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ARAŞTIRMA MAKALESİ **RESEARCH PAPER**

Determination of Intra-population and Inter-cultivar Genetic Similarity of Tea Genotypes in Ordu Province

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Abstract: The establishment of tea gardens and tea production in Turkey began to spread after 1938 and became an important economic sector. The first tea gardens in Ordu province were established in 1960 in Perşembe district due to the expansion of the tea production area with a law enacted in 1951. Since then, the production area and the number of producers began to develop rapidly. The tea production area in this district is between 250-800 m altitude, which is expressed as the middle and high region, and has a topographic area with variable slopes. Since the gardens established in Perşembe, as in other gardens in our country, are established with seeds, the plants differ from each other. This difference in the genetic structure of the plant is reflected in the product, i.e. the quality of the tea. Therefore, determining this difference is essential. This study was conducted to determine the genetic relationship between the tea genotypes grown in Perşembe within the population and the selected cultivars. The plant materials used in the study were taken from different elevations and different gardens. Twenty plant materials were collected to represent the study area. There are three standard varieties as control: Derepazarı7, Fener3 and Tuğlalı10. ISSR method was used in the study. In the study where 10 ISSR markers were used, genetic diversity and related relationships were revealed. According to the cluster analysis performed using UPGMA, significant differences were detected among individuals. As a result of the study; It was determined that the similarity rate among the tea genotypes taken from Ortatepe neighborhood was higher, whereas the similarity rates among the samples taken from İstanbul Boğazı neighborhood were lower. In this context, it can be said that there is higher genetic diversity among the plants found in Ortatepe. The obtained data will help the development of tea cultivation and quality in the region.

Keywords: *Camelia sinensis*, genetic diversity, ISSRs, Perşembe.

Ordu ilindeki Çay Genotiplerinin Populasyon İçi ve Kültüvarlar Arası Genetik Benzerliğinin Belirlenmesi

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Öz: Türkiye'de çay bahçelerinin kurulumu ve çay üretimi 1938 yılında sonra yaygınlaşmaya başlamış olup önemli ekonomik bir sektör haline gelmiştir. Ordu ilinde ilk çay bahçeleri 1951 yılında çıkartılan bir kanunla çay üretim bölgesinin genişletilmesine bağlı olarak Perşembe ilçesinde ilk çay bahçeleri 1960 yılından itibaren kurulmaya başlanmıştır. Bu tarihten itibaren üretim alanı ve üretici sayısı hızlı gelişmeye başlamıştır. Bu ilçede yer alan çay üretim sahası orta ve yüksek bölge olarak ifade edilen 250-800 m rakımlar arasında ve değişken özellikte eğimli topografik alana sahiptir. Perşembe'de kurulan bahçeler, ülkemizdeki diğer bahçelerde olduğu gibi, tohumla kurulmuş olduğundan bitkiler birbirinden farklılık göstermektedir. Bitkinin genetik yapısındaki bu farklılık ürüne yani çay kalitesine yansımaktadır. Bu nedenle bu farklılığın belirlenmesi önem arz etmektedir. Perşembe'de yetişen çay genotiplerinin popülasyon içi ve seçilen kültivarlar ile arasındaki genetik ilişkiyi belirlemek amacı ile bu çalışma yürütülmüştür. Çalışmada kullanılan bitki materyalleri farklı yükseltilerden ve farklı bahçelerden alınmıştır. Çalışma bölgesini temsil edecek şekilde 20 bitki materyali toplanmıştır. Kontrol olarak Derepazarı7, Fener3 ve Tuğlalı10 olmak üzere üç standart çeşit yer almaktadır. Araştırmada ISSRs yöntemi kullanılmıştır. 10 ISSR markörü kullanılan çalışmada genetik çeşitlilik ve buna bağlı olarak ilişkiler ortaya konulmuştur. UPGMA kullanılarak yapılan kümeleme analizine göre bireyler arasında önemli farklılıklar tespit edilmiştir. Araştırma sonucunda; Ortatepe mahallesinden alınan çay genotiplerinin kendi içerisindeki benzerlik oranının daha yüksek olduğu, buna karşın, İstanbul boğazı mahallesinden alınan örneklerin kendi içerisinde benzerlik oranlarının daha düşük olduğu belirlenmiştir. Bu bağlamda Ortatepe'de bulunan bitkiler arasında daha yüksek genetik çeşitlilik olduğu söylenebilir. Elde edilen veriler bölgede çay tarımının ve kalitesinin gelişmesine yardımcı olacak niteliktedir.

Anahtar kelimeler: *Camelia sinensis*, genetik çeşitlilik, ISSRs, Perşembe

INTRODUCTION

Tea, *Camellia sinensis* (L.) Kuntze, Theaceae, is a garden plant that has been growing in our country for a century. It has been important in our country for a century and has a vital place in the economy of the Eastern Black Sea region. Cultivated tea taxa consist of three natural hybrids: *C. sinensis* or Chinese type, *C. assamica* (Masters) or Assam type and *C. assamica* ssp. *lasiocalyx* (Planchon ex Watt.) or Cambodian or Southern type (Beris et al., 2016; Mondal et al., 2004). Two of these genotypes, *C. sinensis* (China) and *C. assamica* (Assam), and their hybrids are well-known and commercially cultivated worldwide (Beris et al., 2016; Ma et al., 2012). Although the tea plant is native to China, Tibet, and Northern India in the world, it also grows well in the humid and temperate climate of our country's Eastern Black Sea Region coast, where annual rainfall is high. The first tea plantation in Turkey was started in Rize planting tea seeds from Georgia in 1924 (Beris et al., 2005; 2016; Çaykur, 2024). Currently, the tea production area in Turkey is from Sarp village of Kemalpaşa district of Artvin province in the east to Perşembe district in the west. Tea is grown in the entire Rize province, in a significant part of Trabzon and Artvin provinces, and a partial area of Giresun and Ordu provinces (Koday, 2000). According to FAO statistics, China ranks first in world tea production with 41%, while Turkey ranks fifth with 4% (FAO, 2022).

The tea plant is a garden plant grown especially for its shoots and is widely consumed as a hot or cold beverage (İslam, 2019). Black tea constitutes a large portion of tea production in Turkey. In addition to improving cultivation techniques, increasing the production, efficiency and quality of tea is possible by vegetatively propagating high-quality individuals and using them in production. This method of production has been traditionally maintained for a long time. Tea, which started to be produced in 5 neighbourhoods in Perşembe district in the 1960s to earn additional income in addition to hazelnuts, has continued to be made for approximately 60 years. The district produces 150 tons of tea annually in approximately 95 decares of land (Anonymous, 2023).

Morphological and biochemical features used in traditional plant identification methods may be insufficient to determine genetic relationships due to environmental conditions and evolution at the genetic level, as well as due to pollination in plants. However, molecular marker techniques based on DNA markers provide phylogenetic analysis to determine genetic diversity for plant breeding. Many such techniques are available for tea breeding. Many techniques based on molecular markers are available, including tea breeding. Ease of application, stability of results, reproducibility and cost are critical parameters in these techniques. However, the ISSRs-PCR technique is quite advantageous in this respect. The ISSRs technique is a relatively simple, low-cost and rapid method to determine genetic diversity (Zietkiewicz et al., 1994; Mondal, 2002; Dan, 2006; Chen et al., 2015). Therefore, ISSRs markers have been used to reveal genetic relationships within and among populations of the genus *Camellia* as well as in other plants in India, China, Japan, Taiwan, and Turkey (Beris et al., 2016; Mondal, 2002; Dan, 2006; Thomas et al., 2006; Chen et al., 2007; Yao et al., 2008; Roy & Chakraborty, 2009; Ben-Ying et al., 2010).

Yao et al. (2008) studied 48 individuals to differentiate teas grown in China, Japan and Kenya and to determine the importance of selection in the tea program. Lai et al. (2001) performed RAPD and ISSRs analyses on 37 genotypes grown in Taiwan to reveal genetic relationships. Ben-Ying et al. (2010) determined the genetic relationships of 134 tea genotypes from China by ISSR analysis with 18 primers. Freeman et al. (2004) determined that repeat motifs were to be used for microsatellite analyses using 15 tea genotypes. Vijayan et al. (2009) performed a molecular taxonomy study on 112 *Camellia* species using ITS analysis. Beris et al. (2005) determined phylogenetic relationships using RAPD on tea clones obtained from the Rize Tea Research Institute. Again, their study published in Beris et al. (2016) found 46% to 74% genetic similarity between tea genotypes using 15 ISSRs markers of 18 Turkish tea genotypes. Also, Yoğurtçu (2019) compared 18 tea genotypes sampled from the Black Sea region with 15 ISSRs markers and as a result of the study, genetic diversity was revealed similarly.

The results obtained from different studies revealed significant relationships between clones or genotypes. This study was conducted to determine the genetic diversity between 20 tea genotypes grown in Perşembe, located at the westernmost end of the Turkish tea production area and has not been included in genetic similarity studies to date, and three parent genotypes in the main lines.

MATERIAL AND METHOD

Plant Materials: In this study, 20 tea genotypes originated from the Ordu province of the Black Sea Region, Türkiye. In addition, three known tea genotypes, Pazar-20, Tuglali-10, and Zihni Derin, were provided by the Rize Ataturk Tea and Horticulture Research Institute. Information on genotypes is given in Table 1. Young leaves of the genotypes were sampled from fresh terminal shoots in the second shoot period. All leaf samples were immediately frozen to avoid heat damage and stored at -70°C.

DNA Isolation: Genomic DNA isolation was achieved with GeneMATRIX Plant & Fungi DNA

Purification Kit (EUR_X Sp., Poland) according to the manufacturer's protocol. DNA concentration and purity were calculated using a NanoDropR ND-1000 Spectrophotometer (Thermo Fisher Scientific Inc. USA) after checking on 0.7% agarose gel electrophoresis.

Table 1. Tea genotypes information used in this study.

No	Sampling Region	Code No
1	Perşembe Ortatepe 1	P-1
2	Perşembe Ortatepe 2	P-2
3	Perşembe Ortatepe 3	P-3
4	Perşembe Ortatepe 4	P-4
5	Perşembe Ortatepe 5	P-5
6	Perşembe Ortatepe 6	P-6
7	Perşembe Ortatepe 7	P-7
8	Perşembe Ortatepe 8	P-8
9	Perşembe Ortatepe 9	P-9
10	Perşembe Anaç 1	P-10
11	Perşembe Anaç 2	P-11
12	Perşembe Anaç 3	P-12
13	Perşembe Anaç 4	P-13
14	Perşembe Anaç 5	P-14
15	Perşembe İstanbul Boğazı 1	P-15
16	Perşembe İstanbul Boğazı 2	P-16
17	Perşembe İstanbul Boğazı 3	P-17
18	Perşembe İstanbul Boğazı 4	P-18
19	Perşembe İstanbul Boğazı 5	P-19
20	Perşembe İstanbul Boğazı 6	P-20
21	Pazar-20	
22	Tuglali-10	
23	Zihni Derin	

ISSRs Analysis

For ISSRs analysis, PCR trial studies were first performed to select primers, according to Beris et al. (2016). Ten of the primer stocks tested were selected and used in this study, and information about the selected ISSRs primers is given in Table 2. In PCR studies, 15 ng genomic DNA, 1.5 µL of 10 µM ISSRs primer, 4 µL of dNTP (2.5 mM), 4 µL of 25 mM $MgC1_2$, 5 µL of 10X *Taq* DNA polymerase buffer, 1.5 U *Taq* DNA Polymerase (Thermo Fisher Scientific, USA) were used according to Beris et al. (2016) and the reaction mixture was completed to 50 µL with sterile dH_2O. The PCR was performed on the LongGene A300 Thermal cycler system (LongGene Sci. Inst. Co. Ltd., China). The reaction steps were completed as the first denaturation at 94°C for 2 min, followed by 38 cycles as denaturation at 94°C for 1 min, annealing at *Tm* for each primer (Table 2) for 1 min, extension at 72°C for 2 min, final extension step at 72°C for 10 min. The obtained PCR amplicons with 100 bp DNA ladder (New England Biolabs Inc.) were done 2% agarose gel electrophoresis at 100 V and 300 mA for 3 hours in a universal TAE buffer system. The gels were photographed on the UV-transilluminator.

Table 2. Information on ISSRs primers used in the study.

No	Primer Sequences (5'→3')	Tm (°C)
1	$(AC)_8T$	45
2	$(GAA)_6$	44
3	$(GT)_8T$	48
4	$(AC)_8C$	51
5	$(CAA)_6$	44
6	$(CAA)_6G$	48
7	$(CAG)_6$	58
8	$(CT)_8GG$	52
9	$(GT)_8C$	48
10	$(AC)_8TG$	50

Statistical Data Analysis: In the gel images obtained as a result of the electrophoresis process, the numbers (1) were given for the presence of bands, (0) for the absence of bands, and (9) for no amplification. The number of bands formed by each ISSRs primer, the number of polymorphic bands formed, and the effective band frequencies were calculated. The polymorphism information content (PIC), which is an indicator of the success of the primers used in the study in distinguishing genotypes of the tea plant, was calculated. The related dendrogram was created based on the similarity index data by UPGMA cluster analysis using the NTSYS-pc 2.02i computer program (Rohlf, 1988). The relationships between all tea samples were described graphically in the dendrogram.

RESULTS AND DISCUSSION

ISSRs Analysis: To determine the molecular properties of the genotypes of the tea plant, 10 ISSRs primers were used. No amplification occurred in 1 of these primers $(AC)_8TG$), and polymorphic bands were obtained from the other nine primers. The band and polymorphism information obtained for the ISSRs primers are given in Table 3.

Table 3. Band numbers and polymorphism information contents of ISSRs primers.

No	Primer's Name	Polymorphic Band Number	Monomorphic Band Number	Total Band Number	Polymorphism Ratio (%)	Polymorhism Information Content
1	$(AC)_8T$	4	0	4	100	0.41
2	$(GAA)_6$	3	1	4	75	0.18
3	$(GT)_8T$	6	0	6	100	0.35
4	$(AC)_8C$	6	3	9	66	0.23
5	$(CAA)_6$	6	0	6	100	0.41
6	$(CAA)_6G$	7	0	7	100	0.25
7	$(CAG)_6$	10	0	10	100	0.35
8	$(CT)_8GG$	2	0	2	100	0.49
9	$(GT)_8C$	9	0	9	100	0.37
10	$(AC)_8TG$	0	0	0	0	0.00
	Total	53	4	57		
	Average	5.3	0.4	5.7	93.44	0.34

All nine primers used in the study that produced amplification were determined to be polymorphic. The primers produced a total of 57 bands. Fifty-three of these bands were defined as polymorphic. The polymorphism average was found to be 93.44%. The average number of bands per primer was determined to be 5.7, while the average polymorphic band was determined to be 5.3. The primer that produced the most bands was $(CAG)_6$ with ten bands, while the primer that produced the least bands was $(CT)_8GG$ with two bands. The highest polymorphic band number was $(CAG)_6$ with ten polymorphic bands, while the primer with the least polymorphic band number was $(CT)_8GG$ with two polymorphic bands. The polymorphism rate values of the primers varied between 66% and 100% (average 93.44%). The polymorphism rate was found to be 100% in 7 of the primers used. The polymorphism

information content values of the primers used in the study ranged from 0.18 ($(GAA)_6$) to 0.49 ($(CT)_8GG$), and the average polymorphism information content was determined as 0.34.

Principal Component Analysis (PCA): Principal component analysis (PCA) was applied to the data obtained with ISSRs primers in determining the variation between genotypes of tea plants selected from the Perşembe district of Ordu province. The findings obtained are as in Table 4. As seen in the table, the conclusions obtained showed that the variation between genotypes in the first 7 of the principal components was 90.81%. The total variation explained by the 10 axes of the principal components was found to be over 95%. These results show that the genotypes were distributed correctly in the dendrogram created with ISSRs markers. It was concluded that the 23 tea genotypes defined with 9 ISSRs primers were correctly represented in the created planes and clusters.

Table 4. Principal component analysis data of ISSRs primers

Component Axes	Core Value	Variation (%)	Total variation (%)
1	15.78	71.72	71.72
2	1.37	6.23	77.94
3	0.82	3.71	81.65
4	0.62	2.83	84.47
5	0.55	2.50	86.98
6	0.44	1.99	88.96
7	0.41	1.84	90.81
8	0.35	1.59	92.40
9	0.34	1.52	93.92
10	0.25	1.14	95.06

Clustering Analysis: The grouping and correlation values obtained from the data obtained from ISSRs primers used to reveal the genetic relationships between the selected tea genotypes from the Perşembe district of Ordu province and cultivar genotypes, according to the UPGMA method, are given in Figures 1 and 2, respectively. The average correlation coefficient value showing the compatibility of the correlation matrix showing the genetic relationships and the dendrogram, which is the visual expression of this relationship, was calculated as r = 0.75257. It is seen that the similarities between the average correlation coefficient values and the genotypes are high.

When the dendrogram graph obtained as a result of the cluster analysis is examined, it is seen that the Tea genotypes taken from Perşembe district and Rize are gathered in 4 main clusters. It was determined that most of the genotypes were in cluster number 4, and the most different genotypes were P-12 and P-17. The correlation matrix values between the genotypes used in the study varied between 0.40 and 0.95. According to the results obtained, it was seen that the genetically most distant lines were between P-12 and P-17, and the closest lines were between P-1 and P-2. It was determined that most of the genotypes taken from Perşembe district of Ordu province were genetically closer to the Pazar-10 and Fener-3 varieties but more distant from the Tuğlalı-10 variety.

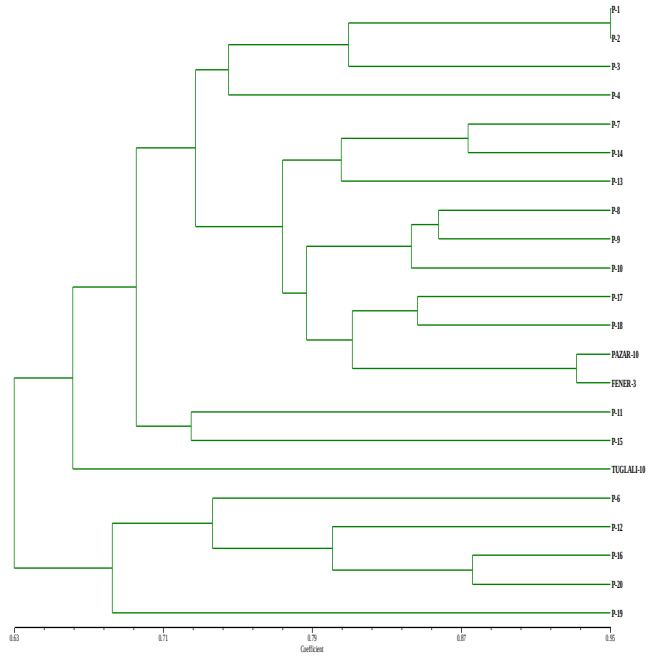

Figure 1. Dendrogram of tea genotypes and varieties obtained from ISSRs data**.**

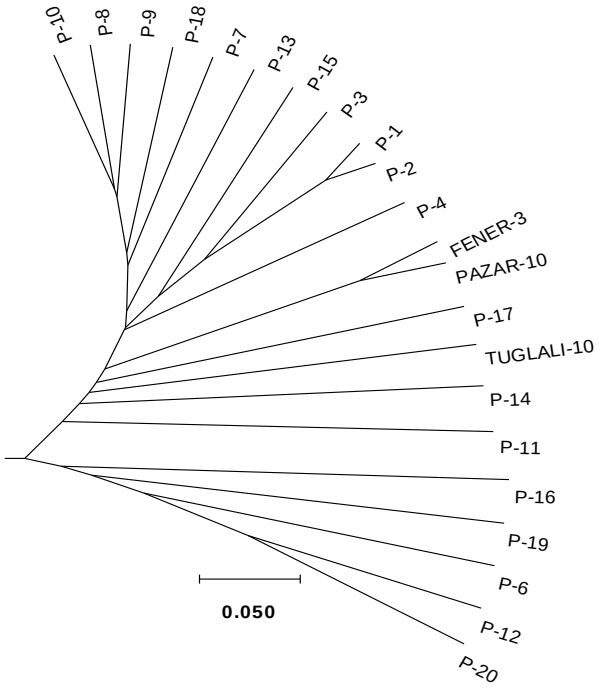

Figure 2. Radial dendogram image obtained from the principal component analysis as a result of ISSRs analysis in Ordu province genotypes and cultivar genotypes.

This study shows the similarities between tea genotypes taken from Ordu province, Perşembe district and Rize province in a three-dimensional plane (Figure 3). When examined in these planes, it was seen that the

farthest lines were between P-12 and P-17, and the closest lines were between P-1 and P-2.

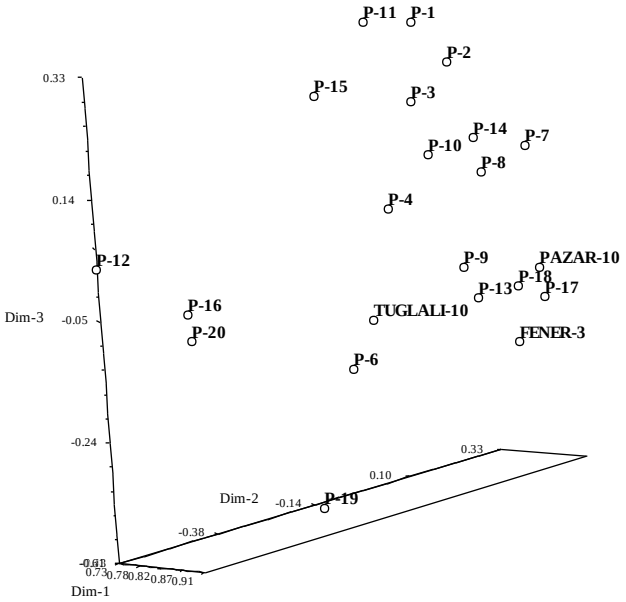

Figure 3. 3D plane graph obtained from the principal component analysis of Ordu province genotypes and cultivar genotypes as a result of ISSRs analysis.

In the interviews conducted with the producers at the beginning of the study, it was reported that in the process of creating the gardens, plants obtained by vegetative means may have been used in some gardens, but in general, plants propagated from seeds were used in creating the garden. As a result of the study, it can be said that the highest similarity rate is between P-1 and P-2, at 95%. Again, the lowest similarity rate was determined at 63%. The Tuğlali-10 variety formed a separate line when all individuals were evaluated together.

CONCLUSION

Like many other places in our country, the tea production areas established in Ordu province are gardens established with seeds. There is genetic variation in gardens established with seeds due to uncontrolled pollination. This difference in the genetic structure of the plant is, therefore, reflected in the product, that is, the quality of the tea consumed.

As a result of this study, It was determined that the similarity rate of the tea genotypes belonging to Ordu province is higher, whereas the similarity rates of the samples taken from the Istanbul Boğazı were lower. This situation may be related to cultivating seeds belonging to different genotypes or at different times in the tea fields in Ordu province. As a result of the study, it was determined that the highest similarity rate was between P-1 and P-2 with 0.95; the most distant lines were between P-12 and P-17 with 0.40. Again, in the comparison made with three standard tea varieties, it was determined that most of the genotypes used in the study were genetically closer to the Pazar-10 and Fener-3 varieties and more distant to the Tuğlalı-10 variety.

The data obtained will help the development of tea cultivation and quality in the region. It can be said that genetic differences are high in individuals propagated by seeds. To ensure high yield and quality in garden establishment, it is recommended that standard varieties be used and propagated by vegetative means.

This study has shown once again that tea varieties and clones in the planting areas have been separated from each other due to hybridization due to sexual reproduction and propagation with seeds obtained from these hybrids. Tea plantation areas in Turkey were established in this way, and as a result, dendrogram differences are seen in similar marker analyses (Beris et al., 2005, 2016; Kafkas et al., 2009). Our results have shown that Turkish tea's genotyping and breeding problems can be overcome by using ISSRs, which have much higher polymorphic amplicon numbers and the highest fragment resolution power compared to other DNA-based markers.

Disclosure statement: The authors declare that there are no conflicts of interest.

Conflict of Interest: The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this paper.

Author Contribution: All authors contributed to the study's conception and design. Material preparation, experiments, analysis, and preparation of the manuscript were performed by Ali İslam, Muharrem YILMAZ, Selim KARAGÖL, and Fatih Şaban BERİŞ contributed to the development of the protocol, writed and reviewed the manuscript. All authors read and approved the final manuscript.

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