Effects of Graphene Oxide on Stress Physiology and Photosynthetic Characteristics of Perennial Ryegrass: Postprint

Authors: Hong Ying, Mao Jianyue, Zhao Shulan, Dorian

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Abstract

To investigate the effects of different concentrations of graphene oxide (GO) on the growth, physiology, and photosynthetic characteristics of perennial ryegrass, a pot experiment was conducted with 0, 10, 20, 30, 40, and 50 mg・g-1 GO added to the soil for perennial ryegrass cultivation. Plant growth indices, photosynthetic pigment contents, protective enzyme activities, malondialdehyde (MDA) content, leaf plasma membrane permeability, soluble protein content, and photosynthetic parameters were measured. The results showed that: 10 and 20 mg・g-1 GO treatments had no significant effect on perennial ryegrass growth; 30~50 mg・g-1 GO treatments inhibited perennial ryegrass growth, with plant height and biomass minimized at 50 mg・g-1 GO concentration, decreasing by 16.8% and 27.1% compared to the control, respectively. When GO concentration reached 30 mg・g-1, total chlorophyll and carotenoid contents decreased significantly, reaching the minimum under 50 mg・g-1 GO treatment. High-concentration GO treatments (40 and 50 mg・g-1) reduced net photosynthetic rate (Pn), stomatal conductance (Gs), and transpiration rate (Tr) in perennial ryegrass leaves, but increased intercellular CO2 concentration (Ci). Low-concentration GO treatments (10 and 20 mg・g-1) had no significant effect on protective enzyme activities, MDA content, leaf plasma membrane permeability, and soluble protein content, whereas high-concentration GO treatments (40 and 50 mg・g-1) significantly increased superoxide dismutase (SOD), peroxidase (POD), catalase (CAT) activities, MDA content, and leaf plasma membrane permeability, while soluble protein content decreased, indicating leaf damage. These results demonstrate that high concentrations of GO (40 and 50 mg・g-1) induce oxidative stress in perennial ryegrass, thereby inhibiting plant growth.

Full Text

Effects of Graphene Oxide on Stress Physiological and Photosynthetic Characteristics of Lolium perenne

HONG Ying, MAO Jianyue, ZHAO Shulan, DUO Li' an*

College of Life Sciences, Tianjin Normal University; Tianjin Key Laboratory of Animal and Plant Resistance, Tianjin 300387, China

Abstract

To investigate the effects of different concentrations of graphene oxide (GO) on the growth, physiological, and photosynthetic characteristics of perennial ryegrass, a pot experiment was conducted by culturing ryegrass in soil amended with GO at concentrations of 0, 10, 20, 30, 40, and 50 mg・g-1. Parameters including plant height, biomass, photosynthetic pigment contents, protective enzyme activities, malondialdehyde (MDA) content, leaf membrane permeability, soluble protein content, and photosynthetic parameters were measured. The results showed that 10 and 20 mg・g-1 GO treatments had no significant effects on perennial ryegrass growth. However, 30–50 mg・g-1 GO treatments inhibited growth, with plant height and biomass reaching minimum values under 50 mg・g-1 GO, decreasing by 16.8% and 27.1% compared with the control, respectively. Total chlorophyll and carotenoid contents decreased significantly when GO concentration reached 30 mg・g-1, with the lowest values observed at 50 mg・g-1. High GO concentrations (40 and 50 mg・g-1) decreased net photosynthetic rate (Pn), stomatal conductance (Gs), and transpiration rate (Tr) but increased intercellular CO2 concentration (Ci). Low GO concentrations (10 and 20 mg・g-1) had no significant effects on protective enzyme activities, MDA content, plasma membrane permeability, or soluble protein content, whereas high GO concentrations (40 and 50 mg・g-1) significantly increased superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) activities, MDA content, and plasma membrane permeability while decreasing soluble protein content and damaging plant leaves. These results indicate that high GO concentrations (40 and 50 mg・g-1) induce oxidative stress in perennial ryegrass, thereby inhibiting plant growth.

Keywords: Lolium perenne, graphene oxide, stress physiology, oxidative stress, photosynthetic characteristics

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As an important derivative of graphene, graphene oxide (GO) contains abundant oxygen-containing functional groups such as carboxyl, hydroxyl, and epoxy groups (Martín et al., 2019). Due to its excellent thermal and electrical conductivity and hydrophilicity, GO has found applications in numerous fields, including pollutant adsorption, energy development, and biomedicine (Chen et al., 2020b). However, with the rapid advancement of nanotechnology and increasing usage of nanomaterials, GO and other nanomaterials can be directly or indirectly released into the environment, posing unpredictable risks to organisms and ecosystems (Fadeel et al., 2018).

In recent years, the effects of GO on plant stress physiology and photosynthetic characteristics have become a focal point of research worldwide. Some studies have reported positive effects, where low doses of GO promoted seed germination and growth in spinach and leek (He et al., 2018), and its high specific surface area enabled strong adsorption capacity, reducing Cd2+ inhibition on maize growth at low concentrations (Yin et al., 2018). However, most research has demonstrated negative impacts of GO on plants (Chen et al., 2018b; Du et al., 2020; Weng et al., 2020). GO' s large specific surface area can increase cellular reactive oxygen species (ROS), which attack DNA, proteins, and cell membranes, leading to cellular damage and toxicity (Hu et al., 2014a; Jia et al., 2019). Additionally, GO' s sharp edges can cause physical damage to cell surfaces through collision (Sengupta et al., 2019).

Hao et al. (2018) found that reduced graphene oxide (rGO) at concentrations of 50 and 500 mg・kg-1 significantly decreased the aboveground dry weight of rice. The effects of GO on plants vary depending on its dosage, size, exposure duration, and plant species. Since plants play a dominant role in ecosystem composition, investigating the impacts of GO on plant growth, physiology, and photosynthetic characteristics is of great significance.

Currently, most studies on GO effects in terrestrial plants have employed hydroponic experiments, with few reports on soil pot experiments (Hao et al., 2018), despite the latter providing a better simulation of GO input into soil and its effects on plant growth. Therefore, this study selected perennial ryegrass as the test plant and added different concentrations of GO to potting soil to investigate the effects of GO on growth and physiological-photosynthetic characteristics of perennial ryegrass, thereby providing a theoretical basis for risk assessment and safe application of GO.

1.1 Materials

Perennial ryegrass seeds (cv. Accent) were purchased from Suqian Huiyi Seed Industry Co., Ltd. The experimental soil was collected from the 0–20 cm surface layer on the campus of Tianjin Normal University, with the following basic properties: pH 7.45, salinity 0.1%, organic matter content 4.68%, alkali-hydrolyzable nitrogen 137.42 mg・kg-1, available potassium 71.63 mg・kg-1, available phosphorus 22.03 mg・kg-1, saturated water content 0.56 mL・g-1, and bulk density 0.87 g・cm-3. Graphene oxide (GO) was purchased from Suzhou Hengqiu Graphene Technology Co., Ltd. as a brownish-yellow powder with an average thickness of 3.4–7 nm, flake diameter of 10–50 μm, and specific surface area of 100–300 m2・g-1.

1.2 Plant Cultivation

Plant cultivation began on July 1, 2019. Perennial ryegrass seeds were placed in trays moistened with distilled water for germination at room temperature. After germination, seedlings were transferred to soil amended with different GO concentrations, with 100 seeds sown per pot.

Specific amounts of GO (4.2, 8.4, 12.6, 16.8, and 21 g) were added to 420 g of non-sterilized soil and thoroughly mixed with a small spatula for 20 minutes, then placed in plastic pots (7 cm diameter, 8 cm height) to achieve GO concentrations of 10, 20, 30, 40, and 50 mg・g-1. A control treatment without GO (0 mg・g-1) was included, with four replicates per treatment. Plants were grown indoors with adequate water supply to maintain soil moisture at 60% of maximum water-holding capacity. Illumination was provided by natural light transmitted indoors (623–33,020 lx). During cultivation, pots were regularly repositioned to ensure consistent light exposure. The environmental conditions were 20–27 °C temperature and 17–54% relative humidity. The cultivation period lasted 40 days, and measurements were taken during the tillering stage of perennial ryegrass.

1.3.1 Growth Index Measurement

Plant height was measured 40 days after sowing by randomly selecting five plants from each pot and calculating the average. Aboveground biomass was determined at 40 days by harvesting the shoots at ground level, then oven-drying at

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