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Moderate ammonium:nitrate alleviates low light intensity stress in mini Chinese cabbage seedling by regulating root architecture and photosynthesis

Linli Hu, Jihua Yu*, Weibiao Liao, Guobin Zhang, Jianming Xie, Jian Lv, Xuemei Xiao, Bingli Yang, Ruohan Zhou, Ruifang Bu

College of Horticulture, Gansu Agricultural University, Lanzhou 730070, PR China

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ABSTRACT

The hydroponic experiment was conducted to study the effects of ammonium (NH_4^+):nitrate (NO_3^-) ratios on growth, photosynthetic responses, chloroplast ultrastructure and root architecture of mini Chinese cabbage under low light intensity condition. Mini Chinese cabbage (*Brassica pekinensis*) seedlings were grown in pots (38 cm × 28 cm × 12 cm) in a greenhouse at photosynthetic photon flux density about 200 μmol m^{-2} s^{-1} (normal light intensity) or 100 μmol m^{-2} s^{-1} (low light intensity) of light intensity. Four ammonium:nitrate ratios were applied: (a) NH_4^+:NO_3^- = 0:100 (5 mM NO_3^-), (b) NH_4^+:NO_3^- = 10:90 (0.5 mM NH_4^+ + 4.5 mM NO_3^-), (c) NH_4^+:NO_3^- = 15:85 (0.75 mM NH_4^+ + 4.25 mM NO_3^-), and (d) NH_4^+:NO_3^- = 25:75 (1.25 mM NH_4^+ + 3.75 mM NO_3^-). Two weeks after treatments started, under normal light, plants treated with 0.75 mM NH_4^+ + 4.25 mM NO_3^- exhibited significantly promoted growth. Simultaneously, these plants showed significantly higher degree of granal stacking and photosynthesis, as well as larger absorption area of root system compared with plants fed with 5 mM NO_3^-. Under shading condition, plants fertilized with 0.5 mM NH_4^+ + 4.5 mM NO_3^- resulted in better growth and had intact chloroplast ultrastructure and a higher degree of granal stacking, chlorophyll contents and net photosynthetic rate, as well as larger root system. Our results indicate that compared with nitrate, adding moderate ammonium concentration in the nutrient could alleviate low light intensity stress in mini Chinese cabbage seedling by regulating root architecture and photosynthesis. The challenge is to determine how to manage ammonium:nitrate ratio according to the various light intensities during plant growth process.

1. Introduction

Light is a predominant source of energy for plant photosynthesis and also an important signal for plant growth and development. It is well known that plants are not only able to respond to light quality but also able to its intensity (Fu et al., 2012; Tu et al., 2012; Wang et al., 2009). Plants in greenhouse are always affected by low light intensity. The light intensity of greenhouse is about 85–150 μmol m^{-2} s^{-1} in higher latitudes during winter seasons. Moreover, as a consequence of recent haze weather, diming and shading have become major challenges to vegetable production in many areas of the world (Mu et al., 2010). Previous studies had shown that weak light or shading could significantly affect growth and development of plants, and hence decreased the yield and quality of plants (Ilić et al., 2012; Li et al., 2010; Mauro et al., 2011; Tabatabaei et al., 2008). Therefore, light intensity is possibly the most noticeable environmental variable with which plants must cope. Finding the technology or method to alleviate detrimental effects of weak light on plants has become a central issue in the field of crop or vegetable production.

It is well known that different plants could endure different light intensity stresses. The solanaceous vegetables were mostly preferred-light vegetables. The light intensity of these vegetables (cucumber, pepper, tomato, etc.) and some crop species (rice, maize, wheat, etc.) considered as stress was about 100 μmol m^{-2} s^{-1} (Bi et al., 2015; Huang et al., 2013). While the leafy vegetables were the shading-tolerated style, so this type could suffer lower light intensity. Fu et al. (2012) investigated effects of different light intensities on chlorophyll fluorescence characteristics and yield in lettuce. They found that lettuce in the 100 and

* Corresponding author. Tel.: +86 13609308343.
E-mail addresses: yujihuagg@163.com, yujihua@gsau.edu.cn (J. Yu).

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800 μmol m^{-2} s^{-1} treatments had lower light use efficiency and yield than in the 200, 400 and 600 μmol m^{-2} s^{-1} treatments.

Nitrogen (N) is one of the nutrients that exert the greatest influence on plant growth and development under different environmental conditions (Cao and Tibbitts, 1993). The form of inorganic nitrogen (nitrate or ammonium) available to plant is another vital environmental variable affecting plant growth (Woolfolk and Friend, 2003). Lots of studies have suggested that plant growth and development were remarkably affected by different N forms, and choosing N forms were dependent on the plant species used. Some upland plants have a preference for nitrate nutrient (Guo et al., 2002; Raab and Terry, 1994). These plants would be subjected to ammonium toxicity when fed with high ammonium concentration. However, there are some ammonium-preferred plants such as rice, having a higher capacity of ammonium assimilation than other plant species (Guo et al., 2007a,b). It has been reported in previous studies that different nitrogen sources could clearly affect growth, yield and quality of many plant species by affecting nitrogen and water-use efficiency (Yin and Raven, 1998), nitrogen metabolism (Tabatabaei et al., 2008), mineral nutrient absorption (Helali et al., 2010; Serna et al., 1992), hormone content (Lu et al., 2009), biomass partitioning (Silva et al., 2013), and photosynthesis (Guo et al., 2006; Tabatabaei et al., 2006). Not only plant shoot growth but also root proliferation may be affected by different nitrogen forms. Some evidences showed the availability of N can strongly regulate root branching, whereas it has a limited effect on primary root growth (Chapman et al., 2011; Lima et al., 2010; Zhang and Forde, 2000). Nitrate (NO_3^-) was reported to significantly affect root growth and development (Gojon et al., 2011; Zhang and Forde, 2000). The extra evidence indicated that NO_3^- and ammonium (NH_4^+) have complementary effects on root architecture when NH_4^+ and NO_3^- were supplied together (Lima et al., 2010). As we known, the optimal proportions of NO_3^- to NH_4^+ for plant growth are related not only to plant species, developmental stage and the total concentration of supplied nitrogen but also to environmental conditions (Serna et al., 1992; Tabatabaei et al., 2008). NH_4^+ has been suggested to play a role in stress tolerance including drought, higher CO_2 concentration, salinity, heavy metals, alkalinity and disease. NH_4^+ was reported to be beneficial to growth and photosynthesis of rice seedlings under drought condition (Guo et al., 2007a; Li et al., 2009), mainly by increasing of the carboxylation efficiency and Rubisco activity. As demonstrated by Cruz et al. (2014), increasing NH_4^+ in the nutrient significantly increased photosynthetic acclimation to CO_2 under elevated CO_2 at beginning growth stage of cassava. Moreover, NH_4^+ could increase photosynthetic rate of sunflower plants under salinity condition (Ashraf, 1999). Furthermore, ammonium nutrition with nitrification inhibitors could improve heavy metals phytoextraction by protecting bulk soil from acidification and presumably from metal leaching (Zaccheo et al., 2006). In addition, there was a counteraction of the bicarbonate-induced growth suppression in strawberry treated with NH_4^+ and NO_3^- simultaneously by increasing SPAD values and quantum yield of PSII photochemistry (F_v/F_m) (Roosta, 2014). There is also evidence that disease severity of Fusarium wilt in tomato was reduced by the action of T34 under increasing concentrations of ammonia (Borrero et al., 2012). Tabatabaei et al. (2008) studied the effects of shading and NH_4^+:NO_3^- ratio on yield, quality and N metabolism in strawberry. They found that the biggest fruits were obtained under the 25:75 and 50:50 (NH_4^+:NO_3^-) treatments in both shaded and unshaded plants. Until now, many studies have focused on the effects of NO_3^- and NH_4^+ fertilization on plant photosynthetic physiology, one of the most important metabolic processes affected by N form (Cruz et al., 2014; Guo et al., 2007a,b). However, there is little literature concerning the alleviation role of NH_4^+:NO_3^- in plant photosynthetic responses, chloroplast ultrastructure and root architecture under low light stress.

Mini Chinese cabbage (*Brassica pekinensis*) originated in China and is one of the most important Brassica vegetables world-wide. The worldwide demand for mini Chinese cabbage grown in plateau is increasing because of its fresh appearance, special crisp texture, and richness in phytochemicals such as carotenoid, vitamin C and zinc element. The annual products from plateau section were exported to Japan, Korea, Canada, the European, Hong Kong and other countries or regions. As a cool season vegetable, mini Chinese cabbage is widely cultivated in greenhouse. They are frequently suffered from low light intensity in the process of growth, especially when cultivated during winter months. This might alter the uptake of N form. Consequently, the adjustment of the ammonium:nitrate ratio in the nutrient solution in accordance with the light intensity should be crucial. Up to now, few studies demonstrated a nutrition-dependent tolerance to light stress of plants. Thus, the effects of NH_4^+:NO_3^- ratio and low light intensity on growth, gas exchange, chloroplast ultrastructure and root architecture of mini Chinese cabbage seedlings has been studied here. The object of this study was to evaluate the alleviation role of NH_4^+:NO_3^- ratio in the growth of mini Chinese cabbage under weak light stress.

2. Materials and methods

2.1. Plant material and growth conditions

The experiment was conducted in a modern climate-controlled greenhouse located at Gansu Agricultural University in Lanzhou, Gansu (northwest China). Mini Chinese cabbage (*Brassica pekinensis* cv. "Jinwa no. 2") seeds were obtained from Gansu Academy of Agricultural Sciences. The seeds were soaked in water for 2 h then placed on moist filter paper in petri dish, and finally kept in the dark to germinate at 25 °C for 16 h. After germination, uniformly geminated seeds were sown in pots filled with clean quartz sand and cultured with half-strength whole Hoagland's nutrient solution for ten days. At the two-leaf stage, uniform seedlings were transferred to containers (38 cm × 28 cm × 12 cm) with 6 L nutrient solutions and aerated intermittently (1 h at 4 h intervals). Seedlings were attached to a plastic plate with a strip of sponge and grown in a greenhouse at 25 ± 2 °C/18 ± 2 °C day/night temperature.

2.2. Shading and NH_4^+:NO_3^- ratios treatments

The plants were subjected to two light intensity treatments (normal and low light intensity) simulated by sunshade nets and incandescent lamps and the photoperiod adjusted to 12 h/12 h day/night with an interval timer. Light intensity under normal and shading conditions was measured with the illuminometer (TES-1332A Electrical Electronic Corp). The average of light intensity under normal light intensity and shading condition were about 200 μmol m^{-2} s^{-1} and 100 μmol m^{-2} s^{-1}, respectively. Treatments commenced after transplanting mini Chinese cabbage seedlings to ammonium:nitrate solutions.

The plants under two light intensity levels were supplied with one of the following NH_4^+:NO_3^- ratios: 0:100, 10:90, 15:85 and 25:75 (for composition, Table 1). The ratios were selected based on the results of a preliminary experiment. The composition of the nutrition solution was as follows: 5 mM N, using $Ca(NO_3)_2{\cdot}4H_2O$, KNO_3 as NO_3^-–N and using $(NH_4)_2SO_4$ as NH_4^+–N, 1 mM P as KH_2PO_4, 3 mM K as KNO_3, K_2SO_4 and KH_2PO_4, 1.5 mM Ca as $Ca(NO_3)_2{\cdot}4H_2O$, $CaSO_4{\cdot}2H_2O$ and $CaCl_2$, 2 mM Mg as $MgSO_4{\cdot}7H_2O$, plus standard micronutrients referred to Hoagland and Arnon (1950). Nitrification inhibitor (DCD, 7 μmol L^{-1}) was added to every container. The initial pH of the nutrient solutions including NH_4^+ and NO_3^- was adjusted to 6.5–7.0 by adding 0.1 M HCl or NaOH. The