Biochemical Diversity of Chenopodiaceae Species: Insights into Adaptation and Biodiversity

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Abstract. The Chenopodiaceae family exhibits great biochemical diversity, which contributes to the successful adaptation of species to diverse and extreme conditions, such as arid, saline and high-temperature climates. The main mechanisms include the control of C4 photosynthesis, which improves water use efficiency and reduces photosynthesis under external conditions. The biochemical adaptation of Chenopodiaceae species provides insight into their ecological significance and potential use in biotechnology, especially for enhancing crop resilience to climate change. Understanding the biochemical diversity of Chenopadiaceae not only sheds light on their evolutionary success, but also provides strategies to address contemporary challenges in agriculture and conservation.

Keywords: C3 photosynthesis, C_4 photosynthesis, *Chenopodiaceae*, enzyme activity.

1 Introduction

A C_3 plant is a type of plant that generates a three-carbon compound, 3-phosphoglyceric acid, through the Calvin-Benson cycle. These plants comprise approximately 95% of all green plants on Earth and are well suited to areas with moderate levels of sunlight, temperature and water.

C_4 plants have higher energy conversion efficiency than C3 plants due to a CO_2 concentration mechanism that reduces photorespiration [2, 28]. Except for single-cell C4 photosynthesis, this mechanism involves two types of reactions [8, 9, 28]. In the mesophyll cells, CO_2 is fixed by PEPC, forming a C_4 acid, which is converted to malate or aspartate (Pick et al., 2011). The C_4 acid is decarboxylated in the bundle sheath cells, concentrating CO2 around Rubisco and reducing photorespiration [15, 24].

One of the most fascinating families is Chenopodiaceae, which contains species with a wide variety of carbon-assimilating organ structures [25]. These species also have different types of photosynthesis, such as C_3-C_4 or C_4-CAM intermediates [20].

The flexibility of Chenopodiaceae species to severe conditions and their importance in ecosystems make them an important group for the research. Further study of diversity,

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biochemical properties and ecological roles may improve our understanding of the resilience of this plants in a changing climate. Research on the Chenopodiaceae species that grow in the flora of Azerbaijan is important to protect this biodiversity and provide for the sustainability of these species

2 Material and methods

The study object consisted of C_3 and C_4 species of the Chenopodiaceae family distributed in the flora of Azerbaijan. *Chenopodium album* was used as the C_3 species, and *Atriplex tatarica* was used as the C_4 species. Samples were collected from the Absheron Peninsula during the active vegetation period. The activities of certain photosynthetic enzymes were determined in the leaves collected from mature plants.

The frozen plant material (0.5 g) was thawed and extracted with 2 ml of chilled extraction buffer (100 mM Tris-HCl, pH 7.8; 10 mM $MgCl_2$; 1 mM EDTA; 10 mM 2-ME; 2 mM phenylmethylsulfonyl fluoride; and 2% insoluble polyvinylpyrrolidone) using a chilled mortar and pestle. The homogenate was centrifuged at 12,000 g for 5 minutes, and the supernatant was collected for enzyme activity assays.

Carbonic anhydrase (CA, carbonate-hydrate lyase, EC 4.2.1.1) activity was measured electrometrically following the Wilbur-Anderson method. The activity of phosphoenolpyruvate carboxylase (PEPC, EC 4.1.1.31) was determined according to Du et al. [7]. The activity of aspartate aminotransferase (AsAT, EC 2.6.1.1) was measured based on the method of Alfonso and Brüggemann [1]. The activities of the NAD-dependent malic enzyme (NAD-ME, EC 1.1.1.39) and the NADP-dependent malic enzyme (NADP-ME, EC 1.1.1.40) were determined according to Pyankov et al. [20].

3 Results and Discussions

The activities of various photosynthetic enzymes were determined in C_3 plants of Chenopodium album and C_4 plants of Atriplex tatarica belonging to the *Chenopodiaceae* family (Table 1).

Table1. Activity of some enzyme in *Chenopodium album* and *Atriplex tatarica*

Species	CA activity	μmol·mg^{-1}protein·min^{-1}			
		PEPC activity	AAT activity	NAD-ME activity	NADP-ME activity
Chenopodium album	91.2± 0.15	0.01±0.004	5.78±0.25	0.04±0.003	0.001±0.0005
Atriplex tatarica	46.3± 0.1	1.14±±0.03	10.19±0.2	2.6±0.07	0.04±0.005

The activity of carboanhydrase enzyme in *Ch. album* was found to be 1.96 times higher than in *A. tatarica.* Carbonic anhydrase (CA) activity varies significantly between C_3 and C_4 plants, influencing their photosynthetic efficiency and adaptation to environmental conditions. In C_3 plants, CA activity is typically higher in the mesophyll cells, which facilitates the conversion of bicarbonate to CO2 for the Calvin cycle [5]. In contrast, in C_4 plants, CA activity is reduced in the bundle sheath cells, which is necessary to maintain high CO_2 concentrations and minimize photorespiration, thereby increasing photosynthetic efficiency. Higher CA activity in the mesophyll cells facilitates CO_2 fixation and necessary for optimal functioning of photosystem II at ambient CO2 levels [26]. Low CA activity in bundle sheath cells is critical for the efficient operation of the C4 pathway. This low activity helps suppress photorespiration, maintaining elevated CO2 levels. While C_3 plants rely on

higher CA activity for efficient carbon fixation, C4 plants strategically minimize CA activity in certain cells to optimize their unique photosynthetic pathway. This adaptation highlights the evolutionary divergence in carbon fixation strategies across plant types.

As shown in Table 1, PEPC enzyme activity was significantly higher in *A. tatarica* compared to *Ch. album*. C_4 plants, like A. tatarica, typically exhibit elevated PEPC activity, which enhances carbon fixation efficiency, particularly under high light and temperature conditions and improves water use efficiency [12]. This reflects the fundamental differences between C_3 and C_4 photosynthetic pathways. PEPC activity is crucial for C_4 plants, allowing them to efficiently fix CO2, with a maximum rate (Vmax) of about 38.0 μmol min^{-1} mg^{-1} chlorophyll in species like A. spongiosa, compared to only 1.48 μmol min^{-1} mg^{-1} chlorophyll in C_3 species such as *A. hastata* [23]. The Km values for phosphoenolpyruvate (PEP) also differ, with C4 plants showing a lower affinity for PEP (0.59 mM) than C_3 plants (0.14 mM), indicating a more efficient use of PEP in C3 species [23]. In addition, C4 PEPC is less sensitive to feedback inhibition by metabolites like l-malate, which allows it to maintain high activity throughout the day [13]. In contrast, C_3 plants primarily use PEPC for anaplerotic CO_2 fixation, synthesizing tetracarboxylic acids from atmospheric CO2 [17]. Thus, while C_4 plants rely on PEPC for efficient carbon fixation, C_3 plants utilize PEPC for various metabolic roles, underscoring the evolutionary specialization of these pathways. However, under certain environmental conditions, the efficiency of C_4 photosynthesis can be compromised, indicating potential trade-offs in plant adaptation strategies.

The activity of aspartate aminotransferase enzyme in *Atriplex tatarica* was approximately 1.76 times higher compared to *Chenopodium album*. Aspartate aminotransferase (AAT) activity differs significantly between C_3 and C_4 plants due to their distinct photosynthetic pathways. C_3 plants primarily rely on the Calvin cycle for CO2 fixation, where AAT is involved in amino acid metabolism and the photorespiratory pathway, which can result in CO_2 loss [3]. This pathway contributes to lower AAT activity in C_3 plants, as they are more dependent on the Calvin cycle, which becomes less efficient under certain environmental stresses, such as high temperatures [18]. In contrast, C_4 plants, which utilize a CO2-concentrating mechanism, exhibit higher AAT activity. This enzyme plays a critical role in converting malate and aspartate, intermediates essential to the CO2-concentrating process that enhances photosynthetic efficiency [3]. The elevated AAT activity in C4 plants supports faster CO_2 assimilation and reduces photorespiration, enabling them to perform better under high light and temperature conditions [3, 18]. These differences in AAT activity reflect evolutionary adaptations, where C_4 plants have developed mechanisms to maximize CO_2 fixation and minimize losses through photorespiration, allowing them to thrive in warmer climates. While C_3 plants possess some mechanisms to mitigate photorespiration, they are less efficient compared to the robust adaptations seen in C_4 plants.

The activity of NAD-ME and NADP-ME enzymes in *A. tatarica* was higher compared to Chenopodium album. However, as can be seen from the table, the activity of NAD-ME enzyme in *A. tatarica* was at a lower level and amounted to 0.04 μmol mg^{-1} protein $min^{-1.}$

The activities of NAD-dependent malic enzyme (NAD-ME) and NADP-dependent malic enzyme (NADP-ME) vary significantly between C_3 and C_4 plants, influencing their metabolic pathways and photosynthetic efficiency. C_3 plants typically exhibit higher NAD-ME activity, which is vital for photorespiration and carbon metabolism. For instance, NAD-ME activity has been found in the mid veins of Arabidopsis, where it plays a role in sugar and amino acid metabolism [4]. NADP-ME activity is lower in C_3 plants since their metabolism focuses more on the Calvin cycle. NADP-ME is predominantly active in C_4 plants, critical for malate decarboxylation in the bundle sheath cells. This enzyme increases CO_2 concentration around Rubisco, enhancing photosynthetic efficiency [14, 27]. In comparison, NAD-ME activity is less prominent in C_4 plants because their CO_2 concentration mechanism reduces the need for photorespiration (Yoshimura et al., 2004).

In C_3 plants, NAD-ME supports photorespiration, a process less efficient in CO_2 fixation. C_4 plants, by contrast, have evolved mechanisms to minimize photorespiration, making NAD-ME less essential in their metabolism [27]. In C_4 plants, NADP-ME is integral to the C_4 cycle, facilitating the conversion of malate to pyruvate and CO_2, which boosts the efficiency of carbon fixation under high light and temperature conditions [6]. The differences between NAD-ME and NADP-ME activities in C_3 and C_4 plants highlight the evolutionary adaptations of these species. C_4 plants have developed a more efficient CO_2 fixation strategy with NADP-ME, while C_3 plants maintain higher NAD-ME activity to sustain photorespiration and associated metabolic processes.

The activity differences between NADP-malic enzyme (NADP-ME) and NAD-malic enzyme (NAD-ME) in C_4 plants are critical in understanding their physiological adaptations to various environmental conditions. NAD-ME species, which are often found in arid regions, display reduced stomatal conductance under high vapor pressure deficit (VPD), suggesting an advantage in hot, dry environments (Gan & Sage, 2024). On the other hand, NADP-ME species show enhanced light-harvesting efficiency, with increased enzyme activity in response to illumination [21, 22]. NAD-ME plants show a significant reduction in stomatal conductance as VPD increases, which helps these species perform better in drought conditions by minimizing water loss [10]. NADP-ME activity is strongly regulated by light, with activity increasing significantly under illuminated conditions, a key adaptation for efficient carbon fixation in high light environments [21]. The NAD-ME subtype has lower photosynthetic efficiency but greater light harvesting plasticity, allowing it to assimilate CO2 across varying light conditions, which may limit the necessity for high NADP-ME activity.

4 Conclusion

Results showing differences in activity between C_3 (Ch. album) and C_4 (A. tatarica) plants highlight the importance of biodiversity in adapting to environmental conditions. C_4 plants exhibit an increased capacity to fix carbon and reduce photosynthesis, as well as increased productivity and ecosystem efficiency. This metabolic diversity is important for agricultural applications and informs crop selection for best performance in different climates. Additionally, understanding these differences can aid conservation efforts by highlighting the need to conserve plant species that contribute to ecosystem stability and functioning.

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