions were grouped into three main groups and nine subgroups or clusters. Two accessions '21702' and '24293' remained in distinct clusters. The results from the present study would assist in the identification and demarcation of elite guar accessions for local consumption, and also for export purposes. This work will significantly add to broaden the scope of selection of diverse parent accessions in order to expand germplasm base for future breeding programs.

Introduction

Guar (*Cyamopsis tetragonoloba* L.) or cluster bean is one of the minor crops of Pakistan. It belongs to the family Leguminosae (Fabaceae) and economically the most important of the four species in the genus *Cyamopsis* (Whistler & Hymowitz, 1979; Sultan et al. 2012). It is vigorous, erect and herbaceous legume grows from 0.4 to 3.0 m with a cycle of 80 to 160 days. Guar beans are termed as a 'non-thirsty crop' in spate irrigation areas. Guar seed contains 78-82% of the endosperm (Das & Arora, 1978) and is extremely valued on industrial scale because of its galactomannan rich endosperm. Guar richness in its gum has made guar one of the most important industrial crops (Hymowitz & Matlock, 1963; Pathak *et al.,* 2010). Guar gum is found to be effective in osteoarthritis, transdermal drug delivery systems (Murthy *et al.,* 2004), as artificial cervical mucus (Burruano *et al.,* 2002) and for anticancer medicine in the treatment of colorectal cancer (Shyale *et al.,* 2006). Guar production from Pakistan is about 15%; and the remainder is mostly from the United States and Sudan. The major world suppliers are Pakistan, India and United States, with limited acreages in Africa and Australia (Jackson & Doughton, 1982). Pakistan is one of the most important guar producing countries. According to local marketers' estimates Pakistan annually produces about 70,000 tons of guar, ranging between 50,000 to 110,000 tons per year. Pakistan earns in million dollars as foreign exchange by exporting guar and its resulting products to about 30 different countries of the world (Anon., 1997). Pakistan possesses an enormous wealth of guar that contains wide variability but until now no such work addressing guar germplasm on molecular basis has been reported in Pakistan. Most of the Pakistani guar germplasm with an extensive range of genetic variability is unidentified and unknown. Characterization and quantification of genetic diversity either within or among closely related crop varieties is indispensable for a balanced and rational use of plant genetic resources and also for determining evolutionary relationships (Turi *et al*., 2012; Vir *et al.,* 2010; Zada et al. 2013). RAPD as a genetic marker is most widely used for germplasm characterization, assessment of genetic diversity (Shinwari *et al.,*2013; Liu *et al.,* 2001; Akbar *et al.,* 2011; Steiger *et al.,* 2002;), cultivar identification, gene tagging and also in genetic purity testing (Hu & Quiros, 1991; Rabbani *et al.,* 2010; Asemota, 1996). The present study was conducted to evaluate the existence of genetic variability among indigenous guar accessions using RAPD markers. This work would assist in the identification and demarcation of elite guar accessions for local consumption, and for export purposes. This will also add to broaden the scope of selection of diverse parent accessions in order to expand the germplasm base for future breeding programs.

Materials and Methods

Plant material comprised of 30 guar accessions collected from diverse areas of Punjab, Pakistan (Table 1). On the basis of agro-morphological performance, the accessions were chosen for molecular analysis. Leaf samples from two weeks old seedlings were used for DNA extraction. Multi-pot plastic trays filled with a commercial peat mix were used to geminate selected guar accessions in the greenhouse. Total genomic DNA was extracted from 2-3 fresh leaves from 2 weeks old seedlings using microprep DNA extraction method (Fulton *et al.,* 1995) with minor modifications in order to save cost, labor and time.

Table 1. List of indigenous guar germplasm used for RAPD analysis.

No.	Accession #	Collection area	No.	Accession #	Collection area
1.	24285	Bahawalpur	16.	27367	Unknown
2.	24272	Bahawalpur	17.	24302	Bahawalpur
3.	24299	Bahawalpur	18.	21702	Pakpattan
4.	24276	Bahawalpur	19.	27365	Unknown
5.	24275	Bahawalpur	20.	24333	Bahawalpur
6.	27345	Bhakkar	21.	24300	Bahawalpur
7.	24315	Bahawalpur	22.	27369	Unknown
8.	24303	Bahawalpur	23.	24293	Bahawalpur
9.	24301	Bahawalpur	24.	24305	Bahawalpur
10.	24298	Bahawalpur	25.	24274	Bahawalpur
11.	24286	Bahawalpur	26.	24319	Bahawalpur
12.	27340	Bhakkar	27.	21929	Lahore
13.	24330	Bahawalpur	28.	27362	Unknown
14.	27351	Unknown	29.	27359	Unknown
15.	27350	Unknown	30.	27347	Unknown

Quality and quantity of total genomic DNA of each genotype was checked by diagram evaluation of band intensity in comparison with lambda DNA molecular standards of known concentrations, i.e., 100, 300 and 500ng using 0.8% agarose gel. For PCR analysis, DNA of each genotype was diluted to a working concentration of 20ng/µl with TE buffer. On the whole 40 arbitrary decamer oligonucleotides, 20 belonging to kit OPA, and 20 belonging to kit OPB, from Operon Technologies Inc (Alameda, California, USA), were used to detect polymorphism. Finally 12 primers were selected to study the genetic diversity and relationship among 30 guar accessions.

For the amplification of DNA of guar genotypes a modified RAPD method based on Williams *et al*., (1990) was used. PCR analysis was accomplished in 0.2ml PCR strip tubes with 20µl total volume of reaction mixture containing 1.0µl of template DNA (25ng/µl), 15.4µl ddH_2O, 2.0µl PCR buffer (1x), 0.4µl dNTPs (0.2mM), primer (Operon Technologies Inc., Alameda, CA) 1.0µl and 0.2µl Taq polymerase (1 unit per reaction) (Fermentas Life Sciences). DNA amplifications were performed in an automated MyGene TM Series Peltier Thermal Cycler (MG96G) and it was programmed to 1 cycle of 4 minutes at 94^{o}C temperature for initial strand separation, followed by 40 cycles of 40 sec at 94^{o}C for denaturation, 40sec at 36^{o}C for annealing and 2 minutes at 72^{o}C for primer extension. Finally 1 cycle of 7 minutes at 72^{o}C was used for final extension, followed by 4^{o}C temperature to hold samples until removed from PCR machine.

After amplification, 3µl of DNA loading dye (0.02% Bromophenol bue, 0.02% xylene cyanol FF, 50% glycerol and 1% SDS) was added to the amplification product tube and spun for few seconds in micro-centirfuge. A 10µl of amplification products in addition with loading dye were then loaded in 1.5% agarose gels for electrophoresis in 1x TBE (10mM Tris-Borate, 1mM EDTA) buffer. Gel was run at 100V for one hour and 30 minutes to separate the amplified products. A 1kb DNA ladder (Fermentas Life Sciences) was used as fragment size standard (Marker) and loaded in the first well of the gel. After electrophoresis the gels were stained with Ethidium bromide for 30 minutes and photographed under UV light in a gel documentation apparatus (Polaroid, Cambridge, Mass., USA).

Each DNA fragment amplified by a certain primer was considered as a unit character. From top of the gel (band number 1) to the bottom, RAPD fragments were scored as 1 for presence or 0 for absence. Since DNA samples consisted of a bulk of DNA, a minute intensity for any specific fragment may be clarified by the smaller symbol of that specific sequence and the fragments with the identical mobility were considered to be the equal fragments. Only key bands were scored and dim bands were not taken into consideration. The molecular size of the amplification products was calculated from a standard curve based on known size of DNA fragments of a 1kb along with molecular size weight marker. Binary data matrix was used to score presence and absence of bands. Pair-wise comparisons of the accessions established on the presence or absence of elite and shared amplification products were used to generate similarity coefficients. Genetic similarity (F) was estimated among all pairs of the accessions by Dice algorithm. The Dice algorithm is corresponding to that of Nei & Li (1979) as under:

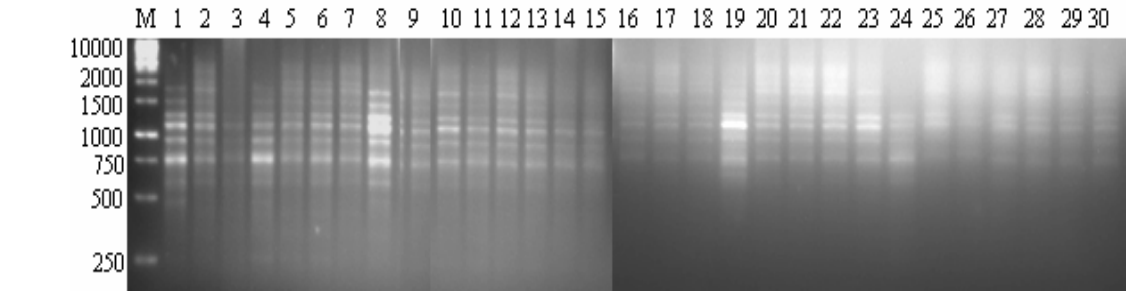
$$\text{Similarity (F)} = 2Nab/ (Na + Nb)$$

where
Na = number of scored fragments of individual ‘a’
Nb = number of scored fragments detected in individual ‘b’ and
Nab = number of shared fragments between individual ‘a’ and ‘b’.

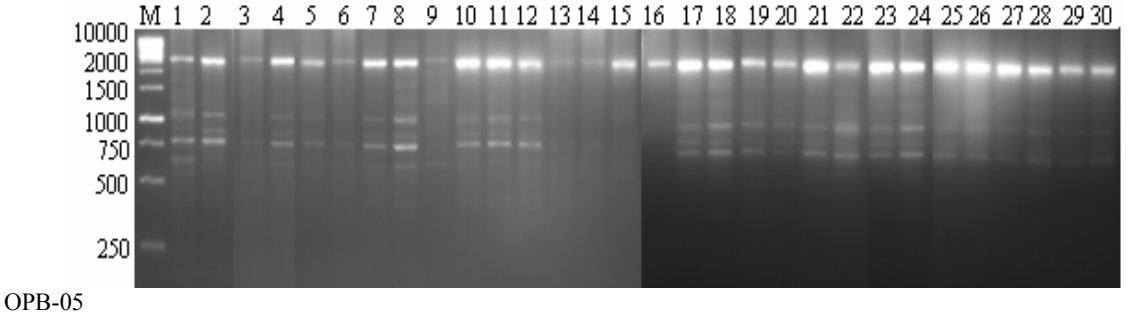
Relations among germplasm accessions were estimated on the basis of the resulting similarity coefficients with cluster analysis using an unweighted pair-group method with arithmetic average (UPGMA) and then plotted in the form of dendrogram. All calculations were performed using the computer program NTSYS, version2.1 (Applied Biostatistics Inc., USA) (Rohlf, 2000).

Results

Overall 30 guar genotypes were differentiated by means of a blend of 12 primers generating polymorphic fragments. A number of polymorphic bands were produced by PCR amplification profiles of primers in guar genotypes. Fig. 1 illustrates the pattern of amplified products in 30 guar accessions generated with the primers OPA-13, OPB-14, OPB-18 and OPB-05, respectively. In some of the guar accessions alike banding patterns were observed, whereas some of the genotypes shared few bands with other accessions, depicting their distinct relationship to them. However, some of the guar accessions showed unique banding pattern as compared to other guar genotypes. PCR amplification profiles of primers OPA-13, OPB-03 and OPB-18 generated a number of polymorphic bands. Considerable variation was noticed in each primer to detect variation among accessions. Some of the primers produced significant number of amplified products and high level of genetic diversity, while minimum number of bands and little variability was produced by rest of the primers.



OPA-13



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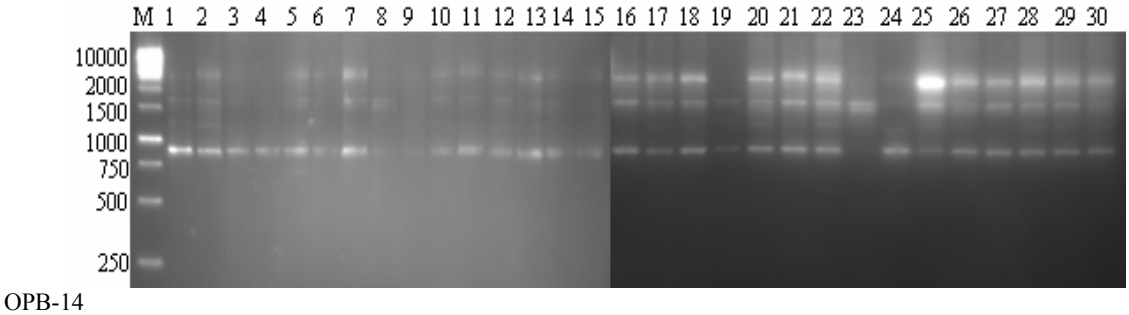

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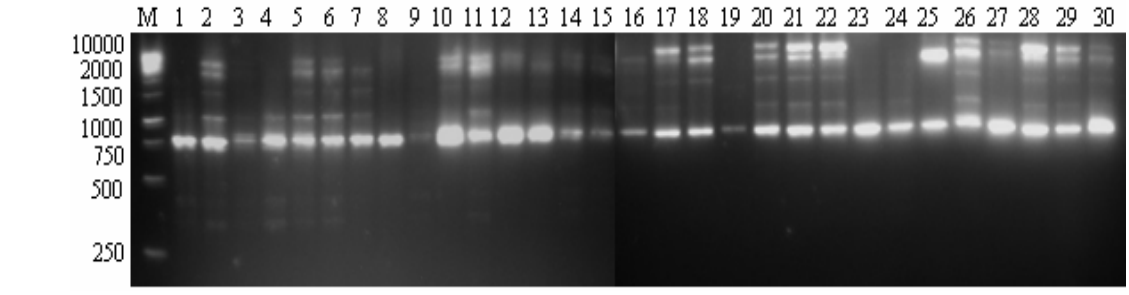

OPB-18

Fig. 1. RAPD banding pattern of guar genotypes generated by random primers OPA-13, OPB-05, OPB-14 and OPB-18. M = 1kb molecular marker, 1 = 24285, 2 = 24272, 3 = 24299, 4 = 24276, 5 = 24275, 6 = 27345, 7 = 24315, 8 = 24303, 9 = 24301, 10 = 24298, 11 = 24286, 12 = 27340, 13 = 24330, 14 = 27351, 15 = 27350, 16 = 27367, 17 = 24302, 18 = 21702, 19 = 27365, 20 = 24333, 21 = 24300, 22 = 27369, 23 = 24293, 24 = 24305, 25 = 24274, 26 = 24319, 27 = 21929, 28 = 27362, 29 = 27359, and 30 = 27347.

Out of total 76 amplification products, 56 bands were polymorphic (Table 2). High degree of polymorphism ranging from 62 to 85% was observed with average proportion of polymorphic loci as 73%. The number of amplified products produced by each primer ranged from 3 to 10 with an average of 6.3 bands per primer. Minimum number of bands was produced by primer OPB-20, whereas OPB-11 generated maximum number of bands across 30 guar genotypes. The highest percentage of polymorphism i.e., 85% was observed by primers OPB-03 and OPB-18. The highest percentage of polymorphism was followed by 83% polymorphism for OPB-19 (5 polymorphic bands out of 6), 80% for OPB-14 (4 polymorphic bands out of 5), 75% for OPB-01 (3 polymorphic bands out of 4) and OPA-13 (6 polymorphic bands out of 8) and 70% polymorphism was noticed by OPB-11 (7 polymorphic bands out of 10). Four primers, OPB-04 (4 polymorphic bands out of 6), OPB-05 (4 polymorphic bands out of 6), OPB-16 (4 polymorphic bands out of 6) and OPB-20 (2 polymorphic bands out of 3) resulted in 66% polymorphism. The lowest percentage of polymorphism was produced by primer OPB-17 (5 polymorphic bands out of 8), i.e. 62%. The size of the amplified fragments ranged from 300 (OPB-17) to 6500bp (OPB-16).

Table 2. Details of RAPD primers showing number of bands and percentage of polymorphism generated from 30 guar genotypes.

No.	Primer names	Sequence (5'-3')	Amplified fragments (a)	Polymorphic fragments (b)	Percentage polymorphism (bx100/a)	Fragment size range (bp)
1.	OPB-01	5'-GTTTCGCTCC-3'	4	3	75%	1000-2500
2.	OPB-03	5'-CATCCCCCTG-3'	7	6	85%	750-4000
3.	OPB-04	5'-GGACTGGAGT-3'	6	4	66%	700-4000
4.	OPB-05	5'-TGCGCCCTTC-3'	6	4	66%	600-3000
5.	OPB-11	5'-GTAGACCCGT-3'	10	7	70%	500-3200
6.	OPB-14	5'-TCCGCTCTGG-3'	5	4	80%	900-3000
7.	OPB-16	5'-TTTGCCCGGA-3'	6	4	66%	900-6500
8.	OPB-17	5'-AGGGAACGAG-3'	8	5	62%	300-300
9.	OPB-18	5'-CCACAGCAGT-3'	7	6	85%	750-4500
10.	OPB-19	5'-ACCCCCGAAG-3'	6	5	83%	1000-6000
11.	OPB-20	5'-GGACCCTTAC-3'	3	2	66%	900-3000
12.	OPA-13	5'-CAGCACCCAC-3'	8	6	75%	1000-2500
		Total	**76**	**56**	**73%**	

A pair-wise similarity matrix based on Dice's similarity coefficients was used to calculate the level of differentiation and relatedness among 30 guar genotypes. The similarity coefficients ranged from 0.49 to 0.93. The highest similarity index of 93% was found between accessions '27350' and '27351' where as guar accession '24293' and '24302' were the most varied accessions with similarity index of 49% . On the basis of Nei & Li's similarity matrix analysis via UPGMA, 30 guar accessions were grouped together into three main groups i.e., A, B, and C. (Fig. 2).

Group A consisted of twenty genotypes and was further subdivided into 6 sub-clusters. Group B comprised of eight accessions and was further divided into three sub-clusters, Last group C was the smallest of all containing only two accessions.

Discussion

Even though guar is a self-pollinated crop, however, 1-9% out crossing has been observed under field conditions in guar (Stafford & Lewis, 1975). According to Hamrick & Godt (1989), 10-20% of the genetic variability is shown by out-crossing species. Present work demonstrated high degree of polymorphism ranging from 62 to 85% with an average proportion of 73% and coefficient of similarity was found in the range of 0.49-0.93. Similar polymorphism i.e., 72.7% was noticed by Punia *et al*., (2009a) in 34 guar varieties with Jaccard's similarity coefficients ranging from 0.52 to 0.95. Present findings were also supported by another study of Punia *et al*., (2009b) on molecular and morpho-physiological characterization of guar by using 37 random primers where genotypic similarity coefficient was estimated in the range of 0.34–0.76. Pathak *et al*., (2010) also monitored parallel degree of polymorphism i.e., 66.6 to 87.5% in guar. Range of amplified products (3-10 with an average of 6.3 bands per primer) observed in present work is in accordance with Mahmood *et al*., (2011) and Jan et al. (2011) findings, who noticed 3-11 RAPD fragments generated per primer in chickpea and *Curcuma* respectively. However Punia *et al*., (2009b) and Pathak *et al*., (2010) noticed 4 to 22 and 5 to 16 bands in guar with an average of 10 and 10.29 bands per primer, respectively. These findings are supported by Kernodle *et al*., (1993) and, Devos & Gale (1992) reports that the variation in the number of bands amplified by different primers is effected by various factors such as sensitivity of the working environment, less number of annealing sites in the genome, primer structure, template quantity and equipment used.

In present work, dendrogram divided 30 guar accessions into three main groups and 9 sub-groups or clusters in the distance range of 0.62-0.1. This division was not based on geographical origin of guar accessions. Brahmi *et al*., (2004) findings on allozyme diversity in guar germplasm are in accordance with present work, who observed three clusters in the distance range of 0–0.28 with no exact separation of accessions according to their place of collection. Similarly, Bisht *et al*., (1998), Lavanya *et al*., (2008), Shinwari *et al*. (2011), Pervaiz *et al*. 2010 and Pathak *et al*., (2010) depicted no relation between geographic location and genetic diversity in guar, other legumes and other species .

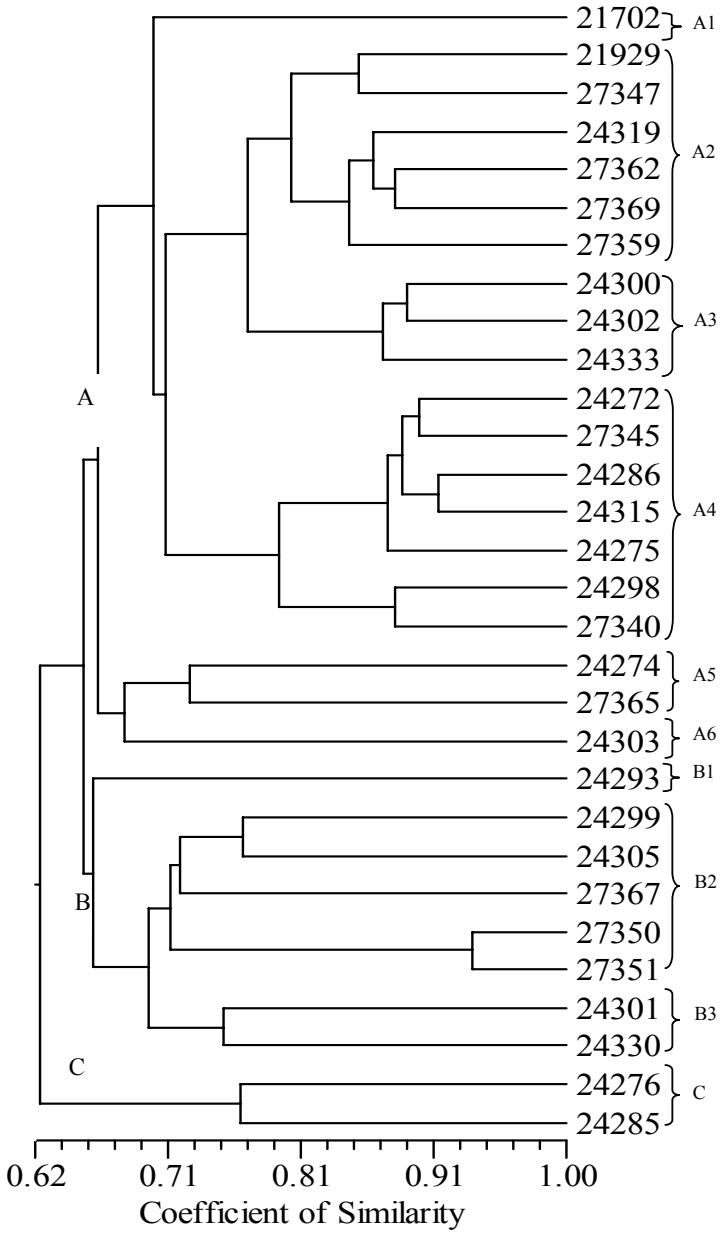

Fig. 2. Dendrogram produced by amplified products of 12 RAPD primers using UPGMA cluster analysis.

The results of the current investigation can contribute to enhance molecular breeding approach for the development of improved indigenous guar germplasm. Present data on the basis of molecular evaluation and assessment of genetic variation of guar accessions through RAPD markers is good enough to verify superior and elite accessions for future use.

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