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Application of Stable Carbon Isotope to Water Use Efficiency of Karst Plants

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Abstract: In the management of karst desertification, water is the limiting factor for plant life. Plant water use efficiency (WUE) reflects the relationship between plant water consumption and dry matter production, serving as an objective evaluation index of drought-resistant characteristics of water use status. This index indicates the ecological drought sensitivity and adaptability of plants when subjected to drought stress. Stable carbon isotope technology is a valuable tool for evaluating water use efficiency (WUE) in plants. This paper provides an overview of the application of stable carbon isotopes in assessing plant WUE. It also examines the impact of environmental water status, CO_2 concentration, and light on plant WUE. The findings can serve as a theoretical reference for the restoration of vegetation in karst desertification management ecosystems.

1. Introduction

Water is a limiting factor for plant life activities and has a significant impact on plant growth, development, distribution, and ecosystem community succession. Plant water use efficiency (WUE) can reflect the relationship between plant water consumption and its dry matter production. It is an objective evaluation index of plant water use status and drought resistance[1]. WUE can intuitively reflect the ecological drought sensitivity of plants under drought stress during the growing period[2]. Additionally, it is an important index for exploring the adaptability of plants to global environmental change and predicting the impacts of global change[3]. Whether plants can survive in extreme environments depends mainly on their ability to effectively coordinate the relationship between carbon assimilation and water dissipation. This implies that plant water use efficiency is a crucial factor for their survival[4]. Plant water use efficiency can be categorized into leaf WUE, individual WUE, and community or ecosystem WUE according to different levels[5]. Traditional measurement methods mainly include direct measurement and the photosynthetic gas exchange method[1]. The direct measurement method needs to be carried out in field experiments, which is detailed and cumbersome and requires a lot of manpower and financial resources. It is mostly used for the control research of crops and saplings. On the other hand, the photosynthetic gas exchange method is easy to operate. However, it is challenging to distinguish between crop transpiration and ground evaporation, leading to difficulty and errors in calculating the effectiveness of plant water. The results mainly provide instantaneous values, representing only the behavior of some leaves of the plant at a specific time. This method is unable to represent the final productivity of the whole plant. The method measures the water use efficiency of the entire plant and the water use efficiency throughout the entire growth period, which is not ideal for determining the plant's long-term WUE [4, 6] .

Stable carbon isotope analysis has been the predominant technique in water use efficiency (WUE) research for the past 30 years. This method is not constrained by temporal or spatial limitations and is valued for its simplicity, accuracy, speed, and efficiency. In the early 20th century, international researchers initiated studies on plant WUE[7]. Building upon this work, Farqhuar (1982) and his contemporaries in the 1980s further elucidated the relationship between carbon isotope ratio ($\Delta^{13}C$) and plant intercellular CO_2 concentration[8]. In the 1980s, Farquhar (1982) and others systematically described the correlation between carbon isotope ratios ($\delta^{13}C$) and plant intercellular CO_2 concentration[8]. They derived formulas for carbon isotope ratios and carbon isotope discrimination values ($\Delta^{13}C$) and investigated the relationship between $\delta^{13}C$, $\Delta^{13}C$, and plant WUE. They found a negative correlation of $\Delta^{13}C$ with Δ and a positive correlation of $\delta^{13}C$ with WUE. In other words, a high $\delta^{13}C$ or low Δ corresponds to a high WUE. Singh B. (1984) and colleagues proposed that plants can enhance water use efficiency by regulating stomata, which in turn affects the drought tolerance mechanism of plants[9]. Unlike the instantaneous WUE derived from the measurement of photosynthetic efficiency/transpiration efficiency in the photosynthetic gas method, the stable carbon isotope technique integrates all the carbon uptake in all tissues during the plant growth cycle. This technique can be used to determine the long-term water use efficiency of plants and provides a more accurate and simpler method for selecting and breeding plants with high water use efficiency[10].

Karst rocky desertification ecosystem is one of the typical fragile ecosystems, accounting for 12% of the global total land area[11]. Due to its unique binary hydrological structure, high rate of rock exposure, poor water storage capacity of the soil, frequency of temporary droughts, and prominent human-land conflicts, the ecological restoration and economic development of rocky desertification areas are seriously constrained. Some studies have shown that plant WUE in karst areas generally increases as rocky desertification deepens, both at the individual and community levels. This indicates that as soil water availability decreases, plant WUE tends to rise. However, in the context of extreme drought and frequent climate extremes, plant WUE continues to decline, sometimes resulting in plant death. Therefore, the study of plant water use efficiency is a crucial technology for vegetation restoration projects in rocky desertification areas. In recent years, numerous scholars have conducted extensive research on the stable carbon isotope technique to determine the WUE of individual plants or ecosystems. They have explored the impacts of various environmental factors such as rainfall, light, climate, and CO_2 concentration on WUE. Additionally, they have investigated how plants respond to WUE under different biotic and abiotic stresses. As a result, significant research findings have been obtained. The purpose of this paper is to review the research on water use efficiency at the leaf level of plants both domestically and internationally. The focus is on the adaptive changes of plant WUE under environmental factors such as water conditions, climate, and CO_2 concentration. The aim is to provide reference value for the further application of stable carbon isotopes in water use and to offer a theoretical basis and guidance for the restoration of vegetation cover and cultivation of forests to manage rocky desertification.

2. Overview of Stable Carbon Isotopes and Water Use Efficiency

Carbon has 15 isotopes in nature, of which ^{12}C and ^{13}C are stable isotopes, and the remaining ones are radioactive isotopes[6]. According to the principle of isotope fractionation, isotope technology can be applied to research in various disciplines. The phenomenon where isotopes of an element are distributed in varying ratios across different substances during physical, chemical, and biological reactions is known as isotope fractionation. The variances in isotope contents before and after the reactions are termed as isotope fractionation effects[12, 13]. Farmer (1974) was the first to utilize stable carbon isotopes in plants to track changes in atmospheric CO_2 concentration, which subsequently broadened the application of stable isotopes[14]. Farqhuar (1982) discovered a strong correlation between carbon isotope abundance $\delta^{13}C$ and discriminant $\Delta^{13}C$ with the ratio of leaf intercellular CO_2 concentration to atmospheric CO_2 concentration (C_i/C_a) in a wheat experiment[8]. C_i/C_a is a physiological and ecological indicator for plants, reflecting the relative amounts of net assimilation rate and stomatal conductance in relation to CO_2 demand and supply. Carbon stable isotopes in plant leaves reflect the effects of changes in environmental factors on C_i/C_a and can provide information about the environment that plants have encountered during their survival and growth. The ratio of $^{13}C/^{12}C$ in plant tissues is not constant, and research has demonstrated that atmospheric $^{13}C/^{12}C$ is typically higher than that found in plant tissues[8, 15]. Thus, as long as there is no carbon loss during plant physiology and biochemistry, the information contained in the variation in carbon isotope ratios is speculative. This variation indicates the fractionation of stable carbon isotopes, providing insights into the physical, chemical, and biometabolic aspects of the carbon transfer and fixation process. Factors affecting the fractionation process are diverse and include both internal plant influences (such as plant species, genetic characteristics, physiological features, and type of photosynthesis) and external environmental factors (such as climate, temperature, rainfall, CO_2 concentration, and light).

Water Use Efficiency (WUE) is the amount of photosynthetic products that can be assimilated per unit of water dissipated through plant leaf transpiration, or the ratio of dry matter produced by vegetative photosynthesis to water dissipated by leaf transpiration[16]. The stable carbon isotope technique not only simplifies the measurement steps compared to traditional methods but also enhances the accuracy and representativeness of WUE measurements. Most importantly, the stable carbon isotope technique overcomes the limitations of time and space, enabling the measurement of water status in plant samples at various time scales and geographical scales simultaneously[17]. This method is now internationally recognized as the most effective approach for determining the long-term WUE of plants[18].

3. Effects of Environmental Factors on Water Use Efficiency

3.1. Effects of Environmental Water Conditions on Water Use Efficiency

In arid and semi-arid regions, particularly in karst desertification areas characterized by high bedrock outcrops, well-developed fissures, and severe soil erosion, moisture plays a significant role in limiting biodiversity and agricultural production. In an increasingly arid climate, plant physiological stress and vulnerability increase, making effective soil moisture a major driver of forest succession processes. When the competition for plant water is intense, plants will reduce leaf stomatal conductance or close some stomata to decrease water consumption from transpiration. As a result, the atmospheric CO_2 concentration entering the leaf through the stomata decreases, leading to an enhancement of $^{13}CO_2$ fixation capacity, a decrease in the $\delta^{13}C$ value, and an increase in the plant's WUE. Conversely, when moisture conditions improve, the plant's capacity to fix $^{12}CO_2$ decreases, the $\delta^{13}C$ value increases, and the plant's WUE decreases. and plant's WUE decreased. Maintaining higher WUE under water scarcity is a strategy to mitigate the effects of water scarcity and improve competition for limited water resources within the habitat[19].

At the beginning of the 20th century, Cai H et al. (2000) conducted wheat experiments and demonstrated that when the soil water content was below 45-50% of the field water holding capacity, there was no significant decrease in crop yields, but the WUE value increased[20]. Jiang

Y et al. (2023) studied the water use efficiency and photosynthetic characteristics of alfalfa under various water gradients through controlled experiments[21]. Their research revealed that as drought intensified, the plant's photosynthetic characteristics (transpiration rate, leaf inter-cellular CO_2 concentration, net photosynthetic rate) exhibited a decreasing trend, while WUE showed an increasing trend. Chen T et al. (2002) found that the WUE values of desert plants in the Tianshan Mountains showed a significant negative correlation with annual precipitation[22]. They observed that plants adopted a more conservative water utilization mode in arid habitats, adapting to drought stress by increasing their WUE values. Akhter et al. (2003) and other researchers also demonstrated that the $\delta^{13}C$ values of plants growing in high humidity were significantly smaller than those of plants in low humidity[23]. Zheng Y et al. (2023) demonstrated that the majority of photosynthetic physiological parameters and WUE of hickory leaves were influenced by soil moisture levels[24]. Most of these parameters and WUE exhibited a pattern of initially increasing and then decreasing with decreasing soil moisture. The transpiration rate was found to be more responsive to drought compared to the net photosynthetic rate of the leaves. This suggests that hickory trees respond to drought stress by enhancing physiological traits for efficient water utilization through reduced transpiration. Jiang G et al. (2000) studied the drought adaptation of plants in northeastern China and found that, as drought intensified, plant WUE values gradually increased to a certain level and then decreased[25]. This suggests that there is a specific threshold value for plant WUE in response to drought severity.

Plant responses to drought stress are categorized into three main groups: 1) stomatal limiting factors; 2) non-stomatal limiting factors; and 3) uneven stomatal closure[10]. Studies have shown that plants primarily regulate WUE by managing the exchange of water and carbon flux in leaf stomata when encountering a water-limited environment. However, the connection between stomatal conductance and carbon sequestration is not linear[26]. Under moderate water deficit conditions, plant water use efficiency increases to sustain normal physiological activities. However, when soil water deficit is severe, the limited water resources cannot support the normal physiological activities of plants to form dry matter and maintain water consumption for plant organ functioning. This can lead to a failure of sugar transport in the plant phloem, limiting carbohydrate utilization, and ultimately contributing to the decline in plant productivity and death.

3.2. Effect of CO_2 Concentration on Water Use Efficiency

Microscopic stomata on plant leaves act as control valves for the entry of CO_2 into the leaf during photosynthesis and the exit of water vapor (H_2O) from the leaf during transpiration. In terms of plant physiological processes, plant WUE is influenced by both the rate of carbon assimilation and the rate of transpiration and dissipation. It can be enhanced by increasing the rate of photosynthesis and reducing stomatal conductance. The primary mechanisms through which CO_2 concentration impacts plant assimilation are twofold: 1) CO_2 serves as the raw material for photosynthesis and as the substrate for photosynthetic carboxylase, and in a high CO_2 environment, the carboxylation reaction speeds up. Carboxylation accelerates the reaction rate. Additionally, increased CO_2 concentration can activate photosynthetic carboxylase, leading to an increase in the number of photosynthetic carboxylase enzymes in the active state, thereby accelerating the carboxylation reaction[1].

Since the Industrial Revolution, the widespread use of fossil fuels has resulted in a significant rise in atmospheric CO_2 concentration from 280 μmol/mol to 419 μmol/mol. Some studies have indicated that the inherent water use efficiency (WUE) of trees has globally increased by approximately 40% since 1901, aligning with the 34% increase in total atmospheric CO_2 levels. Consistent with the results of Ito et al.'s (2012) analysis, likely due to the increase in plant productivity induced by the rise in CO_2 levels, the WUE of global terrestrial ecosystems has also exhibited a consistent upward trend from 1901 to 2004[27]. Feng et al.(1995) conducted a quantitative study on $\delta^{13}C$ values, revealing that with every 10 μL/L increase in atmospheric CO_2 concentration, plant $\delta^{13}C$ values decreased by approximately 0.20% ± 0.01%[28]. By measuring plant carbon isotopes, Rezaie et al.(2018) found that the magnitude of iWUE was positively correlated with CO_2 concentration[29]. They also discovered that CO_2 concentration was the primary driver of water use efficiency and that tree growth provided a positive feedback on the effects of CO_2 fertilization. Liu L(2023) investigated the response mechanism of maize under different CO_2 concentrations[30]. The study revealed that CO_2 concentrations mainly affect the water use efficiency of maize by altering stomata and leaf anatomy. These changes subsequently impact photosynthesis and transpiration processes. Li J et al. (2023) studied the cellular structure and anticorrosive physiology of cereals under drought stress with elevated CO_2 concentration[31]. Their results showed that under elevated CO_2 concentration treatment, leaf thickness, vascular bundle sheath cross-sectional area, net photosynthesis rate, and water-use efficiency of cereals under mild drought conditions were significantly increased. Additionally, the antioxidant enzyme activities of the plant's leaves were enhanced, and the osmotic regulating substances of leaves were altered to mitigate the negative effects of drought on the cereals. It also increased the activity of antioxidant enzymes in plant leaves, altered the osmoregulatory substances in leaves, mitigated the adverse effects of drought on grains, and substantially boosted the grain spikes and grain yield. In addition, the antioxidant effect of high CO_2 concentration can alleviate the effects of drought stress on plants[32] and can also mitigate the oxidative damage caused by ozone on wheat leaves[33].

3.3. Effect of Light on Water Use Efficiency

Photosynthesis is the primary energy source for plant growth, with light being the key factor driving plant photosynthesis. Light significantly influences various processes in plants, such as the distribution of chlorophyll, leaf phototropism, and the activity of photosynthetic carboxylase. These processes are closely linked to light conditions, ultimately impacting the stable carbon isotope composition of plants[34]. Within a certain range of light intensity, photosynthetic efficiency accelerates with increasing light intensity, and after reaching the photosynthetic saturation point, the photosynthetic rate gradually stabilizes[1]. Low light conditions reduce the energy source for plant growth, weakening the ability of plants to undergo photosynthetic carbon fixation. This alteration leads to changes in plant leaf traits, such as an increase in leaf area and the corresponding water loss rate, ultimately affecting the water use efficiency of plants[35]. Under the study on the effect of $\delta^{13}C$ value of plant leaves of Lotus corniculatus under different light conditions, it was found that with the weakening of light intensity, the $\delta^{13}C$ value decreased significantly, and the WUE of the plant was enhanced[36]. Meng F et al. (2023) found that the WUE of alfalfa under different light conditions was higher when exposed to low light compared to high light[37]. Long M et al. (2009) found a highly significant negative correlation between photosynthetically active radiation and alfalfa WUE[38].

4. Conclusions

Numerous scientific achievements have been made in the study of water-use efficiency in plants. However, with the megatrend of climate warming, the frequency of extreme weather events, and the intensification of the water crisis, plants are facing serious challenges like never before. High water-consuming crops can lead to deep soil desiccation and a decrease in groundwater depth, which is harmful to the sustainable development of forest ecosystems and agriculture. Especially for the fragile ecosystems represented by karstic rocky desertification areas, the soil water storage capacity is poor, making it challenging to advance the vegetation restoration project. Therefore, research on plant water use and physiological and ecological aspects is particularly important. The selection of highly efficient water-saving plants is beneficial for enhancing the solid soil water-holding capacity and improving the hydrological cycle in rocky desertification areas. Stable carbon isotope technology offers significant advantages in determining plant water use efficiency, overcoming the limitations of traditional methods, such as high workload and susceptibility to spatial and temporal constraints. The utilization of this technology can efficiently select and cultivate water-saving and drought-resistant plant crops with high effectiveness and consistent yield. Plant WUE is primarily influenced by genetic characteristics, but alterations in environmental factors can also impact the normal physiological activities of plants. Under the backdrop of climate warming, it is crucial to focus on vegetation adaptation strategies in response to environmental factors such as water availability, CO_2 levels, light exposure, temperature, and rainfall. Stable carbon isotope technology offers significant advantages over traditional methods. However, due to the high cost of the equipment and the requirement for precise operation to prevent errors, the measurement process should be conducted by professionals. Exploring alternative indicators for $\delta^{13}C$ values to reduce costs is an important direction that should not be overlooked in future research.

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