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ASSESSING GENETIC DIVERSITY OF SOME POTATO (*Solanum tuberosum* L.) GENOTYPES GROWN IN TURKEY USING THE AFLP MARKER TECHNIQUE

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ABSTRACT

In the present study, the level of polymorphism and the genetic relationships among 26 potato genotypes were studied by means of molecular markers using the amplified fragment length polymorphism (AFLP) technique. DNA was extracted from fresh leaves of the seedlings. Selective amplification products revealed a total of 191 polymorphic bands ranging from 8 to 45 for each combination. Scoring results were used to generate a tree in JMP software. The 26 samples formed six clades with varying number of members between one and eleven. Genetic distances among genotypes were calculated according to Jaccard's formula, in Phylip 3.0 software. According to the results of genetic distance, dendrogram showed that genotypes 6/7-4 and 6/7-2 were the closest genotypes with a genetic distance of 0.13. On the other hand, genotypes Posof-10 and Marabel were the most distinct from each other with a genetic distance value of 0.55. The AFLP marker results showed a great consistency along with their pedigrees indicating the AFLP technique as a useful tool in the calculation of genetic distance of the potato genotypes.

Key words: AFLP, *Solanum tuberosum*, genetic diversity

INTRODUCTION

Over 320 million tones of potatoes are produced yearly and this amount is estimated to be doubled by 2020 (FAO, 2008). Today potato ranks as the fifth most important food crop in the world after rice, wheat, maize, and cassava (International Potato Center, 2010). Potato grown in Turkey is commercial varieties and some farmer-selected tetraploid populations (*Solanum tuberosum* L.) (2*n*=4*x*=48). Potatoes have been introduced over Russia in the Eastern Turkey and Black Sea Region as well as over Balkans in the Western Turkey during the 19th century.

Utilization of genetic variation in potato breeding program is a main factor increasing the success of breeding desired traits. The success of the breeding programs in Potatoes depends largely on the identification of the amount and distribution of genetic diversity in the gene pool. Knowledge of the genetic diversity and relationships among the varieties are very useful in order to recognize gene pools, to identify the gaps in germplasm collections and to develop effective conservation and management strategies (Esfahani et al 2009). Identification of genetic diversity using DNA markers can provide insights into the genetic structure and diversity among varieties from different geographical origins, producers and distributors. When the magnitude and nature of genetic diversity is estimated in advance, a suitable selection strategy is planned in accord with heritability of genetic traits (Yonesawa et al., 1995; Ghislain et al., 1999). A combination of passport data and genetic diversity information from molecular markers would therefore enhance the formation of germplasm stocks.

There are a number of molecular marker techniques to identify genetic diversity such as RAPD (Tanyolac 2003), AFLP (Ozkan et al., 2005), SSR (Kandemir et al., 2010) RFLP (Tanyolac et al., 2003). Among the molecular marker techniques, AFLP is robust and provide a powerful tool in studies of genetic variation, genotype identification and phylogeny (Kafkas et al., 2006). This method is based on the detection of genomic restriction fragments by PCR amplification, and it can be used for DNAs of any origin or complexity (Vos et al., 1995). Large number of loci, high levels of polymorphism, high reproducibility without prior sequence knowledge and genome-wide marker distribution are the most important advantages of this technique (Powell et al., 1996).

The purpose of this study was to characterize genetically 26 potato genotypes collected from certain locations in Turkey using AFLP markers and to explain the genetic relationship among those genotypes. The information obtained will enhance our understanding of the genetic structure of potato genotypes analyzed.

MATERIALS AND METHODS

Plant materials

Twenty-six potato genotypes used in this study and their origin/source were presented in Table 1.

Table 1. Potato genotypes used in this study and their origin.

Sample code	Variety/clone name	Origin/Source
1	Hybrid 93448-15 (Granola x Baraca)	(EAARI-Erzurum) Selection
2	Hybrid 93448-6 (Granola x Baraca)	(EAARI-Erzurum) Selection
3	Samki-15 Land race population	Local selection (EAARI-Erzurum)
4	Samki-17 Land race population	Local selection (EAARI-Erzurum)
5	Posof Central-15 Land race population	Local selection (EAARI-Erzurum)
6	Posof Central -16 Land race population	Local selection (EAARI-Erzurum)
7	Posof-10 Land race population	Local selection (EAARI-Erzurum)
8	Posof-9 Land race population	Local selection (EAARI-Erzurum)
9	ABK-10 (Alabalik) Land race population	Local selection (EAARI-Erzurum)
10	ABK-15 (Alabalik) Land race population	Local selection (EAARI-Erzurum)
11	DC-11 (Dogrucan) Land race population	Local selection (EAARI-Erzurum)
12	DD-25 (Dogrucan) Land race population	Local selection (EAARI-Erzurum)
13	CY-1 (Camyazi) Land race population	Local selection (EAARI-Erzurum)
14	CY-19 (Camyazi) Land race population	Local selection (EAARI-Erzurum)
15	106-1 (R.143xCosima)	Dept of Field Crops, Ege University
16	106-5 (R.143xCosima)	Dept of Field Crops, Ege University
17	6/7-4 (Merrimack x DTO17)	Dept of Field Crops, Ege University (FDR mechanism)
18	6/7-2 (Merrimack x DTO17)	Dept of Field Crops, Ege University (FDR mechanism)
19	Nif-1 (R.143xCosima)	Dept of Field Crops Ege University
20	Nif-3 (R.143xCosima)	Dept of Field Crops Ege University
21	122-5 (R.143xCosima)	Dept of Field Crops Ege University
22	122-1 (R.143xCosima)	Dept of Field Crops Ege University
23	Granola	Aegean Agricultural Research Institute (AARI)-Izmir Turkey
24	Marabel	(AARI)
25	Agria	(AARI)
26	Marfona	(AARI)

DNA Extraction

Doyle (1990) from fresh, young leaves of seedlings. DNA concentrations of each sample were measured in NanoDrop-1000 spectrophotometer (Thermo Co.).

AFLP Analysis

Licor AFLP kit (cat no: 829-06195) was used for AFLP technique. Manufacturer recommendations were followed for digestion, pre-amplification and selective amplification. Isolated DNA samples were diluted to 40 ng/µl. The genomic DNA (100 ng) was restricted with *Eco*R I/*Mse* I enzyme mix in a volume of 12.5 µl containing 1.25 units of each enzyme. After incubation of the mixture at 37° C for 2 hours, an enzyme inactivation step was applied for 15 minutes at 70° C. Adapter mix and 2.5 units of T4 DNA ligase was added to the mixture and ligation was performed at 20° C for 2 hours. After 1:10 dilution of the ligation product, a pre-amplification step was carried out with the primers from the Li-Cor AFLP® Template Preparation Kit.

Thermal cycler (PTC 225, MJ Research) was set to 20 cycles of 30 s at 94° C, 1 min at 56° C, and 1 min at 72° C, and store at 4° C. Five µl of pre-amplification products were diluted 1:40 and unused portions were stored at -20° C. Six different fluorescent labeled selective primer combinations, MCAA-EAAG, MCAA-EACG, MCAG-EAAC, MCAG-EACT, MCAA-EAAC, MCAA-EAGC, were used for selective amplification. Thermal cycler was set to 13 cycles of 30 s at 94° C, 30 s at 65° C (with decrements of 0.7° C at each cycle), 1 min at 72° C and 23 cycles of 30 s at 94° C, 30 s at 56° C 1 min at 72° C and a storage step at 4° C forever. Selective amplification products were resolved in 8% polyacrylamide gel under 1500 V and 40 mA of current at 45° C for 3.5 hours. Fluorescent labeled PCR products were visualized in Li-Cor 4300s DNA analyzer. Gel images (Figure 1) were then transferred from SAGA software in DNA analyzer into computer and used for scoring of the samples for the presence or absence of the polymorphic bands.

Genetic distance (GD) calculation

The polymorphic bands were scored as 1 for presence of the bands and 0 for absence of the bands in Microsoft Excel software. JMP 3.0 software was used to generate a dendrogram among 26 samples according to UPGMA method with the scored matrix. The pairwise comparisons of

Table 2. Genetic distance values of 26 genotypes calculated according to Jaccard's formula (The numbers (1 to 26) indicate sample code in Table 1 and the numbers in bold indicate max and min GD values).

	01.	**02.**	**03.**	**04.**	**05.**	**06.**	**07.**	**08.**	**09.**	**10.**	**11.**	**12.**	**13.**	**14.**	**15.**	**16.**	**17.**	**18.**	**19.**	**20.**	**21.**	**22.**	**23.**	**24.**	**25.**	**26.**
01.	0.00																									
02.	0.18	0.00																								
03.	0.33	0.27	0.00																							
04.	0.34	0.31	0.16	0.00																						
05.	0.42	0.37	0.35	0.42	0.00																					
06.	0.39	0.35	0.37	0.37	0.38	0.00																				
07.	0.42	0.38	0.35	0.37	0.38	0.35	0.00																			
08.	0.38	0.47	0.43	0.43	0.53	0.52	0.30	0.00																		
09.	0.34	0.30	0.41	0.39	0.46	0.40	0.37	0.43	0.00																	
10.	0.33	0.28	0.32	0.28	0.39	0.26	0.33	0.45	0.16	0.00																
11.	0.33	0.34	0.33	0.30	0.42	0.33	0.38	0.50	0.33	0.28	0.00															
12.	0.33	0.27	0.29	0.33	0.30	0.35	0.33	0.40	0.35	0.28	0.30	0.00														
13.	0.35	0.38	0.32	0.36	0.44	0.33	0.43	0.48	0.44	0.29	0.36	0.35	0.00													
14.	0.34	0.34	0.35	0.41	0.44	0.28	0.42	0.45	0.42	0.32	0.33	0.38	0.24	0.00												
15.	0.38	0.36	0.35	0.38	0.45	0.38	0.35	0.35	0.40	0.34	0.38	0.35	0.33	0.27	0.00											
16.	0.39	0.32	0.38	0.38	0.35	0.32	0.41	0.46	0.43	0.35	0.37	0.33	0.35	0.31	0.25	0.00										
17.	0.34	0.31	0.35	0.33	0.39	0.31	0.34	0.48	0.36	0.30	0.33	0.36	0.35	0.33	0.30	0.33	0.00									
18.	0.32	0.28	0.28	0.28	0.34	0.28	0.30	0.44	0.35	0.26	0.30	0.31	0.34	0.27	0.29	0.26	**0.13**	0.00								
19.	0.28	0.31	0.38	0.39	0.46	0.45	0.48	0.45	0.41	0.33	0.33	0.35	0.38	0.38	0.38	0.38	0.32	0.31	0.00							
20.	0.38	0.32	0.37	0.40	0.45	0.39	0.46	0.44	0.42	0.28	0.30	0.34	0.37	0.31	0.29	0.35	0.34	0.28	0.19	0.00						
21.	0.38	0.30	0.33	0.33	0.42	0.36	0.29	0.44	0.28	0.23	0.30	0.31	0.40	0.35	0.28	0.32	0.28	0.21	0.30	0.25	0.00					
22.	0.42	0.30	0.33	0.35	0.41	0.31	0.33	0.42	0.38	0.25	0.38	0.33	0.38	0.32	0.27	0.30	0.32	0.24	0.33	0.26	0.15	0.00				
23.	0.36	0.36	0.37	0.37	0.42	0.36	0.39	0.49	0.37	0.33	0.33	0.34	0.40	0.35	0.38	0.41	0.38	0.35	0.34	0.35	0.30	0.35	0.00			
24.	0.48	0.50	0.51	0.54	0.54	0.52	**0.55**	0.55	0.51	0.44	0.44	0.51	0.47	0.51	0.52	0.48	0.46	0.46	0.44	0.50	0.46	0.51	0.55	0.00		
25.	0.37	0.35	0.30	0.33	0.46	0.37	0.45	0.52	0.38	0.30	0.28	0.39	0.35	0.36	0.38	0.37	0.36	0.30	0.36	0.33	0.31	0.38	0.33	0.42	0.00	
26.	0.35	0.33	0.35	0.34	0.43	0.36	0.44	0.49	0.33	0.33	0.33	0.37	0.40	0.40	0.35	0.38	0.30	0.33	0.37	0.35	0.33	0.40	0.36	0.43	0.24	0.00

the hybridization fragments were used to calculate GD according to Jaccard's formula (1908) in Phylip 3.0 software:

GD=1-Nxy/(N_{XY} + N_X + N_y)

Where:

GD: Genetic distance.

N_{XY}: number of bands common in population x and y.

N_X: number of bands present in population x and absent in population y.

N_Y: number of bands present in population y and absent in population x.

The Polymorphic Information Content (PIC) for each marker was calculated according to Anderson (1993) as:

$$\mathrm{PIC} = 1 - \sum_{i=1}^{n} P_i^2$$

where P_i is the frequency of the *i*th band and n is the number of bands observed.

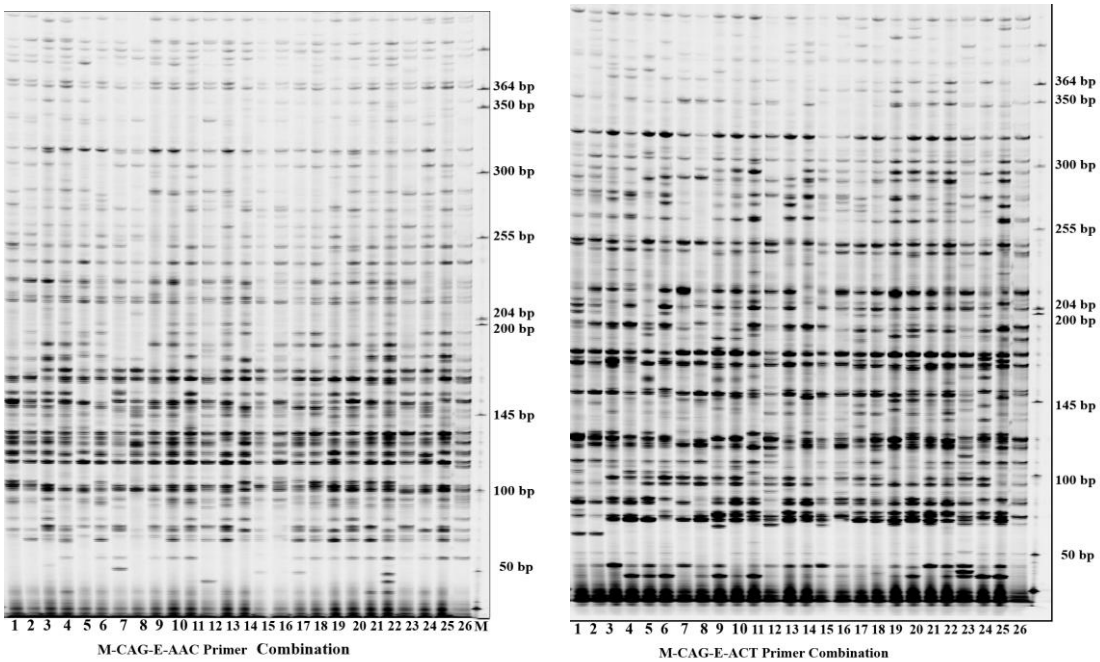

Figure 1. Gel images of AFLP profile obtained from selective primer combinations of M-CAG-E-AAC and M-CAG-E-ACT. M: 50-700bp ladder

Table 3. List of primer combinations used and numbers of polymorphic and monomorphic bands obtained.

Combination order	Primer Combination	Number of polymorphic bands	Number of monomorphic bands	Total	PIC[a]	Min PIC	Max PIC	POL %[b]
1	M-CAA-E-AAG	39	16	55	0.59	0.12	0.96	70.9
2	M-CAA-E-ACG	43	6	49	0.53	0.19	0.88	87.8
3	M-CAA-E-AAC	8	10	18	0.64	0.15	0.96	44.4
4	M-CAA-E-AGC	23	16	39	0.68	0.04	0.96	59.0
5	M-CAG-E-AAC	33	17	50	0.58	0.04	0.96	66.0
6	M-CAG-E-ACT	45	8	53	0.46	0.04	0.96	84.9
	Total	**191**	**73**	**264**	**0.56[c]**	**0.10[c]**	**0.95[c]**	**72.3[c]**

[a]PIC: Polymorphic information content.
[b]POL%: percentage of polymorphic bands to total number of bands
[c]Mean values for PIC and POL%.

RESULTS

A total of 6 primers combination was used and 264 bands were obtained (Table 3). Out of 264 bands in six primer combinations gave a total of 191 clearly scorable polymorphic bands. Some representative gel images were presented in Fig 1. The average number of polymorphic bands per primer was 31.5 bands. The polymorphic bands ratio (POL%) was calculated as an average of 72.3%. The number of polymorphic bands was ranged from 8 to 45 for each combination as shown in Table 3. The highest number of polymorphic bands was obtained in the M-CAG-E-ACT primer combination while the highest POL% value (87.8%) was obtained from primer combination M-CAA-E-ACG and the lowest one was in the M-CAA-E-AAC primer combination as well as the lowest POL% value (44.4%). Presence and absence of the bands were used to calculate genetic distance (GD) among genotypes (Table 2). The max GD was calculated as 0.55 between Posof-10 and Marabel genotypes. The min GD was 0.13 between 6/7-4 and 6/7-2.

The average GD was identified as 0.36. The genetic distance matrix was used to construct a dendrogram for relationship

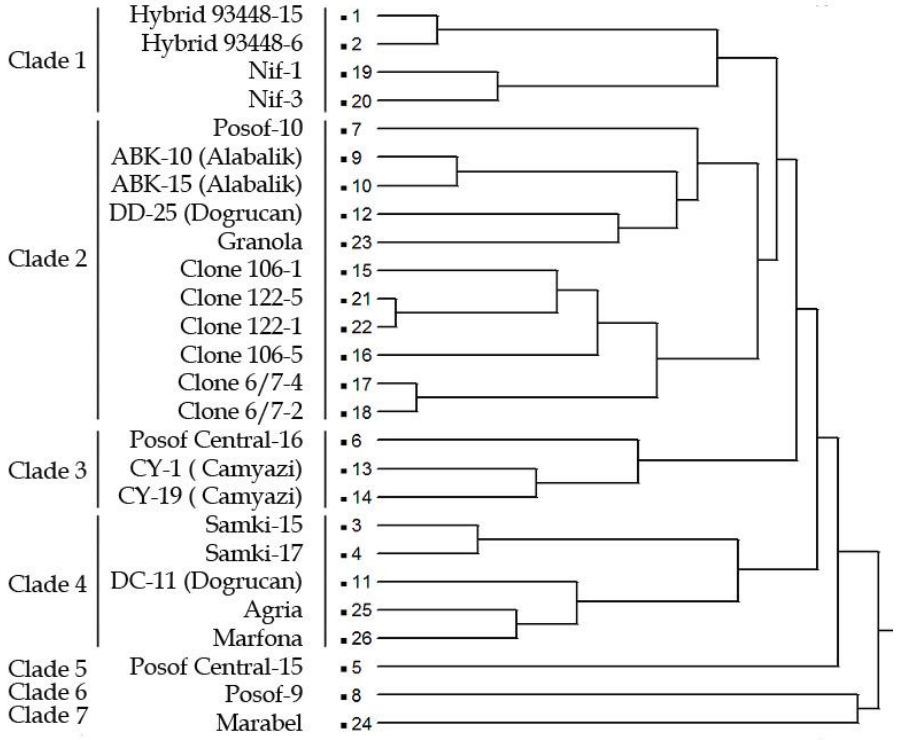

Figure 2. A Dendrogram of 26 genotypes constructed in JMP 3.0 software using 191 polymorphic bands.

among the genotypes. After construction of the dendrogram, 26 genotypes were grouped in seven clades with varying members between 1 and 11 (Figure 2). Clade 1 consisted of 4 genotypes: hybrid 93448-15, hybrid 93448-6, Nif–1 and Nif-3. Clade 2 included Posof-10, ABK-10 (Alabalik), ABK-15 (Alabalik), DD-25 (Dogrucan), Granola, clone 106-1, clone 122-5, clone 122-1, clone 106-5, clone 6/7-4 and clone 6/7-2. Genotypes, Posof Central-16, CY-1 (Camyazi) and CY-19 (Camyazı) were in Clade 3. Genotypes: Samki-15, Samki-17, DC-11 (Dogrucan), Agria and Marfona constituted in the Clade 4. The genotype Posof Central-15, Posof 9 and Marabel formed Clades 5, 6 and 7 individually. Among the commercial varieties used in this study, Agria and Marfona seemed to be close to each other and the rest of genotypes formed relatively close relationship with the Turkish local genotypes.

Table 4. Average GDs among clade members and the most distinct genotypes.

Clade	Average GD	Genotype	Average GD*
1	0.28	Posof Central -15	0.42
2	0.31	Posof-9	0.45
3	0.28	Marabel	0.49
4	0.30		
5	-		
6	-		
7	-		
Overall mean	0.36		

*Average GD of the corresponding genotype to all other genotypes.

Averages of GDs between clade members were evaluated as well as average GD between genotypes Posof Central-15, Posof-9, Marabel to the rest of the samples (Table 4). This table shows the amount of genetic diversity among the individuals in each clade and the most distant genotypes: Posof Central-15 (5), Posof-9 (8) and Marabel (24).

DISCUSSION

In this study 191 clearly scorable, polymorphic bands with an average number of 31.5 were obtained with 6 primer combinations, in 26 genotypes, while Zhang et al. (2000) generated 210 polymorphic bands with 8 primer combinations with an average number of 26.2 polymorphic bands per primer combination. In the study of Zhang et al (2000), they used 69 landraces that could have narrow genetic variability. On the other hand, Esfahani et al. (2009) found 564 polymorphic bands using 16 primer combinations with an average number of 35.5 polymorphic bands per primer combination for the assessment of genetic diversity in the European and North American potato varieties cultivated (in 25 cultivars) in Iran. The higher polymorphism level in the study of Esfahani et al. (2009) may be due to the genotypes from geographically far away from each other as compare to the genotypes used in our study. Polymorphic bands were determined among a total number of 264 bands, which gave the average POL as 72.3%, while Esfahani et al. (2009) evaluated POL as 96.14% with 564 polymorphic bands. Polymorphic information content (PIC) describes discriminatory power of a polymorphic band by giving the frequency information of an allele among the genotypes. In this study, PIC values were distributed between 0.04 and 0.96 with a mean of 0.56 (Table 3) for 191 bands indicating a diverse distribution of polymorphic information throughout the bands. In contrast, Esfahani et al. (2009) calculated a mean value of PIC as 0.61 ranging from 0.48 to 0.72 showing a more uniform distribution of polymorphic bands. Ispizua et al (2007) found the PIC between 0.88 and 0.92 in Argentina Potato landraces using SSR markers. The distant genotypes can be seen on the Fig 1, as Posof Central -15, Posof-9 and Marabel. For the clades number 1 and 4, average GD among the clade members was rather low (0.28-0.30) when compared to the mean GD of all genotypes to each other (0.36) indicating the strength of the clades. However GD between genotypes Posof-9 and Marabel was much higher (0.55) suggesting these genotypes more distant. Esfahani et al. (2009) determined an unreleased accession to be distant to the remaining 24 accessions with a GD value of 0.97 but could not find an extreme dissimilarity with other varieties.

Genotypes used in this study selected from same origin formed in the same clades such as clones 106-1, 106-5, 6/7-4, 6/7-2, 122-5 and 122-1. All these genotypes were selected from the potato breeding program in the Department of Field Crops at Ege University, Izmir, Turkey. Zang et al (2000) could discriminate the sweet potato (*Ipomoea batatas* L. Lam.) cultivars from tropical America according to their origin using AFLP. On the other hand, interestingly, the Posof (9-10-15-16) genotypes were not clustered together but they were distributed in the dendrogram. This indicated that genetic variation could be large in the Posof populations collected in the East Anatolian local potato populations in Turkey.

As information rich marker system AFLP has ability to generate a large number of polymorphic/informative loci (Powell et al., 1996) simultaneously in a single lane with a single-primer combination as compared to RAPDs, RFLPs and microsatellites (Milbourne et al., 1997). The high level of polymorphic bands among the genotypes used in this study suggests that AFLP is powerful markers for classification and diversity analysis in potato (Zhang et al., 2000; Esfahani et al., 2009).

These results discussed indicated that there was significant genetic diversity among 26 potato genotypes. This will contribute to the maintenance of the diversity in potato breeding program. Information about the distribution of the genetic diversity indicates the presence of different genotypes in the department and this is important for the development of in situ conservation and collection strategies. Based on the result of this study, crossing the commercial varieties and clones with local collections could be proposed to increase genetic variability level and to create new breeding populations.

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