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Impact of Bioclimatic Factors on Diversity Patterns in *Quercus brantii* **(Persian Oak) Populations within the Western Iranian Forest Ecosystem**

Adele Rafezi¹, Mohammad Reza Azimi¹, Mehrshad Zeinalabedini^{*2}, Mohammad Reza Ghafari²

¹Department of Plant Production and Genetics, Agriculture Faculty, University of Zanjan 2 Agricultural Biotechnology Research Institute of Iran, Karaj, Iran

K E Y W O R D S A B S T R A C T

Bioclimatic factors; (Geographic Information System) GIS; *Quercus brantii*; Redundancy analysis (RDA); Spatial distribution

The woodlands in western Iran, especially the Zagros forests, are ecologically crucial for their ability to withstand environmental stresses and conserve water and soil. These ecosystems have suffered significant degradation, particularly in Ilam province, where Persian oak trees (*Quercus brantii*) have experienced widespread mortality. Understanding the impact of climatic factors on tree species dominance and forest composition is vital for ecosystem conservation. Despite some knowledge about oak species diversity, research on the influence of bioclimatic parameters on *Q. brantii*, a native Iranian species, is limited. This study aimed to explore how topographical and climatic factors have shaped the population structure and diversity of *Q. brantii* in western Iran. The study collected data from five habitats within three forested regions in the Ilam province, including 183 samples. A total of 20 phenotypic traits, three geographic, and 19 bioclimatic parameters were measured, and statistical analyses revealed distinct oak ecotypes based on leaf and seed characteristics. Based on the morphological characteristics, a clustering analysis identified two distinct groups among all ecotypes. Redundancy analysis (RDA) revealed that three bioclimatic factors namely annual mean temperature, annual precipitation, and altitude significantly influenced population diversity. Specifically, they had a significant impact on fruit formation and seed morphology and together accounted for 86.9% of the observed variability. The spatial distribution analysis based on PCNM indices revealed that the Sarmast, Kochali, and Sirvan locations had the highest morphotypic diversity. This study emphasizes how bioclimatic and geographic factors shape Iranian oak diversity, informing conservation and sustainable management in western Iran.

Introduction

The forests situated in the western and southwestern regions of Iran, notably the Zagros forests, stand as vital and esteemed natural resources within the country. These ecosystems exhibit remarkable resilience against regional degradation, exerting substantial and meaningful impacts on water and soil conservation within forested habitats (Panahi & Jamzad, 2017). The expanse and distribution, species diversity, as well as the assorted by-products and forest commodities render this vegetative expanse an integral component of Iran's biological resources and genetic reserves (Taleshi & Maasoumi Babarabi,

Corresponaing autnor: Email address: m_zeinalabedini@yanoo.com
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2013). The Zagros region is characterized by a Mediterranean climate with cold winters and an extended dry summer season lasting up to four months annually in most areas (Panahi & Jamzad, 2017). Ilam Province alone has around 508,000 hectares of Zagros forests. The Persian oak (*Q. brantii*) is the main tree species in this area. (Ebrahimi *et al*., 2017). Regrettably, these invaluable forests have endured severe damage and substantial depletion in recent decades, particularly within Ilam province, where a significant portion of the Zagros forests has faced the ominous oak decline crisis over the past decade. Presently, the livelihoods of numerous local communities and villagers hinge upon the existence of these forests, supporting activities such as livestock rearing, potable water provision, and agriculture (Vahdati, 2014). The potential loss of these forests would constitute an irreplaceable setback for the region. Multiple factors contribute to this phenomenon, with specific causal agents varying based on regional and ecological conditions. Among the causative factors is the extensive desiccation observed in large segments of Iranian oak forests in the Zagros region (Alikhani *et al*., 2014). In response to recent drought events, *Q. brantii* has emerged as one of the most susceptible tree species, and the substantial mortality of Iranian oak species has inflicted severe structural transformations upon the forest ecosystem, resulting in diminished overall functionality. The formulation of a comprehensive strategy for preserving oak genetic resources and the revitalization and restoration of oak forests through seed propagation and planting represents a critical endeavor in safeguarding the habitats of this genus (Shiran *et al*., 2011). In many instances, afforestation efforts have encountered challenges stemming from genetic incompatibility between planted seedlings and prevailing environmental conditions, leading to diminished survival rates. Consequently, afforestation initiatives necessitate the utilization of seed genotypes that exhibit compatibility with the anticipated climatic conditions of future periods, underscoring the importance of obtaining information concerning morphological traits and their responses to diverse climatic conditions and genetic diversity (Ebrahimi *et al*., 2017).

Understanding the impact of environmental and climatic factors on the dominance of tree species and the composition of communities within ecosystems is crucial for providing insight into the preservation of current forest structures. Research has consistently shown that the influence of environmental conditions on the physical characteristics of plants is greater than that of genetic diversity. Generally, the variability in traits among individuals of a particular species represents the plant's adaptive reaction to different environmental pressures (Li *et al*., 2020 and Alcantara-Ayala *et al*., 2020). Ecologists and evolutionary biologists have placed considerable emphasis on elucidating the relative importance of bioclimatic variables, such as temperature and precipitation, as well as geographic isolation, in influencing the spatiotemporal composition of plant communities (Collart, 2021). Biogeographers have extensively investigated the latitudinal diversity gradient, an empirical phenomenon that delineates the pinnacle of plant species richness in warm and humid tropical areas, steadily decreasing towards frigid and arid polar regions. (Willig, 2003; Das, 2022). The tropical and temperate regions can be distinguished by the predictable alterations in seasonal and daily temperature variations, as well as the variations in precipitation and temperature gradients. (Zanne, 2014; Molina–Venegas, 2022). The ability of plants to withstand extreme ranges and fluctuations in rainfall and temperature serves as a critical determinant of plant species distribution along gradients and reflects phylogenetic niche conservatism (Molina–Venegas, 2022). In conjunction with population structure analysis, the application of distribution modeling for oak species offers valuable insights into population density expectations. In collaboration with the analysis of genetic structure, the utilization of distribution modeling for oak species provides

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significant insights into anticipated population density. The implementation of Geographic Information System (GIS) technology proves to be an effective instrument for monitoring and delimiting distribution patterns of diverse components of biodiversity, thereby facilitating enhanced management and conservation endeavors. (Zeinalabedini, 2019; Vahdati *et al.*, 2019). Phenotypic diversity, in the context of this study, encompasses variations in leaf, flower, fruit, and seed morphology (Thomas, 2016; Danicic *et al*., 2018).

The Persian oak (*Q. brantii*), a keystone species within the Zagros forests of western Iran, occupies a paramount position in these ecosystems (Jazirehi & Rostaghi, 2003). Its economic and ecological significance is underscored by the provision of highquality timber, firewood, and wildlife habitat, along with its pivotal role in rendering various ecosystem services and contributing to biodiversity conservation (Cavender-Bares *et al*., 2018). For example, in a study, the effects of foliar application of oak fruit extracts as plant growth regulators (PGRs) were evaluated to reduce vegetative growth and avoid reproductive growth of evening primrose (*Oenothera biennis* L.) (Ghezel *et al*., 2022). Despite its ecological importance, these forests face a myriad of threats, encompassing habitat loss, invasive species, climate change, pests, and diseases. Additionally, the comprehensive physiological reactions of different *Quercus* species towards temperature variations, instances of drought, or occurrences of flooding have been thoroughly recorded (Cavender-Bares *et al*., 2018; Li *et al*., 2013; Vitasse *et al*., 2014). *Quercus* possesses these attributes that make it a highly suitable taxon for investigating the effects of bioclimatic variables. Altitude fluctuations and diverse environmental circumstances can incite modifications in the physical appearance of oak leaves, contributing to the heterogeneity among oak species. Consequently, researchers have employed phenotypic attributes to formulate identification tools for distinguishing between oak species and

delineating their respective sections. (Panahi *et al.*, 2017; Taleshi and Maasoumi-Babarabi, 2013). Due to its native status in Iran, *Q. brantii* remains understudied on a global scale, and there exists a conspicuous dearth of comprehensive information concerning its status. Our understanding of the impact of bioclimatic and geographic factors on the diversity and structure of oak populations is still limited, despite the growing recognition of oak morphological and molecular diversity.

Numerous studies have highlighted the influence of physical and geographic factors in shaping forest structures (Thammanu, 2021; Hailemariam, 2020; Vacek, 2015; Munishi, 2007). Several investigations have probed the impact of environmental variables on morphological traits (Chen *et al*., 2017; Woz, 2015). Gao *et al*., (2021) conducted a study to examine the impact of geographical and climatic factors on the phenotypic diversity of *Q. variabilis* and proposed that this particular species exhibits substantial levels of phenotypic plasticity in response to geographical and climatic factors. Li *et al*., (2019) shed light on how topography and climate jointly affect the genetic structure and evolutionary history of *Q. chenii* in eastern China. Lin *et al*., (2023) examined the implications of climate change on the spread of *Quercus* section *Cyclobalanopsis* (Fagaceae) in East Asian evergreen broadleaved forests. Additionally, Xu *et al*. (2016) examined the relationship between geographic variables and oak diversity, revealing geographical variations in the impact of climate parameters on oak diversity. They found that water limitation and temperature exert severe effects on oak diversity in arid environments. Similar results has also been reported in walnuts (Arab *et al.*, 2020)

This present study endeavors to elucidate the repercussions of bioclimatic variables on population diversity within *Q. brantii*. Notably, there exists a paucity of research investigating the influence of geographic and climatic conditions on *Q. brantii* within its natural habitat in the Zagros forests, and the potential impacts of climate change on this region

remain ambiguous. In aggregate, these investigations furnish pivotal insights for the preservation and management of the forests in western Iran.

Material and Methods

Sample and data collection

The study focused on Persian oak (*Q.brantii*) ecotypes within various locations across Ilam province's forests (Fig. 1). A total of 183 oak trees were sampled, representing 5 habitats selected from three distinct forested areas within Ilam province

(Table 1). The measurement of 20 traits encompassed morphological characteristics, which included attributes related to leaf, seed, and tree trunk attributes (Table S1). The meticulous recording of geographic data was conducted using GPS devices. To mitigate genetic affinities resulting from vegetative propagation, the minimum distance between trees was maintained at a distance of no less than 100 meters, following guidelines stipulated by Ginwal *et al*. (2005). Nineteen bioclimatic parameters were sourced from the Iran Meteorological Organization (IRIMO) (Table 2).

Forested areas	Habitats	Altitude (approx. Meters)	Longitude (E)	Latitude (N)	Average annual temperature (Celsius)	Average annual rainfall (mm)	Number of samples	Slope (%)
$\mathbf C$	Serawle	1400	46.627222	34.009444	18.5	520.8	12	4%
	Sirvan	1300	46.775833	33.785278	20.8	447.8	14	3.50%
	Gawez	1400	47.035278	33.598333	16.9	537.7	12	15%
	Kochali	1600	46.553056	33.776111	16.9	537.7	11	4%
	Towe Hell	900	46.32234	34.35238	24.6	225.8	13	15%
B	Dare shahr	700	47.262222	33.573333	19	484	12	10%
	1 _O Dare shahr 4	1400	47.123056	33.476038	16.9	537.7	11	46%
	Dare shahr 3	1400	47.321389	33.473056	16.9	537.7	12	12%
	Dare shahr 2	1300	47.151944	33.990278	16.9	537.7	12	7%
	Dare shahr 1	1300	47.089722	33.728333	16.9	537.7	12	3%
\mathbf{A}	Danuk	1400	46.80043	34.46531	17	663	14	3%
	Dolaw-K	1500	47.000278	34.495556	16.7	571	12	2%
	Dolaw- G	1700	47.2175	34.099167	16.7	571	12	4%
	Dolaw	1400	47.178889	34.503333	16.7	571	12	15%
	Sarmast	1900	47.159167	33.965556	13	720	12	12%

Table 1. Sampling locations, climatic characteristics, and ecotype counts at each habitat.

Fig. 1. Sampling locations and natural habitats of *Q. brantii* ecotypes.

Morphologic analysis

Following the assessment of data normality, an analysis of variance (ANOVA) was carried out within a stratification statistical design. Subsequently, multiple comparisons were executed using Duncan's test, facilitated through the SPSS statistical software. Cluster analysis of the sampled populations, based on morphological traits, was performed utilizing the kmeans method and the Clustering Tree analysis. Statistical analyses and plot generation were executed through R software version 4.0.4 and XLSTAT software (Version 2016.1), by methodologies described by Kassambara (2017) and Zappia and Oshlack (2018).

Table 2. Bioclimatic parameters evaluated as effective in geographical areas of *Q. brantii* distribution.

Spatial analysis

To evaluate the correlation between bioclimatic variables within geographical regions, the Pearson correlation approach was employed, utilizing R software 4.0.4 and the Corrplot package. Subsequently, climatic parameters exhibiting low to moderate correlations ($|r| \leq$ 0.70) were selected for further analyses. The Mantel test was then conducted to determine the relationships between observed morphotype diversity, bioclimatic parameters, and geographical locations (latitude, longitude, and altitude) of sampling using the Kulczynski and Gower dissimilarity matrix. For this purpose, vegan R package CC211 was used. To assess the combined influence of geography and climate in shaping morphotype variation patterns, redundancy analysis (RDA) was performed using R package *rda* (Oksanen *et al.,* 2018). Geographic variables were quantified using eigenvectors corresponding to positive eigenvalues of the principal coordinates of the neighbor matrix (PCNM), as outlined by Borcard and Legendre (2002). PCNM was

derived from a truncated matrix, incorporating great circle distances between sampled individuals. Model significance was determined through 999 permutations. Furthermore, the spatial distribution of PCNMs was incorporated into the analysis, representing species distribution across geographical locations. This was achieved using the *pcnm* function within the R package 'vegan.'

Results

Population trait variation

Upon confirming the assumptions of variance analysis and appropriate data transformation, the results of variance analysis, based on a stratified statistical design, indicate significant variation ($p \leq$ 0.005) in morphological characteristics within and among *Q. brantii* populations across sampling geographical locations. Furthermore, significant

differences were observed among all studied morphological traits at a five percent probability level. To group populations based on geographical locations, a k-means clustering analysis (k=2) was performed, utilizing mean standardized variables and Euclidean distance. Fig. 2 illustrates the distinct separation of the two clusters, suggesting the presence of two distinct oak populations in the sampled forest

areas of Ilam province. These populations exhibit disparities in characteristics related to leaf and seed size and shape, with the first group featuring larger leaves and smaller seeds, and the second group demonstrating the opposite traits. The information regarding every group and the ecotypes within each cluster can be found in Table S2.

Fig. 2. Distribution of oak samples in groups resulting from K-means clustering in two groups (LL: Leaf length, LW: Maximum width of the leaflet, DBW: Distance from leaf base to maximum leaf width, LP: Petiole length, LR: Leaf shape, BW90: Leaf width at 90% of leaf length, BW50: Leaf width at 50% of leaf length, LN: Number of leaf teeth, VN: Number of veins, LOD: Leaf teeth distance, LOL: Leaf teeth length, LDE: Leaf teeth depth, SL: Seed length, SW: Seed width, VS: Seed volume, 1000SW: Weight of 1000 seeds, D130: Breast height, DC: Collar diameter, TH: Total tree height, CH: Crown height)

Following the outcomes of the Clustering tree analysis, as presented in Fig. 3, the groupings are established through a clustering methodology grounded on the first principal component. Notably, the most pronounced level of dissimilarity among these groupings is manifest when a dichotomous division is applied, resulting in two distinct clusters, comprising 51 and 132 ecotypes, respectively (as illustrated in Fig. S1). The initial cluster encompasses ecotypes No. 1, 2, 7, 8, and 13 of the Danuk habitat, ecotypes No. 2, 4, and 9 of the Dare shahr1 habitat, ecotypes No. 4, 11, 14, 15, and 17 of the Dare shahr3 habitat, as well as all ecotypes within Dare shahr18, ecotypes No. 3, 6, 7, 8, and 10 of the Towe Hell, ecotypes No. 5 and 7 of the Kochali, ecotypes No. 1

and 2 of the Gawez, and the entire ecotypes within the Sirvan habitat. The remaining ecotypes are classified within the second cluster. This analysis underscores the pivotal determinants that exert substantial influence on the clustering process, which include the Maximum width of the leaflet, leaf teeth distance, and Leaf width at 50% of leaf length. It is evident that, when the division results in two distinct clusters, there is a decreased incidence of ecotypes that are found in both groups. As the number of clusters increases, the likelihood of ecotypes being shared between groups also increases. This diversity may be attributed to hybridization and protected hereditary polymorphism within *Q. brantii* and potentially other oak species.

Fig. 3. Clustering diagram based on k-means clustering of ecotypes using morphological traits and the first principal component

The contribution of bioclimatic factors to phenotypic

variation

Following correlation analysis, climatic parameters exhibiting high correlations ($|r| \ge 0.70$) were excluded, while the remainder were retained for further analysis. For instance, annual precipitation (BIO 12) demonstrated a significant and negative correlation with temperature-related parameters at a 5% probability level. Notably, BIO 2 exhibited a positive and significant correlation with BIO 3, and BIO 13 correlated positively with BIO 8 (Fig. 4).

Fig. 4. Spearman correlation coefficients among 19 bioclimatic parameters in the studied locations.

The Mantel's test, performed between all characteristics in two dissimilarity matrices (Kulczynski and Gower), revealed a significant correlation (r statistic = 0.1788, significant at 5% level) between morphotype diversity and all climate variables.

Redundancy analysis (RDA) was employed as a robust tool for detecting multivariate genotypeenvironment associations, outperforming traditional methods like the Mantel test (Legendre and Fortin, 2010; Forester *et al*., 2018). The results of the RDA test indicated that the first two factors accounted for 86.9% of the variance. The utilization of the reduction model (0.012*) in conjunction with the Permutation test (999) elucidated the presence of a notable proportion of diversity that can be attributed to a restricted quantity of RDA factors, thereby underscoring the superior impact exerted by morphotype, environmental parameters, and ecotypes (Fig. 5). The analysis unveiled that annual mean temperature (BIO 1), annual precipitation (BIO 12), precipitation of the driest quarter (BIO 17), and geographical altitude (BIO 14) had the most substantial impact, particularly on traits associated

with seed shape such as seed width, seed length, weight of 1000 seeds, and seed volume (Fig. 6). These parameters were identified as limiting factors for forest development and restoration. Conversely, precipitation of the wettest quarter and warmest quarter exhibited minimal effects on morphotype diversity.

Fig. 5. The redundancy analysis (RDA) biplot illustrates the correlation between morphological diversity and geographical as well as climatic factors.

Small red crosses at the center represent the principal coordinates of the neighbor matrix (PCNM). Eigenvectors corresponding are positive eigenvalues of the bioclimatic variables. Black or gray dots indicate *Q.brantii* individuals from sampling locations

Fig. 6. Biplot from redundancy analysis (RDA) illustrating the association of morphological variation with geographic and climatic variables.

To investigate the spatial distribution of the studied oak ecotypes, latitude and longitude were utilized in conjunction with Principal Coordinates of Neighbor Matrices (PCNMs), as depicted in Fig. 7.

The spatial distribution of the specimens was additionally illustrated according to the initial six factors of PCNMs (Fig. 8).

Fig. 7. Spatial distribution of studied oak samples based on latitude and longitude.

Fig. 8. Spatial distribution of studied *Q. brantii* specimens based on longitude and latitude, emphasizing the first six PCNM indices.

In Fig. 5, the distribution of the examined oak specimens, categorized by their respective latitude and longitude, alongside the PCNM indices within the designated sampling locations, are presented. Consequently, the samples with data points exhibit a color spectrum spanning from red to blue, wherein the blue color signifies the locations or plant communities that bear resemblance to one another in comparison to their neighboring individuals. The blue-colored oaks, in essence, exhibit the lowest level of biodiversity, whereas the red-colored oaks are associated with plant communities that possess the highest level of diversity. Therefore, it can be inferred that the oak trees situated in the locations of Kochali, Sarmast, and Sirvan exhibit the most substantial morphotypic variation, while the oak trees associated with the regions of Dolaw, Dolaw-K, and Danuk demonstrate a minimal level of diversity.

Discussion

This research demonstrates a novel approach for analyzing the respective influences of geographical and environmental factors on the observed variation in a population of a species that is widely distributed. The findings emphasize the intricacy of genetic variation, necessitating its assessment alongside geographic and climatic factors when constructing evolutionary scenarios, especially for plants like oak in Iran. Different factors have influenced the variation observed in distinct morphological traits, underscoring the importance of evaluating various hypotheses for geographical variation (Ficetola *et al*., 2010). Environmental factors are found to significantly impact the phenotypic variation among populations of the study species. Li *et al*. (2019) similarly reported the influence of topography and climate on the genetic variation of *Q. chenii*. Climatic

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factors, in particular, play a pivotal role in shaping plant distributions, and using geographic information systems (GIS) and geographic analysis were instrumental in enhancing the understanding and monitoring of spatial patterns of Persian oak diversity in western Iranian forests. Spatial analysis of 19 bioclimatic variables on 183 *Q. brantii* individuals identified several limiting factors for *Q. brantii* afforestation in western Iranian forests. Notably, temperature (annual mean temperature), precipitation (annual mean precipitation and precipitation of the driest quarter), and geographical altitude emerged as significant limiting factors. These findings align with studies on other plant species, such as *Malus fusca* and *Prunus scoparia*, where climatic variables played a crucial role in defining climatic clusters and influencing plant distribution (Routson *et al*., 2012; Zeinalabedini *et al*., 2019). The findings of this study have the potential to provide valuable guidance for efforts aimed at conserving *Q. brantii*.

The study highlights the substantial impact of bioclimatic variables and altitude on morphological traits related to seed shape in Persian oak populations, emphasizing traits like seed length, seed width, seed volume, and 1000 seed weight (Fig. 6). This impact suggests the adaptability and high phenotypic plasticity of *Q. brantii*, as seed phenotypic diversity provides insights into plant adaptation mechanisms under varying climatic conditions. Similar responses have been observed in studies on other species, including *Q. variabilis*, *Pinus tabuliformis* Carr, and others (Li *et al*., 2020; Gao *et al*., 2021). Mantel tests confirm the influence of bioclimatic variables on the structure of *Q. brantii* populations in the studied regions, paralleling findings in studies on other species like *Alnus incana* (L.) Moench subsp. *incana* (Poljak *et al*., 2018), where phenotypic divergence was associated with both geographic distance and environmental factors.

According to the results obtained from the spatial distribution analysis based on 6 PCNM indicators, the oaks of the Dolaw, Dolaw-K, and Danuk areas had the least diversity. The oaks of Kochali, Sarmast, and Sirvan regions had the highest diversity, these regions have an altitude of more than 1500 meters and had the highest amount of annual precipitation among the rest of the sampling regions, which can be a confirmation of the identification of climatic variables affecting diversity. On the other hand, Kochali is a protected area that has been spared from factors that destroy forest diversity, such as cutting down trees, feeding livestock with oak seeds, and collecting oak seeds to prepare a type of local bread by forest dwellers and villagers. This issue can be another important reason for the high diversity of oaks in this region. This shows that the Environment Protection Agency's measures to protect the endangered Persian oaks in this region have been successful and have led to an increase in genetic and morphological diversity in this region. According to the identification of areas with high and low diversity among the sampling areas of Ilam province, the seeds of Kochali, Sarmast, and Sirvan areas can be used in reforestation and afforestation programs of areas with low diversity such as Dolaw, Dolaw-K, and Danuk.

In this study, our evaluation was limited to a morphologic classification of the populations under investigation. This approach overlooked other genetic discrepancies that may exist within or among individuals. Additionally, our examination of the impact of environmental factors on phenotypic variation only took into account the most evident climatic elements. However, we are currently planning future research that aims to delve into the genetic and environmental factors in a more detailed manner. By doing so, we hope to gain a deeper understanding of the influence these factors have on population variation.

Conclusions

Our research outcomes indicate a comprehensive analysis of the genetic and environmental elements that shape the phenotypic diversity of *Q. brantii* populations in the forests of western Iran. The

identified constraining variables, such as annual mean temperature, annual precipitation, precipitation of the driest quarter, and geographical altitude, along with determining areas of high population diversity, provide valuable insights for conservation strategies. Although this study represents a significant advancement in comprehending these influences, future investigations are planned to further explore the genetic and environmental factors, thus offering a more nuanced understanding of their contributions to the phenotypic variation within this particular species.

Conflict of interests

No Conflict.

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