

Effects of Soil Moisture Content on Protective Enzymes and Osmotic Adjustment Substances in *Phalaris* Seedlings (Postprint)

Authors: Cao Yun, Ji Xinsheng, Guo Zhichang, Wu Haiying

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Abstract

Using reed canary grass seedlings as experimental material, a 50-day indoor pot experiment with controlled water was conducted, establishing seven water gradients based on soil volumetric water content: 10% (soil water saturation), and 51% (flooded with 2 cm water), to investigate the effects of different soil water contents on plant antioxidant enzyme activity, malondialdehyde content, and soluble protein content. The results demonstrated: (1) Superoxide dismutase (SOD) activity was relatively high when soil water content ranged from 20% to 35%, decreasing when below 20% or above 35%, with SOD activity at 50 days exceeding that at 25 days across all soil water content treatments; (2) Catalase (CAT) and peroxidase (POD) activities were extremely significantly correlated ($p < 0.01$), both exhibiting a trend of sharp initial decline followed by stabilization and subsequent slight increase with rising soil water content; (3) Malondialdehyde (MDA) content displayed an opposite trend to SOD activity, continuously decreasing with increasing soil water content, though the flooded treatment showed a slight increase at 25 days; (4) Soluble protein content was relatively high between 20% and 40% soil water content, where plants exhibited better growth status, while decreasing under both drought and waterlogging stress conditions. These findings indicate that reed canary grass seedlings activate their antioxidant enzyme system to protect plant tissues when subjected to drought stress; however, prolonged stress or stress exceeding their tolerance threshold (soil water content $< 20\%$ or waterlogged conditions with soil water supersaturation) compromises the plant defense system to varying degrees, consequently affecting growth and development.

Full Text

Preamble

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Effects of Soil Moisture Content on Antioxidase and Osmoregulation Substrate Content of *Phalaris arundinacea* Seedlings

CAO Yun^{1, 2}, JI Xin-sheng¹, GUO Zhi-chang¹, WU Hai-ying¹

(1 College of Geography and Environment, Jiangxi Normal University, Nanchang 330022, Jiangxi, China;

2 Key Laboratory of Poyang Lake Wetland and Watershed Research, Ministry of Education, Jiangxi Normal University, Nanchang 330022, Jiangxi, China)

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Corresponding author: CAO Yun (1974-), E-mail: yun.cao@163.com

Abstract: Soil moisture is an important environmental factor that affects plant growth and physiological metabolism. Under water stress conditions, plants can activate protective enzyme/antioxidant enzyme systems and adjust the content of osmotic adjustment substances to respond to environmental challenges. To investigate the response mechanisms of the antioxidant enzyme defense system in *Phalaris arundinacea* to different soil moisture levels, we selected healthy *P. arundinacea* seedlings with well-preserved rhizomes and uniform germination as experimental materials. Through a 50-day indoor pot experiment, we established seven moisture gradients: $(10\pm 0.5)\pm 0.5\pm 0.5\pm 0.5\pm 0.5\pm 0.5\%$ (saturated soil moisture), and 51% (2 cm flooded), based on volumetric water content. Soil moisture was controlled through manual watering, measured daily at 20:00 using an HH2 soil moisture meter, with calculated water volumes added to maintain target levels. The effects of different soil moisture contents on malondialdehyde (MDA), superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), and soluble protein content were studied using the nitroblue tetrazolium method for SOD, guaiacol method for POD, potassium permanganate titration for CAT, thiobarbituric acid method for MDA, and Coomassie brilliant blue G-250 staining for soluble protein. The results showed: (1) SOD activity was higher when soil moisture content was between 20% and 35%, and decreased when moisture was below 20% or above 35%. SOD activities at 50 days were higher than those at 25 days across all treatments. (2) CAT and POD activities were extremely significantly correlated ($p < 0.01$), both showing an initial sharp decrease, followed by stabilization, then a slight increase with rising soil moisture content. (3) MDA content showed a trend opposite to SOD, decreasing with increasing soil moisture content. The C7 group (2 cm flooding) showed a slight increase at 25 days. (4) Soluble protein content was higher between 20%

and 40% moisture, where plants grew well, but showed a declining trend under drought or flooding stress. These findings indicate that when *P. arundinacea* seedlings experience drought or other stress, they trigger the antioxidant enzyme system to protect plant tissues. The antioxidant defense system enhances stress resistance and reduces or eliminates damage through coordinated protective mechanisms. However, long-term stress or conditions exceeding the plant's tolerance threshold (soil moisture <20% or oversaturated) will progressively compromise the defense system, affecting plant growth and development.

Keywords: *P. arundinacea*; antioxidant enzyme; malondialdehyde; soluble protein; soil water content

1. Introduction

Soil moisture is a critical environmental factor influencing plant growth and physiological metabolism. Under water stress conditions, plants initiate protective enzyme/antioxidant enzyme systems and adjust osmotic adjustment substance content to cope with environmental challenges [?]. Previous research has demonstrated that drought stress can enhance antioxidant enzyme activity in plants [?]. The antioxidant defense system comprises key enzymes including superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT), which work synergistically to protect plants from oxidative damage [?]. However, when stress exceeds the plant's tolerance limits, the defense system itself can be damaged, affecting growth and development [?].

2. Materials and Methods

2.1 Experimental Design and Plant Materials

The experiment utilized *Phalaris arundinacea* seedlings with well-developed rhizomes and uniform germination as test materials. Seven soil moisture treatments were established based on volumetric water content: $(10 \pm 0.5) \pm 0.5 \pm 0.5 \pm 0.5 \pm 0.5 \pm 0.5 \pm 0.5$ % (saturated), and 51% (2 cm flooded). Soil moisture was manually controlled and measured daily at 20:00 using an HH2 soil moisture meter. The required water volume was calculated based on current moisture status and quantitatively supplemented to maintain target levels throughout the 50-day experimental period.

2.2 Biochemical Measurements

Antioxidant enzyme activities and biochemical parameters were measured using standard protocols: SOD activity by the nitroblue tetrazolium method, POD activity by the guaiacol method, CAT activity by potassium permanganate titration, MDA content by the thiobarbituric acid method, and soluble protein

content by Coomassie brilliant blue G-250 staining [?]. Data were analyzed using Excel and SPSS 19.0 software. Pearson correlation analysis was used to examine relationships between variables, and LSD test was used for multiple comparisons.

3. Results and Discussion

3.1 Antioxidant Enzyme Responses

SOD activity exhibited distinct patterns across moisture gradients, with significantly higher values observed when soil moisture content ranged between 20% and 35%. When moisture levels fell below 20% or exceeded 35%, SOD activity decreased markedly. Furthermore, SOD activities measured at 50 days were consistently higher than those at 25 days across all treatments, indicating a temporal response to sustained moisture conditions.

CAT and POD activities demonstrated extremely significant correlation ($p < 0.01$). Both enzymes displayed similar response patterns: an initial sharp decline, followed by a period of stabilization, and subsequently a slight increase with rising soil moisture content. This coordinated response suggests functional complementarity within the antioxidant enzyme system [?].

3.2 MDA and Soluble Protein Content

MDA content showed a trend inverse to that of SOD, decreasing progressively with increasing soil moisture content. The flooded treatment group (C7, 2 cm flooding) exhibited a slight increase in MDA content at 25 days, suggesting early oxidative stress under waterlogging conditions [?].

Soluble protein content was highest when soil moisture ranged between 20% and 40%, corresponding to optimal growth conditions. Under both drought and flooding stress, soluble protein content declined, indicating impaired metabolic function and protein synthesis under extreme moisture conditions [?].

These results demonstrate that *P. arundinacea* seedlings activate their antioxidant enzyme defense system in response to water stress, enhancing stress resistance through coordinated protective mechanisms. However, prolonged stress or conditions exceeding the plant's tolerance threshold—specifically soil moisture content below 20% or above saturation—can progressively compromise the defense system, ultimately affecting growth and development [?]. The optimal soil moisture range for *P. arundinacea* appears to be 20-40%, where antioxidant systems function most effectively and soluble protein content supports healthy growth.

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