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The effects of ploy (γ-glutamic acid) on spinach productivity and nitrogen use efficiency in North-West China

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ABSTRACT

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Recently, with the problem of low utilization rate of nitrogen fertilizers in farmland, agriculture practices have shifted towards the development of environmentally friendly nitrogen fertilizers. Ploy (γ-glutamic acid) is a new plant growth regulator with characteristics of water and fertilizer conservation. In this study, pot experiments were conducted to investigate the effects of ploy (γ-PGA) on the yield, nitrogen use efficiency and soil aggregate of spinach (*Spinacia oleracea* L.). The results indicated that γ-PGA (0.1%) increased plant growth (as measured by fresh and dry plant weight). However, increasing γ-PGA significantly decreased spinach quality parameters. The nitrogen utilization and use efficiency were increased with increasing γ-PGA. Ploy was stronger in water coagulation which effectively increased soil porosity and improved soil structure. The results suggest that 0.1% of γ-PGA has positive effects on spinach growth.

Keywords: soil amelioration; spinach N uptake; spinach yield; N fertilization; soil water-stability of aggregates

In recent years, several studies with various new materials were developed to improve soil structure and restoration. Although various soil remediation technologies have contributed to the remediation of contaminated soil, there are many problems such as high cost of remediation, long period of remediation, and soil secondary pollution (Li et al. 2011, Lu et al. 2011). Additionally, spinach is one of the major leafy vegetables, which is widely cultivated in spring, autumn, and winter seasons (Abdelraouf 2016). The global planting area for spinach was reported to reach 893 494 ha with 14 044 816 t of spinach production in 2007 (FAO Statistical Yearbook 2008).

Ploy (γ-glutamic acid, γ-PGA) is a polyamino acid consisting of L-and D-glutamic acids linked by γ-amide bonds (Shih and Van 2011). Ploy is a water-soluble biopolymer material which is biodegradable and can be synthesized by a variety of microorganisms. Furthermore, ploy has good water retention, adsorption, biodegradability and biocompatibility. Moreover, ploy was first isolated from the cellular

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membrane of *Bacillus anthracis* by Ivanovics in 1937 (Ivanovics and Bruckner 1937a,b). Ploy has strong water absorbability and high viscosity of water solution because there is a large amount of dissociate carboxyl on the molecular side chain of γ-PGA (Zhang 2014). Recently, ploy was used in hydrogel, humectant, thickener, film-forming agent, dispersant, genophore, cosmetics, fertilizer synergist and food additives (Wang and Xu 2014).

Nitrogen is one of the most important nutrients limiting plant growth (Mengel and Kirkby 1980). Currently, nitrogen use efficiency (NUE) was at a low level, and the average utilization rate of nitrogen fertilizer in field crops in the whole country is only about 30% in China (Zhu 2008). Therefore, improving the NUE, reduction of nitrogen leaching, prolonging fertilizer efficiency and reducing environmental pollution have become the key problems, needed to be resolved in the future. Chuai et al. (2016) repeated that γ-glutamic acid urea could accelerate the release of nitrogen, slow down the loss of nitrogen, and significantly increase the fresh weight and dry weight and root length of pak choi. Thus, the objective of this study is to evaluate the effects of ploy on growth, yield, and quality of spinach and soil structure.

MATERIAL AND METHODS

Site description. Field experiments were conducted in the Agricultural Water and Soil Test field of the Xi'an University of Technology at Xi'an, Shaanxi province in north-west China (34°27'N, 108°95'E, and elevation of 415 m a.s.l.). Soil texture is silt loam (clay (< 0.002 mm) 13.58%, silt (0.002~0.02 mm) 71.55% and sand (0.02~2 mm) 14.87%) and pH is 7.5, electrical conductivity is 167 µs/cm, containing 1.6 g/kg total nitrogen, 1.22 g/kg total phosphorus and 40.21 g/kg total potassium. The soil field capacity moisture was 33.92% and the bulk density was 1.21 g/cm^3.

The spinach (cv. Lvjian 2618) seeds were manually sown on October 18, 2017 in the soil of a pot (80 × 80 × 40 cm), and harvested on March 7, 2018. The number of seeds per pot was 30, the amount of soil per pot was 309.76 kg, pouring enough water and application of basal fertilizer (90 kg N/ha of fertilizer, 39.6 kg P/ha as monoammonium phosphate and 74.7 kg K/ha as potassium sulfate) into the soil at depth of about 10 cm before sowing. The rates of emergence of CK, No. 1, No. 2, No. 3 and No. 4 were 73.33, 96.67, 76.67, 73.33 and 70.00%, respectively. The planting density of CK, No. 1, No. 2, No. 3 and No. 4 was 343 750, 453 125, 359 375, 343 750 and 328 125 plants/ha, respectively. The maximum and minimum air temperature were 26°C and –1°C, in 2017 to 2018 growing season, respectively.

Experimental design. The experiments were set in a completely randomized design with three replications. The ploy was mixed into the soil at a proportion of 0.0, 0.1, 0.2, 0.3 and 0.4%. The number of pots was 15. The N fertilization was top-dressed on each pot after 22, 40 and 91 days from planting. The plants were flood-irrigated and fertilized by topdressing with 300 kg/ha urea. All treatments had the same irrigation quota with 60 m^3/ha and the irrigation interval of 7 days was used for the experiment. All experiment pots were placed under canopy (pervious to light) without regard for precipitation.

Soil sampling and analysis. The spinach was harvested 140 days after planting and weighed. The leaf area index (LAI) was derived from the leaf area of one plant obtained with a LI-3100 Area Meter (LI-COR; Lincoln, USA) (Abdelraouf 2016). The fresh weight (FW); dry weight (DW); moisture content and dry matter content of plant were calculated. The yields (fresh and dry matter weights in t/ha) were also calculated. The total nitrogen values of leaf, stem and root were analysed after harvesting by improved H_2SO_4-H_2O_2 digestion method (Li et al. 2014) at the Northwest Agriculture and Forestry University in China. The calculation of total N uptake was the sum of the total N of leaf, stem and root. On the basis of these measurements and according to procedures of Delogu et al. (1998), Montemurro et al. (2006) and López-Bellido et al. (2005), the N utilization efficiency (NUtE; ratio of dry matter yield to N uptake, in kg/kg) and the N use efficiency (NUsE; ratio of dry matter yield to N applied) were calculated. After harvest, soil samples of 0–5, 5–10, 10–15 and 15–20 cm soil depths were collected and mixed, then the soil water-stability of aggregates was measured by the wet sieving apparatus (wet screening device) produced by the Holland Eijkelkamp Company (Giesbeek, the Netherlands). Moreover, according to sample quartering, the 200 g natural soil sample was taken, and the soil mechanical stability aggregates were measured by artificial screening method through a set of sieves

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of 10, 7, 5, 3, 2, 1, 0.5 and 0.25 mm, respectively. Percentage of aggregate destructions (PAD) was calculated by formula (1) and (2) which was obtained by using the soil water-stability of aggregates (especially > 0.25 mm) and mechanical stable aggregates (> 0.25 mm) (Tisdall and Oades 1982).

$$WR_{0.25} = \frac{M_{r>0.25}}{M_T} \times 100\% \quad (1)$$

Where: $M_{r>0.25}$ – weight of the aggregate (> 0.25 mm) (g); M_T – total weight of the aggregate (g).

$$PAD = \frac{(DR_{0.25} - WR_{0.25})}{DR_{0.25}} \times 100\% \quad (2)$$

Where: $DR_{0.25}$ – mechanical stable aggregates (> 0.25 mm) (%); $WR_{0.25}$ – water stable aggregates (> 0.25 mm) (%).

Statistical analysis. Statistical analysis was conducted by one-way ANOVA followed by the least significant difference (*LSD*) test at α = 0.05 level of significance using SPSS 23.0 (New York, USA). All experiment data were preliminary analysed using Excel 2010 (Redmond, USA).

RESULTS AND DISCUSSION

Effects of ploy on growth of spinach. The plant weight and LAI significantly increased with the application of γ-PGA. There were significant differences in plant fresh weight and dry weight ($P < 0.05$) (Table 1). CK with no γ-PGA and No. 2 (0.2% γ-PGA) treatments had the lowest and the highest plant fresh weight and dry weight among the treatments, respectively. The plant fresh weight and dry weight of No. 1 (0.1% γ-PGA) were similar to No. 3 (0.3% γ-PGA), and No. 4 (0.4% γ-PGA) was similar to CK. The relative increase in plant fresh weight with increasing γ-PGA was 128.07, 184.21, 145.61 and 5.26% at 0.1, 0.2, 0.3 and 0.4% of γ-PGA, respectively as compared to CK. On the other hand, the relative increase in plant dry weight with increasing γ-PGA were 109.68, 190.32, 101.61 and 61.29% at 0.1, 0.2, 0.3 and 0.4% of γ-PGA, respectively as compared to CK. That is attributed to the application of γ-PGA that can promote the growth and development of the stems and leaves of the spinach. There were significant differences in leaf area ($P < 0.05$) (Table 1). CK and No. 1 had the lowest and highest leaf area. Leaf area of No. 2 was similar to that of No. 3, and No. 4 and CK provided similar leaf area. The relative increase in leaf area with increasing γ-PGA was 149.68, 62.51, 51.61 and 4.92% at 0.1, 0.2, 0.3 and 0.4% of γ-PGA, respectively as compared to CK. Therefore, increasing γ-PGA can improve the biomass accumulation which is mainly due to the leaf area increases.

There were significant differences in leaf area index ($P < 0.05$) (Table 1). The rule of LAI changes was similar to that of leaf area. The relative increase in leaf area with increasing γ-PGA was 99.53, 91.51 and 44.81% at 0.1, 0.2 and 0.3% of γ-PGA, respectively as compared to CK. While comparing to No. 4, the relative increase in leaf area of CK was 27.36%. The reason is the application of γ-PGA that can enhance soil particles' capacity of water absorption and γ-PGA becomes hydrogel after absorbing water, which increases the viscosity of water (Zeng et al. 2018). Moreover, γ-PGA improves the availability of soil moisture and soil nutrient leaching is slowed down by γ-PGA, which contributes to continuous and steady supply of nutrients to spinach. On the contrary, high application of γ-PGA reduces the proportion of soil capillary pores and leads to the inhibition of spinach root respiration (Chu et al. 2016).

To summarize, appropriate amount of γ-PGA is useful to increase the fresh plant weight, dry plant

Table 1. Effect of ploy (γ-glutamic acid, γ-PGA) on growth period for spinach

Treatment	γ-PGA (%)	Plant weight (g/plant)		Leaf area (cm^2/plant)	Leaf area index (m^2/m^2)
		fresh	dry		
CK	0	5.57 ± 0.18^e	0.62 ± 0.02^e	24.81 ± 0.11^e	2.12 ± 0.09^d
No. 1	0.1	12.97 ± 0.05^c	1.31 ± 0.03^b	61.84 ± 0.05^a	4.25 ± 0.02^a
No. 2	0.2	16.12 ± 0.08^a	1.81 ± 0.02^a	40.27 ± 0.01^b	4.04 ± 0.03^b
No. 3	0.3	13.95 ± 0.07^b	1.25 ± 0.03^c	37.62 ± 0.07^c	3.06 ± 0.03^c
No. 4	0.4	5.94 ± 0.09^d	1.00 ± 0.02^d	26.00 ± 0.01^d	1.55 ± 0.02^e

Values with the same letters are not significantly different at $P < 0.05$ by *LSD* (least significant difference) test

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Table 2. Effects of ploy (γ-glutamic acid, γ-PGA) on yield (t/ha), moisture and dry matter (%)

Treatment	γ-PGA (%)	Fresh yield	Dry yield	Plant dry matter
CK	0	1.95 ± 0.05^{d}	0.22 ± 0.02^{d}	9.97 ± 0.40^{b}
No. 1	0.1	6.00 ± 0.04^{a}	0.61 ± 0.03^{a}	9.19 ± 0.36^{bc}
No. 2	0.2	5.80 ± 0.03^{b}	0.65 ± 0.05^{a}	10.09 ± 0.61^{b}
No. 3	0.3	4.90 ± 0.06^{c}	0.44 ± 0.07^{b}	8.20 ± 1.29^{c}
No. 4	0.4	2.01 ± 0.02^{d}	0.34 ± 0.02^{c}	14.41 ± 0.85^{a}

Values with the same letters are not significantly different at $P < 0.05$ by *LSD* (least significant differences) test

weight and leaf area. The appropriate amount of γ-PGA is 0.1%.

Effect of γ-PGA on yield of spinach. There were significant differences in spinach yield ($P < 0.05$) (Table 2). No. 1 (0.1% γ-PGA) obtained the highest fresh yield (6.00 t/ha) among all the treatments, which was not in the same trend as fresh yield per plant. That is mainly because there were differences in rates of emergence and plant density in different treatments. However, increasing γ-PGA obviously decreased fresh yield of spinach. The relative increase in fresh yield with increasing γ-PGA was 66.51, 65.37 and 59.01% at 0.1, 0.2 and 0.3% of γ-PGA, respectively as compared to 0.4% of γ-PGA. In addition, the highest dry yield of spinach (3.36 t/ha) was in No. 2 (0.2% γ-PGA) (Table 2). The relative increase in fresh yield with increasing γ-PGA was 84.31, 190.2, 77.45 and 15.68% at 0.1, 0.2, 0.3 and 0.4% of γ-PGA, respectively, as compared to CK. This study shows that the spinach yield is strongly influenced by γ-PGA applied and 0.1% of γ-PGA is in favour of enhancing spinach yield.

Chuai et al. (2016) found that urea with 1.00 g γ-PGA can significantly increase the fresh weight, dry weight and root length of pak choi. Chu et al. (2016) reported that the chlorophyll content of tomato seedlings increased gradually with the increase of γ-PGA and chlorophyll content of 3 kg/m^3 γ-PGA was the highest; they also reported that γ-PGA can enhance soil moisture, the capability of nutrient supply, promote the growth and development of tomato.

Effects of γ-PGA on quality of spinach. Table 2 showed significant differences ($P < 0.05$) in plant moisture content with increasing γ-PGA, which was similar to that in plant dry matter. Zhai et al. (2013) reported that γ-PGA can strengthen fertilizer and increase the dry matter accumulation, especially the dry matter accumulation of reproductive organs after bud stage of cotton. To summarize, considering the effects of γ-PGA on plant moisture, dry matter and the agricultural economic performance, 0.1% of γ-PGA applied was the optimal treatment.

Nitrogen uptake and utilization efficiency of spinach. Increasing γ-PGA significantly ($P < 0.05$) increased the N uptake of spinach (Table 3). That might be attributed to γ-PGA that can enhance the amount of soil microbial N at the early growth

Table 3. Effects of ploy (γ-glutamic acid, γ-PGA) on total nitrogen (N) content, N uptake and N utilization efficiency

Treatment	γ-PGA (%)	Leaf and stem total N	Root total N	N uptake (kg/ha)	NUtE	NUsE
		(g/kg)			(kg/kg)	
CK	0	49.30 ± 0.01^{b}	27.29 ± 0.01^{a}	52.91 ± 0.08^{e}	21.85 ± 0.05^{d}	3.84 ± 0.18^{e}
No. 1	0.1	48.81 ± 0.02^{c}	29.48 ± 0.03^{b}	103.27 ± 0.03^{b}	22.57 ± 0.03^{b}	7.85 ± 0.05^{b}
No. 2	0.2	45.04 ± 0.01^{e}	28.51 ± 0.02^{ab}	147.68 ± 0.12^{a}	24.01 ± 0.03^{a}	11.80 ± 0.14^{a}
No. 3	0.3	50.71 ± 0.04^{a}	28.23 ± 1.40^{a}	102.13 ± 0.17^{c}	21.92 ± 0.01^{cd}	7.45 ± 0.19^{c}
No. 4	0.4	47.70 ± 0.07^{d}	27.85 ± 0.34^{a}	56.89 ± 0.13^{d}	21.96 ± 0.03^{c}	4.13 ± 0.09^{d}

Values with the same letters are not significantly different at $P < 0.05$ by *LSD* (least significant differences) test. NUtE – ratio of dry matter yield to N uptake; NUsE – ratio of dry matter yield to N applied

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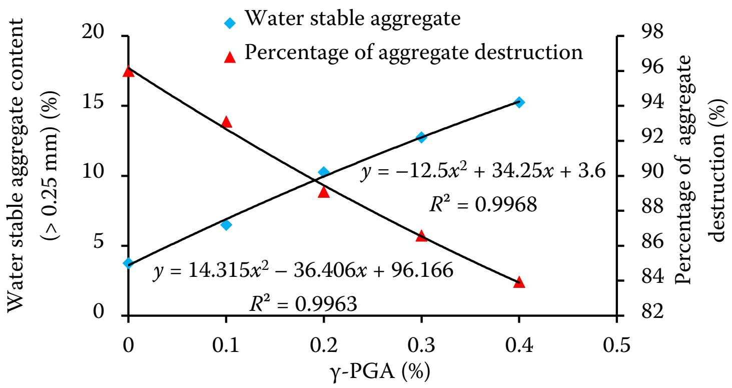

Figure 1. Effects of ploy (γ-glutamic acid, γ-PGA) on water stable aggregate and percent of aggregate destruction

stage of spinach, and then the N immobilized by soil microbes that can be mineralized during the late growth stage of spinach and increase the soil available N. Moreover, the activities of urease, sucrose and catalase in the soil increased after γ-PGA application (Xu et al. 2013). The highest N uptake was observed with 0.2% of γ-PGA (147.66 kg/ha), and N uptake of 0.1% and 0.3% of γ-PGA were similar. However, the highest total N of leaf, stem and root was observed with 0.1% of γ-PGA. Table 3 showed that increasing γ-PGA slightly decreased NUtE and NUsE. However, our results showed a higher spinach efficiency in terms of yield when lower γ-PGA (0.1%) was applied.

Effects of γ-GPA on soil agglomeration structure. It is generally considered that aggregates with diameters greater than 0.25 mm have the ability to resist destruction caused by water and have important influence on soil fertility. According to Figure 1, the content of water stable aggregates (> 0.25 mm) increased by 73.33~306.7% with increasing γ-PGA application; however, there was a negative correlation between PAD and γ-PGA content which increased with increasing γ-PGA application due to the aggregates. PAD decreased by 3.03~12.58% with increasing γ-PGA application. It was reported that γ-PGA could reduce the number of unstable aggregates in the soil and increased the stability of soil structure. That was mainly attributed to γ-PGA that becomes hydrogel after water saturation, which can bind tiny soil particles into large and stable soil micro-aggregates and they are not easy to disintegrate when exposed to water. Moreover, small condensed aggregates caused by γ-PGA were stronger in water coagulation and the soil bulk density decreased, which effectively increased soil porosity and improved soil structure. To summarize, γ-PGA can help increase the structure of stable soil aggregates, thereby promoting the development and growth of crop roots, and improving soil fertility and texture.

In summary, γ-PGA with a large number of carboxyl groups and acylamino can chelate or adsorb cation mineral nutrients and prevent nutrient leaching (Chu 2016). The study indicated that the nitrogen utilization and nitrogen use efficiency were increased with increasing γ-PGA. Additionally, γ-PGA can be used as a fertilizer synergist to increase crop productivity, improve soil structure and decrease excessive chemical fertilizer use and environmental pollution. Moreover, γ-PGA is not only beneficial for improving soil fertility but also for increasing bioavailability of phosphate radicals and calcium ions and for reducing the loss of ammonium nitrogen (Ho et al. 2006, Xia et al. 2008, Li et al. 2009, Xu et al. 2013). This study is related to the effects of γ-PGA on spinach growth, soil structure and fertility of nitrogen fertilizer in arid areas in Western China. The influence of γ-PGA on available nitrogen, available phosphorus and exchangeable calcium and magnesium content and microbial activity in soil has not been studied.

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