



Determination of genetic diversity of edible-seeded watermelon genotypes using SRAP markers

Çerezlik karpuz genotiplerinin genetik çeşitliliğinin SRAP markırları kullanılarak belirlenmesi

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ARTICLE INFO	ABSTRACT
<p>Article history: Recieved / Geliş: 14.09.2023 Accepted / Kabul: 07.10.2023</p> <p>Keywords: <i>Citrullus lanatus</i> Edible-seeded watermelon Molecular characterization SRAP</p> <p>Anahtar Kelimeler: <i>Citrullus lanatus</i> Çerezlik karpuz Moleküler karakterizasyon SRAP</p> <p>✉Corresponding author/Sorumlu yazar: Ömer Faruk COŞKUN omerfaruk.coskun@mku.edu.tr</p> <p>Makale Uluslararası Creative Commons Attribution-Non Commercial 4.0 Lisansı kapsamında yayınlanmaktadır. Bu, orijinal makaleye uygun şekilde atıf yapılması şartıyla, eserin herhangi bir ortam veya formatta kopyalanmasını ve dağıtılmasını sağlar. Ancak, eserler ticari amaçlar için kullanılamaz. © Copyright 2022 by Mustafa Kemal University. Available on-line at https://dergipark.org.tr/pub/mkutbd</p> <p>This work is licensed under a Creative Commons Attribution-Non Commercial 4.0 International License.</p> <p> </p>	<p>The watermelon (<i>Citrullus lanatus</i> L.) is one of the most commonly grown and consumed vegetables in the world. Some genotypes of watermelon, which have significant variations, have a snack potential due to their seed characteristics. In this study, SRAP (Sequence Related Amplified Polymorphism) marker technique was used to determine the genetic relationship between some edible-seeded watermelon genotypes. A total of 166 bands were obtained in 24 genotypes and the polymorphism rate was calculated as 97.4%. Four main clusters were observed in the cluster analysis. It was determined that genotypes 2 and 7 clustered separately from the others. Structure analysis revealed that the genotypes consisted of two subpopulations. It was concluded that the edible-seeded watermelon genotypes can be genetically differentiated by the SRAP techniques. The results of this study can be used in breeding strategies for the improvement of the edible-seeded watermelon cultivars.</p> <p>ÖZET</p> <p>Karpuz (<i>Citrullus lanatus</i> L.) dünyada en çok yetiştirilen ve tüketilen sebzelerden biridir. Önemli bir varyasyona sahip olan karpuzun bazı genotipleri, tohum özelliklerinden dolayı çerezlik potansiyele sahiptir. Bu çalışmada, bazı çerezlik karpuz genotipleri arasındaki genetik ilişkinin belirlenmesi amacıyla SRAP (Sequence Related Amplified Polymorphism) markır tekniği kullanılmıştır. Toplam 24 genotipte, toplam 166 bant elde edilmiş ve polimorfizm oranı %97.4 olarak hesaplanmıştır. Kümeleme analizinde dört ana küme ortaya çıkmıştır. 2 ve 7 numaralı genotiplerin diğerlerinden ayrı kümelendiği belirlenmiştir. Structure analizi sonuçları genotiplerin iki alt popülasyondan oluştuğunu ortaya çıkarmıştır. Çerezlik karpuz genotiplerinin SRAP teknikleri ile genetik olarak ayırt edilebileceği sonucuna varılmıştır. Bu çalışma sonuçları çerezlik karpuz çeşit ıslahına yönelik ıslah stratejilerinde kullanılabilir.</p>
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INTRODUCTION

Türkiye is one of the countries with the richest biodiversity in Europe and the Middle East. The variability in the geographical structure in Türkiye is the most important reason for the emergence of high endemism and genetic diversity in plants (Demirayak, 2002). *Citrullus lanatus*, which is grown in tropical and subtropical regions of the world, is the most diverse species in the genus *Citrullus* (Maynard, 2001). Watermelon is produced with a total amount of 101.634.720 tons in the world and 3.468.717 tons in Türkiye (FAO, 2021). Türkiye is the second largest watermelon producing country in the world after China.

The demand for fruit and fruit products, which are important for human nutrition and health, is increasing in the world (Scheerens, 2001). Watermelon is a type of vegetable grown mainly for fruit flesh consumption in tropical and subtropical regions of the world. The watermelon, which is widely consumed in the world, often yields a significant amount of seeds that are thrown away as waste. In recent years, the demand for watermelon seeds has been increasing day by day due to public health concerns and changing dietary habits. Besides being produced for its fruit, watermelon is also grown for its edible seeds in many Asian and African countries. Edible seeds of watermelon are consumed as a snack (Wehner, 2008; Coşkun et al., 2019; Toprak et al., 2023). Watermelon seeds have also been consumed as a snack in the south and southeast of Türkiye for a long time (Gökseven, 2013). In order to consume the seed as a snack, large seeds of watermelon are selected and consumed after roasting or boiling (Köçeroğlu, 2018). Watermelon seeds have an important place in human nutrition due to the nutrients it contains. Watermelon seeds contain minerals such as calcium, magnesium, iron and zinc (Lakshmi & Kaul, 2011) as well as significant amounts of protein and fat (Braide et al., 2012).

Analyzing genomic variations in plants is the most important part of plant genetics and product development programs. Genotyping can be used in plant characterization, gene mapping, species identification and evolution, and selection studies with the help of markers (MAS) and seed purity determination. With the help of molecular markers, successful studies are carried out in the field of identification of cultivars in horticultural crops, phylogenetic analysis, determination of genetic relatedness, marker assisted selection, QTL (Quantitative Trait Locus) analysis and genetic mapping. Many marker techniques have been used in genetic characterization studies in vegetables (Aslan et al., 2021; Morilipınar et al., 2021; Coşkun, 2022; Kamaşak et al., 2022; Coşkun, 2023). In the SRAP marker technique, open reading regions in DNA are amplified. The SRAP technique is used effectively in the investigation of genetic relationships and genetic diversity (Uzun et al., 2009; Oruç, 2012). High reproducibility and low cost are the important advantages of this technique (Li & Quiros, 2001). The SRAP technique has been used previously in genetic diversity studies (Levi et al., 2007) and mapping studies (Levi et al., 2006) in watermelons. Studies carried out for the genetic characterization of the confectionery watermelon genotypes distributed in Türkiye are insufficient. Therefore, this study was carried out to determine the molecular characterization of some edible-seeded watermelon genotypes with the SRAP marker technique.

MATERIALS and METHODS

In the study, 24 edible-seeded watermelon genotypes with high snack potential obtained from seven different provinces of Türkiye (Hatay, Diyarbakir, Şanlıurfa, Adana, Aydın, Mardin, and Batman) were used as genetic material (Table 1).

Table 1. Edible-seeded watermelon genotype numbers and origins used in the study

Çizelge 1. Çalışmada kullanılan çerezlik karpuz genotip numaraları ve orjinleri

No	Genotype	Origin, Province	No	Genotype	Origin, Province
1	HMKU-KR-1	Hatay	13	HMKU-KR-13	Adana
2	HMKU-KR-2	Hatay	14	HMKU-KR-14	Aydın
3	HMKU-KR-3	Hatay	15	HMKU-KR-15	Mardin
4	HMKU-KR-4	Hatay	16	HMKU-KR-16	Batman
5	HMKU-KR-5	Hatay	17	HMKU-KR-17	Mardin
6	HMKU-KR-6	Hatay	18	HMKU-KR-18	Mardin
7	HMKU-KR-7	Hatay	19	HMKU-KR-19	Adana
8	HMKÜ-KR-8	Diyarbakır	20	HMKU-KR-20	Adana
9	HMKU-KR-9	Diyarbakır	21	HMKU-KR-21	Adana
10	HMKU-KR-10	Şanlıurfa	22	HMKU-KR-22	Adana
11	HMKU-KR-11	Şanlıurfa	23	HMKU-KR-23	Adana
12	HMKU-KR-12	Adana	24	HMKU-KR-24	Adana

DNA isolation was performed according to the Cetyltriethylammonium bromide (CTAB) method by using the first true leaves for edible-seeded watermelon genotypes. DNA quality and quantity were determined by running on 0.8% agarose gel. The 16 SRAP primers with the best amplification were used in the study. For PCR (15 µl final volume), target DNA (20 ng), 1.5 mM MgCl₂, 0.2 µM primers, 0.5 mM dNTPs, 1x PCR buffer, 0.5 units of Taq DNA polymerase enzyme were used. A 1.5% agarose gel stained with ethidium bromide in 0.5X TBE solution was used for PCR products. Agarose gels were recorded in the UV gel imaging system and 1, 0 and 9 data were obtained. Clustering analyzes were performed using NTSYS (Numerical Taxonomy Multivariate Analysis System, NTSYS-pc version 2.1, Exeter Software, Setauket, N.Y., USA) package program (Rohlf, 2000). Similarity indexes between individuals were calculated according to the DICE method (Dice, 1945). UPGMA (Unweighted Pair Group Method with Arithmetic Mean) dendrogram based on DICE similarity matrix and Principal Component Analysis (PCA) based on variance-covariance matrix were performed from the similarity index. Two-dimensional and three-dimensional graphics were obtained by using eigen vectors in the PROJ module for the PCA. Structure program (Version 2.3.4) (Pritchard et al., 2000) was used to determine the population structure of the edible-seeded watermelon genotypes. In each analysis step, 100.000 burn-in cycles and 100.000 repetitions for each K value were performed and the analysis was repeated 10 times. "Structure Harvester" (Earl & Vonholdt, 2012) software was used to calculate the ΔK value of the populations and the number of subpopulations was determined. For the K and ΔK values obtained for this purpose, the optimum K value was reached based on the probability value of the data (Evanno et al., 2005).

RESULTS and DISCUSSIONS

Some characteristics of SRAP primers and genetic diversity, clustering analyzes and population structures in edible-seeded watermelon genotypes were determined in the study. Sixteen SRAP primers were used for genetic characterization in a total of 24 edible-seeded watermelon genotypes. A total of 166 bands and 10.4 bands per primer were obtained from the primers (Table 2). A total of 162 polymorphic bands and an average of 10.1 polymorphic bands per primer were obtained. The total number of bands in the primers ranged from 5-20 and the most bands were obtained from the EM12-ME9 primer (20). The number of polymorphic bands in the primers was between 5-20 and the most polymorphic bands were obtained from the EM12-ME9 primer (20). The rate of polymorphism varied from 70 to 100% and was calculated as 97.4% on average. Polymorphization rate was 70% in EM2-ME6 primer, 88.9% in EM5-ME2 primer and 100% in the other primers (Table 2). The band gap in all primers

was determined between 220-1330. Although the previous molecular studies for the edible-seeded watermelon genotypes were insufficient, successful genetic studies were carried out with the SRAP technique in edible-seeded watermelon genotypes. In this study, it was determined that this primer technique could provide amplification and detect polymorphism in watermelon genotypes, confirming previous studies (Zhang et al., 2008; Wang et al., 2015).

Table 2. Polymorphism and band informations obtained from SRAP primers

Çizelge 2. SRAP primerlerinden elde edilen polimorfizm ve bant bilgileri

Primer Name	Primer Sequence 5'-3'	Number of Polymorphic Bands	Number of Total Bands	Polymorphism Rate	Band Range
EM14-ME13	GCA TGC GTA CGA ATT ATT CTT-TGA GTC CAA ACC GGA AG	13	13	100.0	220-1150
EM5-ME2	GCA TGC GTA CGA ATT ATT AAC-TGA GTC CAA ACC GGA GC	8	9	88.9	325-1330
EM12-ME13	GCA TGC GTA CGA ATT ATT CTC-TGA GTC CAA ACC GGA AG	10	10	100.0	250-1350
EM10-ME10	GCA TGC GTA CGA ATT ATT CAT-TGA GTC CAA ACC GGA AA	10	10	100.0	280-1270
EM9-ME11	GCA TGC GTA CGA ATT ATT CAG-TGA GTC CAA ACC GGA AC	15	15	100.0	250-1240
EM11-ME5	GCA TGC GTA CGA ATT ATT CTA-TGA GTC CAA ACC GGA AG	5	5	100.0	550-1300
EM1-ME10	GCA TGC GTA CGA ATT ATT ATT-TGA GTC CAA ACC GGA AA	7	7	100.0	300-1210
EM8-ME9	GCA TGC GTA CGA ATT ATT CAC-TGA GTC CAA ACC GGA GG	17	17	100.0	300-1150
EM7-ME2	GCA TGC GTA CGA ATT ATT CAA-TGA GTC CAA ACC GGA GC	8	8	100.0	390-1250
EM15-ME13	GCA TGC GTA CGA ATT ATT GAT-TGA GTC CAA ACC GGA AG	9	9	100.0	300-1200
EM7-ME10	GCA TGC GTA CGA ATT ATT CAA-TGA GTC CAA ACC GGA AA	6	6	100.0	350-1160
EM11-ME10	GCA TGC GTA CGA ATT ATT CTA-TGA GTC CAA ACC GGA AA	10	10	100.0	510-1270
EM2-ME6	GCA TGC GTA CGA ATT ATT TGC-TGA GTC CAA ACC GGA CA	7	10	70.0	410-1200
EM4-ME5	GCA TGC GTA CGA ATT ATT TGA-TGA GTC CAA ACC GGA AG	8	8	100.0	460-1180
EM3-ME4	GCA TGC GTA CGA ATT ATT GAC-TGA GTC CAA ACC GGA CC	9	9	100.0	300-1190
EM12-ME9	GCA TGC GTA CGA ATT ATT CTC-TGA GTC CAA ACC GGA GG	20	20	100.0	290-1330
	Total	162	166	1558.9	300-1280
	Mean	10.1	10.4	97.4	

According to the Dice similarity index, the similarity coefficient values between the genotypes were determined between 0.25 and 0.76. The most distant genotypes were found to be genotype 1 and genotype 2 with a similarity coefficient of 0.25. Genotypes closest to each other were genotype 17 and genotype 18 with a similarity coefficient

of 0.76. Four main clusters were observed in the UPGMA dendrogram. There were fifteen genotypes in cluster A, seven genotypes in cluster B and two genotypes in cluster C. Two genotypes (genotypes 2 and 7) in cluster D clustered further away from the others. Genotypes clustered closest to each other are genotypes 2 and 7 (Figure 1). In some previous studies (Solmaz et al., 2010; Uluturk et al., 2011), it was determined that the genetic distance was low. In some other studies similar to this study, on the other hand, higher genetic distance was obtained (Dje et al., 2010; Kwon et al., 2010).

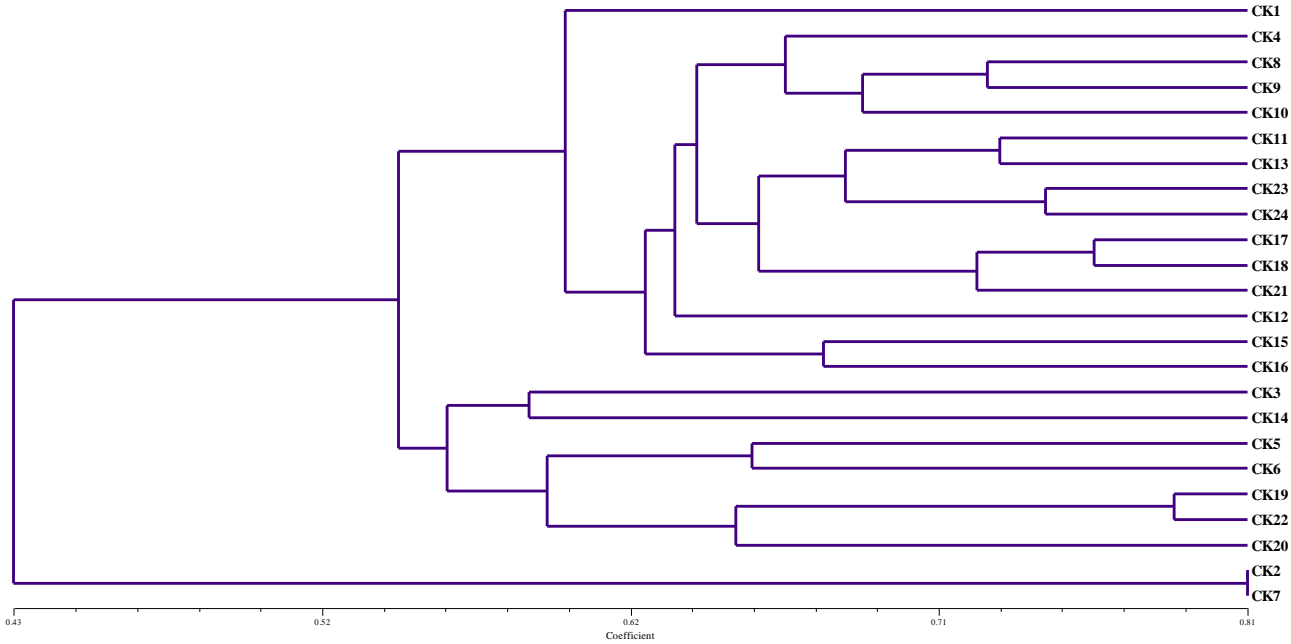


Figure 1. Dendrogram obtained by using similarity indices in the SHAN module

Şekil 1. SHAN modülündeki benzerlik indeksleri kullanılarak elde edilen dendrogram

NTSYS package program was employed to obtain two-dimensional and three-dimensional PCA graphics (Figure 2). The first three eigen values explain about 75% of the total genetic variant, meaning that PCA may be usable (Mohammadi & Prasanna, 2003). The data compose of three groups in the two-dimensional PCA graph. Three genotypes were included in A cluster and six genotypes in B cluster, while other genotypes were collected in C cluster. In the three-dimensional PCA graph, some genotypes were clustered together and few genotypes were located in separate clusters (Figure 2). Using UPGMA, 2, 5 and 7-end genotypes form different clusters in two-dimensional and three-dimensional PCA graphics. Although both genotypes are of similar origin (Hatay), they cluster differently from other genotypes (1, 3, 4 and 6) of similar origin. This difference may be due to the module configurations used in the package programs.

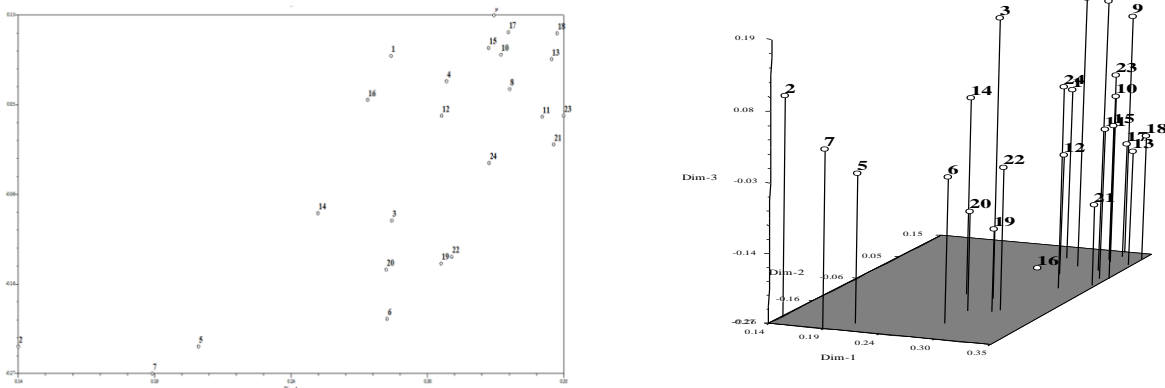


Figure 2. Two and three dimensional graphics obtained as a result of principal component analysis (PCA)

Şekil 2. Temel bileşenler analizi (PCA) sonucunda elde edilen iki ve üç boyutlu grafikler

For population structure determination studies, the optimum number of subpopulations was determined by using the 'Structure Harvester' software. It was determined that the examined genotypes consisted of two subpopulations. By using the Structure program, the belonging values of the genotypes to the subpopulations were determined. Individuals with a membership coefficient of 0.80 and above in a subpopulation are considered pure genotype (Fukunaga et al., 2005) and individuals with a lower membership coefficient are considered mixed genotype. In the present study, 22 genotypes have membership coefficients of 0.80 and higher and are therefore likely to be pure. The remaining 2 genotypes (3 and 21) are a mixture of the two populations. Of the 22 genotypes, 14 were in the 1st subpopulation and 8 were in the 2nd subpopulation (Figure 3).

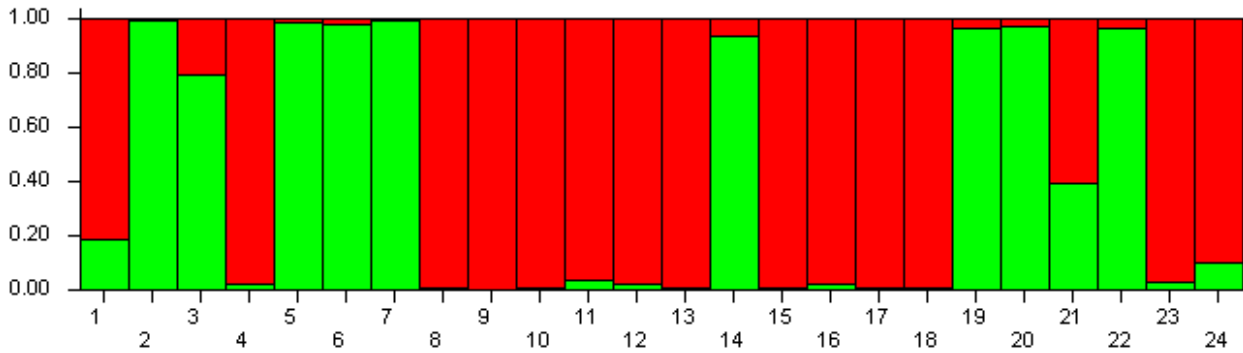


Figure 2. Ratios of edible-seeded watermelon genotypes based on $K = 2$ (where K is the number of subpopulations, each represented by a different color)

Şekil 2. $K = 2$ 'ye dayalı çerezlik karpuz genotiplerinin oranları (K , her biri farklı bir renkle temsil edilen alt popülasyonların sayısıdır)

In the current study, SRAP markers were used to determine the genetic diversity of 24 watermelon genotypes with high snack potential. This technique has been successfully used previously to determine the genetic structure of different watermelon genotypes. While some studies used fewer genotypes than our study (Yan & Chunging, 2005; Zhao et al., 2010), some studies compared a higher number of genotypes (Aras et al., 2012; Wang et al., 2015). There is also significant variation in the number of primer pairs used. Some studies used fewer primer pairs than the present study (Aras et al., 2012), and some studies used more primer pairs than the current study (Zhang et al., 2008). However, in present study, the number of bands per primer is higher than some other studies (Yan &

Chunging, 2005; Zhang et al., 2008; Ulutürk et al., 2011; Aras et al., 2012; Yağcıoğlu et al., 2016). This may be due to the higher efficiency of the selected primer pairs. Wang et al. (2015) and Solmaz et al. (2016) obtained a similar number of bands per primer compared to the present study. The polymorphism value obtained from the current study (97.4%) is high, similar to the values determined by Zhao et al. (2010), Solmaz et al. (2016) and Yağcıoğlu et al. (2016). The similarity coefficient values determined in present study (0.25-0.76) are narrower than the genetic distance determined by Yağcıoğlu et al. (2016). However, larger similarity coefficient values were obtained in the present study than in many other studies (Zhang et al., 2008; Ulutürk et al., 2011). The reason for this difference may be due to the genetic background of the genotypes examined.

There is a significant amount of local edible-seeded watermelon genotypes in Türkiye. Some of them are considered as snacks. There is a significant variation in morphology and seed characteristics in watermelon. Molecular studies on edible-seeded watermelon in Türkiye are scarce. In general, it can be concluded that there is genetic diversity among the edible-seeded watermelon genotypes and that the SRAP marker technique can be used due to its ability to show polymorphism. The results of the present study are important for breeding studies to distinguish some genotypes (genotypes 2, 5 and 7) from others in similarity coefficient, UPGMA dendrogram and PCA graphs. At the same time, it was determined that some primers are more important and can be more effective in differentiating the edible-seeded watermelon genotypes. This study provides important information to breeders to improve the characteristics of new cultivars in edible-seeded watermelon in breeding programs.

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STATEMENT OF CONFLICT OF INTEREST

The authors declare no conflict of interest for this study.

AUTHOR'S CONTRIBUTIONS

The contribution of the authors is equal.

STATEMENT OF ETHICS CONSENT

Ethical approval is not required as there are no studies with human or animal subjects in this article.

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