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Full Length Research Paper

Effect of 5-aminolevulinic acid on yield and quality of lettuce in sunlit greenhouse

Fuli Xu[1,2], Weiling Wang[1]* and Dan Yu[1]

[1]College of Life Science, Northwest A and F University, Yanling, China.
[2]Institute of Soil and Water Conservation, Chinese Academy of Sciences, Yangling, China.

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The role of 5-aminolevulinic acid (ALA) as a precursor of chlorophyll and heme is well documented. Low concentration of exogenous ALA has been found to regulate plant growth and increase crop yield, but there is little information on how ALA influences the yield and quality of lettuce in sunlit greenhouse. Here, we report the effects of ALA on photosynthetic rate, yield and quality of lettuce in sunlit greenhouse.5-aminolevulinic acid and 5-aminolevulinic acid with nitrogen fertilizer (ALA+N) were applied by foliage and soil. The results showed that application of ALA improved the photosynthetic rate of lettuce leaves by 23.9 to 34.7% and by 35.3 to 41.6%. Moreover, exogenous ALA increased vitamin C and soluble sugar content, reduced nitrate and crude fiber content and lead to better quality and taste of lettuce.

Key words: 5-aminolevulinic acid, lettuce, plant growth, promotive effects, yield, vegetable quality.

INTRODUCTION

Plant growth regulators (PGRs) are widely applied on crops as a means of improving productivity, increasing stress resistance and regulating plant growth (Ke-fu et al., 1986; Ramesh and Kumar, 1975; Watanabe et al., 2000; Zhang et al., 2006). 5-Aminolevulinic acid (ALA) is a biosynthetic precursor of porphyrins, such as chlorophyll and heme (Von Wettstein et al., 1995). ALA is effective in increasing stress resistance of plants (Watanabe et al., 2000; Zhang et al., 2008). It is involve in photosynthesis and respiration in higher plants (Wang et al., 2004; Youssef and Mohamed, 2008; Naeem et al., 2010). Studies have shown that ALA is not simply an intermediate of metabolism in plants. High concentration of exogenous ALA can be used as non-polluting, non-residual photosensitive herbicides; in low concentration, it can regulate plant growth and development, increase productivity, and enhance plant resistance (Watanabe et al., 2000). Thus, ALA has an important potential to be applied in agricultural production. This growth promotion is probably related to the photosynthesis-enhancing effects of ALA, which have been demonstrated in various plant species. In this work, we report the effect of application of ALA and ALA+N on the physiological characteristics, yield and quality of lettuce (*Lactuca sativa* L.), and explore the efficiency of various application methods of exogenous ALA in vegetables grown in sunlit greenhouse.

*Corresponding author. E-mail : ylwwl@nwsuaf.edu.cn.

MATERIALS AND METHODS

Study site description

The field experiments were carried out on a typical vegetable production farm in Jingjiawan County, Hezhuangping Town, Baota District, Yan'an City,Shaanxi Province china (36°39′29″N, 109°26′25.3″E, Elevation 987 m). The soil parent material is loess and loessial soils account for > 90% of the region's soils (Gang Liu et al., 2011). A plastic greenhouse 55 m in length and 7.5 m in width was constructed with a steel frame structure in 1999. The preceding crop was tomato. The fertilization rate was 617 kg N/ha and the manure rate was 27,000 kg/ha. During November 2006 to

February 2007, a randomised complete block design field experiment was carried out on a typical vegetable production farm in Jingjiawan County, Hezhuangping Town, Baota District, Yan'an City, in north of Shaanxi province, where the typical loess hill-gullied region of Loess Plateau is (36°39′29″N,109°26′25.3″E, altitude 987 m). The soil characteristics were: organic matter (6.332 g/kg), total N (0.401 g/kg), available N (135.6 mg/kg), total P (0.611 g/kg), available P (39.4 mg/kg), available K (76.11 mg/kg).

Seeds of the lettuce cultivar Star were sown in 6.5 m^2 (1.2 m × 5.5 m) plots. The crops were fertilized at sowing with 134 t/ha of organic fertilizer (cattle manure containing total N 4.216 g/kg, available N 154.8 mg/kg, total phosphorus 2.11 g/kg, available P 325.1 mg/kg, available K 3299 mg/kg), urea 1340 kg /ha, phosphoric acid diamine 900 kg /ha. ALA was applied once every 15 days (4 times) from seedling establishment to harvest at the rate of 3000 L/ha. Four treatments with four replications were set up:T1,Untreated control;T2, ALA (application);T3,ALA+N (ALA with nitrogen fertilizer, foliar application); T4:ALA (soil application).Method for foliar application: first spray 1kg/ha, 2 to 4 times 800 g/ha, diluted 800-fold; ALA + N 1st 5kg/ha 2 to 4 times 3 kg/ha, diluted 800-fold. Method for soil application: first application 2 kg/ha, 2 to 4 times 1.2 kg/ha.

Data acquisition

Throughout the growth process of lettuce, from planting to harvest, leaf chlorophyll content (CCI = Chlorophyll Content Index)were measured three times by chlorophyll meter(CCM-200), photosynthetic rate (Pn, μmol m^{-2} s^{-1}) was measured by LC pro+ portable photosynthesis open systems. Lettuce were harvested and weighted for each plot, and vitamin C, soluble sugar, crude fiber leaves, nitrate content in plants were determined. Vitamin C was measured by 2,6–dichloro indophenol titration method, soluble sugar, crude fiber content of leaves were measured by anthrone colorimetric method, nitrate content was measured by UV Spectrophotometer .

Data analysis

For statistical analysis of the data collected, the general linear model developed by the SAS Institute (version 9.1; Cary, NC) was constructed, and means were compared using the least significant difference (LSD) method; P ≤0.05 and P ≤0.01 were considered significant.

RESULTS

Effect of ALA application on the photosynthetic rate of lettuce

Photosynthetic rate is the most intuitive parameter of plant growth, which can directly reflect the assimilation capacity of crops (Naeem et al., 2010). To elucidate the physiological effects of ALA on lettuce, photosynthetic rate was measured throughout the whole growth process. The photosynthetic rate of lettuce leaves has improved on application of ALA by different methods. The maximum photosynthetic improvement is ca. 34.7% (P<0.01) using ALA+N (foliar application) compared with control, supply of ALA without N also increased photosynthetic rate by 23.9 and 25.8% (P<0.01), respectively. However, values did not significantly differ between different treatments. This indicated that foliar application and soil application with ALA had similar effect in photosynthetic activity (Figure 1).

Effect of ALA application on the chlorophyll content of lettuce

Chlorophyll content of lettuce was measured three times in this experiment. It can be seen that total chlorophyll content was gradually increased with the frequency of ALA application, and the average chlorophyll content of all treatments were significantly higher than the control, ALA+N Treatment showed that ALA +N was more effective in promoting chlorophyll content contrasted with simply application of ALA in lettuce (Table 1)

Effect of application of ALA on the yield of lettuce

The effect of ALA application on yield of lettuce was tested. Applying exogenous ALA through both foliage and root uptake from soil significantly promoted lettuce productivity. Compared with the control, the yield of lettuce treated with ALA (foliar application), ALA+N, ALA (soil application) increased by 38.8, 41.6, 35.3%, respectively. There was no significant difference of the values between foliar application and soil application, which was consistent with the results about the photosynthetic rate (Table 2).

Effect of application of ALA on the quality of lettuce

As can be seen from Table 3, compared with the control, exogenous ALA application increased vitamin C content of lettuce by 4.4 to 17.0%. Foliar ALA spray treatments increased in the soluble sugar content (7.85 and 10.6%). Moreover, treated with ALA+N, lettuce had 15.4% lower value for the lowest crude fiber content than the control. Application of only ALA by foliage and soil also significantly reduced crude fiber content by 12.8 and 8.3%, respectively. The NO^{3-} content in lettuce leaves was reduced by 11.0 to 15.3% after ALA application.

DISCUSSION

ALA is a key precursor in the biosynthesis of tetrapyrroles, such as chlorophyll and heme. ALA has been shown to be involved in regulating plant growth and development processes, but ALA levels are maintained at very low concentrations *in vivo* (Bindu and Vivekanandan, 1998; Kefu et al., 1986; Ramesh and Kumar, 1975; Watanabe

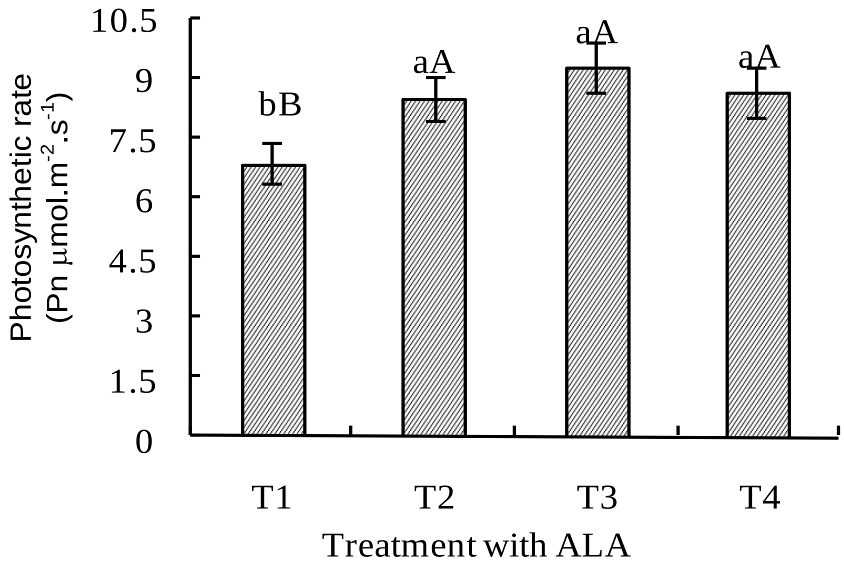

Figure 1. Effect of ALA application on photosynthetic rate of lettuce.

Table 1. Effect of application of ALA on the chlorophyll of lettuce.

Treatment	Test time			
	2007/01/05	2007/01/19	2007/02/03	Average(CCI)
T1	6.94 ± 0.35^{aA}	7.34 ± 0.35^{bB}	7.26 ± 0.23^{bB}	7.18^{bB}
T2	7.31 ± 0.42^{aA}	8.30 ± 0.42^{aAB}	8.73 ± 0.31^{aA}	8.11^{aA}
T3	7.19 ± 0.37^{aA}	8.64 ± 0.31^{aA}	9.03 ± 0.22^{aA}	8.29^{aA}
T4	7.36 ± 0.18^{aA}	8.62 ± 0.34^{aA}	8.80 ± 0.33^{aA}	8.26^{aA}

Note: a, b indicated P< 0.05; A, B indicated P<0.01.

Table 2. The yield of lettuce with the application of ALA.

Treatment	Plant height (cm)	Leaf width (cm)	Leaf length (cm)	Mean yield (kg/m^2)	Yield add (%)
A	26.1 ± 1.3^{bB}	3.55 ± 0.21^{bB}	23.5 ± 1.32^{bB}	4.33 ± 0.23^{bB}	0
B	32.2 ± 2.1^{aA}	4.46 ± 0.32^{aA}	29.7 ± 1.65^{aA}	6.01 ± 0.32^{aA}	38.80
C	31.5 ± 2.5^{aA}	4.68 ± 0.35^{aA}	28.6 ± 2.26^{aAB}	6.13 ± 0.29^{aA}	41.60
D	31.8 ± 1.7^{aA}	4.52 ± 0.41^{aA}	29.3 ± 2.03^{aA}	5.86 ± 0.41^{aA}	35.30

Note: a, b indicated P< 0.05; A, B indicated P< 0.01.

Table 3. Quality of leaf-used lettuce with application of ALA.

Treatment	Vc (mg/kg)	Add (%)	Disaccharide (mg/kg)	Add (%)	Fiber(mg/kg)	Add (%)	NO_3^-(mg/kg)	Add (%)
T1	20.38 ± 1.01^{bA}	-	8.57 ± 0.41^{bA}	-	15.6 ± 0.8^{aA}	-	209 ± 11^{aA}	-
T2	21.27 ± 1.12^{abA}	4.40	9.15 ± 0.39^{abA}	7.8	13.6 ± 0.6^{bB}	-12.8	186 ± 13^{abA}	-11.0
T3	23.84 ± 2.13^{aA}	17.00	9.48 ± 0.44^{aA}	10.6	13.2 ± 0.8^{bB}	-15.4	177 ± 15^{bA}	-15.3
T4	23.40 ± 1.54^{aA}	14.80	8.49 ± 0.37b^{A}	-0.1	14.3 ± 0.7^{abAB}	-8.3	182 ± 18^{abA}	-12.9

Note: a, b indicated P< 0.05; A, B indicated P< 0.01.

et al., 2000; Zhang et al., 2006; Watanabe et al., 2000). ALA has been reported also in relation to chlorophyll biosynthesis and plant greening. ALA formation is the rate limiting step in chlorophyll biosynthesis (Ayumi et al., 1994). Study on *Euonymus japonicus* shows that in greening leaves, the ALA synthesis activity is 2 times higher in green parts than in white part. In this study, exogenous ALA remarkably enhanced the chlorophyll content of lettuce (Table 1), this is similar to Hotta's findings on several vegetables.

Large numbers of experiments show that photosynthesis and crop yield is closely related (Castelfranco and Beale, 1983; Hotta et al., 1997). It was found that application of exogenous ALA significantly improved photosynthetic efficiency of lettuce leaves by 23.9 to 34.7% (Figure 1). Furthermore, applying ALA and ALA + N, yield of lettuce was significantly higher than the control (Table 2). And ALA+N (foliar application) showed the maximum promotive effect in both photosynthesis and yield. This is not only explained by the role of ALA, but also because of the provided additional nitrogen source.

Exogenous ALA has been found to be able also to enhance the quality of some medicinal plants (Xu and Zhu, 2010; Xu and Cheng, 2011). When living standards rise, consumers have increasing requirements in high quality vegetables. Therefore, both high yield and good quality should be considered for the cultivation of vegetable. In this work, besides photosynthetic rate, chlorophyll content and yield, exogenous ALA and ALA + N also improved the quality of lettuce (Table 3). Its content is considered to be a relevant indicator for vegetable safety. Lower nitrate and crude fiber content can improve the quality of lettuce.

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