







Adaptive physiological traits of *Artemisia absinthium* L. seedlings grown in vitro for establishment in arid environments

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ABSTRACT

This study investigates the physiological responses of *Artemisia absinthium* L. seedlings propagated in vitro and acclimatized under arid zone conditions, with a focus on transpiration dynamics and the accumulation of photosynthetic pigments across key phenological stages. Diurnal transpiration rates were monitored during the vegetative, flowering, and seed formation stages, revealing a distinct midday peak in water loss. The highest transpiration rate (903.7 ± 12.4 mg/g·h) was recorded at the vegetative stage, indicating elevated water use during early development. In contrast, chlorophyll a and b contents exhibited a progressive decline from the vegetative to the seed formation stage, suggesting a reduction in photosynthetic efficiency under advancing drought stress. Meanwhile, carotenoid levels increased across the developmental stages, reflecting their role in photoprotection and oxidative stress mitigation. These physiological adjustments demonstrate the adaptive strategies employed by *A. absinthium* to cope with arid environmental pressures. The results support the potential application of microclonally propagated *A. absinthium* in ecological restoration of degraded rangelands in water-limited regions.

Keywords: *Artemisia absinthium*, microclonal propagation, physiological traits, arid conditions, photosynthetic pigments, ecological restoration.

INTRODUCTION

Arid and semi-arid ecosystems, which account for more than 40% of the Earth's terrestrial surface, are increasingly threatened by the combined impacts of climate change and land degradation (O'Mara, 2012). These regions are characterized by low and irregular precipitation, high evapotranspiration rates, and extreme temperature variability all of which impose significant limitations on plant establishment, growth, and survival (Parmesan, 2006).

Desertification, defined by the United Nations Convention to Combat Desertification (UNCCD, 1994) as land degradation in arid, semi-arid, and dry sub-humid areas driven by climatic and anthropogenic factors, represents a particularly

pressing environmental issue in Central Asia. In Uzbekistan, where more than 70% of the territory falls within arid and semi-arid zones, desertification poses serious ecological and socioeconomic challenges. These include biodiversity loss, declining agricultural productivity, water scarcity, and negative impacts on rural livelihoods (UNEP, 2016; Valiyev et al., 2024; Akramov et al., 2025). The drivers of desertification in the region are complex and multifaceted. Climatic trends – such as increasing temperatures, reduced rainfall, and prolonged drought periods – have been widely reported (Valiyev et al., 2023; Mukhtorova et al., 2024). At the same time, unsustainable land-use practices, including excessive irrigation, overgrazing, deforestation, and poor resource management, have exacerbated soil degradation,

particularly in ecologically fragile zones near the Aral Sea (Rajabov et al., 2020; Liu et al., 2003; Gorai et al., 2011). The desiccation of the Aral Sea, one of the most striking examples of human-induced environmental degradation, further underscores the urgency of addressing desertification through science-based strategies.

Understanding the physiological and ecological responses of native plant species is central to developing sustainable land restoration and vegetation management practices in arid regions. Recent research has increasingly focused on plant traits such as transpiration rate, water-holding capacity, and drought tolerance as indicators of ecological fitness and adaptation (Valiyev et al., 2023). In particular, field studies in the semi-desert rangelands of Samarkand and Kashkadarya have shown that plant species growing on gypseous soils exhibit higher water retention than those on sandy substrates, highlighting the importance of soil–plant interactions in arid environments (Valiyev et al., 2022). These findings contribute to a growing body of knowledge guiding species selection and grazing management for rangeland restoration.

Artemisia absinthium L., a species of the Asteraceae family, is widely recognized for its medicinal value and its ecological resilience in xeric environments. While its bioactive compounds and pharmacological properties – including antimicrobial, antioxidant, and anti-inflammatory effects—have been extensively studied (Ivanova et al., 2008), less attention has been paid to the species' ecophysiological characteristics, especially its water-use strategies under natural field conditions. Transpiration plays a pivotal role in plant water relations, particularly in arid zones where water availability determines vegetation dynamics and ecosystem productivity. Species within the *Artemisia* genus are known for xerophytic adaptations such as reduced leaf area, thick cuticles, and efficient stomatal control (Sreenivasulu et al., 2012; Tohidi et al., 2017). For instance, studies on *A. tridentata* and *A. herba-alba* have revealed their ability to regulate transpiration in response to drought, maintaining photosynthetic activity under stress (Panayotova et al., 2021). However, comparable data for *A. absinthium* under field conditions remain scarce.

Microclonal propagation (in vitro culture) has emerged as a valuable technique for conserving and mass-producing medicinal and ecologically significant plant species. It enables the generation of genetically uniform and pathogen-free plantlets

with stable biochemical properties (Gonçalves et al., 2013; Biasi et al., 2014). In the case of *A. absinthium*, successful protocols have been established using nodal and shoot-tip explants, with emphasis on optimizing growth and secondary metabolite production under controlled conditions (Ahmad et al., 2010; Malik et al., 1992). However, despite these advancements, the ecophysiological behavior of microclonally propagated seedlings after transfer to field conditions – especially their water-use efficiency, photosynthetic performance, and stress tolerance – has not been thoroughly studied.

Among the most informative physiological traits are transpiration rate and photosynthetic pigment content (chlorophyll a, chlorophyll b, and carotenoids), which reflect both the plant's hydration status and its ability to perform photosynthesis under stress. These traits are dynamic and vary across developmental stages – vegetative, flowering, and seed maturation – particularly in response to drought conditions (Taiz et al., 2015; Flexas et al., 2004). Yet, their combined influence on the adaptation of in vitro-grown *A. absinthium* seedlings to arid environments remains largely unexplored.

This study aims to address this knowledge gap by assessing transpiration rates and pigment composition in microclonally propagated *A. absinthium* L. across key phenological stages under natural arid conditions. By linking physiological responses to environmental stressors, the findings will contribute to a deeper understanding of adaptive mechanisms in xerophytic species and support the development of effective strategies for rangeland rehabilitation and medicinal plant cultivation in arid regions.

MATERIALS AND METHODS

Study area

The field-based part of this study was conducted in the semi-arid grasslands of Surkhandarya region, located in Uzbekistan. The specific site is located near the village of Denov, at geographical coordinates 38°14'57.5"N and 67°54'30.3"E.

The area is representative of the semi-desert ecosystems of Central Asia and is characterized by the following features:

- continental desert climate: hot, dry summers and cold winters;

- average annual precipitation: up to 200–250 mm, mainly in spring and autumn;
- average summer temperature: from +30 °C to +38 °C;
- soil types: mainly sandy and gypsum, typical of shrublands dominated by *Artemisia*.

The natural vegetation consists of drought-tolerant subshrubs, especially *Artemisia absinthium*, *A. diffusa*, *Anabasis* species, with sparse grass and sedge layers. The site experiences moderate grazing pressure, making it ecologically relevant for the study of desertification and plant water use.

In contrast, the *in vitro* component of the study was conducted in the Plant Tissue Culture Laboratory of the Bog‘bon *In vitro* Laboratory, where controlled greenhouse conditions allowed comparison with natural field conditions. In field experiments, regenerant seedlings that had undergone a 28-day adaptation phase in a greenhouse were used. Based on generally accepted requirements, physiological parameters were evaluated in seedlings that were kept in the field for 3 months after transplanting.

Data collection

To investigate the transpiration activity of *Artemisia absinthium* L. propagated by microclonal methods, data were collected under both natural field conditions and *in vitro* conditions. The study focused on three key phenological stages (Figure 1):

- vegetative,
- flowering,
- seed formation.

Experimental design

Microclonally propagated plants of *A. absinthium* were grown in two environments:

- in natural conditions, plants were cultivated in open field plots exposed to ambient climate in a semi-arid region of Uzbekistan, under typical soil and weather conditions;
- *in vitro* plants were grown in greenhouse conditions with controlled humidity, temperature (25 ± 2 °C), and photoperiod (16 h light/8 h dark), in sterilized potting soil.

For both environments, a randomized block design was used with three biological replicates per treatment.

Transpiration rate measurement

Transpiration was measured using the gravimetric method (weight loss per unit fresh mass per hour) as described by (Taiz and Zeiger 2010). Measurements were taken at six time points during the day: 7:00, 9:00, 11:00, 13:00, 15:00, and 17:00, which reflect diurnal changes in environmental parameters.

- For each plant, fully expanded leaves were excised and immediately weighed, then placed

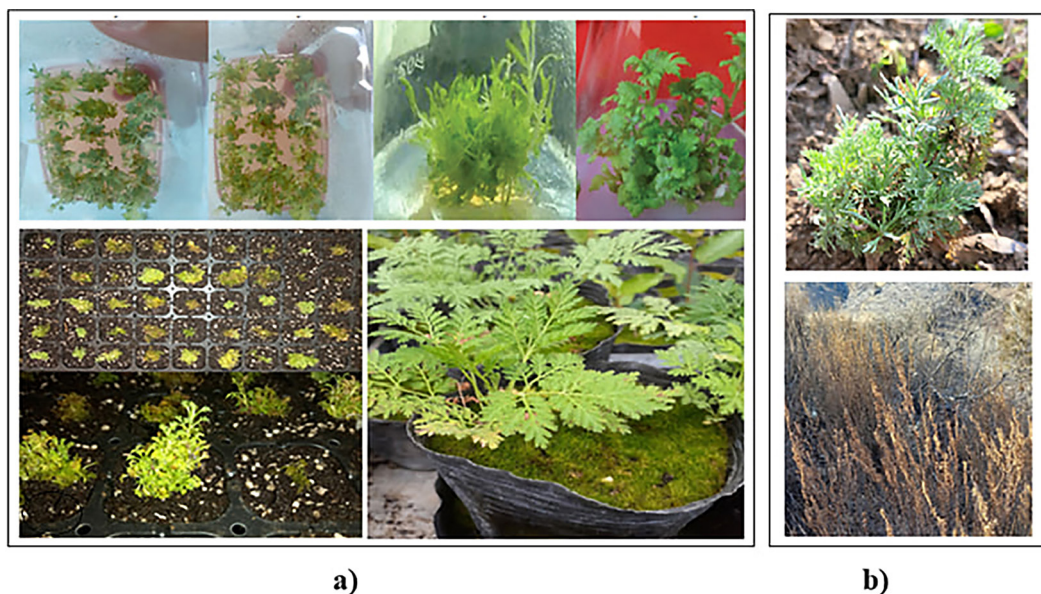


Figure 1. *Artemisia absinthium* L.: (a) grown *in vitro*; (b) under arid conditions

under identical ambient light and temperature for one hour, and reweighed;

- The transpiration rate was calculated in mg of water loss per gram of fresh leaf weight per hour (mg/g·h).

Determination of photosynthetic pigments

Photosynthetic pigments, including chlorophyll a, chlorophyll b, and total carotenoids, were extracted and quantified using the spectrophotometric method described by Lichtenthaler and Wellburn (1983), with minor modifications.

Fresh leaf samples (0.1 g) were homogenized in 80% acetone (v/v) using a chilled mortar and pestle. The homogenate was centrifuged at 10,000 rpm for 10 minutes at 4 °C, and the supernatant was collected for pigment analysis.

Absorbance was measured at three wavelengths using a UV-Vis spectrophotometer:

- 663 nm for chlorophyll a (Chl a),
- 646 nm for chlorophyll b (Chl b),
- 470 nm for total carotenoids.

Data analysis

All experimental data were statistically analyzed using Microsoft Excel 2016. A multi-way analysis of variance (ANOVA) was performed to assess the significance of differences between developmental stages (vegetative, flowering, and seed production) for each physiological parameter, including transpiration rate, chlorophyll a, chlorophyll b, and total carotenoid content. Where significant differences were found ($p < 0.05$), a one-way analysis of variance (ANOVA) was performed.

- F-statistic: 10.09.
- p-value: 0.0017.

This indicates a significant difference ($p < 0.05$) in transpiration among the stages, confirming that water use varies according to plant developmental phase (Ergasheva et al., 2024).

RESULTS

Transpiration patterns across phenological stages and growing conditions

The ANOVA results showed a statistically significant difference in transpiration rates among the three phenological stages ($p < 0.05$), confirming

that water use in *Artemisia absinthium* L. is not uniform throughout its developmental cycle. This suggests that transpiration is tightly regulated in relation to phenological transitions, with environmental exposure playing a crucial role.

Compares the diurnal transpiration rates of *A. absinthium* grown under natural arid zone conditions and in vitro environments. In both conditions, transpiration peaked at 11:00, reflecting the time of maximum stomatal conductance and photosynthetic activity. The highest recorded rate was 903.7 mg/g·h in arid-grown plants, slightly exceeding the peak of 888.2 mg/g·h in vitro. This modest difference points to a more responsive water transport system in field-acclimated plants, likely driven by environmental stimuli such as wind, temperature gradients, and solar radiation.

Across all time intervals, transpiration rates were consistently higher in arid-grown plants, indicating better-developed water regulation mechanisms. The largest disparity occurred at 13:00, where transpiration remained high (894.1 mg/g·h) under field conditions but declined sharply in vitro (770.9 mg/g·h), likely due to midday stomatal closure in the absence of real-world stress cues indoors. Both conditions showed a marked decline in transpiration by 17:00, indicating stomatal closure and reduced photosynthetic demand in the afternoon. These findings demonstrate that while in vitro plants retain baseline physiological function, their water-use efficiency and stress responsiveness are inferior to those of naturally acclimated plants (Table 1).

Phenological influence on transpiration dynamics

When transpiration was analyzed by phenological stage, clear distinctions emerged. During the vegetative phase, the highest transpiration rates were recorded, with a peak of 934.7 mg/g·h at 13:00 (Figure 2).

This intense activity likely corresponds to vigorous physiological processes such as leaf expansion and rapid biomass accumulation. High stomatal conductance and elevated photosynthetic demand underpin this phase's water usage strategy. Early morning values (e.g., 637.1 mg/g·h at 7:00) also indicated rapid stomatal activation following sunrise. In the flowering phase, transpiration values remained moderately high (627.9 mg/g·h peak at 13:00), suggesting continued physiological activity despite a shift in energy

Table 1. Average daily transpiration rates of *Artemisia absinthium* L. under arid zones (a) and in vitro (b) conditions

Times	Transpiration rate mg/g.h (A)	Transpiration rate mg/g.h (B)
7:00	667.3±4.6	598.1±6.1
9:00	728.2±3.3	674.6±5.7
11:00	903.7±12.4	888.2±3.6
13:00	894.1±5.3	770.9±7.1
15:00	618.5±2.4	577.5±4.5
17:00	357.6±0.45	306.3±3.5

allocation toward reproductive development. The seed production phase showed the most conservative water-use pattern, with transpiration peaking at only 428.5 mg/g·h and declining steeply to 77.6 mg/g·h by 17:00. This decrease reflects physiological senescence and enhanced stomatal closure mechanisms during late development stages, consistent with xerophytic strategies aimed at conserving water during seed maturation (Figure 3).

Photosynthetic pigment dynamics

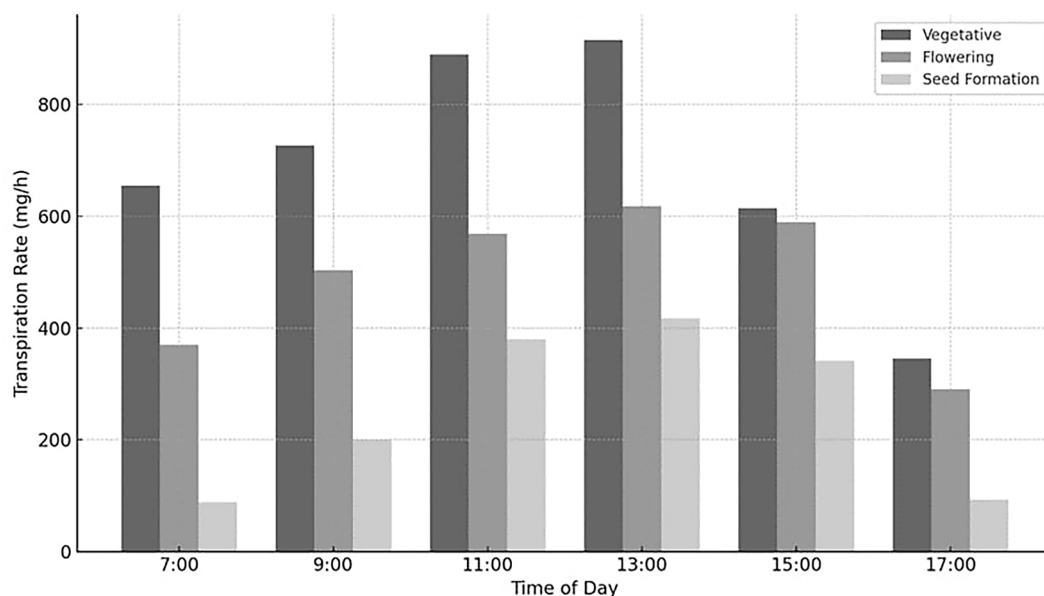
Changes in photosynthetic pigments—chlorophyll *a*, chlorophyll *b*, and carotenoids—across the growth cycle of *A. absinthium* also reflect physiological adaptation strategies. Under in vitro conditions, chlorophyll *a* and *b* concentrations were highest during the vegetative phase (4.1 mg/g and 3.1 mg/g, respectively), coinciding

with active leaf development and photosynthesis (Figure 4). As plants progressed to flowering and seed formation, chlorophyll levels declined to 3.2 and 2.8 mg/g (chlorophyll *a*), and 2.4 mg/g and 2.2 mg/g (chlorophyll *b*), respectively. This trend illustrates the redirection of metabolic resources from chlorophyll synthesis to reproductive development (Figure 4).

Carotenoid content, however, followed an inverse pattern. Lower levels were observed during early growth (2.5 mg/g), while concentrations increased progressively during flowering (3.1 mg/g) and peaked during seed formation (3.7 mg/g) (Figure 5).

This increase suggests a compensatory mechanism enhancing photoprotection as chlorophyll content declines, allowing the plant to minimize oxidative damage under high light and temperature stress.

In arid-grown plants, chlorophyll *a* peaked slightly lower than in vitro (3.9 mg/g vs. 4.1 mg/g), but showed a sharper decline during flowering (2.8 mg/g) and seed formation (2.2 mg/g). Similarly, carotenoid levels were consistently higher under arid conditions, underscoring their protective role in harsh environments. The rapid degradation of chlorophyll *b* may reflect enhanced sensitivity to oxidative stress, particularly in microclonally propagated plants exposed to field conditions without extended acclimatization. The observed transpiration and pigment dynamics highlight the drought-adaptive strategy of *A. absinthium*, characterized

**Figure 2.** Transpiration rate during phenological phases of *A. absinthium* grown in vitro

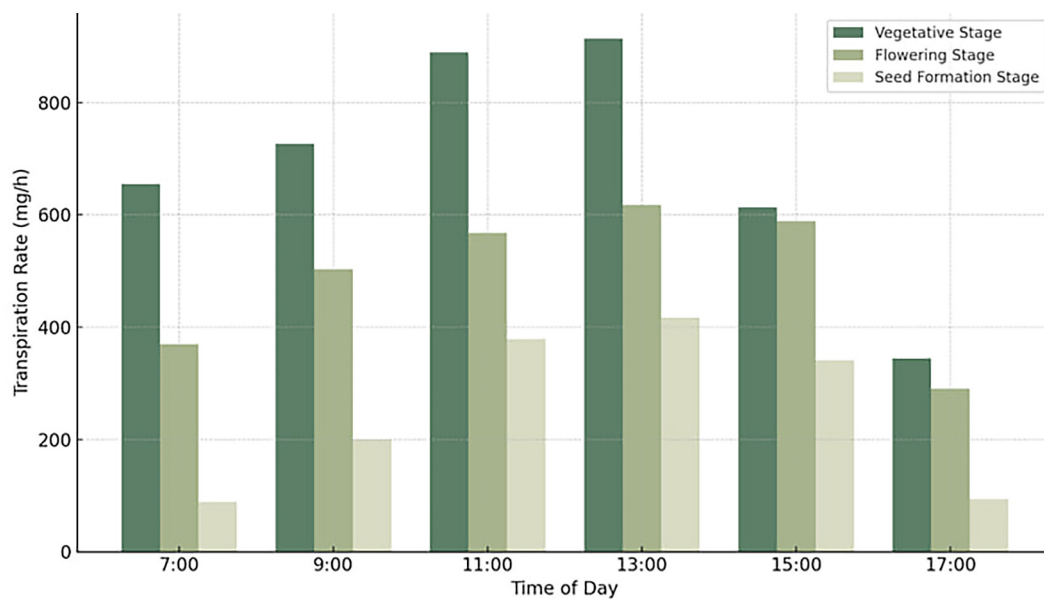


Figure 3. Indicators of transpiration rate during phenological phases of *A. absinthium*, distributed in arid zones

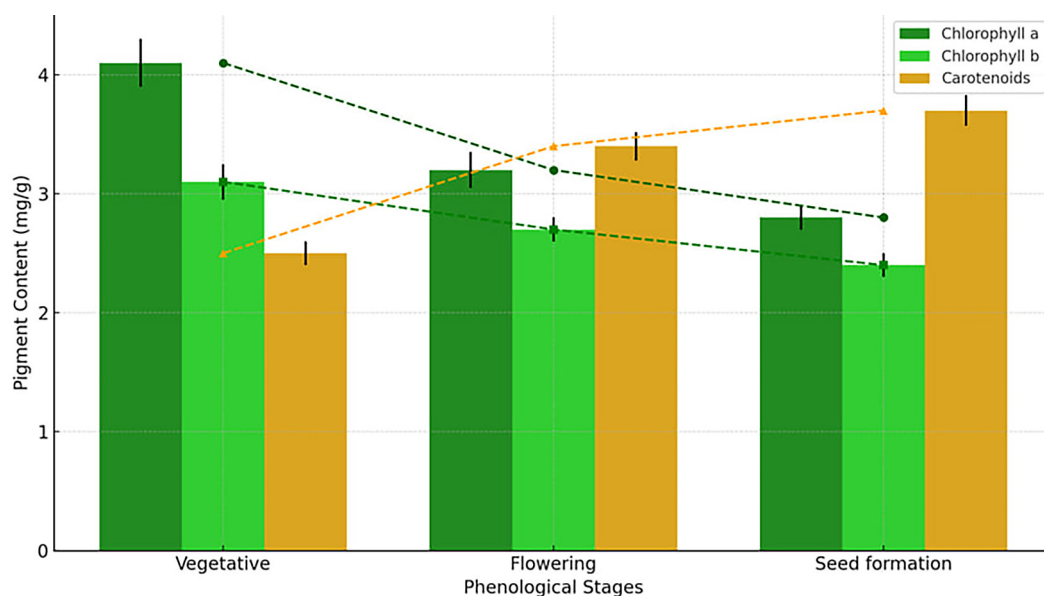


Figure 4. Changes in the amount of photosynthetic pigments during the vegetation period of *A. absinthium* under in vitro conditions

by high water-use during vegetative growth and reduced transpiration during reproductive stages. This developmental modulation of water use is typical of perennial xerophytes and essential for survival in arid ecosystems.

DISCUSSION

Stomatal control appears to be well coordinated with environmental cues, particularly light intensity and vapor pressure deficit (VPD). The

midday peak in transpiration coincides with optimal photosynthetic conditions, while the afternoon decline reflects stomatal closure to prevent excessive water loss. These patterns likely involve hormonal regulation (e.g., abscisic acid-mediated responses) and structural adaptations such as sunken stomata or reduced stomatal density (Zobayed et al., 2015). Phenological stage had a significant effect on transpiration, with the vegetative stage showing the highest water loss across all time points. This can be attributed to increased leaf surface area, higher stomatal

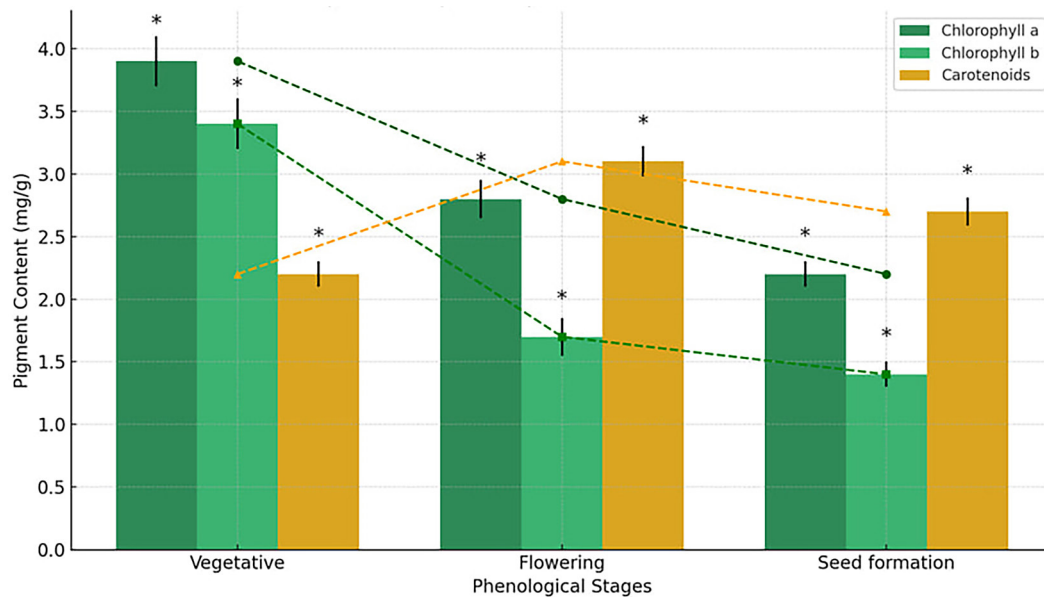


Figure 5. Changes in the amount of photosynthetic pigments during the vegetation period of *A. absinthium* under arid conditions

density, and elevated metabolic activity associated with rapid biomass accumulation (Taiz et al., 2015). The flowering and seed production stages showed progressively lower transpiration, reflecting a shift in resource allocation toward reproductive processes and a reduction in active water loss – an adaptive strategy commonly observed in drought-tolerant perennials (Jones, 2014; Tohidi et al., 2017).

The sharp reduction in transpiration during the seed formation stage, especially in the afternoon, suggests enhanced stomatal sensitivity and tighter regulation under water-deficit conditions. This behavior aligns with reports of other *Artemisia* species where reproductive development is accompanied by a conservative water-use strategy to maximize reproductive success under stress (Panayotova et al., 2021; Sreenivasulu et al., 2012). The dynamics of photosynthetic pigments further support the species' adaptive plasticity. Chlorophyll *a* and *b* concentrations peaked during the vegetative stage, indicating high photosynthetic capacity during early development. As the plant transitioned to flowering and seed formation, both pigments declined, suggesting a reduction in photosynthetic activity and a reallocation of energy toward reproductive growth (Lichtenthaler 1983; Taiz et al., 2015).

Interestingly, carotenoid levels increased during the later stages of development, particularly during seed formation. Carotenoids function as photoprotective pigments, scavenging reactive

oxygen species and protecting the photosynthetic apparatus from photooxidative damage (Demmig-Adams and Adams, 1996; Niyogi, 1999). The elevated carotenoid content in late phenophases suggests a compensatory mechanism against stress induced by high light and temperature, especially under arid conditions.

In vitro plants showed slightly higher chlorophyll levels during early stages, likely due to stable temperature, humidity, and absence of photoinhibition. However, their carotenoid levels were generally lower during vegetative stages, indicating reduced need for photoprotection in controlled environments. These differences further highlight the importance of environmental stimuli in developing full photoprotective capacity in micropropagated plants (Zobayed et al., 2005). Microclonally propagated *A. absinthium* seedlings exhibited overall physiological responses aligned with field-grown counterparts, particularly after acclimatization. Despite slightly lower transpiration and pigment variability, the retention of diurnal water-use patterns and stage-dependent adjustments confirms their suitability for transplantation into arid landscapes. This supports previous studies advocating for the use of micropropagation in conservation, especially for medicinal and xerophytic species (Gonçalves et al., 2013; Biasi et al., 2014).

Importantly, these findings demonstrate that micropropagated *A. absinthium* maintains the physiological plasticity necessary for water-use

regulation and stress resistance – traits essential for survival and productivity in desertified and semi-arid ecosystems. This reinforces its potential role in dryland agriculture, restoration ecology, and sustainable biomass production.

CONCLUSIONS

The physiological responses of *Artemisia absinthium* L. seedlings grown in vitro demonstrate strong potential for adaptation to arid environments. Transpiration patterns varied significantly across phenological stages, with the highest rates during vegetative growth and sharp declines during flowering and seed formation an adaptive trait for water conservation. Photosynthetic pigment dynamics further confirmed high photosynthetic activity in early growth and increased photoprotection during later stages via carotenoid accumulation. These results confirm that micropropagated *A. absinthium* seedlings retain eco-physiological functions necessary for survival and productivity under water-limited conditions. The study validates microclonal propagation as a viable method for producing robust planting material for use in dryland agriculture, ecological restoration, and the sustainable cultivation of medicinal plants in arid zones.

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