Vol. 17(12), pp. 354-366, December, 2023
DOI: 10.5897/JMPR2023.7324
Article Number: 4CA286371640
ISSN 1996-0875

http://www.academicjournals.org/JMPR

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Journal of Medicinal Plants Research

Full Length Research Paper

Effects of different nitrogen forms and concentration combinations on American ginseng seedling growth

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Received 5 September, 2023; Accepted 30 October, 2023

This study investigated the impact of various nitrogen forms on American ginseng (*Panax quinquefolius*) seedling growth. Thirteen treatments of inorganic and organic nitrogen combinations were tested in the first year and growth indicators were measured. In the second year, treatments with significant growth promotion effects were further verified. The results showed improved growth of American ginseng seedlings when the concentrations of nitrate and ammonium nitrogen were 1.5 and 0.5 mM, respectively. The addition of 0.5 mM tyrosine and 0.1 mM glycine as organic nitrogen to this combination of high nitrate and low ammonium further improved seedling performance. However, American ginseng seedlings treated with high ammonium (1.5 mM) and low nitrate (0.5 mM) concentrations combined with organic nitrogen did not exhibit any growth-beneficial responses and showed a negative response at high organic nitrogen doses. This study suggests that American ginseng is a nitrate-loving plant and that the combination of higher nitrate with lower ammonium nitrogen concentrations, added with an appropriate amount of organic nitrogen, may greatly promote seedling growth. These results may be beneficial for implementing management strategies to effectively overcome the challenges encountered by attempts to continuously crop American ginseng with success.

Key words: *Panax quinquefolium*; inorganic nitrogen, organic nitrogen, continuous cropping, seedling growth, nitrate, ammonium, survival rate.

INTRODUCTION

Panax quinquefolium (American ginseng, AG) is a perennial herbaceous species within the Araliaceae family that is native to North America and most low-latitude and high-altitude mountainous regions in China, including the northeast and north. Although AG thrives in these regions exhibiting a considerable preference for specific soil conditions making its cultivation more challenging (Haq et al., 2023) and posing serious constraints on the development of China's AG industry (Wang, 2017). Previous research on the problems encountered when attempting continuous cropping of AG has mainly focused on four aspects: allelopathic

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autotoxicity, rhizospheric microorganisms, soil deterioration, and nutrient shortage due to disease and pests (Wang et al., 2016). These studies have reported that excessive β-coumaric acid in the soil can inhibit the growth of AG roots (Yang et al., 2022a) and that with the increase in risk of plant infection, considerably hindering AG growth (Luan et al., 2017; Yu et al., 2018). Moreover, replant disease in AG is often immediately reflected in seedling growth (Chen et al., 2023). Nevertheless, the mechanisms underlying the challenges of continuous AG cropping have not been elucidated.

Nitrogen (N) is a vital constituent of proteins, enzymes, chlorophyll, auxins, and cytokinins, plays a critical role in plant growth and development (Sun et al., 2014; Li et al., 2017), and is in considerably higher demand than other elements (Boudsocq et al., 2012). While the relationship between available nitrogen and plants has been established (Tang et al., 2022; Yang et al., 2022b), studies on AG responses to the availability of N are scarce.

Owing to advances in technology, it has been revealed that plants cannot only absorb inorganic N but also a certain amount of small molecule organic-N (Wang et al., 2015). Inorganic N sources include nitrate, ammonium, amino acids, small molecular-weight peptides, and proteins, all of which are important N sources provided by fertilizers (Gioseffi et al., 2011; Paungfoo-Lonhienne et al., 2012). Fan et al. (2017) found that in the presence of ammonium, plant nitrate uptake was inhibited compared to when only one inorganic nitrogen species was present. This phenomenon is probably due to the involvement of NH_4^+ or amino acids in mediating root transport of amino acids and N assimilation (Takushi and Hitoshi, 2017). Nitrate promotes ammonium uptake to some extent (Fan et al., 2017), and nitrate can mitigate the inhibitory effect of ammonium-N on plant growth and development. Furthermore, the appropriate proportions of nitrate and ammonium may significantly increase crop yields (Ye et al., 2022), enhance soluble protein content, reduce nitrate content (Boudsocq et al., 2012), and improve crop quality (Lu and Guo, 2006).

Organic N comprises numerous natural compounds containing basic amines with an acidic carboxyl group that are used in the synthesis of proteins, and many physiologically important low molecular weight compounds (Paungfoo-Lonhienne et al., 2012; Wu, 2015). In plants, amino acids are involved in several important processes, including the biosynthesis of important substances, stress resistance, signal transduction, and stress responses (Park et al., 2014; Jones and Bari, 2013). Foliar spraying with amino acids increases biomass and yield components and effectively promotes plant growth (Liu et al., 2020). Seed treatment with amino acids and foliar application of amino acids can help mitigate the effects of water deficit and increase dry biomass and soybean yield (Teixeira et al., 2019). Furthermore, exogenous proline application can promote the absorption of K^+, Ca_2^+, P, and N by different cultivars of the same crop during water stress (He et al., 2019). Similarly, the application of glycine to plants growing under heavy metal stress effectively enhances growth and development (Alexopoulos et al., 2021; He et al., 2019), photosynthetic capacity, and antioxidant enzyme activity, thereby reducing heavy metal absorption and oxidative stress (Hernandez-Leon and Valenzuela-Soto, 2022).

Soil inorganic and organic nutrient contents are important factors affecting the growth and quality of AG (Xu et al., 2017). Notably, most of the nutrients in the soil that are essential for AG growth cannot be restored to their original levels after the first planting, especially inorganic N (Li et al., 2022). It has previously been shown that most of the organic N in the soil decreases after planting AG. For example, N-acetylornithine, serine, leucine, glycine, and tyrosine levels were reduced with AG cultivation, and restoring them to pre-AG cultivation levels proved challenging in the subsequent 10 to 20 years (Li et al., 2021). In the present study, we hypothesized that the form and concentration of N may play an important role in improving the growth and stress resistance of AG. Glycine and tyrosine, whose contents are difficult to restore due to their marked reduction after the first harvest of AG, were selected as sources of organic N, while $Ca(NO_3)_2$ and $(NH_4)_2SO_4$ were selected as nitrate-N and ammonium-N sources, respectively (Lobit et al., 2007).

Bioassay experiments were conducted using different N sources and concentration ratios. The specific aims of the study are to explore the effects of different N forms, concentrations, and combinations on AG, identify the beneficial combinations with significant effects on AG, and further investigate the potential role of such treatments in the elimination of the issues faced by attempts to continuously crop AG.

MATERIALS AND METHODS

Materials and cultivation

Two-year-old AG-rooted seedlings of similar height (3.5 ± 0.25 cm) and weight (3 ± 0.25 g) were supplied by Weihai DongXu Ginseng Co., Ltd. Sand and vermiculite were mixed in a 3:1 ratio to create the substrate for cultivation, and 700 ml seedling cups (bottom diameter 5.5 cm, top diameter 8 cm, height 16.5 cm) were used as cultivation containers. The AG-rooted seedlings were kept upright in the cup at planting; the top was 2 cm away from the cup mouth, and the substrate was added to the top of the rooted seedlings to cover the soil at a depth of 1 cm. After planting, the cups were placed on ridges under a shade and were watered appropriately to ensure that the entire growth process occurred under conditions as close to the natural growth environment as possible.

Experimental design

The experiment was divided into two planting stages. For the first planting stage, a total of 13 N treatments, divided into three groups,

Table 1. Nitrogen forms, concentrations, and ratio in the treatment groups.

	Treatments	Reagent composition
Group 1	T1	1 mM $(NH_4)_2SO_4$ + 1 mM $Ca(NO_3)_2$
	T2	2 mM $(NH_4)_2SO_4$
	T3	1.5 mM $(NH_4)_2SO_4$ + 0.5 mM $Ca(NO_3)_2$
	T4	0.5 mM $(NH_4)_2SO_4$ + 1.5 mM $Ca(NO_3)_2$
	T5	2 mM $Ca(NO_3)_2$
Group 2	T3	1.5 mM $(NH_4)_2SO_4$+0.5 mM $Ca(NO_3)_2$
	T3-1	0.1 mM Tyr + 0.1 mM Gly + 1.5 mM $(NH_4)_2SO_4$ + 0.5 mM $Ca(NO_3)_2$
	T3-2	0.1 mM Tyr + 0.5 mM Gly + 1.5 mM $(NH_4)_2SO_4$ + 0.5 mM $Ca(NO_3)_2$
	T3-3	0.5 mM Tyr + 0.1 mM Gly + 1.5 mM $(NH_4)_2SO_4$ + 0.5 mM $Ca(NO_3)_2$
	T3-4	0.5 mM Tyr + 0.5 mM Gly + 1.5 mM $(NH_4)_2SO_4$ + 0.5 mM $Ca(NO_3)_2$
Group 3	T4	0.5 mM $(NH_4)_2SO_4$+1.5 mM $Ca(NO_3)_2$
	T4-1	0.1 mM Tyr + 0.1 mM Gly + 0.5 mM $(NH_4)_2SO_4$ + 1.5 mM $Ca(NO_3)_2$
	T4-2	0.1 mM Tyr + 0.5 mM Gly + 0.5 mM $(NH_4)_2SO_4$ + 1.5 mM $Ca(NO_3)_2$
	T4-3	0.5 mM Tyr + 0.1 mM Gly + 0.5 mM $(NH_4)_2SO_4$ + 1.5 mM $Ca(NO_3)_2$
	T4-4	0.5 mM Tyr + 0.5 mM Gly + 0.5 mM $(NH_4)_2SO_4$ + 1.5 mM $Ca(NO_3)_2$

Group 1: Inorganic N; Group 2: Organic N with high ammonia and low nitrate; Group 3: Organic N with high nitrate and low ammonium. High ammonium and low nitrate concentrations indicate that the ratio of ammonium to nitrate in the treatment solution was 3:1. High nitrate and low ammonium concentrations indicate that the ratio of ammonium to nitrate in the treatment solution was 1:3.

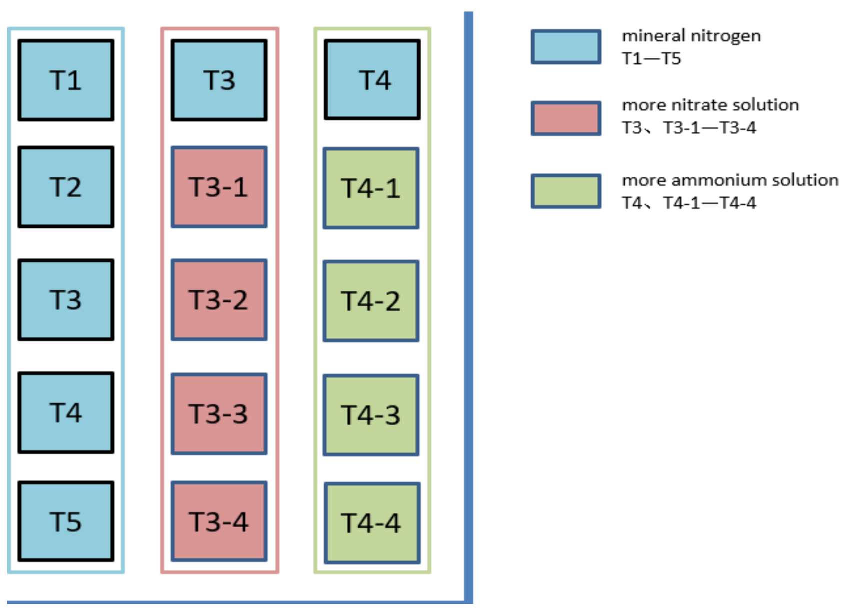

Figure 1. Schematic representation of the grouping of different nitrogen treatments.

were designed as follows: Group 1, inorganic N in different ratios (T1-T5, among which T1 served as control treatment); Group 2, organic N combined with different ammonium-N concentrations (T3, T3-1, T3-2, T3-3, and T3-4, among which T3 served as control treatment), and group 3, organic N combined with different nitrate-N concentrations (T4, T4-1, T4-2, T4-3, and T4-4, among which T4 served as control treatment). Twenty biological replicates were planted for each treatment group. The N forms, concentrations, and combinations of each treatment are listed in Table 1, and the specific placement of each treatment group in the shed is shown in Figure 1.

In the second year of planting, treatments with significant differences were further verified according to the data results of the first year. In total, there were five nitrogen treatments: control group TT0 (no nitrogen treatment); two groups of inorganic nitrogen treatment with specific concentrations TT1 and TT3 (combination of nitrate nitrogen and ammonium nitrogen with different concentrations); and combined treatment of inorganic nitrogen and organic nitrogen TT2 and TT4 (different concentrations of nitrate nitrogen combined with specific concentrations of ammonium

Table 2. Nitrogen forms, concentrations, and ratio in the treatment groups in second-year planting cycle.

Treatment	Reagent composition
TT0	Distilled water
TT1	1 mM $(NH_4)_2SO_4$ + 1 mM $Ca(NO_3)_2$
TT2	1 mM $(NH_4)_2SO_4$ + 1 mM $Ca(NO_3)_2$ + 0.5 mM Tyr + 0.1 mM Gly
TT3	0.5 mM $(NH_4)_2SO_4$ + 1.5 mM $Ca(NO_3)_2$
TT4	0.5 mM $(NH_4)_2SO_4$ + 1.5 mM $Ca(NO_3)_2$ + 0.5 mM Tyr + 0.1 mM Gly

High ammonium and low nitrate concentrations indicate that the ratio of ammonium to nitrate in the treatment solution was 3:1. High nitrate and low ammonium concentrations indicate that the ratio of ammonium to nitrate in the treatment solution was 1:3.

nitrogen). The N forms, concentrations, and combinations of each treatment are listed in Table 2.

Treatment methods and daily management

Two weeks after transplanting the rooted seedlings, the sprouts had grown to approximately 3 cm, and treatments were initiated. The experiment lasted for 90 days. Each treatment was added at 7-day intervals as 20 ml of the corresponding solution with 40 ml of 50% N-deficient Hoagland nutrient solution prepared as previously described. Each liter of this solution contained 616.2 mg $MgSO_4 \cdot 7H_2O$, 555 mg $CaCl_2$, 372.8 mg KCl, 272.2 mg KH_2PO_4, 7.45 mg Na_2-EDTA, 5.57 mg $FeSO_4 \cdot 7H_2O$, 2.86 mg H_3BO_3, 1.015 mg $MnSO_4$, 0.9 mg H_2MoO_4, 0.22 mg $ZnSO_4 \cdot 7H_2O$, and 0.079 mg $CuSO_4 \cdot 5H_2O$. The solution was added slowly around the root perimeter to avoid contact between the reagent and the leaves of the plant. During the treatment period, water in the pot was observed daily and was added as necessary.

Plants were moved every two weeks to eliminate position effects. To prevent nutrient accumulation, the growth substrate was rinsed with water before each addition of nutrient solution (Zhao et al., 2020).

Data collection and statistical analysis

Data were collected on days 31 and 52 after transplanting. At the end of the experiment, the growth and performance of all seedlings were measured; plant height, crown width, aboveground biomass, and seedling survival rates were recorded. Root surface impurities were washed off the AG seedlings with water; fresh weights were recorded, plant height was measured, and the fibrous root number was counted. Finally, seedlings were placed in envelopes, labeled, and placed in an oven. Dry weight was recorded after drying at 80°C to a constant weight.

Excel 2016 and SPSS 27.0 software were used to record and analyze the data. One-way ANOVA followed by Duncan's Multiple Range test was used to examine the significance of the treatment differences (Khan et al., 2019), and GraphPad Prism 8 was used to visualize the data. Adobe Illustrator 2020 was used for photography and chart processing.

RESULTS

Effects of nitrate-N and ammonium-N concentration on survival and growth of AG seedlings

The results showed marked differences in the survival rates and growth of AG seedlings treated with different concentrations of nitrate and ammonium. As shown in Figure 2a, on days 31 and 52 after treatment initiation, the survival rates of seedlings in the T4 and T5 groups were higher than those in the T1 group ($P<0.05$), and the survival rate in the T4 group remained the highest at the time of final harvest. As shown in Figure 2b and c, the aboveground height and breadth of plants in the T4 group were both higher than those in any other group on both days 31 and 52 after treatment initiation ($P<0.05$). As shown in Figure 2d to g, fresh and dry weights were the highest in the T4 group and the lowest in the T3 group, and there was no significant difference among the other groups. Only the T3 group had a lower number of fibrous roots, and the difference among the other groups was not significant. Combined with the analysis of survival rate and growth, the ratio of inorganic N in the T4 treatment (nitrate: ammonium = 3:1) had a significant positive effect on the survival and growth of AG seedlings.

Effects of combined inorganic and organic N treatments on the survival and growth of AG seedlings

Effects of organic N concentration combined with high ammonium and low nitrate on the survival and growth of AG seedlings

The survival rates of AG seedlings treated with high ammonium and low nitrate combined with different concentrations of organic N differed significantly. As shown in Figure 3a, on days 31 and 52 after treatment initiation, the survival rates of the T3-2 and T3-3 groups were similar to those of T3 and higher than those of the remaining treatment groups ($P<0.05$). Particularly, the T3-3 group had the highest breadth on day 31 after treatment initiation. Additionally, although the breadth of all groups increased on day 52 after treatment initiation, the differences among the groups were not significant (Figure 3b). Figure 3c shows that on day 31 after treatment initiation, the aboveground height of plants in the T3-2 group was higher than that of plants in the other treatment groups ($P<0.05$). On day 52 after treatment

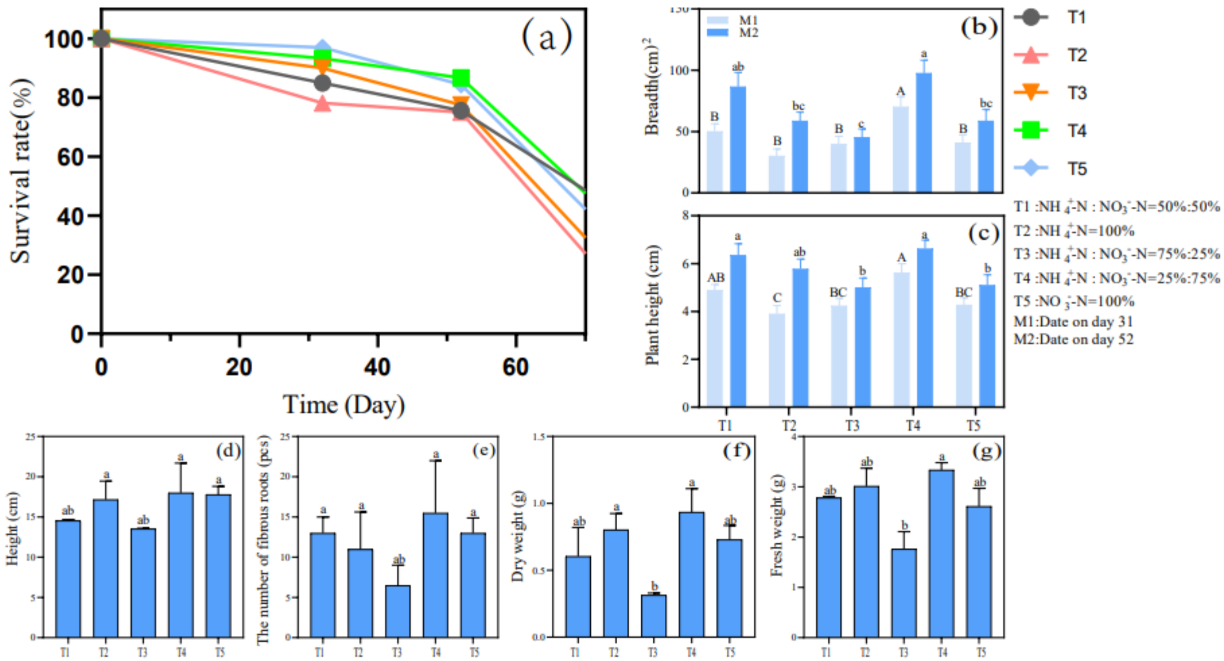

Figure 2. The effects of different concentrations of inorganic N on the growth of AG seedlings. (a) Seedling survival rate; (b) and (c) Breadth and aboveground height measurements on the 31st and 52nd day, after treatment initiation, respectively. The same capital and lowercase letters indicate no significant difference among samples (P >0.05). Panels (d), (e), (f), and (g) show plant height, fibrous root number, dry weight, and fresh weight of harvested plants, respectively. There was no significant difference among samples with the same lowercase letter (P >0.05).

initiation, there was an increase in plant height, but no significant differences were observed among groups. The number of fibrous roots was significantly (P<0.05) higher in the T3-3 and T3-4 groups than in the other treatment groups (Figure 3(e)). As shown in Figure 3f and g, the fresh and dry weights of the T3-1 group were the highest, and there were no significant differences among the other groups. Combining the survival rate and growth analysis, only treatment T3-1 (that is, 0.1 mM tyrosine + 0.1 mM glycine combined with high concentration ammonium-N and low concentration nitrate-N) had a significant effect in promoting the survival and growth of AG seedlings.

Effects of different concentrations of organic N combined with high nitrate and low ammonium on the survival and growth of AG seedlings

The survival rate of AG seedlings showed significant differences among groups treated with high nitrate and low ammonium combined with different concentrations of organic N. As shown in Figure 4a, the seedling survival rate in the T4 group was the highest on days 31 and 52 after treatment initiation, and that of seedlings in groups T4-3 and T4-4 were significantly higher than those of seedlings in the other groups at harvest (P<0.05).

No significant difference was observed in plant breadth among treatment groups 31 days after treatment; however, on day 52, group T4-3 showed the highest value (Figure 4b). Additionally, on day 31 after treatment initiation, the aboveground height of seedlings in group T4-1 was significantly lower than that of seedlings in the other groups, and there were no significant differences among these groups (Figure 4c). However, on day 52 day after treatment initiation, the seedlings in group T4-3 were the tallest (P<0.05). Figure 4f and g shows that the fresh and dry weights were the highest for groups T4-2 and T4-3, which were markedly higher than those recorded for seedlings in the other treatment groups (P<0.05). Finally, the number of fibrous roots in the seedlings of group T4-3 was the highest among group 3 variants, but lower than that in the internal control (T4). Overall, treatment T4-3 (0.5 mM tyrosine + 0.1 mM glycine combined with high nitrate-N and low ammonium-N) showed the best results with respect to the survival and growth of AG seedlings.

Effects of different concentrations of inorganic and organic N ratio on seedling survival and growth in the second-year planting cycle

As shown in Figure 5a, group TT2 had the highest seedling survival rate on days 31 and 52 after the start of treatment, and the seedling survival rate under all treatments, except TT4, was higher than that of the

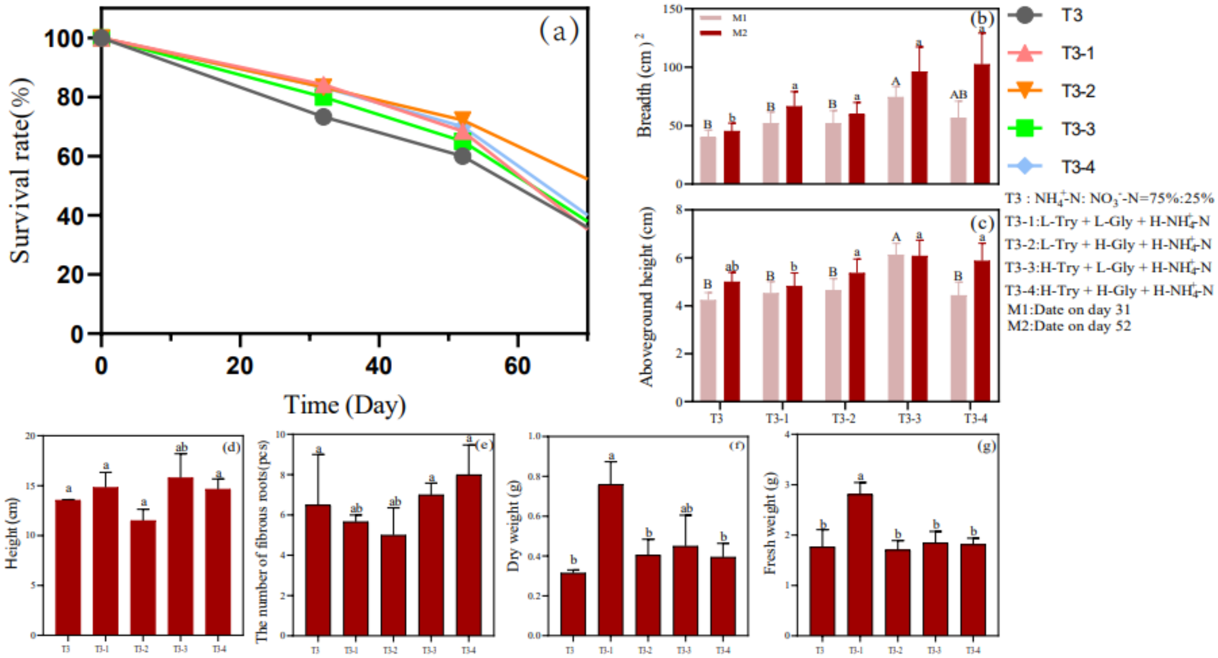

Figure 3. The effects of high ammonium and low nitrate combined with different concentrations of organic N on the growth of AG seedlings. (a) Seedling survival rate; (b) and (c) Breadth and aboveground height on the 31st and 52nd day after treatment initiation, respectively. The same capital and lowercase letters indicate no significant difference among samples (P >0.05). Panels (d), (e), (f), and (g) show plant height, fibrous root number, dry weight, and fresh weight of harvested plants, respectively. There was no significant difference among samples with the same lowercase letter (P >0.05). H and L denote high and low concentrations, respectively.

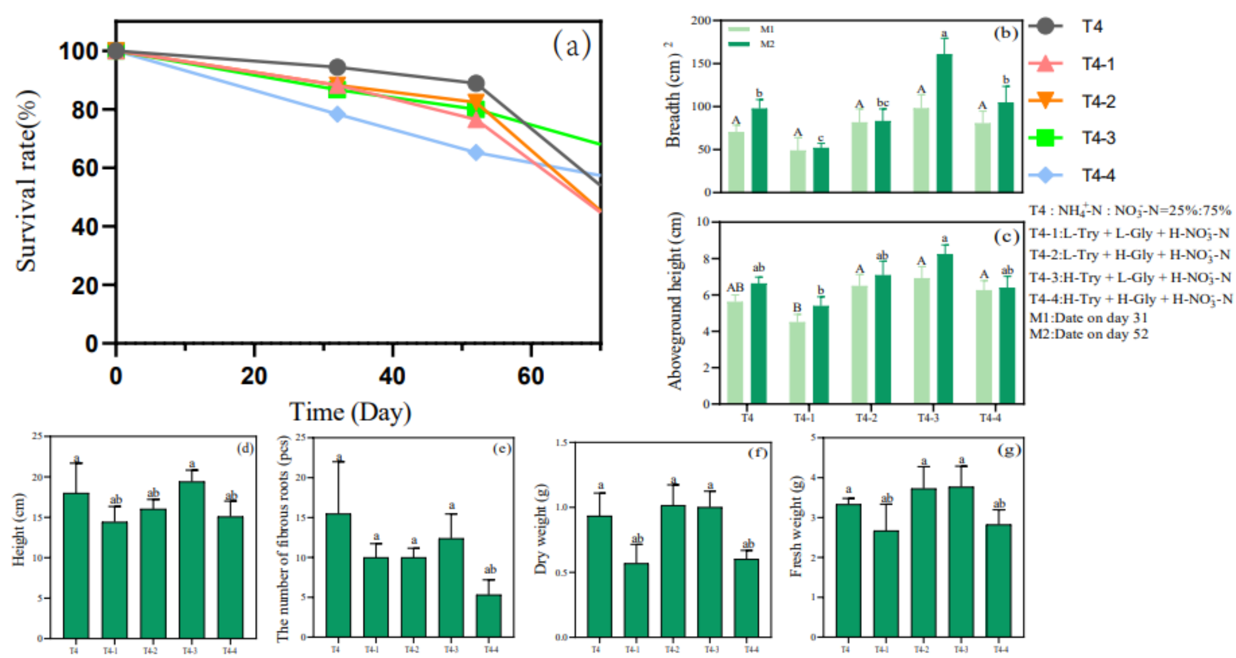

Figure 4. Effects of high nitrate and low ammonium combined with different concentrations of organic N on the growth of AG seedlings. (a) Seedling survival rate, (b) and (c) breadth and aboveground height on the 31st and 52nd day after treatment initiation, respectively. The same capital and lowercase letters indicate no significant difference among samples (P >0.05). Panels (d), (e), (f), and (g) show plant height, fibrous root number, dry weight, and fresh weight of the plants harvested, respectively. There was no significant difference among samples with the same lowercase letter (P >0.05). H and L denote high and low concentrations, respectively.

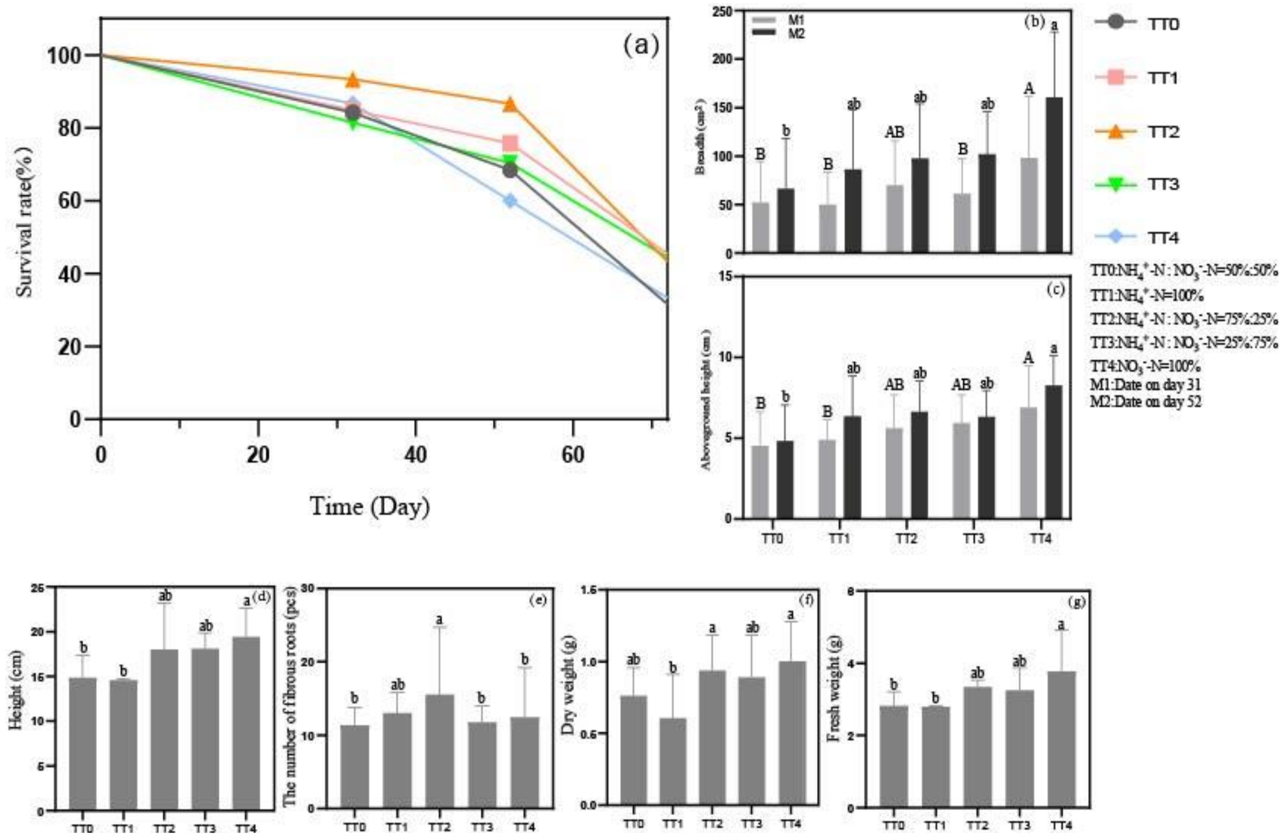

Figure 5. Effects of different concentrations of inorganic and organic N ratio on seedling survival and growth in the second-year planting cycle. (a) Seedling survival rate; (b) and (c) Breadth and aboveground height on the 31st and 52nd day after treatment initiation, respectively. The same capital and lowercase letters indicate no significant difference among samples (P >0.05). Panels (d), (e), (f), and (g) show plant height, fibrous root number, dry weight, and fresh weight of the plants harvested, respectively. There was no significant difference among samples with the same lowercase letter (P >0.05). H and L denote high and low concentrations, respectively.

control group at harvest.

In terms of breadth, the TT4 group had significantly higher breadth than other treatment groups on days 31 and 52 (Figure 5b). In addition, the plant aboveground height of seedlings in the TT4 group was significantly higher on day 31 after the initiation of treatment, and similar results were observed on day 52 (Figure 5c). As shown in Figure 5d, the whole plant height of seedlings under nitrogen treatment showed a similar trend to that of the aboveground part. The number of fibrous roots of seedlings under TT2 treatment was the highest, which was significantly higher than that of the control group (Figure 5e). Figure 5f and g show that the fresh and dry weights of seedlings were the highest in the TT4 group, which were significantly higher than those in the control group. In conclusion, TT4 treatment (0.5 mM tyrosine + 0.1 mM glycine combined with high nitrate-N and low ammonium-N) exhibited the best effect on the growth and survival of AG seedlings. This result is consistent with the results obtained from the T4-3 treatment in the first year of the experiment. The differences among AG seedlings under different treatment conditions are as shown in Figures 6 to 11.

DISCUSSION

Nitrogen is an essential element for plant growth and development (Estrada-Ortiz et al., 2016); consequently, the demand for N in plants is substantially higher than that of other elements. Changes in N content in the growing substrate have a significant impact on plant growth (Wright and Dorken, 2014). In the absence of essential nutrients, particularly N, the biosynthesis of the necessary metabolites that constitute most of the plant body is blocked, and cell elongation and division are inhibited (Kong et al., 2020). Thus, N deficiency can lead to a decrease in chlorophyll content and consequently, to reduced production of photoassimilate, causing leaf yellowing, which seriously affects plant growth and development, ultimately reducing crop yield (Chen et al., 2021). Conversely, an excess of N can cause nutrient imbalances, changes in plant growth mode, and even toxicity, which results in the burning of seeds and

Figure 6. The real object pictures of different concentrations of inorganic N on the growth of AG seedlings. The upper part is the top view, and the lower part is the main view. The processing information is shown in labels.

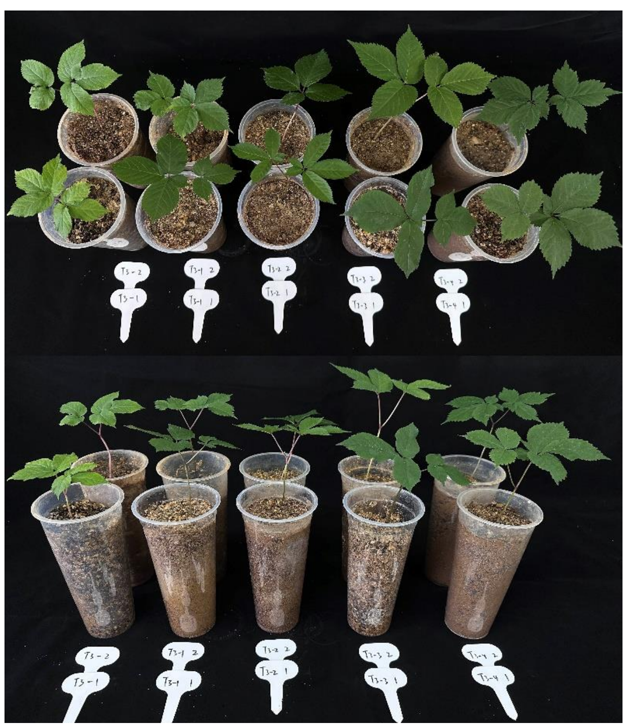

Figure 7. The real object pictures of high ammonium and low nitrate combined with different concentrations of organic N on the growth of AG seedlings. The upper part is the top view, and the lower part is the main view. The processing information is shown in labels.

Figure 8. The real object pictures of high nitrate and low ammonium combined with different concentrations of organic N on the growth of AG seedlings. The upper part is the top view, and the lower part is the main view. The processing information is shown in labels.

Figure 9. The real object pictures of different concentrations of inorganic and organic N ratio on the growth of AG seedlings. The upper part is the top view, and the lower part is the main view. The processing information is shown in labels.

Figure 10. Figure of seedling growth of American ginseng under different treatments on day 32.

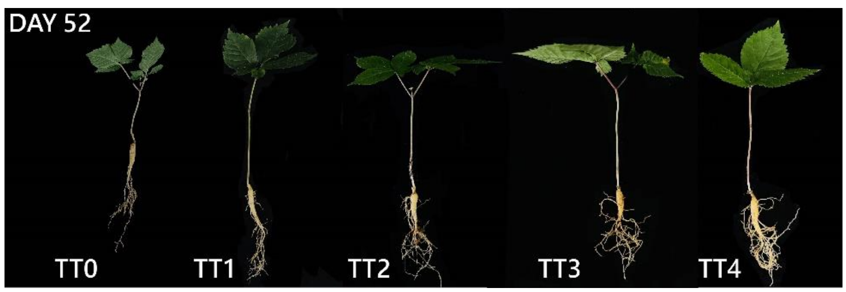

Figure 11. Figure of seedling growth of American ginseng under different treatments on day 52.

seedlings at the early stages of growth. Therefore, N plays a role of paramount importance in plant metabolic activities, which makes it a major limiting factor for plant growth in producing the best agricultural products (Nendel et al., 2019).

Different forms of N regulate root growth by modulating the levels and spatial distribution of growth hormones to optimize nutrient uptake and assimilation (Lorinda et al., 2022). Additionally, different forms of N can affect the rate of N uptake and regulate shoot growth and plant biomass accumulation (Guo et al., 2022). The present study demonstrated that increased nitrate and low ammonium concentrations increased plant height, crown width, and biomass accumulation. We found that treatment with high nitrate and low ammonium concentrations significantly promoted the survival rate, growth, and biomass accumulation of AG seedlings. As an essential part of N nutrition, the effects of inorganic N on plants are reflected in many aspects, mainly in response to varying soil nitrate-N and ammonium-N contents (Hasbullah and Marschner, 2016). Suitable ammonium-N uptake can increase the leaf chlorophyll content, delay leaf senescence, and preserve a high degree of plant photosynthetic capability (Carlisle et al., 2012; Mengesha, 2021). Therefore, the application of an appropriate amount of ammonium-N may increase the level of ammonium-N in plants and improve photosynthetic efficiency (Nasar et al., 2021). Moreover, the ability of AG to accumulate nutrients via photosynthesis can be improved by the application of an appropriate level of ammonium-N, which is consistent with our previously reported results (Wei et al., 2019). However, in the present study, as the concentration of ammonium-N increased, adverse effects on plants were observed. Thus, excessive NH_4^+ uptake can result in the inactivation of plant leaves and an imbalance of cations and anions, which affects the accumulation of amino acids in the plant (Zhang et al., 2022). Furthermore, excessive absorption of NH_4^+ can be toxic, and higher ammonium-N concentrations can directly lead to a reduction in the capacity of the soil to sustain nitrification (Burger and Jackson, 2005), concomitantly with a reduction in soil temperature, which can render plants more vulnerable to pathogen and pest attack and abiotic stress (Pidlisnyuk and Zgorelec, 2022). In addition to the toxic effects, the assimilation of excess NH_4^+ reduces the available carbon sources (that is, sugars) for plant growth and increases energy consumption, which lowers the survival and growth quality of seedlings (Coskun et al., 2013). The application of an appropriate amount of nitrate-N can enhance leaf vitality and plant growth. Plants with less nitrate accumulation do not grow well during the period of vegetative growth; the accumulation of nitric N in stems and leaves decreases significantly and finally leads to the weakening of plant growth, leaf yellowing, shortening of the growth period, and the inability to accumulate sufficient photosynthates (Li et al., 2020). In contrast, with an increase in nitrate-N accumulation, plants grow vigorously at the vegetative stage, accumulate nitrate-N in the plant body in the late growth period, and maintain high growth momentum and greater leaf freshness (Li et al., 2009). These previous reports are consistent with the results reported in our study.

While a single addition of an appropriate amount of nitrate-N or ammonium-N can have a positive impact on plants, the effects of a mixed application are considerably greater than those of a single addition. For example, hydroponic culture experiments have shown that adding a small amount of ammonium-N into the nitrate-N culture solution can increase the total N and protein contents of the plant, increase the rates of growth and development, and contribute to higher yields (Huang et al., 2022). Some studies have suggested that the simultaneous application of nitrate-N and ammonium-N can help plants maintain more N with much lower energy consumption (Hachiya and Sakakibara, 2017).

Ammonium-N is generally assimilated in the root whereas nitrate-N is transported to the aerial plant body through the vascular tissues for assimilation in the leaves, in addition to root assimilation. When both cooperate, the carbohydrates accumulated in all components of the plant can be reasonably and effectively utilized (Liu et al., 2021). Other studies have highlighted the fact that it is difficult for plants to obtain ample available reductive N when nitrate-N is used alone.

Instead, adding a small amount of ammonium-N can accelerate the reducing ability of plants, and a small amount of ammonium-N is preferably absorbed instead of producing ammonium toxicity (Ferrón-Carrillo et al., 2021). Moreover, the application of ammonium-N or nitrate-N alone changes the soil pH (Pahalvi et al., 2021), and AG is known to grow better under acidic conditions. When nitrate-N and ammonium-N are applied together, the rhizosphere pH is adjusted to a suitable range in balance with the cellular pH, which provides an environmental guarantee for the growth of AG seedlings. The results of these experiments confirm that, when fertilizing plants with nitrate-N, adding a small amount of ammonium-N (that is, maintaining a high nitrate-to-ammonium ratio) can significantly promote the growth and quality of AG seedlings.

In addition to inorganic N, plant growth is also affected by organic N. Within a certain concentration range, amino acids are positively correlated with plant biomass (Li et al., 2022). Sauheitl et al. (2009) found that the efficiency of amino acid absorption in plants increases significantly as the amino acid concentration increases within a certain range (Sauheitl et al., 2009). Moreover, plant absorption of soil amino acids is affected by plant N levels and soil inorganic N concentrations, among other factors. Under low N concentrations, the uptake of amino acids by plants is lower than that by microorganisms (Jonasson et al., 1999). Conversely, under conditions of high N concentrations, N uptake by microorganisms tends to saturate, and a large number of amino acids in the soil are absorbed by plant roots (Kebede, 2001). In the present study, the effects of amino acid addition on AG seedlings were mainly observed in the length and number of roots. The addition of a specific concentration of amino acids combined with nitrate-N and ammonium-N in the soil can synergistically promote the growth of taproots and lateral roots, thus promoting the absorption and utilization of soil nutrients (Owen et al., 2001). Therefore, the mixed application of organic and inorganic N changes the plant nutrient-absorption mode and improves absorption efficiency. The results reported in the present study showed that the seedling survival rate and biomass accumulation under mixed N application were significantly greater than those of seedlings treated with inorganic N alone, under conditions of a high concentration of nitrate-N and a low concentration of ammonium-N, supplemented with moderate concentrations of organic N. In contrast, high ammonium and low nitrate concentrations supplemented with organic N had either no effect or a negative effect on AG seedling growth.

Overall, the present study demonstrates that the growth-promoting effect of the combined application of the different forms of N was considerably higher than that of the addition of any single form of N. Moreover, treatment with high nitrate and low ammonium concentrations combined with amino acids (tyrosine and glycine), at levels that are difficult to restore in AG-cultivated soils, had a significant effect on the growth of AG seedlings. Our findings and those of previous studies suggest that N nutrition may affect disease resistance in plants (Bi et al., 2023). Therefore, it was speculated that environmental stress and pathogen infection of *P. quinquefolium* during the growth period would be affected by N addition. Future work should focus on the effect and mechanism of different N forms and combinations on the disease resistance of AG in the later growth stages to provide a theoretical basis for overcoming the obstacles that currently hamper the continuous cropping of AG.

This study had several limitations. First, the growth process of AG seedlings in these experiments was not observed in a natural soil environment. Second, our experiments did not include measuring any of the changes that surely affect the physicochemical indexes of AG.

In conclusion, our experiments confirmed that AG is a plant that thrives when supplemented with nitrate-N. Further, when comparing a variety of N supply options, an inorganic N supply containing high nitrate and low ammonium, combined with an appropriate amount of organic N, led to an improved survival rate and growth quality of AG seedlings.

In the future, according to the existing experimental data and results analysis, further experiments will performed to investigate the resistance of nitrogen form to pathogen infection of AG seedlings. By observing the display of seedling form, differences in plant-related resistance indexes, and changes in soil microbial environment, the disease-resistance effect and specific mechanism of nitrogen action on AG in the growth stage will be comprehensively explored. Moreover, further studies are required to provide supporting data and a theoretical basis for mitigating the issues encountered in the continuous cropping of medicinal crops such as AG.

ACKNOWLEDGEMENTS

The authors thank all their colleagues for their valuable contributions, and their help with this research. They also thank Editage (www.editage.cn) for English language editing. This study was funded by the Fundamental Research Projects of Science and Technology Innovation and Development Plan in Yantai City (No. 2022JCYJ029), the Natural Science Foundation of Shandong Province (ZR2023MC077), the Major Science and Technology Innovation Project of Shandong Province (2017GGH5129), and by the Shandong University Students Innovation and Entrepreneurship Training Project (2021030225).

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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