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HYDROGEN PEROXIDE DYNAMICS IN RIPARIAN HERBACEOUS SPECIES UNDER VARYING SOIL MOISTURE

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Abstract. Understanding plant responses to varying soil moisture is crucial for effective riparian zone management and restoration. This study investigates the relationship between hydrogen peroxide (H_2O_2) concentration and soil moisture levels in six herbaceous species within riparian zones. Given the critical role of environmental conditions in plant colonization, we analyzed how H_2O_2 serves as a biomarker for stress induced by varying soil moisture. Field samples were collected from multiple riparian sites over several years, focusing on six plant species: *Pueraria lobata*, *Fallopia japonica*, and *Persicaria longoseta.* Healthy leaves from selected herb species were collected, along with soil samples to measure moisture content. The methods ($TiSO_4$ assay) was employed to determine H_2O_2 levels in plant tissues. The study revealed a general trend where H_2O_2 concentrations initially increase with soil moisture, peaking at around 5%, and subsequently decrease as moisture levels rise. However, a secondary increase in H_2O_2 concentration was observed with some species under near-saturated soil moisture conditions. Species-specific responses were noted, with differences attributed to factors such as root depth, aeration systems, and plant morphology. These findings highlight the potential of H_2O_2 measurement as a practical tool for assessing the physiological status and habitat suitability of riparian vegetation, contributing to improved management and restoration strategies in these critical ecosystems.

Keywords: Abiotic stress response, Colonization patterns, Drought resistance, Herbaceous plants, Reactive oxygen species, Riparian vegetation, Soil moisture, Water stress

1 INTRODUCTION

Plant species require specific environmental conditions to successfully colonize an area. The riparian zone exhibits a distinct gradient of environmental factors, including sediment particle size, soil moisture, and nutrient concentration. Additionally, shallow groundwater seepage creates a unique water-related environment (Uria-Diez, 2013; González et al., 2015; Asaeda et al., 2022a). Depending on these site-specific conditions, different plant species establish themselves in different locations (Dybkjær et al., 2012).

Several factors influence species colonization, each contributing differently to overall feasibility (Asaeda et al., 2010). Thus, a comprehensive indicator that integrates these factors would be highly valuable in restoration projects. This is typically done by monitoring the distribution patterns, growth rates, or propagation rates of target species in natural or artificially induced environments. While this method provides reliable results, it requires a long time to yield conclusive findings. Furthermore, maintaining stable conditions over extended periods in field studies is challenging due to environmental fluctuations. Therefore, a faster method for determining feasible colonization conditions is necessary (Richardson et al., 2007).

Living organisms and biological systems play a crucial role in mitigating stress by preventing or repairing damage. When plants experience environmental stress, they undergo metabolic and physiological adjustments, leading to the production of reactive oxygen species (ROS) in different organelles, depending on the type of abiotic stress (e.g., anoxia, drought) (Mittler, 2002). Some ROS are scavenged quickly by antioxidants, maintaining ROS balance within tissues (Sharma et al., 2012), however, in the process of scavenging these ROS, hydrogen peroxide (H_2O_2) is either directly or indirectly generated (Asada, 2006). Compared to other ROS, H_2O_2 in plant tissues can be analyzed with minimal loss, making it a useful indicator of stress (Jana and Choudhuri, 1982).

The H_2O_2 concentration in plant tissues could be quantitatively evaluated, even in field samples, offering a potential method to measure ROS-induced damage and environmental stress levels (Pospíšil, 2016). Combined with stress intensity monitoring, H_2O_2 concentration may serve as an indicator of a plant's physiological status in response to specific environmental conditions, making it a valuable tool for assessing habitat suitability (Asaeda et al., 2019, 2020, 2022b; Barnuevo and Asaeda, 2018). However, the species-specific characteristics of H_2O_2 concentration in response to environmental stress remain unclear. Thus, the current study aims to analyze the trends of H_2O_2 concentration in typical herbaceous species in riparian environments under varying soil moisture conditions. Additionally, it seeks to understand the underlying mechanisms of these trends through eco-physiological insights.

2.MATERIALS AND METHODS

2.1 Sampling and treatment of samples

Across various years and months, riparian zone sites along different rivers were selected for observation. On a cloudless day, after maintaining nearly identical conditions for more than a week, sampling was conducted between 10 AM and 3 PM. The sampling was conducted in different rivers, years and seasons. At each site, major herb species were selected. Five healthy plants were then chosen from the center of several colonies. A fully expanded, sun-exposed, healthy leaf was carefully collected from the upper part of each plant. Typical root depth was estimated by excavated deeper. Soil samples were collected at the root depth from three different locations within each plant colony, sealed in plastic bags, and analyzed for soil moisture content using the gravimetric method. At all sites, soil particles were predominantly categorized as fine sand. During sampling, solar radiation exceeded 1,000 mmol/m²/s, and the temperature was close to the monthly average. The sampled leaves were placed in sealed plastic bags, immediately stored in a freezing box with dry ice, and then kept at –80°C in the laboratory until analysis.

2.2 Determination of H_2O_2 content with $TiSO_4$ assay

The Brennan and Frenkel (1977) method was used to measure H_2O_2. In an acidic solution, titanium (II) metal ions form a peroxide complex with H_2O_2, producing a yellow-colored compound. For the assay, 0.25mL of the extract was placed in a 15 mL round centrifuge tube. Then, 0.875 mL of a 0.1% $Ti(SO_4)_2$ solution in 20% H_2SO_4 was added. The mixture was centrifuged at 10,000 rpm for 15 minutes at room temperature. After centrifugation, 1 mL of the supernatant was transferred to a 1 mL spectrophotometer cell, and its absorbance was measured at 410 nm by spectrophotometry (UVmini-1240, Shimadzu, Japan). For the blank, a mixture of 0.25 mL of 0.05 M phosphate buffer (pH 6.0) and 0.835 mL of 0.1% $Ti(SO_4)_2$ in 20% H_2SO_4 was used.

3 RESULTS

The investigated species included typical herbs with different morphologies in the riparian zones, *Pueraria lobata* (Fabaceae, perennial), *Fallopia japonica* (Polygonaceae, perennial), and *Persicaria longoseta* (Polygonaceae, annual) (Asaeda et al. 2011; Waal, 2001). The stem heights of sampled plants were as follows: *F. japonica*: approximately 50 cm; *P. longoseta*: less than 20 cm across all sites. *P. lobata* is a liana species and grew several meters long. Leaves were sampled from a height of approximately 1 meter. Root and rhizome depths were *F. japonica*: about 30 cm; *P. lobata*: 40 to 50 cm; and *P. longoseta*: about 15 cm.

Although the studied areas cover different river sites and months, the species-specific trend of the highest H_2O_2 concentration in response to soil moisture remained relatively consistent. The variation in the highest H_2O_2 concentration exhibited distinct patterns across soil moisture levels, ranging from nearly dry to nearly saturated conditions (Fig. 1). At very low soil moisture levels (less than 5%), H_2O_2 concentration was low for some plants. However, it increased significantly at around 5% of soil moisture. Beyond this point, it gradually decreased as soil moisture levels increased (Fig. 1).

Interestingly, beyond a certain soil moisture level, approximately 20 % of soil moisture, H_2O_2 concentration began to rise again with *P.lobata* (Fig.1A).

In contrast, *F. japonica* (Fig. 1B) and *P. longoseta* (Fig. 1C) displayed a continuous decline in H_2O_2 concentration, with no significant increase observed even under near-saturated conditions.

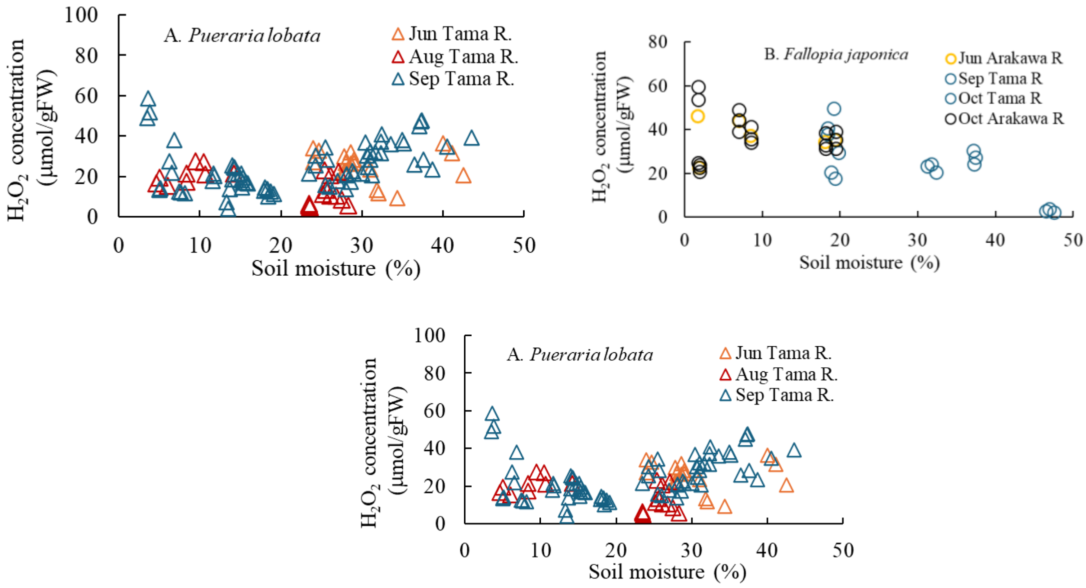

Figure 1. The H_2O_2 concentration of leaves.

4.DISCUSSION

4.1 The effect of photosynthesis on the variation of H_2O_2 concentration

In the photosynthetic system, solar energy is captured by antenna pigments in photosystem II (PSII) and transported to the reaction center with minimal energy loss. At the reaction center, this energy is used to split water molecules into protons, electrons, and oxygen. The electrons are then transferred to photosystem I (PSI), where they participate in the reduction of $NADP^+$ to NADPH in the stroma via the action of ferredoxin (Fd) and the flavoprotein ferredoxin–$NADP^+$ reductase (FNR). Finally, in the Calvin cycle, these electrons reduce ribulose 1, 5-bisphosphate to 3-phosphoglycerate (RuBp), facilitating the conversion of carbon dioxide (CO_2) into carbohydrates. In this process, CO_2 is electron accepter, and carbon is reduced from oxidation state IV to 0. Therefore, the presence of CO_2 is essential for electron consumption, consuming four electrons per one mole CO_2.

The exchange of CO_2 between the mesophyll and the ambient air primarily occurs through the stomata. Water is transported through both the stomata and the xylem. While the amount of supply through xylem is higher, therefore, the amount of water in the mesophyll depends on the soil moisture content. Under high water stress, stomata close to prevent water loss from the mesophyll, which inhibits CO_2 to diffuse from the ambient ultimately into the chloroplast, where it is utilized for carbohydrate synthesis, consuming electrons, although CO_2 transport via stomata is approximately 500 times smaller than that of water molecules (Ögren and Öquist, 1985; Asaeda et al., 2025).

At low soil moisture levels, reduced mesophyll water content leads to stomatal closure, inhibiting CO_2 absorption. As a result, carbohydrate synthesis is impaired, preventing the normal consumption of electrons in PSII. The excess electrons are instead accepted by oxygen molecules, leading to the formation of H_2O_2. This explains the high H_2O_2 concentration observed at sites with low soil moisture (Goltsev et al., 2012). As soil moisture increases, mesophyll water content rises, leading to stomatal opening. This enhances CO_2 supply, and carbohydrate synthesis using electrons, which in turn reduces H_2O_2 concentration. These processes contribute to the trend of highest H_2O_2 concentration at very low soil moisture levels, followed by a decline as soil moisture increases. In low-growing herbs with short stems, the transport of soil moisture to the mesophyll via the xylem depends heavily on the site's soil moisture content. Consequently, mesophyll water content directly reflects soil moisture conditions. However, under low CO_2 concentrations relative to O_2, photorespiration intensifies during carbohydrate synthesis, producing significant amounts of H_2O_2 in the peroxisomes. The H_2O_2 concentration in relation to soil moisture is influenced by the electron generation rate during photosynthesis. This rate reflects the surplus electrons produced by solar energy beyond those required for carbohydrate synthesis. Electron consumption is primarily determined by the CO_2 concentration in the mesophyll, which is regulated by stomatal activity and the water content of the mesophyll. Water content, in turn, is shaped by the morphological and anatomical traits of leaves, stems, roots, and other plant structures. Notably, similar trends were observed across different herb species.

4.2 Effect of respiration

In the mesophyll, excessive water content results in a thicker water film forming on the surface of mesophyll cells (Syvertsen et al. 1995). Since oxygen has low solubility in water, its supply to mesophyll cells as dissolved oxygen through the stomata remains limited. Dissolved oxygen in water transported via the xylem likely plays a significant role in cellular respiration. Therefore, at lower soil moisture levels than saturation, the water in the soil appears to be in an anoxic condition, and the oxygen supply to mesophyll cells becomes insufficient.

During respiration, carbohydrates undergo oxidation, converting carbon into CO_2. In the process, carbon's oxidation state changes from 0 to +4, releasing four electrons. Therefore, the low oxygen levels cause excess electrons to react with water molecules, leading to the production of H_2O_2 under excessively high soil moisture (Dumont and Rivoal, 2019).

5 CONCLUSIONS

The H_2O_2 concentration in the leaves of typical riparian herbs was investigated in response to soil moisture levels. Although H_2O_2 concentration varies widely below its highest values due to multiple factors, the peak concentration at each soil moisture level follows a species-specific pattern. In the photosynthetic system, solar energy is captured by photosystem II (PSII) and used to split water molecules into protons, electrons, and oxygen. Under sufficient CO_2 concentrations in the mesophyll, these electrons drive the conversion of CO_2 into carbohydrates. However, when stomatal closure limits CO_2 availability, surplus electrons are redirected to produce H_2O_2. During respiration, carbohydrates undergo oxidation, converting into CO_2. Under anoxic conditions, low oxygen levels cause excess electrons to react with water molecules, resulting in H_2O_2 production. In species with well-developed aeration systems in stems and rhizomes, such as *P. longoseta* and *F. japonica*, H_2O_2 concentrations, high at around 5% soil moisture, uniquely decrease as soil moisture increases, even up to saturation levels of water. Under high soil moisture conditions, the soil becomes anoxic, leading to an increase in H_2O_2 concentration. These understandings can aid in managing riparian vegetation and assessing habitat suitability.

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