Scientia Horticulturae 227 (2018) 117–123

Contents lists available at ScienceDirect

Scientia Horticulturae

journal homepage: www.elsevier.com/locate/scihorti

Research paper

Exogenous GABA application improves the NO_3^--N absorption and assimilation in $Ca(NO_3)_2$-treated muskmelon seedlings

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ARTICLE INFO

Keywords:
Cucumis melo L.
$Ca(NO_3)_2$ stress
N metabolism
γ-Aminobutyric acid
Plant growth

ABSTRACT

The potential of exogenous γ-aminobutyric acid (GABA) application to improve the NO_3^--N absorption and assimilation in $Ca(NO_3)_2$-treated muskmelon seedlings was investigated in 'Yipintianxia 208', a salt-sensitive melon cultivar grown in a deep-flow hydroponic system. Plants were treated under control or 80 mM $Ca(NO_3)_2$ stress conditions with or without foliar spraying 50 mM GABA. We found that under $Ca(NO_3)_2$ stress, the activities of nitrate reductase (NR), glutamate synthetase (GS), and glutamate amino-transferase (GOGAT) in muskmelon seedlings were significantly reduced, while the activities of glutamate dehydrogenase (GDH), glutamate oxaloacetate transaminase (GOT), and glutamate pyruvate aminotransferase (GPT) were enhanced. These changes lead to a decrease in the content of NO_3^--N and an increase in that of NH_4^+-N in muskmelon roots and leaves, which further severely inhibited the plant growth. Exogenous GABA application effectively improved the absorption of NO_3^--N in muskmelon roots and leaves under $Ca(NO_3)_2$ stress and the NH_4^+-N assimilation by enhancing the activities of nitrogen assimilation enzymes (NR, GS, and GOGAT) in muskmelon seedlings. Exogenous GABA also reduced the NH_4^+ release by limiting the GDH deamination, thus further alleviating the ammonia toxication induced by $Ca(NO_3)_2$ stress. Our results suggest that exogenous GABA applications can relieve the nitrogen metabolic disorders caused by $Ca(NO_3)_2$ stress and eventually promote plant growth. In addition, Exogenous GABA regulated the transaminase (GOT and GPT) activities, maintaining the balance of the amino acid metabolism. Furthermore, the accumulation of the functioning as a nitrogen source endogenous GABA was promoted, eventually improving the resistance of muskmelon seedlings to $Ca(NO_3)_2$ stress.

1. Introduction

The issue with the excessive application of nitrogen fertilizers is becoming increasingly prominent in greenhouse crop production in China, which leads to a serious waste of fertilizers and secondary salinization (Yuan et al., 2014). Muskmelon (*Cucumis melo* L.) is an important vegetable crop that is sensitive to salinity, which has consequently restricted the production of muskmelon in China in recent years.

Nitrate is a major source of the most important plant nutrient nitrogen (N), and thus its availability is a rate-limiting factor in the growth and development of many plant species (Masclaux-Daubresse et al., 2010; Hirel et al., 2011; Krouk et al., 2011; Andrews et al., 2013). Despite the existence of several forms of nitrogen, nitrate nitrogen (NO_3^--N) is the most bioavailable and most assimilated by plants. Calcium nitrate [$Ca(NO_3)_2$] is the main compound of nitrogen fertilizers. However, previous reports have shown that Ca^{2+} accounts for > 60% of the total cations, and NO_3^- accounts for 67%–76% of the total anions in secondary salinized soil (Li et al., 2004). Therefore, a high level of $Ca(NO_3)_2$ accumulation is one of the main characteristics of secondary salinization in Chinese agricultural greenhouses. Salinity could induce various biochemical and physiological responses in plants and affects almost all plant functions, including growth, physiological metabolism, and development (Li et al., 2010; Zhen et al., 2011, 2012; Yuan et al., 2014; Hu et al., 2015), which eventually severely reduces the productivity of greenhouse vegetables. Of these effects, the imbalance in the nitrogen metabolism is one of the most serious factors that limit plant growth and development (Surabhi et al., 2008; Piwpuan et al., 2013).

Using plant growth substrates to promote plant adaptation to salt

Abbreviations: GABA, γ-aminobutyric acid; NO_3^--N, nitrate nitrogen; NH_4^+-N, ammonium nitrogen; NR, nitrate reductase; GS, glutamate synthetase; GOGAT, glutamate amino transferase; GDH, glutamate dehydrogenase; GOT, glutamate oxaloacetate transaminase; GPT, glutamate pyruvate aminotransferase

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http://dx.doi.org/10.1016/j.scienta.2017.09.025
Received 9 May 2017; Received in revised form 20 September 2017; Accepted 24 September 2017

stress is one of the approaches to improve crop yields. Recently, considerable research effort has been dedicated to determining the effect of the application of exogenous γ-aminobutyric acid (GABA) on plant responses to saline conditions. Importantly, the use of exogenous GABA was found to be a critical strategy for increasing the salt tolerance of soybean (Yin et al., 2014), *Arabidopsis* (Renault et al., 2010), and tobacco (Zhang et al., 2011). The results of our previous study on muskmelon also suggest that exogenous GABA implementation improves plant adaptation to salt stress (Hu et al., 2015; Xiang et al., 2016).

γ-aminobutyric acid (GABA) is a four-carbon non-proteinamino acid that is found in animals, plants and bacteria. In animals, GABA is known as a major inhibitory neurotransmitter in vertebrates and functions in preventing certain forms of cancer and reducing the risk of cardiovascular diseases (AlWadei et al., 2011; Yang et al., 2013a). In plants, GABA is well recognized as an endogenous plant signaling molecule and involved in various physio-biochemical responses to biotic and abiotic stresses (Faës et al., 2015; Wang et al., 2014b). In addition, there has been special interest in GABA as a health related compound, and the tomato and soybean sprouts have being targeted for producing γ-aminobutyric acid (GABA)-enriched functional food (Saito et al., 2008; Guo et al., 2012).

Exogenous application of GABA promoted morphological growth, functioning of photosynthetic machinery, gas exchange capacities, chlorophyll biosynthesis, enzymatic, and non-antioxidant responses and membrane stabilization in tomato (Luo et al., 2011). GABA is also reported that can be used as a nitrogen source for the nitrogen metabolism, storage, and transportation (Barbosa et al., 2010). Although we previously reported that foliar spraying with 50 mM of GABA effectively improved muskmelon seedling tolerance to $Ca(NO_3)_2$ stress (Hu et al., 2015), little is known about the influence of exogenous GABA treatment on nitrogen metabolism in muskmelon seedlings experiencing salinity stress. The aims of the current study are to determine the impact of exogenous GABA application on NO_3^--N uptake and assimilation under $Ca(NO_3)_2$ stress and to elucidate the physiological mechanism of GABA-mediated tolerance to $Ca(NO_3)_2$ stress in muskmelon seedlings. The results of this study have important guiding significance in overcoming the soil secondary salinization and the promotion of more sustainable nitrogen fertilizer application.

2. Materials and methods

2.1. Plant material and experimental treatments

The experiment was conducted from March 2015 to August in a research greenhouse of the Northwest Agricultural and Forestry University in Northwest China (34°16′N, 108°4′E). A salt-sensitive muskmelon (*Cucumis melo* L.) cultivar, 'Yipintianxia No. 208′ was used as plant material. Seeds were surface sterilized in 10% Na_3PO_4 for 20 min, immersed in distilled water for 6 h, and germinated at 27 °C in the dark. After 2 d, germinated seeds were sown in washed commix medium (Xintiandi Co., Yangling, Shaanxi, China), and placed in a seedling greenhouse with an average day/night temperature of 26–30 °C/16–18 °C, a 12 h light and 12 h dark photoperiod, and 50–90% relative humidity. When the fourth leaves were fully expanded, all seedlings with equivalent growth and development were transplanted into troughs containing 40 L of half-strength Japan Yamazaki muskmelon special nutrient medium (pH 6.3 ± 0.1, electrical conductivity 1.3 ± 0.1 mS cm^{-1}), with 6 seedlings per trough and 24 thoughs. Seedlings fully expanded with four true leaves were grown in a nutrient medium with or without 80 mM $Ca(NO_3)_2$, which was administered in two steps of 40 mM $Ca(NO_3)_2$ per day to prevent salt shock. GABA was applied by daily spraying of the leaves with 50 mM of GABA dissolved in water at 08.00 h; this concentration was chosen based on our previous experiment (Hu et al., 2015). Four treatments were implemented: untreated control plants (CK), plants treated with GABA only (G), plants under $Ca(NO_3)_2$ stress only (S), and plants treated with GABA under $Ca(NO_3)_2$ stress (SG). Seedlings were treated with same amount of GABA and H_2O at 9:00 am every day. After seven days of stress, the third fully expanded leaf and roots were harvested for analysis and determination of nitrate nitrogen (NO_3^--N) and ammonium nitrogen (NH_4^+-N) contents, enzyme activities related to nitrogen assimilation, and endogenous GABA content. Six plants per treatment were harvested to measure plant growth. Each treatment was replicated three times with 12 plants per replicate (Hu et al., 2015).

2.2. Analysis of plant growth

The total fresh weight was determined by collecting leaves and roots, washing in sterile distilled water, blotting dry, and weighing. Dry weight was determined in shoots and roots prepared as above, which were dried in an oven at 75 °C for 72 h, and then weighed. The data of root and shoot fresh weight were further used to calculate the root/shoot ratio (root/shoot ratio = root fresh weight/shoot fresh weight). The leaf area was determined by scanning leaves with a desktop scanner (Epson Expression 1680, Heraeus Co. Ltd., Germany) and then using Image-J software to calculate the leaf area.

2.3. Determination of NO_3^--N and NH_4^+-N contents

Tissue samples were dried to constant weight in an air-forced oven at 80 °C. The dried material (200 mg) was ground to a powder and extracted in 10 mL of distilled water for 2.5 h. The NO_3^--N content was determined spectrophotometrically after mixing 0.2 mL of the solution with 10% (w/v) salicylic acid in 96% sulfuric acid. The values were quantified after generating a standard curve (Cataldo et al., 1975). The NH_4^+-N content was determined by the colorimetric assay described by Krom (1980).

2.4. Analysis of nitrogen assimilation enzyme and transaminase activities

Assay for determination of nitrate reductase (NR) activity was performed in accordance with the procedures described by Datta and Sharma (1999). The activity was assayed by monitoring the absorbance at 540 nm. The consumed NO_2^- was expressed as nmol per hour per milligram of protein.

Assay for glutamate synthetase (GS) activity was carried out using the method described by Brun et al. (1992). The GS activity was expressed as μmol γ-glutamyl-hydroxamate (γ-GHM) produced per hour per milligram of protein.

Glutamate amino-transferase (GOGAT) activity was evaluated as reported by Chiu and Shargool (1979). The activity was expressed as μmol NADH consumed per hour per milligram of protein based on the absorbance at 340 nm.

Glutamate dehydrogenase (GDH) activity was analyzed according to the method of Groat and Vance (1981) and expressed as μmol NADH consumed per hour per milligram of protein based on the absorbance at 340 nm.

In addition, the activities of glutamate oxaloacetate transaminase (GOT) and glutamate pyruvate aminotransferase (GPT) were determined by the method described by Liang et al. (2011). Aspartic acid was used in the assay for GOT activity determination, whereas alanine was utilized for the measurement of GPT activity. Enzyme activities were calculated by measuring their absorbance changes at 500 nm. The control treatments were conducted with inactivated enzymes using the same methods. GOT and GPT activities were calculated using a standard curve prepared with pyruvic acid, and expressed as μmol pyruvic acid formed per gram fresh weight for 30 min under assay conditions.

2.5. Analysis of endogenous GABA concentration

The concentration of GABA in the leaves and roots was estimated