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Enhancing growth rate of lettuce by mutating lettuce seeds with nuclear irradiation

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ABSTRACT

To improve growth rate of hydroponics lettuce with CO_2 aeration, lettuce seeds were mutated by nuclear irradiation (γ rays from ^{137}Cs) and domesticated with high concentration of CO_2. When lettuce seeds were mutated with 30 Gy dosage, wet weight of lettuce increased by 22.3% (to 52.24 g) on the 30th day and grana volume in the chloroplasts of leaf cells increased by 244%. After the mutant (irradiated by 50 Gy dosage) was domesticated with elevated CO_2 concentration, wet weight of lettuce increased by 74.6% (to 48.7 g) on the 24th day under 3 vol% CO_2, compared with wet weight of lettuce under air condition. Results showed that mutagenesis by nuclear irradiation made it possible to improve CO_2 fixation rate in lettuce in a gene-modified way.

1. Introduction

Global warming caused by extensive CO_2 emissions has become a significant concern in both environmental and energy aspects [4]. Improving lettuce growth rate and biomass productivity, can not only enhance grain production, but also mitigate atmospheric CO_2, thus alleviating greenhouse effect. Traditional methods to improve lettuce growth rate and biomass productivity were limited to the improvement of external conditions, such as nutrient solution, light conditions, temperature and carbon dioxide ventilation. There was little research about improving lettuce productivity at genetic level.

At present, a lot of work was done on the enhancement of lettuce biomass yield. Santos et al. [16] showed that the vinasse solution (a nutritive solution composed of 10% vinasse supplemented with nutrients) promoted the growth of leaves in lettuce. Khoshgoftarmanesh et al. [8] proved that addition of Ni to the nutrient solution significantly increased the leaves and root growth. Mohammadi and Khoshgoftarmanesh [15] found that $Zn(Gly)_2$ was more effective than other Zn sources at improving root and shoot growth of lettuce at saline conditions. In addition, a lot of research reported the optimal illumination condition, temperature and CO_2 concentration for lettuce growth. Shen et al. [18] demonstrated that red–blue LEDs (RBL) and 24 h illumination time were the best for lettuce cultivation in a controlled environment. McKeehen et al. [13] found that nitrate content in lettuce leaves (a percentage of total NPK) decreased with the increase of CO_2 concentration. He et al. [7] found that elevating root-zone CO_2 could enhance leaf area, shoot and root productivities, because increasing root-zone CO_2 could alleviate midday depression of photosynthesis and thereby increase the productivity of lettuce plants. However, none of these methods changed the metabolic pathway by shifting lettuce genes, as a result, nothing was improved in the lettuce itself, improving CO_2 mitigation by lettuce was limited. Marcu et al. [12] found that an irradiation dose between of 2–30 Gy could enhance the growth parameters (final germination percentage, germination index, root and hypocotyl length), compared with untreated plants. However, the mutants were still cultivated under air condition after mutation, effects of elevated CO_2 concentration were not studied in the research.

Although changing gene expression by nuclear irradiation was adopted in the study of other plants such as fruits, wheat, and rosemary [5,9,10], it was not common in lettuce. Moreover, optimal CO_2 concentration for lettuce root-zone growth was unknown. To improve growth rate of lettuce at genetic level, lettuce seeds were irradiated by γ rays from ^{137}Cs and a mutant with higher biomass productivity was obtained. Then the lettuce mutant was cultivated at various CO_2 conditions to explore the optimal CO_2 concentration for lettuce. Results proved that it is a feasible way to improve CO_2 fixation rate of lettuce by irradiating lettuce seeds with γ rays from ^{137}Cs.

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2. Materials and methods

2.1. Mutation, breeding and screening of lettuce seeds

A total number of 800 lettuce seeds (Lactuca sativa L. cv. Hebei Vegetable Base, China) were irradiated with 20, 30, 40 and 50 Gy dosages of γ-rays from ^{137}Cs (200 lettuce seeds at each dosage) at a dose rate of 1 Gy min^{-1} in the Academy of Agricultural Sciences, Zhejiang Province, China. Theγ rays were generated from a ^{137}Cs source (a single linear source with a diameter of 52 mm and a length of 480 mm, the radiation intensity was 25,000 Ci) routinely stored in a double stainless steel protection layer filled with helium and moved out to irradiate microalgae cells in a special laboratory with thick walls (>1 m). Many genes in lettuce seed cells were broken down and recombined with high-energy free radicals activated by γ rays to dramatically accelerate natural evolution of cells themselves and generate non-directional mutants with special functions. These mutants which were not transgenic products between different species can be screened for targeted purposes (e.g. high growth rate) and directly cultivated in the field. After nuclear mutation, the mutants were basked for twelve hours and soaked for twelve hours to accelerate their germination speed. Afterwards, the mutants were all germinated in a nursery seedling plate (2.5 cm × 2.5 cm × 3.0 cm; width [W] × length [L] × height [H]) and hydroponically grown for 6 days in an environmentally controlled room. Temperature was 24 °C, light intensity was 2000 Lux with cool white fluorescent lamps. Uniform-sized seedlings of lettuce at 3-leaf stage were individually raised in hollow pots (12 cm × 20 cm; Diameter [D] × [H]) filled with 600 ml nutrient solution, exposed to air. The nutrient solution was as following: $Ca(NO_3)_2{\cdot}4H_2O$, 354 mg/L; $NH_4H_2PO_4$, 76 mg/L; $Fe{\cdot}(C_{10}H_{16}N_2O_8)$, 25 mg/L; KNO_3, 303 mg/L; $MgSO_4{\cdot}7H_2O$, 185 mg/L; $MnCl_2{\cdot}4H_2O$, 1 mg/L; H_3BO_3, 1.5 mg/L; $ZnSO_4{\cdot}7H_2O$, 0.09 mg/L; $CuSO_4{\cdot}5H_2O$, 0.04 mg/L; $NaMnO_4{\cdot}2H_2O$, 0.01 mg/L.

2.2. Ultrastructure observation of lettuce leaves

The mutants were cultivated in an artificial greenhouse at 24 °C with 24 h of illumination at 12,000 Lux. Illumination was provided by LED lamps. According to Shen et al. [18], red LED light (primary light source) and blue LED light was the optimal light source for nutrient accumulation in lettuce, so we used the red LED light and blue LED light as light source in this experiment. The proportion of red and blue light was 4:1 (Fig. 1). Fresh leaves were obtained from the eighteen-day-old plants mutated by γ rays. The diameter and thickness of grana lamella in the chloroplast of each leaf cell sample were observed under a transmission electron microscope (TEM, H-7650, Hitachi, Japan). The grana volume of chloroplast on each sample was calculated as following:

$$V = 4^{-1}\sum \pi D_i^2 * h_i \tag{1}$$

where D_i is the diameter of a grana lamella (μm), h_i is the thickness of a grana lamella and i = 1, 2, . . ., n (n is the number of grana visible on the section).

2.3. CO_2 fixation rate and growth indexes of the mutants

The initial volume of nutrient solution was 600 ml, the solution was replaced to replenish nutrient elements and water on day 24. The net photosynthetic rate was calculated every six days.

CO_2 fixation rate was calculated as following: the lettuce was transferred into a glass jar containing 400 ml of nutrient solution. A closed loop was made, consisted of a glass jar, a CO_2 gas analyzer (LI-840A, US) and a circulation pump. The temperature was kept at 25 °C and light intensity was 5000 Lux controlled by RBL. At the beginning, the glass jar was aerated with 1000 PPM CO_2 for twenty minutes to generate an initial CO_2 concentration of 1000 PPM, CO_2 fixation rate was the reducing speed of CO_2 concentration, which was measured by the CO_2 gas analyzer.

Leaf number, plant height, maximum leaf width and leaf chlorophyll content (measured by plant nutrition tester TYS – 3N, China) were measured every six days.

2.4. Cultivating the mutant (irradiated at 50 Gy dosage) under various concentration of CO_2

The lettuce mutant which was irradiated at 50 Gy dosage was seeded onto the nursery seedling plate and grown hydroponically

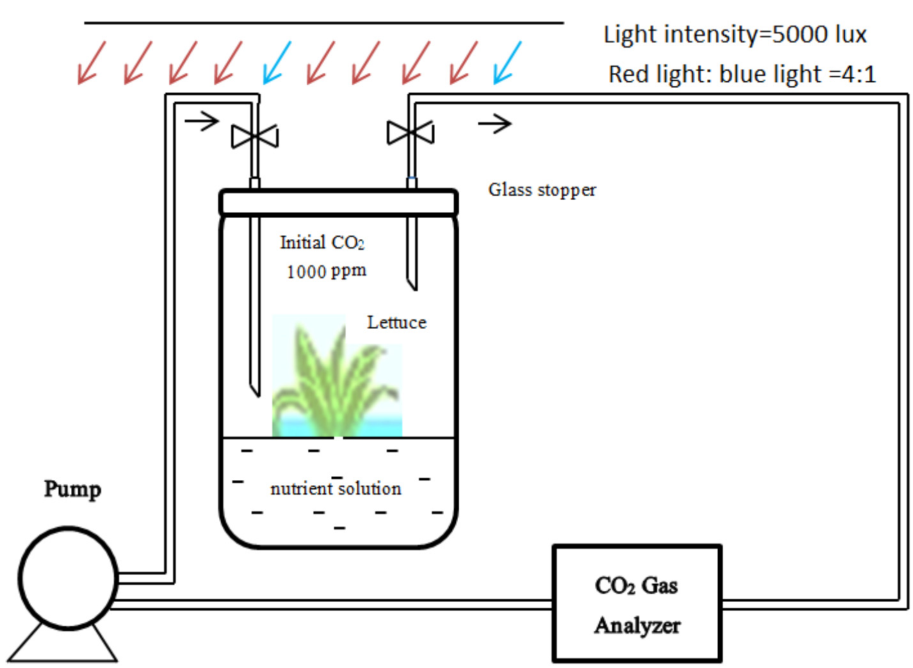

Fig. 1. Experimental apparatus for lettuce growth with CO_2 fixation.

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