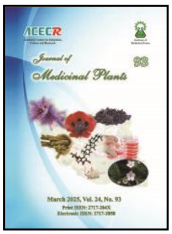
J. Med. Plants 2025; 24(93): 27-39

Institute of
Medicinal Plants

Journal of Medicinal Plants

Journal homepage: www.jmp.ir



Research Article

Adaptation of *Carum carvi* L. to high altitudes: investigating the effect of altitude on seed physiological dormancy

Mohsen Zafaranieh[1,*], Seyed Fazel Mirahmadi[1], Majid Ghorbani Nohooji[2]

[1] *Department of Agriculture and Natural Rescores, Technical and Engineering Faculty, Velayat University, Iranshahr, Iran*

[2] *Medicinal Plants Research Center, Institute of Medicinal Plants, ACECR, Karaj, Iran*

ARTICLE INFO

Keywords:
Apiaceae
α and γ – tocopherol
HPLC analysis
Plant regulators
Seed germination parameters

ABSTRACT

Background: The relationship between altitude and seed dormancy in high-mountain plants, particularly in *Carum carvi* L., reveals significant insights into how environmental conditions influence seed viability and germination. **Objective:** This research investigated the role of different *C. carve* habitats, with an emphasis on how altitude variations affect seed dormancy parameters. **Methods:** Three seed populations from altitudes of 650, 1200, and 2200 m a.s.l. were collected. Germination and viability tests, as well as analyses of biochemical parameters (primarily plant hormonal and antioxidant components), were carried out. **Results:** Seed germination rates were significantly lower at the highest altitude compared to lower elevations. This reduction was linked to enhanced seed dormancy, which correlated with elevated abscisic acid (ABA) concentrations, suggesting ABA's key role in altitude-dependent dormancy regulation. Concurrently, gibberellins (GAs) levels rose at higher altitudes, implying their contribution to improved germination traits and survival adaptation. Among endogenous compounds, xanthophylls (e.g., lutein, neoxanthin, violaxanthin, and antheraxanthin) exhibited a positive altitudinal trend, whereas α-tocopherol levels remained unchanged. **Conclusion:** These findings suggest that the lower temperatures and harsher conditions at higher altitudes significantly influence seed viability, germination, and dormancy. The physiological mechanisms, particularly the accumulation of ABA and the increase in xanthophylls, indicate an evolutionary adaptation that enhances survival under extreme environmental conditions. This adaptation is crucial for the persistence of high-mountain plant populations in Iran, where climatic challenges are pronounced.

1. Introduction

Mountain ecosystems are incredibly diverse, supporting a wide range of plant life. This biodiversity is crucial for many reasons, including climate regulation and the existence of unique species. However, these ecosystems are threatened by climate change and human activities,

---

*Abbreviations:*m a.s.l., metres above sea level; ROS, Reactive oxygen species; N and E, North and East; µmol m-2 s-1, micromol per square meter measured in a second; Gas, gibberellins; ABA, abscisic acid; ACC, ethylene precursor 1-aminocyclopropane-1-carboxylic acid; SA, salicylic acid; JA, jasmonic acid; Z, zeatin; ZR, zeatin riboside; 2-iP, 2-isopentenyl adenine; IPA, isopentenyl adenosine; IAA, auxin indole-3-acetic acid; Mel, melatonin; HPLC, High-performance liquid chromatography; ng. gDW-1, nanogram per gram, dry weight; vs, voucher specimen

*Corresponding author:m.zafaranieh@velayat.ac.ir

doi:

Received 29 December 2024; Received in revised form 26 February 2025; Accepted 11 March 2025

highlighting the urgent need for conservation efforts [1]. Our understanding of how mountain plants adapt to change is limited by complex ecosystems, data gaps, and research challenges. Moreover, climate change and human activities further complicate this issue [2].

Carum carvi L., commonly known as caraway, is a biennial aromatic herb widely cultivated for its seeds, which are valued as a culinary spice. It grows best in sunny conditions and well-drained soil. All parts of the plant are edible, offering culinary and potential medicinal benefits [2]. Nevertheless, the species faces threats from climate change and human activities in these fragile ecosystems [3]. Evidence indicates that *Carum carvi* L. demonstrates remarkable adaptation to high-altitude conditions, showcasing physiological plasticity and evolutionary fitness at population, whole-plant, and likely cellular scales [4].

Caraway plants growing at higher altitudes exhibit distinct physical characteristics (e.g., height, branching), which are likely adaptations to the colder, drier conditions found in mountainous environments. [4, 5]. Although cellular-level adaptations are not explicitly mentioned in the cited sources, the documented morphological differences between low- and high-altitude populations suggest the potential for physiological and biochemical adaptations at the cellular level to mitigate the stresses of high-altitude environments [6].

Seed dormancy represents a key evolutionary strategy that ensures germination occurs only under optimal environmental conditions, thereby maximizing seedling survival and fitness. This prevents seeds from germinating during unfavorable periods, such as drought or extreme temperatures, which would likely lead to seedling mortality [7]. This control over germination timing is crucial for survival and allows plants to thrive in challenging environments, especially in harsh, variable high-mountain ecosystems, thereby ensuring successful reproduction [8, 9].

Physiological seed dormancy, particularly the cold stratification requirement, is predominantly regulated by temperature regimes and moisture availability during quiescence. Notably, seeds from lower altitudes exhibit greater sensitivity to increases in stratifying temperatures compared to those from higher altitudes. This adaptive response is particularly evident in *Halenia elliptica* D. Don, where low-altitude populations exhibit significantly greater sensitivity to thermal elevation during dormancy break compared to their high-altitude counterparts [10]. Lower temperatures generally break seed dormancy more effectively. Low-altitude populations often require higher soil moisture for germination, which is likely an adaptation to drier conditions. Seed germination depends on a delicate balance between temperature and soil moisture, with higher temperatures often requiring higher moisture levels [11]. Seed dormancy at high altitudes is influenced by various factors, including temperature, soil moisture, and environmental conditions during seed development. This dormancy ensures germination occurs under favorable conditions for seedling survival. Plant hormone responses, particularly ABA sensitivity, also play a crucial role, with plants in humid climates exhibiting higher sensitivity to conserve water [12, 13].

Given these factors, seed longevity is significantly impacted by oxidative stress during storage. This study investigates the interplay between plant hormones and antioxidants in maintaining seed viability, which is crucial for optimizing storage and predicting plant resilience to climate change. Hormonal and antioxidant balance governs physiological dormancy, which is essential for the survival and reproductive success of plants under environmental stress conditions.

2. Materials and methods

2.1. Plant material and data collection

Identification of *C. carvi* plant was carried out using Flora Iranica by the authors [14].The voucher specimens (VS) of the herbarium samples were deposited at the Herbarium of the Velayat University central laboratory. In addition, the samples of collected seeds deposit in the seed bank of Velayat University central laboratory. Three populations were selected for this study. The first population was located at 650 m a.s.l. on Sirch Mountain (30°10'56" N, 57°24'49" E) (VS: VUH00104; seed bank number: VUS0101), the second population was found on Lalehzar Mountain at 1200 m a.s.l. (46°29'48" N, 56°24'49" E) (VS: VUH00105; seed bank number: VUS0102), and the third one occurred in Sarbanan Mountain at 2200 m a.s.l. (50°27'48" N, 56°24'49" E) (VS: VUH00106; seed bank number: VUS0103). Mature seeds from 10 different individuals per population were collected on June 18, 25, and 30, 2023. To preserve their biochemical properties, particularly phytohormones, and antioxidants, seeds were flash-frozen in liquid nitrogen to preserve biochemical integrity, then transferred under cryogenic conditions to the laboratory for long-term storage at -80°C pending subsequent analyses. Ultra-low temperature storage like -80 °C is effective for preserving seed viability by minimizing moisture content and metabolic activity. Following a two-month dark incubation at room temperature (25 ± 2°C), seed germination capacity and viability were assessed using standardized testing protocols. This method of storage is essential for maintaining seed viability over time, as it minimizes metabolic activity and degradation

2.2. Germination and viability tests

From each sampled plant across study populations, fifty seeds were randomly selected for processing. Seeds underwent surface sterilization via immersion in a 50% commercial sodium hypochlorite solution (v/v in distilled water) for five minutes with constant gentle agitation (25 ± 1°C). Following three successive sterile distilled water rinses, seeds were aseptically transferred to filter paper-lined Petri dishes.

For cold stratification, plates were maintained at 4°C in darkness for 14 days. Post-stratification, seeds were exposed to controlled conditions simulating diurnal alpine rhythms: 16/8-hour photoperiod with 500 μmol $m^{-2} s^{-1}$ PPFD provided by full-spectrum LEDs, and corresponding thermoperiod of 20/10°C (day/night). For the viability assessment, seeds were imbibed (allowed to absorb water) for 48 hours at 4 °C in darkness. After imbibition, seeds were submerged in a 1% (v/v) solution of 2,3,5-tetrazolium chloride (TTC) at room temperature and incubated in darkness at 40 °C for 24 hours. Embryos were then carefully separated from their seed coats. Embryos with strong, uniform red staining were classified as viable; those with patchy or weak (pink or orange) staining were classified as dying; and unstained (white) embryos were classified as non-viable.

2.3. Hormone and antioxidant extraction and analysis

Fifty milligrams of frozen seeds were homogenized in liquid nitrogen and extracted with 0.2 mL of ice-cold methanol using ultrasonication (Bransonic 2800, 40 kHz, 10 min, 4°C), followed by centrifugation (12,000 × g, 10 min, 4°C; Jouan MR18-22). The supernatant was collected, and the extraction repeated until the pellet became colorless. Pooled supernatants were filtered (0.22 µm PTFE) and analyzed via UHPLC-MS/MS (Waters Acquity with Kinetex C18 column, 50 × 2.1 mm, 1.7 µm) coupled to a triple quadrupole mass spectrometer (API3000, SCIEX). Quantified compounds included ABA, GAs, IAA, JA, SA, ACC, cytokinins (Z, ZR, 2-iP, IPA), and melatonin, using isotope-labeled internal standards and a 10-point calibration curve (Analyst™ 1.6.3 software). Antioxidants were simultaneously analyzed under identical chromatographic conditions [15].

Tocopherols and carotenoids were analyzed using HPLC with UV detection at 283 nm [16] and a reverse-phase C30 column with photodiode array detection [17], respectively, following established extraction protocols.

2.4. Statistical analysis

Data were analyzed using one-way ANOVA with Bonferroni correction for multiple comparisons to examine differences among the three populations (Sirch and Lalehzar: n = 10 each; Sarbenan: n = 19). Relationships between seed viability/germination percentages and hormonal/antioxidant profiles were assessed using Spearman's rank correlation. All analyses were performed in SPSS 20.0 (IBM Corp.), with statistical significance threshold set at $P < 0.05$.

3. Results

3.1. Seed vigor parameters

The study of *C. carvi* L. populations in central Iran, spanning an altitudinal range of 1550 meters (from 650 to 2200 meters above sea level) over a straight-line distance of 145 kilometers, reveals significant environmental variation within a relatively compact geographic area. This variation is likely to influence the timing of flowering and fruit set among the different populations (Fig. 1).

Seed Viability: Tetrazolium analyses revealed a significant effect of altitude on seed viability. Maximum seed viability and the lowest proportion of dead and dying seeds were observed in the highest-altitude population (Sarbanan Mountain). In contrast, seed viability decreased in the lowest-altitude population (Sirch Mountain). No significant differences in the proportion of dead or dying seeds were found between the two lowest-altitude populations (Table 1).

Seed Germination: Maximum germination rates were observed in seeds collected from the intermediate-altitude population (Lalehzar Mountain) (Table 1).

Seed Dormancy: In the highest-altitude population (Sarbanan), the increased physiological dormancy observed in seeds is likely influenced by the prevailing low temperatures experienced during fruit maturation (Table 1).

3.2. Seed vigor physiological parameters

3.2.1. Hormonal analysis

Dormancy hormones (ABA and GAs): Endogenous ABA and GA concentrations were critical regulators of physiological dormancy, particularly in high-altitude populations. Quantitative analysis revealed significantly elevated levels of both hormones in Sarbanan seeds (Table 2), showing a parallel altitudinal increase in their accumulation. This coordinated hormone pattern suggests their synergistic role in altitude-dependent dormancy regulation.

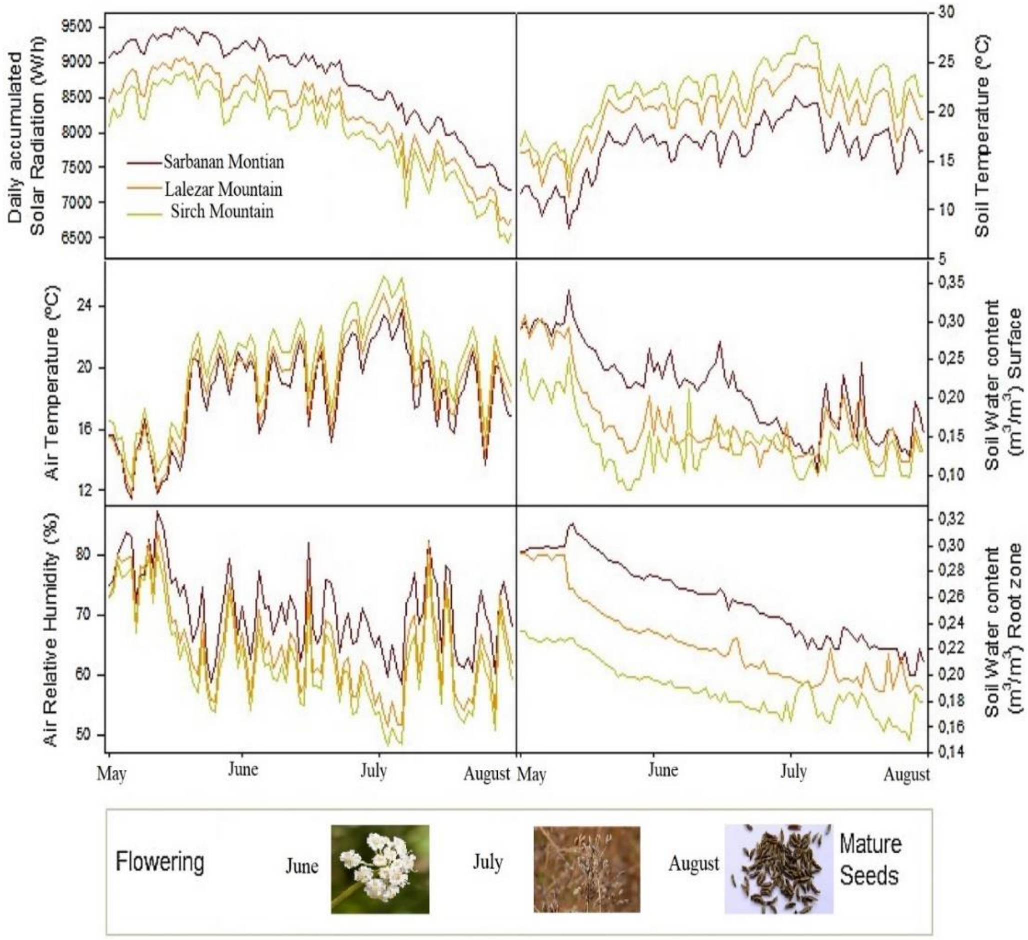


Fig. 1. Climatological conditions from fruit set to fruit ripening in the three studied populations

Table 1. Germination characteristics, viability, and dormancy status of *C. carvi* L. seeds across three altitudinal populations

		Viability	Dying	Dead
Seed Viability	Sirch	$62\%^{b}$	$20\%^{ns}$	19^{ns}
	Lalehzar	$75\%^{a}$	21^{ns}	20ns
	Sarbanan	$80\%^{a}$	$20\%^{ns}$	$17\%^{ns}$
See germination		**30 days**		**60 days**
	Sirch	$38\%^{ab}$		$51\%^{ab}$
	Lalehzar	$53\%^{a}$		$64\%^{a}$
	Sarbanan	$26\%^{b}$		$32\%^{b}$
Seed dormancy		Sirch	Lalehzar	Sarbanan
		$12\%^{b}$	$7\%^{b}$	$46\%^{a}$

The data indicate the mean of seed measurements. Different letters in each column shows statistically significant differences at $P < 0.05$. "NS" (not significant)

Table 2. Endogenous concentrations of total gibberellins (GAs), abscisic acid (ABA), and GAs/ABA ratio in *C. carvi* L. seeds across three altitudinal gradients.

	GAs (ng.gDW^{-1})	ABA (ng.gDW^{-1})	GAs/ABA (ng/ng^{-1})
Sirch	703^{b}	202^{b}	3.4^{ab}
Lalehzar	810^{a}	217^{b}	3.7^{a}
Sarbanan	815^{a}	293^{a}	2.7^{b}

The data indicate the mean of seed measurements. Different letters in each column shows statistically significant differences at $P < 0.05$. "NS" (not significant)

The endogenous contents of gibberellins showed different patterns: GA7 did not vary significantly across the altitudinal gradient; however, GA4 increased with increasing altitude, while the levels of GA1 and GA3 increased with decreasing altitude (Table 3).

GA4 (808 ng/gDW) was identified as the most abundant gibberellin in the seeds, indicating its primary role in promoting growth and breaking seed dormancy. Meanwhile, GA3 (58 ng/gDW) significantly less abundant than GA4, it remained functionally important in regulating both seed germination and subsequent growth processes.GA1 (22 ng/gDW) is present in lower concentrations than GA4 and GA3, suggesting a lesser but still relevant role in the hormonal regulation of seed development. GA7 (6 ng/gDW) is the least abundant gibberellin measured, indicating that its influence on seed physiology may be minimal compared to the other GAs.

Stress hormones (other hormones): The study identified four cytokinins in the seeds: zeatin (Z), zeatin riboside (ZR), 2-isopentenyl adenine (2-iP), and isopentenyl adenosine (IPA). However, Z and 2-ip showed significant differences in cytokinin content at high altitudes among the three populations, whereas ZR and IPA exhibited higher levels at lower altitudes, suggesting that Z and 2-iP play more primary roles in stress control than the others (Table 3).

The results in Table 4 also show that IAA and melatonin levels did not vary significantly with altitude.ACC, an ethylene precursor, increased at higher altitudes. Salicylic acid (SA) levels were elevated at higher elevations, Whereas Jasmonic acid (JA) levels remained consistent across populations.

3.2.2. Photosynthetic and Antioxidant biochemicals

According to the data presented in Table 5 that arrived from spectrums of the different chromatograms Fig. 2, *C. carvi* L. seeds exhibited an increase in xanthophyll content at higher altitudes, with lutein being the most abundant. Its concentration increased significantly with altitude, indicating a potential adaptive response to the harsher conditions found at higher elevations. In addition, no significant correlations were observed between tocopherol isoforms (*α*- and *γ*-tocopherol) and either seed viability or germination percentages (P > 0.05, Pearson correlation analysis).

Table 3. Gibberellin contents and cytokinin contents, including in *C.carvi* L. seeds collected at three different altitudes

	GA1(ng.gDW^{-1})	**GA3(ng.gDW^{-1})**	**GA4(ng.gDW^{-1})**	**GA7(ng.gDW-1)**
Sirch	19^{a}	58^{a}	659^{b}	5^{ns}
Lalehzar	22^{a}	44^{b}	802^{a}	4^{ns}
Sarbanan	13^{b}	43^{b}	808^{a}	6^{ns}
	Z(ng.gDW^{-1})	**ZR(ng.gDW^{-1})**	**2-iP(ng.gDW^{-1})**	**IPA(ng.gDW^{-1})**
Sirch	1.2^{b}	5^{a}	1.4^{b}	3.2^{a}
Lalehzar	1.5^{ab}	2.9^{b}	1.2^{b}	2.2^{b}
Sarbanan	1.7^{a}	3.3^{b}	1.9^{a}	3.1^{a}

The table presents quantitative measurements of gibberellins (GA_1 , GA_3 , GA_4 , GA_7) and cytokinins (Z, ZR, 2-iP, IPA) in seed samples. Within each column, values marked with distinct superscript letters differ significantly (P < 0.05), while "NS" indicates non-significant comparisons

Table 4. Stress-related phytohomone contents, in *C.carvi* L. seeds collected at three different altitudes

	ACC (ng. gDW^{-1})	**SA (ng. gDW^{-1})**	**JA (ng. gDW^{-1})**
Sirch	240^{b}	600^{ab}	200^{ns}
Lalehzar	275^{b}	320^{b}	190^{ns}
Sarbanan	620^{a}	730^{a}	210^{ns}
	CKs(ng.gDW^{-1})	**IAA(ng.gDW^{-1})**	**Mel(ng.gDW^{-1})**
Sirch	10.4^{ns}	43^{ns}	1.9^{ns}
Lalehzar	6.7^{ns}	41^{ns}	2^{ns}
Sarbanan	9.4^{ns}	41^{ns}	2.1^{ns}

The table displays mean concentrations of the ethylene precursor ACC, SA, JA, endogenous CKs, IAA, and Mel. Different superscript letters within columns indicate significant differences ($p<0.05$, one-way ANOVA with Bonferroni post-hoc test). NS = not significant.

Table 5. Photosynthetic and antioxidant biochemicals in *C.carvi* L. seeds collected at three different altitudes

	Car µmol/gDW	**Lut µmol/gDW**	**VAZ µmol/gDW**	**Nx µmol/gDW**	**Ax µmol/gDW**	**Zx µmol/gDW**	***α*-tocopherol µmol/gDW**	***γ*-tocopherol µmol/gDW**
Sirch	0.014^{ns}	0.015^{b}	0.009^{ns}	0.00050^{a}	0.0042^{ns}	0.0081^{ns}	0.019^{b}	0.053^{ns}
Lalehzar	0.012^{ns}	0.020^{ab}	0.011^{ns}	0.00052^{a}	0.0030^{ns}	0.0100^{ns}	0.043^{a}	0.063^{ns}
Sarbanan	0.017^{ns}	0.028^{a}	0.010^{ns}	0.00023^{b}	0.0023^{ns}	0.0079^{ns}	0.026^{b}	0.058^{ns}

Data represent mean of carotenoids (Lut, Vx, Ax, Nx, Zx) and tocopherols (*α*-tocopherol, *γ*-tocopherol). Different superscript letters within columns indicate significant differences ($P < 0.05$, one-way ANOVA with Bonferroni correction). NS = not significant.

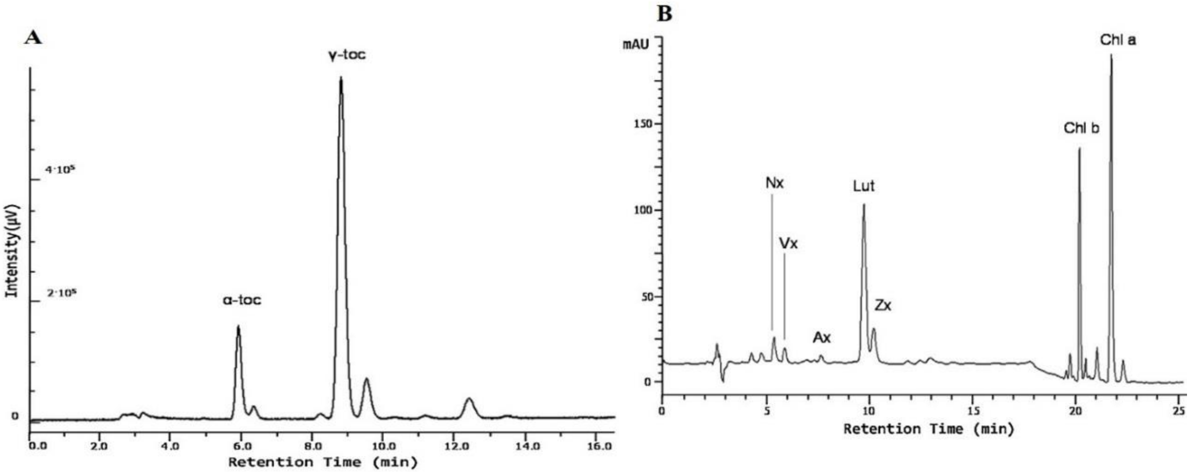


Fig. 2. Representative UHPLC chromatograms of seed antioxidant compounds: (A) Tocopherol isoforms (*α*-toc, *γ*-toc) and (B) Xanthophyll pigments (Nx, Vx, Ax, Lut, Zx) with chlorophyll derivatives (Chl a, Chl b). Peaks are labeled using standard biochemical abbreviations.

4. Discussion

4.1. Seed Vigor Parameters

The findings on seed viability suggest consistent factors may be influencing seed mortality at lower altitudes, potentially indicating a threshold for conditions that negatively impact seed viability in these environments. Findings on seed germination contrast with those reported for *Heracleum persicum* Desf., where germination rates and speeds decreased with increasing altitude from 1700 to 2700 meters above sea level [12].

Increasing altitude, coupled with decreasing temperatures and increased radiation, can create stressful conditions that may inhibit germination, potentially leading to slower and less successful germination [18].

4.2. Physiological parameters of seed vigor

The survival of plant seeds in harsh mountain environments is influenced by various factors, including physiological seed dormancy. This adaptive strategy, most evident in high-altitude ecotypes, enhances plant survival by maintaining dormancy until environmental conditions optimize seedling establishment and subsequent growth [19]. The observed altitudinal gradient in seed dormancy appears mediated by elevation-dependent environmental variables - including thermal regimes, moisture availability, and soil nitrate concentrations - which collectively modulate the critical ABA-to-GA hormonal balance governing dormancy maintenance and release. Specifically, these changes likely increase the ABA/GA ratio, inducing stronger physiological dormancy through the regulation of key dormancy genes. This prevents germination under unfavorable conditions and enhances seed survival [20].

4.2.1. Hormonal analysis

Hormonal profiling data strongly implicate ABA as a key regulator of seed dormancy, with its concentration dynamics showing significant correlation with dormancy maintenance across altitudinal gradients. The study found that the endogenous contents of gibberellins, except for GA7, varied significantly across the altitudinal gradient. These variations indicate simultaneous increases in ABA and GA levels, suggesting a complex interplay between these hormones. The increased concentration of ABA at higher altitudes resulted in a decreased GAs/ABA ratio. This hormonal shift suggests that ABA assumes greater regulatory dominance in mediating plant growth and developmental responses under the abiotic stresses characteristic of high-altitude environments (e.g., temperature fluctuations, reduced oxygen availability, and increased UV exposure). The findings suggest that higher ABA levels may enhance stress resistance during early seedling growth, as ABA is known to be involved in various plant defense mechanisms. This is particularly relevant in environments where stress factors such as drought or temperature extremes are prevalent. The lack of significant altitudinal-dependent patterns in gibberellin levels indicates that their role may be more stable across different environmental conditions, contrasting with the observed variability in ABA levels [21, 22].

The hormonal profiling of gibberellins reveals critical information about their roles in seed physiology. The significant abundance of GA4 relative to other GAs suggests its primary importance in regulating growth and germination processes, while also indicating that other GAs contribute to a complex hormonal network essential for optimal seed development (Table 3). The observed elevation-dependent increase in ABA accumulation appears driven by two coordinated molecular mechanisms: (1) upregulation of ABA biosynthesis genes (particularly NCED family members), and (2) downregulation of ABA catabolic enzymes (e.g., CYP707As). This dual regulation establishes a pronounced ABA-GA imbalance that strongly favors dormancy maintenance under high-altitude stress conditions. The lower GAs/ABA ratio inhibits germination and maintains seeds in a dormant state adapted to the harsh high-altitude environment [23].

Previous studies have established that morphophysiological seed dormancy, which combines morphological and physiological dormancy mechanisms, exists across various plant families. This type of dormancy is characterized by seeds that possess underdeveloped embryos (morphological dormancy) and physiological mechanisms that prevent germination until certain environmental conditions are met. This type of dormancy is characterized by the presence of both morphological and physiological barriers that prevent seed germination [10]. The interplay between low temperatures and ABA accumulation is pivotal in enhancing seed dormancy in high-altitude plants. This physiological adaptation ensures seeds remain viable until environmental conditions are optimal for germination, highlighting the importance of hormonal regulation in plant survival strategies. Deciphering these altitudinal hormonal dynamics offers critical insights into plant adaptive strategies, with direct implications for predicting species resilience under climate change scenarios [10].

The findings from Sarbanan support the hypothesis that low temperatures can induce physiological dormancy in seeds through ABA accumulation during maturation. This relationship underscores the importance of environmental factors in regulating seed dormancy and highlights the adaptive significance of ABA in ensuring seed survival under varying climatic conditions. Further research could focus on the specific biochemical pathways involved in ABA synthesis and its role in seed dormancy across different plant species. At the same time, gibberellins (GA) and other phytohormones antagonize ABA to promote germination. Low temperatures during seed development can likely increase dormancy through maternal temperature signaling, which regulates seed coat properties and hormone pathways, ultimately influencing seed dormancy [7].

The analysis of stress-related hormones suggests that there is a relatively stable hormonal environment for these compounds across different elevations, potentially indicating a consistent physiological response in the plants regardless of their growing conditions. At higher altitudes, the levels of ACC, a precursor to ethylene, increased. Ethylene is known to regulate various plant responses to stress, including biotic stress from pathogens and herbivores. Salicylic acid, integral to plant defense against pathogens, was also elevated at higher elevations. This suggests an enhanced readiness of plants to combat biotic stresses, potentially due to increased exposure to environmental stressors like UV radiation and temperature fluctuations. Jasmonic acid (JA), crucial for mediating plant responses to both biotic and abiotic stresses, showed higher concentrations at elevated locations. This indicates that plants likely adapt their defense strategies in response to the increased incidence of herbivory and other stress factors encountered in such environments.

Seeds from the Sarbanan population exhibited significantly elevated levels of 1-aminocyclopropane-1-carboxylic acid (ACC), the ethylene precursor, and salicylic acid (SA), both crucial for plant defense mechanisms and stress tolerance. In contrast, jasmonic acid (JA) levels remained consistent across different populations, suggesting that while ACC and SA may contribute to enhanced stress responses in the Sarbanan population, JA may not be as influential in this specific context. This hormonal profiling suggests a potential adaptive advantage for the Sarbanan population in facing environmental stresses, highlighting the importance of these compounds in seed physiology and plant health. The elevated levels of ACC and SA in Sarbanan seeds likely enhance stress resistance during early seedling growth. Their roles in promoting plant defense mechanisms could provide a significant adaptive advantage in challenging environments.

While the accumulation of ACC and SA in seeds from the Las Blancas population may also represent an additional mechanism for stress tolerance, complementing the effects of seed dormancy, this requires further investigation. This highlights the multifaceted strategies that *C. carvi* L. employs to survive and thrive in varying environmental conditions. Ethylene, known for its role in seed dormancy regulation, also acts as a key mediator of environmental stress responses in seeds. This phytohormone modulates the expression of stress-related genes (e.g., ERF1 and RD29B) while enhancing the biosynthesis of protective compounds such as antioxidants (e.g., glutathione) and osmolytes (e.g., proline). Through these coordinated mechanisms, ethylene activates adaptive responses to abiotic stresses like drought and high temperatures, ultimately supporting seed viability under challenging conditions[24].

4.2.2. Photosynthetic and Antioxidant Biochemicals

In connection with the analysis of the data in Table 5 regarding biochemical compounds related to antioxidant properties, it is necessary to mention the following points:

The environmental conditions associated with higher elevations may stimulate the biosynthesis of these important compounds. Data in Table 5 indicate that the highest concentration of lutein was observed in seeds from the highest elevation, measuring 0.029 μmol/g dry weight (DW). This finding underscores the importance of altitude in influencing the metabolic pathways responsible for xanthophyll production. The consistent concentrations of violaxanthin, neoxanthin, and antheraxanthin in the two lowest plant populations highlight their importance in maintaining photosynthetic efficiency and protecting against light-induced stress. Understanding these dynamics can provide insights into how plants adapt to their environments and manage biotic and abiotic stresses effectively.

Table 4 indicates that lutein and zeaxanthin are the two predominant xanthophyll carotenoids found in the analyzed seeds, with lutein being the most abundant overall. However, the populations did not show statistically significant variations in their zeaxanthin content.The study identified the presence of two forms of tocopherols in *C. carvi* L. seeds: α-tocopherol and γ-tocopherol. α-tocopherol was found to be the most abundant form among the populations studied. Although tocopherols are crucial for oxidative stress protection in seeds, their measured levels showed no significant correlation with viability or germination parameters, implying either functional redundancy with other antioxidants or threshold-dependent effects not captured in this study. This suggests that while tocopherols contribute to the overall antioxidant capacity of the seeds, their levels may not be the sole determinant of seed performance under the tested conditions.

Xanthophylls, particularly neoxanthin, demonstrated a strong positive correlation with seed viability ($r = 0.520$, $P < 0.01$). This indicates that higher levels of neoxanthin are associated with better seed viability. Conversely, xanthophyll content was negatively correlated with germination rates, suggesting that these compounds play a crucial role in maintaining seed viability until conditions are favorable for germination. The findings emphasize the importance of neoxanthin in preserving seed viability during dormancy. While xanthophylls provide photoprotection and contribute to plant fitness, their accumulation appears to be strategically beneficial for seeds to remain viable until germination conditions are optimal. Empirical evidence demonstrates a significant positive correlation between xanthophyll accumulation (notably neoxanthin) and seed viability, while showing an inverse relationship with germination rates. These findings suggest xanthophylls function as key regulators of dormancy maintenance, particularly in high-altitude environments where extended seed viability is essential to withstand abiotic stressors including temperature fluctuations, UV exposure, and oxidative pressure [25-29].

The findings suggest that the accumulation of neoxanthin, along with other xanthophylls like lutein and antheraxanthin, plays a crucial role in preserving seed viability during dormancy in *C. carvi* L. This protective mechanism has been documented in multiple plant species, demonstrating the critical role of xanthophylls in preserving seed viability under stressful environmental conditions.

5. Conclusions

C. carvi L., a plant species commonly found in mountain environments, has evolved seed dormancy as a mechanism to enhance its survival probability in the face of environmental heterogeneity. The study found that increased seed dormancy correlates with higher seed viability. This relationship suggests that dormancy is a crucial survival strategy for *C. carvi* L., allowing seeds to remain viable under adverse environmental conditions.The mechanism governing this dormancy is linked to the biosynthesis of neoxanthin, an important carotenoid that functions as an antioxidant. Neoxanthin is also a precursor for abscisic acid (ABA), a hormone known to induce seed dormancy. This dual role helps maintain seed viability while promoting dormancy, particularly in challenging environments like high altitudes.The findings indicate that the combination of increased xanthophylls (like neoxanthin) and ABA accumulation is vital for *C. carvi* L. to thrive in fluctuating conditions, ensuring that seeds can remain dormant until favorable conditions for germination arise.Understanding these mechanisms not only enhances the knowledge of *C. carvi* L. biology but also has practical implications for the cultivation and preservation of seed stocks in other horticultural plants, particularly Apiaceae species.

Author contribution

All authors contributed to the study conception and design. Material preparation, exact plant identification, data collection, and analysis were performed by [MGN], [MZ] and [SFM] respectively. The first draft of the manuscript was written by [MZ] and all authors critically reviewed and approved the final version.

Conflicts of interest

The authors declare no conflicts of interest, financial or otherwise, related to this work.

References

1. Hammami H, Saadatian B and Aliverdi A. Geographical variation in breaking the seed dormancy of Persian cumin (*Carum carvi* L.) ecotypes and their physiological responses to salinity and drought stresses. *Industrial Crops and Products* 2018; 124: 600-606. doi: 10.1016/j.indcrop.2018.08.040.

2. Zhai B, Hu Z, Sun S, Tang Z and Wang G. Characteristics of photosynthetic rates in different vegetation types at high-altitude in mountainous regions. *Sci. Total Environ*. 2024; 907: 168071. doi: 10.1016/j.scitotenv.2023.168071.

3. Morcia C, Tumino G, Ghizzoni R and Terzi V. Carvone (*Mentha spicata* L.) oils. *Essential Oils in Food Preservation, Flavor and Safety*. 2016; 309-316. doi: 10.1016/B978-0-12-416641-7.00035-3.

4. Zehra M, Razaq A and Khan IA. Molecular analysis in medicinally important species *Carum carvi* and *Bunium persicum* (family Apiaceae) from district Astore. *Pak. J. Bot*. 2018; 50(1): 301-305.

5. Zakharova EA. Morphological evidence of polyphyletic nature of traditional *Carum* (Apiaceae-Apioideae). *Plant Diversity and Evolution*. 2010; 128(3): 409-21. doi: 10.1127/1869-6155/2010/0128-0019.

6. Cabrera E, Hepp J, Gómez M and Contreras S. Seed dormancy of *Nolana jaffuelii* IM Johnst.(Solanaceae) in the coastal Atacama Desert. *Flora*. 2015; 214: 17-23. doi: 10.1016/j.flora.2015.05.004.

7. Klupczyńska EA and Pawłowski TA. Regulation of seed dormancy and germination mechanisms in a changing environment. *Int.J.Mol.Sci*. 2021; 22(3): 1357. doi: 10.3390/ijms22031357.

8. Koornneef M, Bentsink L and Hilhorst H. Seed dormancy and germination. *Curr.Opin. Plant Biol*. 2002; 5(1): 33-36. doi: 10.1016/s1369-5266(01)00219-9.

9. Willis CG, Baskin CC, Baskin JM, Auld JR, Venable DL, Cavender-Bares J, Donohue K, Rubio de Casas R and Group NGW. The evolution of seed dormancy: environmental cues, evolutionary hubs, and diversification of the seed plants. *New Phytol*. 2014; 203(1): 300-309. doi: 10.1111/nph.12782.

10. Cotado A, Garcia MB and Munne-Bosch S. Physiological seed dormancy increases at high altitude in Pyrenean saxifrage (*Saxifraga longifolia* Lapeyr.). *Environmental and Experimental Botany*. 2020; 171: 103929. doi: 10.1016/j.envexpbot.2019.103929.

11. Chen DL, Luo XP, Yuan Z, Bai MJ and Hu XW. Seed dormancy release of *Halenia elliptica* in response to stratification temperature, duration and soil moisture content. *BMC Plant Biology*. 2020; 20(352): 1-8. doi: 10.1186/s12870-020-02560-8.

12. Salehani MK, Mahmoudi J, Mahdavi SK and Habibzadeh R. The effect of altitude on breaking seed dormancy and stimulation of seed germination of Persian hogweed (*Heracleum persicum*). *Afr. J. Tradit. Complement. Altern. Med*. 2013; 10(6): 555-558. doi: 10.4314/ajtcam.v10i6.29.

13. Heilmeier H, Schulze E-D, Fan J and Hartung W. General relations of stomatal responses to xylem sap abscisic acid under stress in the rooting zone–A global perspective. *Flora-Morphology, Distribution, Functional Ecology of Plants*. 2007; 202(8): 624-636. doi: 10.1016/j.flora.2007.06.002.

14. Rechinger KH. Flora Iranica. Graz-Austria: Akademic Druck-u; 1987.Vol. 162.

15. Pociecha E, Janeczko A, Dziurka M and Gruszka D. Disturbances in the biosynthesis or signalling of brassinosteroids that are caused by mutations in the *HvDWARF*, *HvCPD* and *HvBRI1* genes increase the tolerance of barley to the deacclimation process. *J. Plant Growth Regulation*. 2020; 39: 1625-1637.doi: 0.1007/s00344-020-10183-4.

16. Amaral JS, Casal S, Torres D, Seabra RM and Oliveira BP. Simultaneous determination of tocopherols and tocotrienols in hazelnuts by a normal phase liquid chromatographic method. *Anal. Sci*. 2005; 21(12): 1545-1548. doi: 10.2116/analsci.21.1545.

17. Munné-Bosch S and Alegre L. Changes in carotenoids, tocopherols and diterpenes during drought and recovery, and the biological significance of chlorophyll loss in *Rosmarinus officinalis* plants. *Planta*. 2000; 210(6): 925-931. doi: 10.1007/s004250050699.

18. Dostálek T, Rokaya MB and Münzbergová Z. Altitude, habitat type and herbivore damage interact in their effects on plant population dynamics. *PLoS One*. 2018; 13(12): e0209149. doi: 10.1371/journal.pone.0209149.

19. Metz J, Liancourt P, Kigel J, Harel D, Sternberg M and Tielbörger K. Plant survival in relation to seed size along environmental gradients: A long-term study from semi-arid and Mediterranean annual plant communities. *J. Ecol*. 2010; 98(3): 697-704. doi: 10.1111/j.1365-2745.2010.01652.x.

20. Shu K, Liu X-d, Xie Q and He Z-h. Two faces of one seed: hormonal regulation of dormancy and germination. *Molecular Plant*. 2016; 9(1): 34-45. doi: 10.1016/j.molp.2015.08.010.

21. Finch-Savage WE and Leubner-Metzger G. Seed dormancy and the control of germination. *New Phytologist*. 2006; 171(3): 501-523. doi: 10.1111/j.1469-8137.2006.01787.x.

22. Bewley JD. Seed germination and dormancy. *The Plant Cell*. 1997; 9(7): 1055-66.doi: 10.1105/tpc.9.7.1055.

23. Sajeev N, Koornneef M and Bentsink L. A commitment for life: Decades of unraveling the molecular mechanisms behind seed dormancy and germination. *The Plant Cell*. 2024; 36(5): 1358-1376. doi: 10.1093/plcell/koad328.

24. Mafakheri M, Kordrostami M and Al-Khayri JM. Plant abiotic stress tolerance mechanisms. Nanobiotechnology: Mitigation of abiotic stress in plants. Springer. 2021, pp: 1-31. doi: 10.1007/978-3-030-73606-4_2.

25. Tonguç M, Önder S, Gülcemal N and Tonguç F. Seed, germination, and seed-reserve traits differ along an altitudinal gradient. *Journal of Forestry Research*. 2022; 33(6): 1903-1912. doi: 10.1007/s11676-022-01467-4.

26. Bailly C. Active oxygen species and antioxidants in seed biology. *Seed Sci.Res*. 2004; 14(2): 93-107. doi: 10.1079/SSR2004159.

27. Kranner I and Birtić S. A modulating role for antioxidants in desiccation tolerance. *Integr.Comp.Biol*. 2005; 45(5): 734-740. doi: 10.1093/icb/45.5.734.

28. Waterworth WM, Bray CM and West CE. The importance of safeguarding genome integrity in germination and seed longevity. *Journal of Experimental Botany*. 2015; 66(12): 3549-3558. doi: 10.1093/jxb/erv080.

29. Bailly C and Kranner I. Analyses of reactive oxygen species and antioxidants in relation to seed longevity and germination. *Methods Mol.Biol.* 2011; 773: 343-367. doi: 10.1007/978-1-61779-231-1_20.

How to cite this article: Zafaranieh M, Mirahmadi SF, Ghorbani Nohooji M.Adaptation of *Carum carvi* L. to high altitudes: investigating the effect of altitude on seed physiological dormancy. ***Journal of Medicinal Plants*** 2025; 24(93): 27-39.
doi: