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Genome-wide Runs of Homozygosity Islands Uncover Candidate Genes Involved in Stress Response in *Pistacia vera*

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ABSTRACT

Pistacia vera L. is a perennial tree species of significant economic importance, exhibiting natural adaptation to arid and semi-arid environments. Elucidating the genomic underpinnings of this adaptation is crucial for enhancing stress resilience and facilitating breeding programs. This study employed whole-genome sequencing data from 31 Iranian pistachio samples to characterize runs of homozygosity (ROHs) and identify genomic regions potentially shaped by selective pressures. A total of 3597 ROHs were detected, predominantly comprising short segments (<200 kb), which reflect both the scaffold-level genome assembly and historical recombination patterns. The overall genomic inbreeding coefficient (FROH) was low (mean = 0.026), suggesting limited recent inbreeding within the studied accessions. Utilizing SNP frequency-based criteria, 48 ROH islands were identified across 44 scaffolds, representing localized regions of elevated homozygosity. Functional annotation of these regions revealed 589 protein-coding genes, many of which are associated with stress responses, hormonal regulation, metabolism, and development. Notably, genes such as *HDAC9*, implicated in epigenetic regulation under heat stress; *NCED6* and *PSY2*, key components of abscisic acid and carotenoid biosynthesis pathways, respectively; *AAE3*, linked to oxalate metabolism and tolerance to biotic and abiotic stresses; *TPR1*, associated with drought response; and *ATG5*, a central regulator of autophagy under diverse stress conditions, were localized within ROH islands. Collectively, these findings indicate that ROH-based analyses can effectively delineate genomic regions and candidate genes potentially involved in environmental adaptation and trait optimization in pistachio. This study provides a genomic framework for comprehending selective processes in *P. vera* and offers valuable targets for future functional investigations and molecular breeding initiatives.

Keywords: Abiotic Stress, Functional Annotation, Genomic Selection, Pistachio, Runs of Homozygosity (ROH)

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INTRODUCTION

The genus *Pistacia* (Anacardiaceae) encompasses a minimum of 11 dioecious tree and shrub species, exhibiting a broad distribution throughout North Africa, Central Asia, the Middle East, and the Mediterranean basin [1]. Among these, *P. vera* L. stands as the sole species commercially cultivated for its edible nuts, whereas its wild relatives, such as *P. atlantica*, *P. integerrima*, and *P. terebinthus*, function as crucial genetic reservoirs for breeding programs and rootstock improvement [2-4]. Pistachio trees are globally recognized for their ecological adaptability, economic

significance, and diverse applications in nutrition, medicine, and ornamental horticulture [5, 6]. Pistachio nuts are particularly rich in bioactive compounds, offering high levels of protein, dietary fiber, monounsaturated fatty acids, essential vitamins (E and C), carotenoids, minerals, and phenolic constituents [7]. This unique nutritional profile contributes to human health and supports the prevention of chronic diseases.

Despite these advantages, commercial pistachio production encounters several significant agronomic challenges. These include alternate bearing, shell splitting, empty seeds, pronounced sensitivity to abiotic stressors such as drought and salinity, and susceptibility to fungal pathogens [8]. Traditional breeding approaches are inherently slow and inefficient, primarily due to the species' extended juvenile phase and complex reproductive biology. Consequently, the application of modern genomic and molecular breeding technologies offers promising avenues to accelerate cultivar development, enhance stress resilience, and improve nut quality [9].

Whole-genome sequencing has yielded substantial genomic resources, facilitating the identification of selection signatures. Pinpointing such regions offers critical insights into the genetic underpinnings of adaptation, domestication, and agronomically valuable traits, thereby supporting the targeted enhancement of crops and plant species [10]. Runs of homozygosity (ROHs), defined as continuous homozygous segments within the genome, serve as a valuable framework for investigating the relationship between these regions and natural or artificial selection pressures. Analyzing ROHs aids in identifying genes potentially under selection and associated with important traits [11, 12], rendering them a crucial resource for breeding and genetic improvement initiatives.

ran is a leading global producer of pistachios, with an average annual output of approximately 286 thousand tons between 2000 and 2023, positioning it as the top worldwide producer [13]. Pistachio also represents Iran's primary agricultural export commodity. The economic importance of this crop has spurred considerable research interest from diverse perspectives. A more profound understanding of *Pistacia vera* and the genetic basis of its key traits can significantly bolster conservation efforts and breeding programs. Given the pivotal role of pistachios in Iran's agricultural economy and the increasing focus on elucidating the genetic basis of valuable traits, genome-wide data present a robust approach to examining patterns influenced by evolutionary and breeding processes. This study is among the first to map runs of homozygosity islands in Iranian *Pistacia vera*, linking them to stress-adaptive genes like HDAC9, NCED6, and ATG5. By combining whole-genome data with functional annotation, it offers a practical genomic framework for breeding climate-resilient pistachio. It aimed to identify ROHs and ROH islands in Iranian *P. vera* and to detect genes within these regions that may be under positive selection. To achieve this, we analyzed whole-genome sequences from Iranian pistachio samples to identify ROHs.

MATERIALS AND METHODS

Samples and Quality Assessment

Whole-genome sequencing (WGS) data for *P. vera* were obtained from the National Center for Biotechnology Information (NCBI) Sequence Read Archive (SRA), comprising 31 samples originating from Iran (Table 1). Raw sequencing reads underwent initial quality assessment utilizing FastQC (v11.9). Adapter sequences and low-quality bases were subsequently trimmed using Trimmomatic (v0.39) (Bolger et al., 2014) with the following parameters: TRAILING:3, LEADING:3, SLIDINGWINDOW:5:20, and MINLEN:40. Supplementary trimming options, including HEADCROP and ILLUMINACLIP, were applied to specific samples as necessitated to enhance read quality.

Table 1 WGS data accessions for 31 *P. vera* samples

No.	Cultivar	SRA/SRR ID	No.	Cultivar	SRA/SRR ID
1	Khanjari	SRR8772685	2	Italiaei	SRR8772686
3	Mohseni	SRR8772687	4	Ebrahimi	SRR8772688
5	Akbari	SRR8772689	6	Ahmad Aqaei	SRR8772690
7	Jandaqi	SRR8772691	8	Qazvini	SRR8772692
ss9	Shahpesand	SRR8772693	10	Fandoqi Riz	SRR8772694
11	Khanjari Damghan	SRR8772705	12	Mikegi	SRR8772706
13	Joon	SRR8772707	14	Hasan Abbasi	SRR8772708
15	Badami Zarand	SRR8772709	16	Accession 108	SRR8772710
17	Accession 107	SRR8772711	18	Accession 106	SRR8772712
19	Accession 105	SRR8772713	20	Accession 103	SRR8772714
21	Accession 102	SRR8772715	22	Accession 99	SRR8772716
23	Accession 98	SRR8772717	24	Accession 97	SRR8772718
25	Accession 96	SRR8772719	26	Ketani Qermez	SRR8772720
27	Sabz Pesteh Nooq	SRR8772722	28	Ohadi	SRR8772727
29	Seifadini	SRR8772728	30	Fandoqi Zoodras	SRR8772730
31	Accession 94	SRR8772731			

Mapping to Reference and Variant Calling

Sequencing reads that passed quality filtering were aligned against the pistachio reference genome (GCF_008641045.1) using BWA-MEM (v0.7.17) [14]. The resulting SAM files were converted into BAM format with SAMtools [15], then sorted and deduplicated using Picard. These finalized BAM files served as the input for subsequent variant detection and genomic analyses.

Variant calling was performed on the processed BAM files using GATK HaplotypeCaller, with ploidy parameters adjusted appropriately, in accordance with the GATK best-practice

pipeline. To ensure high confidence in the detected variants, raw calls were filtered using GATK VariantFiltration and BCFtools [16]. Filters included common quality metrics such as QD < 2.0, FS > 60.0, MQ < 40.0, MQRankSum < −12.5, ReadPosRankSum < −8.0, and SOR > 3.0, thereby removing variants that did not meet the established quality criteria.

Runs of Homozygosity (ROH) Islands

In the present study, ROHs were identified using the --homozyg function implemented in PLINK (v1.9), with the following parameters: --homozyg-window-het 2, --homozyg-window-missing 5, --homozyg-snp 40, --homozyg-kb 100, --homozyg-density 50, and --homozyg-gap 1000.

To detect ROH islands, we first calculated the frequency of each SNP within the identified ROHs across all individuals. This was done by dividing the number of samples carrying a particular SNP in a ROH by the total number of samples in the present study. Finally, ROHs harboring SNPs that ranked within the top 1% in frequency were classified as ROH islands.

Reciprocal Best Hit-Based Functional Annotation

To obtain reliable functional information for genes identified within runs of homozygosity (ROH) islands in the *P. vera* genome, an orthology-based strategy was employed due to the absence of comprehensive Gene Ontology (GO) annotations in current pistachio gene models. To address this limitation, putative orthologs were inferred by comparing pistachio protein-coding sequences with the extensively annotated *Populus trichocarpa* proteome using a Reciprocal Best Hit (RBH) BLAST approach. High-confidence orthologous relationships were established by applying stringent filtering criteria, specifically a minimum amino-acid identity of 50%, an alignment coverage exceeding 60% for both query and subject sequences, and an E-value threshold of 1e−5. The *Populus* orthologs identified via this procedure served as proxies for assigning GO terms to pistachio genes within ROH intervals, thereby facilitating functional interpretation despite limited native annotation resources. GO analysis of the identified genes was performed using g:Profiler, with terms exhibiting an FDR-corrected p-value <0.07 considered statistically significant.

RESULTS

ROHs in *P. vera*

In the present study, a total of 3,597 ROHs were identified across all samples. These ROHs were categorized into eight size classes. The mean length of all identified ROHs was 150.13 kb, with ROH sizes ranging from 100 to 531.08 kb.

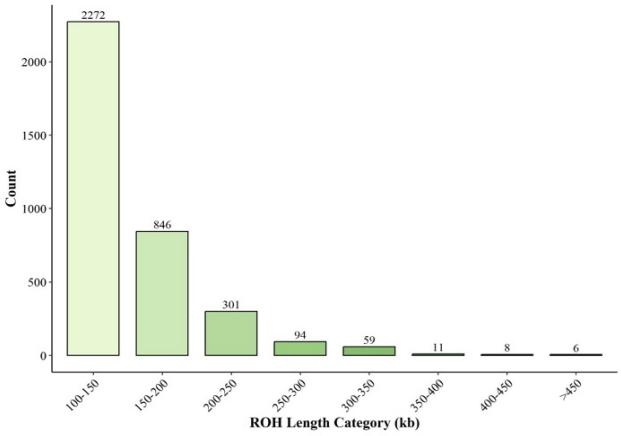


Fig. 1 The total number of identified ROHs in the eight size categories. The number on each bar represents the number of ROHs in that category.

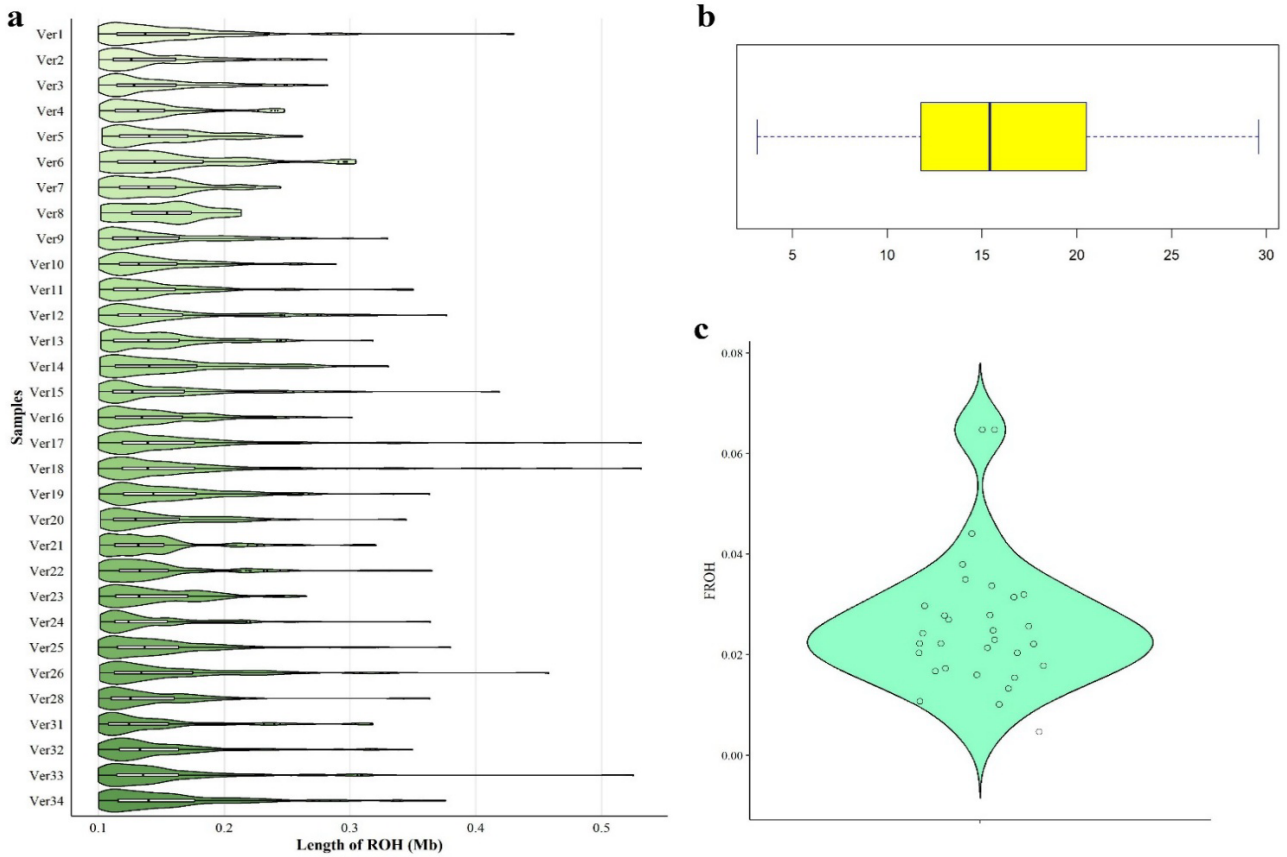


Fig. 2 Distribution of ROH and genomic inbreeding across samples. a) Violin plots showing the distribution of lengths of identified ROHs (in Mb) per samples. b) Boxplot summarizing the total length of ROHs (in Mb) identified across all samples. c) Violin plot showing the distribution of genomic inbreeding coefficients (FROH) among individuals, with dots representing individual sample values

The results showed that 86.68% of the ROHs were shorter than 200 kb, and only 6 ROHs exceeded 450 kb (Fig. 1). The relatively short ROHs may be attributed to the fact that the pistachio genome is assembled at the scaffold level.

Figure 2A illustrates the distribution of Runs of Homozygosity (ROH) lengths across 31 pistachio accessions through individual violin plots. A pronounced concentration of ROH lengths was observed within the shorter size classes across the majority of samples, signifying that short ROHs constitute the predominant component of homozygous segments in the analyzed genomes. In several individuals, the density of ROHs exhibited a rapid decline with increasing length, while a limited number of longer ROHs extended into higher length values. This pattern was not uniformly distributed among samples, reflecting substantial inter-individual variability in the extent and distribution of homozygosity. Overall, the total ROH length demonstrated a median of 15.41 Mb (Figure 2B). Approximately 50% of the detected ROH segments ranged from 3 to 15 Mb in length. Only a few individuals presented higher total ROH values (with the longest ROH measuring 43.45 Mb), indicating elevated genomic homozygosity relative to the other samples.

Genomic inbreeding coefficients (FROH), representing the proportion of the genome covered by runs of homozygosity (ROHs), are presented in Figure 2C as a violin plot with superimposed individual data points. The distribution of FROH values demonstrated moderate inter-sample variation, with the majority of individuals exhibiting low to intermediate levels of genomic inbreeding. A subset of samples displayed comparatively elevated FROH values, indicative of an increased contribution of homozygous segments within their genomes. The overall mean FROH across the investigated *P. vera* samples was determined to be 0.026, signifying a generally low level of genomic inbreeding. It is important to acknowledge that the pistachio reference genome employed in this study is assembled at the scaffold level rather than the chromosome level, a factor that may contribute to an underestimation of extended ROHs and, consequently, lower FROH values.

ROH Islands

In the present study, a total of 48 runs of homozygosity (ROH) islands were identified across 44 distinct scaffolds in the *P. vera* genome. The shortest ROH segment spanned 130.49 kb on scaffold NW_022196357.1, while the longest extended to 493.87 kb on scaffold NW_022196434.1 (Figure 3). The mean length of ROH islands across all scaffolds was 238.06 kb. Notably, among all scaffolds harboring ROHs, only four scaffolds each contained two ROHs, whereas the remaining scaffolds exhibited a single ROH. These scaffolds with paired ROHs suggest localized regions of elevated homozygosity that may be consistent with signatures of selection. Specifically, scaffold NW_022196080.1 harbored two ROHs measuring 171.4 kb and 283.9 kb; NW_022196295.1 included ROHs of 169.6 kb and 197.8 kb; NW_022196774.1 contained ROHs of 150.4 kb and 220.0 kb; and NW_022196165.1 featured two homozygous segments of 162.2 kb and 133.9 k

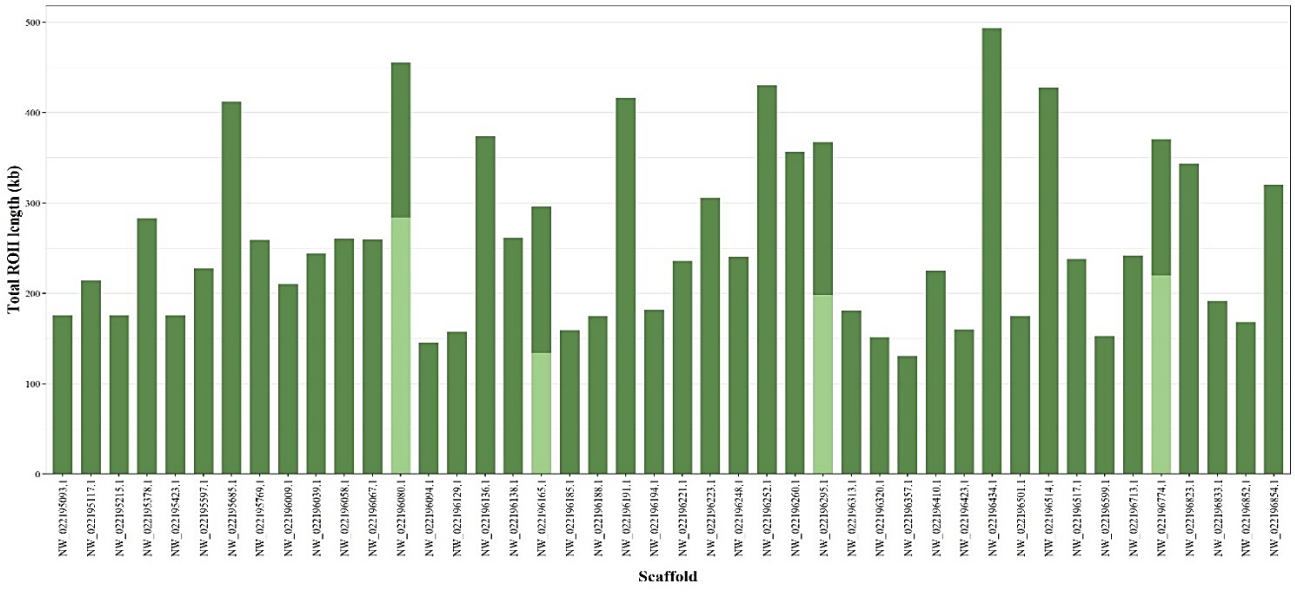


Fig. 3 Total length of ROHs per scaffold. Each bar represents the cumulative ROH length on a given scaffold. Scaffold bars that contain multiple ROH segments are displayed in stacked colors, with each color representing an individual ROH.

ROH-Linked Genes

Annotation of runs of homozygosity (ROH) islands in the *P. vera* genome revealed 589 protein-coding genes, suggesting these loci as potential targets of positive selection. To explore their functional significance, these genes were further analyzed through gene ontology (GO) and KEGG pathway enrichment. GO analysis identified 45 statistically significant terms, comprising 11 biological process (BP) terms, 10 cellular component (CC) terms, and 15 molecular function (MF) terms, in addition to 9 KEGG pathways (Supplementary Table 1).

DISCUSSION

The formation of Runs of Homozygosity (ROH) is a recognized consequence of positive selection and the fixation of advantageous mutations within a genome [17], thereby serving as valuable markers of selective pressure. Consequently, investigations into these regions can enhance our comprehension of evolutionary processes, including selection and environmental adaptation. Furthermore, the analysis of ROH can elucidate genomic loci associated with economically significant traits and overall population genetic structure. This study aimed to identify genomic regions under selective pressure that contribute to the adaptation of

P. *vera* to extreme arid environments. Through a genome-wide analysis of ROH, we characterized the genomic architecture and detected signatures of selection, identifying 44 genomic regions as ROH islands. Within these regions, numerous genes were identified, some of which exhibited evidence consistent with selective pressure and functional adaptation.

Among the notable genes identified within the ROH islands in the present study was *Histone deacetylase 9*(*HDAC9; LOC116129021*in *P. vera*). Histone deacetylases (HDACs) are critical mediators of epigenetic flexibility, a key mechanism in environmental adaptation. HDACs catalyze histone deacetylation, which leads to chromatin compaction and transcriptional repression. Under conditions of heat stress, this reversible chromatin remodeling facilitates the rapid activation and subsequent silencing of stress-inducible genes, including Heat Shock Proteins (HSPs). The delicate balance between histone acetyltransferase (HAT)-mediated activation and HDAC-mediated repression, exemplified by *HDAC9* in *Arabidopsis thaliana*, is essential for energy-efficient stress recovery [18]. The selection of HDAC family members in *P. vera*, a perennial crop indigenous to arid and semi-arid regions characterized by hot, dry climates, likely reflects an evolutionary optimization of gene-expression plasticity in response to thermal and water stress. Notably, *HDA9* has been demonstrated to interact with ABI4 to repress *CYP707A* gene expression, thereby maintaining abscisic acid (ABA) levels during drought stress [19]. The transduction of heat-induced signals within plant cells is a fundamental component of the plant's response to thermal stress. Evidence suggests that *HDA9* mediates the transfer of these signals from the cytoplasm to the nucleus. Under elevated temperatures, HDA9 interacts with PP2AB'β, undergoes dephosphorylation, and is protected from 26S-proteasome–mediated degradation. This dephosphorylated form subsequently translocates into the nucleus, where it deacetylates and represses genes involved in plant growth. This mechanism helps to balance growth with heat-stress responses and underscores the positive regulatory role of *HDA9* in enhancing plant thermotolerance [20].

Pistacia vera is inherently adapted to the arid and semi-arid landscapes of the Middle East and Central Asia, regions characterized by high temperatures, intense solar radiation, and prolonged periods of water scarcity that exert substantial selective pressures. One of the central hormonal pathways involved in drought adaptation is abscisic acid (ABA) biosynthesis, in which 9-cis-epoxycarotenoid dioxygenase (NCED) enzymes play a rate-limiting role. While *NCED6* (LOC116106273 in *P. vera*) in *Arabidopsis thaliana* primarily contributes to seed ABA biosynthesis, its rice homolog *OsNCED4* plays a key role in ABA-mediated responses to salinity and cold stress [21]. Evidence from *Populus davidiana* indicates that NCED family members are upregulated in drought-tolerant genotypes [22], suggesting their critical role in mediating adaptive responses to water-deficit stress. Similarly, *Ipomoea batatas* exhibits elevated NCED6 expression in flood-tolerant cultivars [23]. *NCED6* has emerged as a key regulator of ABA accumulation under water-deficit conditions. As highlighted by Cardoso et al [24], upregulation of *NCED* genes under drought stress leads to increased ABA levels, promoting stomatal closure, reducing transpiration, and enhancing drought tolerance. This mechanism is particularly vital for perennial crops like pistachio, which must endure prolonged periods of environmental stress. Furthermore, *NCED*-mediated ABA biosynthesis is closely associated with the regulation of growth–stress trade-offs, enabling plants to prioritize survival under extreme environmental conditions [25]. The localization of *NCED6* within runs of homozygosity (ROH) islands in *P. vera* likely represents a signature of positive selection for drought tolerance, suggesting that historical selection pressures favored alleles enhancing water-stress resilience in pistachio populations. Studies in *Arabidopsis* indicate that *NCED6* is a major determinant of ABA biosynthesis during seed development. Disruption of this gene leads to reduced ABA accumulation and measurable shifts in dormancy behavior. Its pronounced, endosperm-specific expression, together with the elevated ABA levels observed in *NCED6*-overexpressing lines, underscores the gene's essential contribution to the hormonal regulation that governs seed dormancy and germination [26].

Phytoene Synthase 2 (*PSY2*; *LOC116121143* in *P. vera*), a key enzyme in carotenoid biosynthesis, complements NCED6 by catalyzing the first committed step in carotenoid formation and supplying precursors for ABA synthesis, while enhancing photoprotection [27, 28]. The co-selection of *PSY2* and *NCED6* in *P. vera* suggests a finely tuned regulatory network that links primary metabolism to hormonal stress responses.

The role of arogenate dehydrogenase 1 (*ADT1*; *LOC116120521* in *P. vera*) in aromatic amino acid biosynthesis, particularly phenylalanine, extends beyond its classical enzymatic function. Recent findings demonstrated that *ADT* gene expression is regulated by light during the seed-to-seedling transition in *Arabidopsis thaliana*, confirming its involvement in developmental and metabolic coordination. This light-responsive regulation directly influences nitrogen metabolism and protein synthesis, positioning *AtADT1* as a key integrator of environmental signals and core biosynthetic pathways [29]. Aromatic amino acids, such as phenylalanine, are key precursors of volatile aroma compounds and play a crucial role in determining the flavor and aroma of nuts and seeds [30]. Pistachio has been reported to contain relatively high levels of phenylalanine [31], providing the biochemical capacity to produce aromatic compounds that underlie its distinctive flavor. Furthermore, studies characterizing the volatile profile and sensory attributes of pistachio have demonstrated that these compounds, produced both naturally and during processes such as roasting, are major contributors to its flavor [32]. Taken together, these findings suggest that pathways related to aromatic amino acids may have been subject to selective pressures to enhance flavor in pistachio. However, direct evidence for intentional genetic selection of these pathways in pistachio has not yet been reported.

Minichromosome maintenance (MCM) proteins are integral to DNA unwinding during replication, and their elevated expression has been linked to enhanced stress tolerance in plants [33]. In *Arabidopsis thaliana*, the disruption of *AtMCM2* (orthologous to *LOC116131426* in *P. vera*) leads to early embryonic lethality, while its overexpression modulates endoreduplication levels, underscoring its pivotal role in both embryonic development and root meristem function [34].

AAn oxalate–CoA ligase-like gene (*LOC116136921*) was identified within runs of homozygosity (ROH) regions in the *P. vera* genome. Its functional ortholog in *Arabidopsis thaliana*, *AAE3*, encodes an oxalyl-CoA synthetase that catalyzes the ATP- and CoA-dependent conversion of oxalate to oxalyl-CoA. *AAE3* deficiency results in oxalate accumulation, reduced germination rates, and compromised seed-coat mucilage [35]. Furthermore, *AAE3* in *Arabidopsis*is essential for proper seed development and defense against oxalate-producing fungal pathogens [36]. In *Vigna umbellata*, *AAE3* is critical for aluminum (Al) tolerance through

the regulation of oxalate levels. This enzyme exhibits specific oxalate-degrading activity and is predominantly expressed in root tips. Oxalate accumulation under Al stress inhibits root growth, a detrimental effect mitigated by *AAE3* activity. Overexpression of *AAE3* in *Nicotiana tabacum* also enhances Al tolerance and reduces oxalate accumulation, suggesting that AAE3-dependent oxalate turnover is a key mechanism for Al tolerance in plants [37]. Subsequent research on wild soybean corroborated the role of *AAE3* in augmenting plant resistance to cadmium and Al stresses [38]. Activation of *AAE3* expression by the transcription factor HubHLH36 in pitaya confers resistance to both salt and oxalate stresses, simultaneously lowering reactive oxygen species (ROS) levels and alleviating salt-induced oxidative stress [39]. Given that calcium is a widely recognized mediator of cellular signaling in plant responses to environmental stressors [40], the *AAE* gene, which may indirectly influence calcium homeostasis via oxalate catabolism, could significantly modulate these processes. The identification of a gene implicated in both biotic and abiotic stress responses within an ROH in pistachio, a species renowned for its resilience to harsh environmental conditions, supports the hypothesis of positive selection on this gene in the *P. vera* genome. Members of the tetratricopeptide repeat (TPR)–like superfamily, characterized by conserved TPR motifs, are known to participate in plant growth and adaptation to abiotic stress [41]. We identified a member of this family, *TPR1* (orthologous to *LOC116143780* in *P. vera*), located within an ROH in the pistachio genome. Elevated expression of *ZmTPR1* (TPR1 in maize) was observed in a drought-resistant maize inbred line compared to a drought-sensitive line under drought stress. Functional analysis of *ArabidopsisTPR1* knockout mutants revealed reduced drought tolerance, accompanied by inhibited growth and declines in physiological parameters under drought conditions, indicating a crucial role for *ZmTPR1* in drought stress response [42]. Additionally, another member of the TPR gene superfamily, *TPR16*, has been shown to contribute to drought tolerance in apple, a process mediated by enhanced expression of drought-responsive genes and reduced accumulation of reactive oxygen species (ROS) under stress [41]. The presence of *TPR1* within ROH regions in *the P. vera* genome suggests its potential involvement in stress-related processes, such as drought tolerance, in pistachio.

ATG5-mediated autophagy plays a critical role in plant development and stress responses. Knockout of *ATG5* (orthologous to *LOC116140294* in *P. vera*), along with *ATG2*, *ATG7*, or *ATG10*, disrupts pollen development and anther dehiscence under high-temperature stress [43]. In *Arabidopsis*, the loss of *ATG5* impairs tolerance to drought, heat, salt, and oxidative stresses [44], delays growth and reduces protein synthesis under carbon starvation [45], and accelerates senescence under zinc deficiency [46], thereby emphasizing its significance in nutrient recycling and stress adaptation.

CONCLUSION

This study presents a genome-wide analysis of runs of homozygosity (ROH) in Iranian *Pistacia vera* accessions, offering insights into genomic regions potentially influenced by selection within a species adapted to arid and semi-arid environments. Several genes located within identified ROH islands are associated with key biological functions, including stress response, hormonal regulation, metabolism, and development. Notably, genes such as *HDAC9*, *NCED6*, *PSY2*, *AAE3*, *TPR1*, and *ATG5* are linked to epigenetic regulation, abscisic acid biosynthesis, metabolic adaptation, and stress tolerance pathways, suggesting their putative involvement in pistachio adaptation to challenging environmental conditions. While functional validation is required, the established roles of these genes in other plant species support their relevance as candidate targets of selection. Overall, these findings demonstrate the efficacy of ROH-based analyses for identifying candidate genomic regions associated with adaptation and agronomically important traits in perennial crops. This work contributes genomic information that may be valuable for future functional investigations and could inform genomics-assisted approaches in pistachio breeding and conservation programs.

Conflict of Interests

All authors declare no conflict of interest.

Ethics Approval and Consent to Participate

No human or animals were used in the present research.

Ethical Issues

Ethical issues (including plagiarism, data fabrication, double publication) have been completely observed by the authors. The authors have adhered to ethical standards, including avoiding plagiarism, data fabrication, and double publication.

Consent for Publication

All authors read and approved the final manuscript for publication.

Availability of Data and Material

All the data is included in the manuscript. The data that support the findings of this study are available from the corresponding author upon reasonable request.

Authors' contributions

Conceptualization: S. J-G., G. D-O.
Data curation: G. D-O.
Formal analysis: R. Kh-E.
Investigation: N. H., B. F-N.
Methodology: S. J-G., G. D-O.
Project administration: B. F-N.
Resources: All authors.
Supervision: S. J-G.
Validation: N. H.
Visualization: S. J-G., G. D-O.
Writing–original draft: S. J-G., G. D-O.
Writing–reviewing & editing: All authors.

Informed Consent

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