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EDITED BY
Mohammad Bagher Hassanpouraghdam,
University of Maragheh, Iran

REVIEWED BY
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Foad Fatehi,
Payame Noor University, Iran
Omer Ismail,
University of Anbar, Iraq

*CORRESPONDENCE
Huifeng Xu
xhfzj@163.com

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Effects of phosphorus and potassium fertilization on leaf senescence and yield of *Echium vulgare* L.

Xiukun Li, Jingying Yu, Aihua Yin and Huifeng Xu*

Agricultural College of Jilin Agricultural University, Changchun, Jilin, China

Phosphorus (P) and potassium (K) fertilization rates are key determinants of crop yield. *Echium vulgare* L. is a valuable medicinal and industrial oilseed crop rich in ω-3 polyunsaturated fatty acids, while its roots synthesize shikonin, a bioactive compound with antibacterial and anti-inflammatory properties. However, the interactive effects of P and K fertilization on leaf senescence, photosynthetic performance, antioxidant defense, and yield remain unclear. A two-year field experiment was conducted using a randomized complete block design with a factorial arrangement. Four P levels (0, 80, 120, and 160 kg ha^{-1}) and four K levels (0, 60, 90, and 120 kg ha^{-1}) were applied, resulting in 16 treatment combinations. Most physiological traits and yield components exhibited a quadratic response to increasing P and K supply. Moderate fertilization significantly enhanced chlorophyll content, photosynthetic rate, antioxidant enzyme activities, thousand-seed weight, and seed yield. Intercellular CO_2 concentration (Ci) decreased initially and partially recovered at later growth stages. Malondialdehyde content generally declined, although the rate of reduction slowed under higher fertilizer levels, with slight increases observed under excessive application. Among all treatments, the combination of 120 kg ha^{-1} P and 90 kg ha^{-1} K (P3K3) was the most effective, significantly improving photosynthetic performance and antioxidant capacity, and resulting in the highest seed yield. Overall, balanced P and K fertilization enhances plant physiological performance and yield through coordinated regulation of photosynthesis and antioxidant defense. This fertilization strategy provides a practical approach for high-yield cultivation of *E. vulgare*.

KEYWORDS
***Echium vulgare* L., phosphorus and potassium fertilize, photosynthetic characteristics, physiological characteristics, yield**

1 Introduction

Echium vulgare L. (Boraginaceae), commonly known as viper's bugloss, is a herbaceous dicotyledonous plant (Zhu et al., 2017). *E. vulgare* is widely distributed in the Mediterranean region and has also been introduced to Australia, New Zealand, South Africa, southern South America, and the western United States (Molinelli et al., 2020). The seeds of *E. vulgare* are rich in polyunsaturated fatty acids, including linoleic acid (~14%), γ-linolenic acid (10%), α-linolenic acid (33%), and stearidonic acid (14%). Nearly half of the total fatty acids consist of

ω-3 fatty acids, which cannot be synthesized endogenously by humans (Gray et al., 2010). ω-3 fatty acids have been associated with a reduced risk of cancer, insulin resistance, dementia, and psychiatric disorders (Pires et al., 2020). In addition, the roots of *E. vulgare* produce bioactive naphthoquinone compounds, such as shikonins, which possess antibacterial, anti-inflammatory, and wound-healing properties (Sheydaei et al., 2025). These characteristics highlight the considerable potential of *E. vulgare* as a medicinal and industrial oilseed crop.

During the growth and reproductive development of *E. vulgare*, vigorous metabolic activities are accompanied by the production of reactive oxygen species (ROS), which can induce leaf senescence. ROS are highly reactive oxygen-containing molecules generated during electron transport processes in chloroplasts, mitochondria, and plasma membranes, and mainly include superoxide anion ($O_2\bullet^-$), hydrogen peroxide (H_2O_2), hydroxyl radical (•OH), and singlet oxygen ($^1O_2$) (Waszczak et al., 2018). Excessive ROS can react with proteins, carbohydrates, nucleic acids, lipids, and other cellular constituents, resulting in oxidative damage and impaired plant growth and metabolism (Khan et al., 2025). During leaf senescence, the balance between ROS generation and antioxidant scavenging systems is progressively disrupted, resulting in membrane lipid peroxidation and the accumulation of malondialdehyde (MDA), a widely used indicator of oxidative stress (Islayem et al., 2022). To eliminate excess ROS, superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD) constitute the major enzymatic antioxidant defense system and function cooperatively to detoxify ROS, thereby alleviating oxidative damage to cellular components (Zheng et al., 2025).

Mineral nutrients play a crucial role in regulating plant growth, delaying senescence, and improving crop productivity. Phosphorus (P) and potassium (K) are essential macronutrients that influence both yield formation and leaf senescence through the regulation of physiological metabolism and antioxidant defense systems (Berg et al., 2021). Adequate phosphorus supply has been shown to enhance antioxidant enzyme activities, reduce MDA accumulation, and maintain photosynthetic capacity and soluble protein content (Duan, 2013). Potassium fertilization activates key photosynthetic enzymes, increases chlorophyll content and net photosynthetic rate, and enhances antioxidant enzyme activity, effectively prolonging the green leaf period (Pettigrew, 2008). Potassium has also been shown to enhance antioxidant enzyme activities and strengthen the antioxidant defense system under environmental stress, as demonstrated in sesame and tobacco leaves. Increasing potassium supply has been reported to improve the activities of SOD, POD, and CAT, as well as total chlorophyll content in leaves, as observed in *Fritillaria thunbergii* (Che et al., 2022; Fang et al., 2022). Wright (1999) reported that potassium deficiency in cotton is a major cause of premature leaf senescence, closely related to source–sink imbalance. Previous studies have suggested that combined P and K application may improve nutrient use efficiency, photosynthetic performance, and crop productivity more effectively than the application of either nutrient alone. Therefore, appropriate P and K fertilization may synergistically enhance antioxidant defense, delay leaf senescence, and improve yield and industrial potential of *E. vulgare*. However, information regarding the interactive effects of P and K fertilization on leaf senescence, photosynthetic performance, antioxidant defense, and yield formation in *E. vulgare* remains limited.

Previous international research on *Echium vulgare L.* has predominantly focused on its bioactive ingredients and derived by-products, including seed oil, bee pollen, honey, and shikonin derivatives (Terzieva et al., 2025). Domestic studies have mainly examined the effects of planting density, germination temperature, and sowing date on crop yield (Xu et al., 2014a, 2014; Wang et al., 2023). Fertilization trials related to phosphorus (P) and potassium (K), have only recorded yield variation to date, without further exploring the underlying physiological responses of *E. vulgare*. Accordingly, we proposed the following research hypothesis: rational combined application of P and K exerts synergistic effects on enhancing photosynthetic performance and antioxidant capacity, delay leaf senescence, and ultimately improve yield formation in *E. vulgare*. In contrast, insufficient or excessive P and K supply may impair plant physiological metabolism and restrict yield potential. To date, few experimental studies have been conducted to verify this hypothesis. The objectives of this study were to: (1) clarify the single and interactive effects of P and K fertilization on leaf chlorophyll accumulation and photosynthetic characteristics of *E. vulgare*; (2) reveal how combined P–K application regulates antioxidant enzyme activities and membrane lipid peroxidation during leaf senescence; and (3) determine the optimal P–K fertilization formula for high-yield and cost-efficient cultivation of *E. vulgare*. Findings from this work will provide a theoretical basis and practical guidance for standardized regional cultivation, yield improvement, and quality enhancement of commercial *E. vulgare* production.

2 Materials and methods

2.1 Experimental site description

The field experiments were conducted in 2024 and 2025 at the experimental station of Jilin Agricultural University (Changchun, Jilin Province, 43°53′ N, 125°10′ E). The region has an annual accumulated effective temperature of 2860 °C, an average annual temperature of 4.6 °C, an annual precipitation of 567 mm, and a frost-free period of approximately 140 days. The soil is classified as black soil, with an organic matter content of 23.5 mg·kg^{-1}, alkaline-hydrolyzable nitrogen of 176.25 mg·kg^{-1}, available phosphorus of 24.3 mg·kg^{-1}, available potassium of 111.9 mg·kg^{-1}, total nitrogen of 1.645 g·kg^{-1}, and a pH of 6.3. The preceding crop was *Echium vulgare* L. During the two-year growth period, temperature conditions were relatively stable (Figure 1). In 2024, the average temperatures in spring, summer, and autumn were 28.0, 29.0, and 30.0 °C, respectively, and in 2025 they were 28.0, 29.0, and 31.0 °C, showing a similar trend between the two years, which met the normal growth requirements of *E. vulgare* L.

2.2 Experimental materials, design, and crop management

The seeds of *Echium vulgare* L. were obtained from the germplasm collection preserved at the College of Agriculture, Jilin

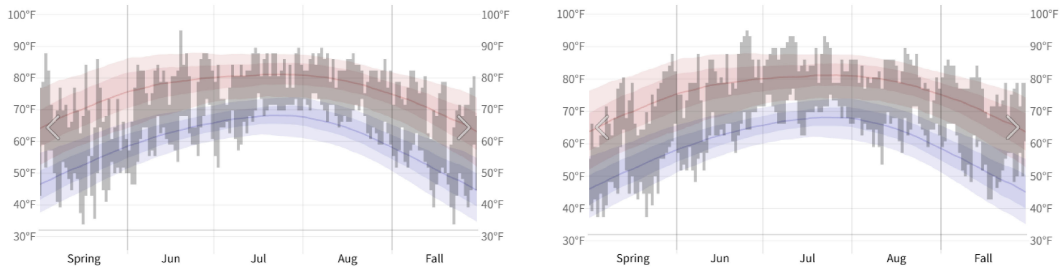


FIGURE 1
Historical temperature during the growth period. The daily range of reported temperatures (indicated by gray bars) and 24-h high (red checkmark) and low temperature (blue check) are placed above the daily average high (light red line) and low temperature (light blue line) and have 25–75% and 10–90% percentile bands. [Data quoted from: Weather Spark, website: https://zh.weatherspark.com].

Agricultural University. The experiment was conducted using a two-factor randomized block design, with phosphorus (P) and potassium (K) as factors. Four P levels were applied: 0 kg ha^{-1} (P1), 80 kg ha^{-1} (P2), 120 kg ha^{-1} (P3), and 160 kg ha^{-1} (P4), and four K levels were applied: 0 kg ha^{-1} (K1), 60 kg ha^{-1} (K2), 90 kg ha^{-1} (K3), and 120 kg ha^{-1} (K4). Each replicate consisted of three raised beds (65 cm wide, 10 cm high, 5 m long), separated by 1 m alleys. Seeds were sown in holes spaced 35 cm apart, with three seeds per hole. Buffer rows were established around the experimental plots to minimize edge effects. Seedlings were thinned after establishment, leaving one uniform seedling per hole, resulting in 42 plants per subplot. Conventional field management practices were followed throughout the growing season. Nitrogen (N) fertilizer was applied uniformly as a basal dose, with a total N application rate of 180 kg ha^{-1}, split in a ratio of 6:4 between basal application and top-dressing at the bud stage. The fertilizers used were urea (46% N), calcium superphosphate (16% P_2O_5), and potassium sulfate (52% K_2O), with application rates calculated according to their nutrient concentrations. The detailed P and K fertilizer application rates for each treatment are listed in Table 1.

2.3 Sample collection

Sample collection was conducted at the full flowering stage, when over 50% of the plants in each plot reached peak bloom. At this time, approximately one-third of the basal flowers on the main racemes had completed pollination and begun senescing, while the upper inflorescences were still budding. Three healthy and uniform *E. vulgare L.* plants were randomly selected per plot, and fully expanded mature leaves were harvested for the determination of chlorophyll content, leaf gas exchange parameters, malondialdehyde (MDA) concentration, and antioxidant enzyme activities. All samples were collected between 9:00 and 11:00 AM. Samples were immediately transported to the laboratory and pretreated to prevent water loss and oxidative damage.

2.4 Photosynthetic pigment (chlorophyll) measurement

Chlorophyll content was determined using the acetone–ethanol (1:1, v/v) extraction method. During the flowering stage, three uniform *Echium vulgare* L. plants were selected from each plot. From each plant, fully expanded functional leaves in the middle canopy with the same light exposure were collected, immediately frozen in liquid nitrogen, and stored at –80 °C until analysis. Leaf samples were completely immersed in 30 mL of the mixed extraction solution and extracted in the dark at room temperature for 24 h. The optical densities (OD) of the extracts were measured at 663, 645, and 470 nm using a UV–visible spectrophotometer, with untreated original extracts as the control. Chlorophyll content was calculated according to the method of Arnon.

2.5 Gas exchange parameter measurement

The photosynthetic rate (Pn), stomatal conductance (Gs), transpiration rate (Tr), ambient CO2 concentration (Ca) and intercellular CO2 concentration (Ci) of the leaves were measured from 10:00 to 12:00 on a clear and windless day at the mid-flowering stage using a LI-6400 portable photosynthetic apparatus (LI-6400 Inc., Lincoln, NE, USA). For each plot, three uniform plants, excluding those at the edges, were selected, and fully

TABLE 1 Fertilization treatment.

Treatments	Fertilizer application rate	
	P_2O_5 (kg ha^{-1})	K_2O (kg ha^{-1})
P1K1	0	0
P1K2	0	60
P1K3	0	90
P1K4	0	120
P2K1	80	0
P2K2	80	60
P2K3	80	90
P2K4	80	120
P3K1	120	0
P3K2	120	60
P3K3	120	90
P3K4	120	120
P4K1	160	0
P4K2	160	60
P4K3	160	90
P4K4	160	120

expanded functional leaves from the middle canopy with the same light exposure were used for measurements (Ren et al., 2021).

2.6 MDA content determination

MDA content was determined using the thiobarbituric acid (TBA) method (Li, 2000). Briefly, 1.0 g of leaf tissue was homogenized in 2 mL of 10% trichloroacetic acid (TCA). An additional 8 mL of 10% TCA was added, and the sample was further homogenized. The homogenate was then centrifuged, and 2 mL of the supernatant was mixed with 2 mL of 0.6% TBA solution. The mixture was incubated in a boiling water bath for 15 min, rapidly cooled, and centrifuged again. Absorbance was measured at 532, 600, and 450 nm.

2.7 Antioxidant enzyme activity determination

Antioxidant enzyme activities were determined according to Li Hesheng (Li, 2000). Fresh leaf tissue (0.5 g) was homogenized in 1 mL of phosphate buffer (0.05 mol·L^{-1}, pH 7.8) on ice. The homogenate was brought to a constant volume with buffer and centrifuged at 10,000 × g for 20 min at 4 °C. The supernatant was collected and stored on ice for subsequent assays.SOD activity was determined using the nitroblue tetrazolium (NBT) photoreduction method. A 50 μL aliquot of the supernatant was added to 3 mL of SOD reaction solution (containing phosphate buffer, NBT, EDTA-Na, riboflavin, and methionine) and exposed to 4000 lx light for 20–30 min. A dark control was used to calibrate the baseline. Absorbance was measured at 560 nm. One unit of enzyme activity (U) was defined as the amount of enzyme causing 50% inhibition of NBT photoreduction, and activity was expressed as U·g^{-1} FW.POD activity was determined using the guaiacol method. A 20 μL aliquot of supernatant was added to 3 mL of reaction solution (containing 0.1 mol·L^{-1} phosphate buffer, pH 6.0, and guaiacol), and the absorbance at 470 nm was recorded immediately every minute for 3 min. One unit of enzyme activity (U) was defined as an absorbance change of 0.01 per minute, expressed as U·g^{-1} FW.CAT activity was determined by UV absorption. A 100 μL aliquot of supernatant was added to 3 mL of reaction solution (containing 0.1 mol·L^{-1} phosphate buffer, pH 7.0, and 0.1 mol·L^{-1} H_2O_2), and absorbance at 240 nm was recorded immediately every minute for 3 min. One unit of enzyme activity (U) was defined as an absorbance change of 0.01 per minute, expressed as U·g^{-1} FW.

2.8 Yield

At maturity, ten *Echium vulgare* L. plants per plot were randomly selected and carefully uprooted, and the samples were brought to the laboratory. Seeds were air-dried, threshed, and weighed to determine the seed yield per plant, which was then used to estimate plot yield (Wang et al., 2023). For the determination of thousand-seed weight, the seeds from each plot were thoroughly mixed, and three random samples of 500 seeds each were weighed. After each 500-seed measurement, the seeds were returned to the bulk and mixed again before the next measurement. The average weight was then converted to thousand-seed weight (g).

2.9 Statistical analysis

All experimental data were initially organized in Microsoft Excel 2016. The Grubbs test at a 95% confidence level was applied to screen outliers to identify and remove outliers potentially arising from measurement or field variation; observations beyond the critical threshold were discarded, whereas valid raw data were reserved. Following outlier removal, all statistical analyses were conducted with SPSS 27.0, and results were expressed as mean ± standard error (SE). Three-way ANOVA (Year × P × K) was performed to detect the main and interactive effects of experimental year, phosphorus and potassium application rates. One-way ANOVA combined with Duncan's multiple range test ($P < 0.05$) was used to separate treatment differences in chlorophyll content (CC), gas exchange parameters (GEP), malondialdehyde content (MDA), antioxidant enzyme activities (AEA), thousand-grain weight (TGW) and grain yield (GY), including comparisons of varied K fertilization rates at a fixed P level and varied P fertilization rates at a fixed K level. Pearson's correlation analysis was carried out to quantify pairwise correlations among all physiological and yield indices, and all figures were plotted using Origin 2024.

3 Results

3.1 Effects of phosphorus and potassium fertilizer application on chlorophyll content of *Echium vulgare* L.

Chlorophyll is the primary photosynthetic pigment in plants and its content is a key physiological indicator of the photosynthetic capacity and functional status of *E. vulgare* leaves. At the same level of potassium (K), the chlorophyll content was significantly higher in the P3 treatment than in the other treatments (Figure 2). No significant difference was observed between the P2 and P4 treatments, which were both higher than the P1 treatment. At the same phosphorus level, the K3 and K4 treatments showed no significant difference, but both had significantly higher chlorophyll content than the K1 and K2 treatments. Notably, under the no-K application condition (K1), additional phosphorus fertilization failed to significantly increase chlorophyll content. However, the promoting effect of phosphorus fertilization was observed only when potassium was applied. These results suggest that the application of potassium fertilizer during the flowering stage is a key factor limiting chlorophyll accumulation and that the interaction between phosphorus and potassium significantly affects chlorophyll content in *Echium vulgare* at flowering.

3.2 Effects of phosphorus and potassium fertilization on photosynthesis of *Echium vulgare* L.

Phosphorus and potassium fertilization significantly enhanced the photosynthetic performance of *Echium vulgare* L. during the flowering stage (Figure 3). Net photosynthetic rate (Pn), stomatal conductance (Gs), and transpiration rate (Tr) were significantly higher in all fertilized treatments compared to the unfertilized control

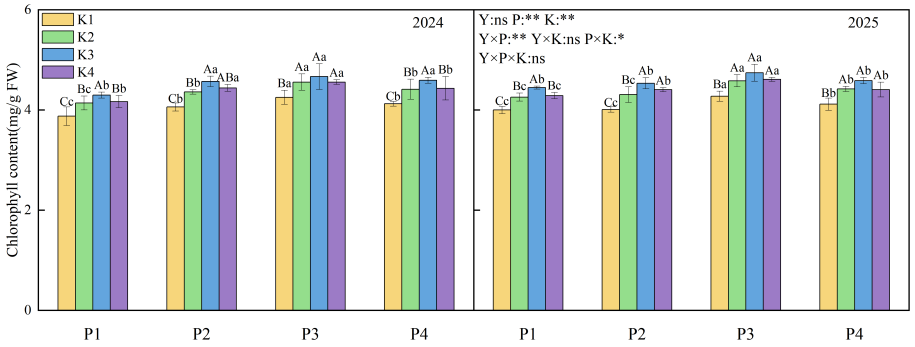


FIGURE 2
Effects of phosphorus and potassium fertilizer application on chlorophyll content of *Echium vulgare*. In the figure, 2024 and 2025 represent two independent experimental years. P1, P2, P3, and P4 represent phosphorus application rates of 0 kg ha^{-1}, 80 kg ha^{-1}, 120 kg ha^{-1}, and 160 kg ha^{-1}, respectively; K1, K2, K3, and K4 represent potassium application rates of 0 kg ha^{-1}, 60 kg ha^{-1}, 90 kg ha^{-1}, and 120 kg ha^{-1}, respectively. The vertical bars represent the standard error (SE). Different lowercase letters above the error bars indicate significant differences among different phosphorus treatments at the same potassium level ($P < 0.05$), and different uppercase letters above the error bars indicate significant differences among different potassium treatments at the same phosphorus level ($P < 0.05$). Y, C, and T represent year, cultivar, and treatment, respectively. "*" indicates a significant difference at the 0.05 probability level, and "*" indicates a significant difference at the 0.01 probability level.

(P1K1), while intercellular CO_2 concentration (Ci) decreased. At a given phosphorus level, Pn, Gs, and Tr generally increased with increasing potassium application, reaching a peak at K3, and slightly declining at K4. At a given potassium level, these parameters first increased and then decreased with increasing phosphorus application, with P3 showing the optimal effect, significantly higher than P1 and P2. Compared with the control (P1K1), the P3K3 treatment exhibited the most pronounced increases in Pn, Gs, and Tr, all exceeding 30%, whereas low-phosphorus or low-potassium treatments produced only modest increases. Analysis of variance indicated that year, phosphorus, and potassium had highly significant main effects on all four parameters, with a significant interaction between phosphorus and potassium. Interactions between year and phosphorus, and year and potassium, showed inconsistent patterns, and the three-way interaction was not significant. Overall, the combined application of phosphorus and potassium enhanced stomatal conductance and transpiration, promoting CO_2 supply and water metabolism, thereby significantly increasing net photosynthetic rate and demonstrating a positive causal relationship between fertilization treatment and photosynthetic performance.

3.3 Effects of phosphorus and potassium fertilization on malondialdehyde content of *Echium vulgare* L.

Malondialdehyde (MDA) content is an important physiological indicator reflecting the degree of membrane lipid peroxidation and the progression of leaf senescence in plants. As shown in Figure 4, in 2024, phosphorus or potassium application significantly reduced MDA content compared to treatments without phosphorus or potassium (P1, K1). During the flowering stage, MDA content showed no significant differences among P2, P3, and P4, but all were significantly lower than P1. Moderate phosphorus application (P3) was sufficient to maintain low oxidative damage throughout most of the growth period. Among potassium treatments, MDA content during flowering was significantly lower at intermediate potassium levels. Regarding combined P and K applications, under no phosphorus (P1) or low phosphorus (P2) conditions, increasing potassium application significantly decreased MDA content. Phosphorus alone did not reduce MDA content in the absence of potassium; only combined application effectively decreased MDA levels. In 2025, a similar trend was observed, except that under P1 and P2, increasing potassium initially decreased MDA content but showed a slight increase at the K4 level. Analysis of variance indicated that phosphorus, potassium, and their interaction significantly affected flowering-stage MDA content. No significant interactions were observed between year and phosphorus, or year and potassium. However, a significant three-way interaction among year, phosphorus, and potassium was detected.

3.4 Effects of phosphorus and potassium fertilization on antioxidant enzyme activities of *Echium vulgare* L.

Superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) play crucial roles in the antioxidant system of *Echium vulgare* L. Significant interannual differences were observed in the activities of SOD, POD, and CAT during the flowering stage, with overall higher enzyme activities in 2025 compared to 2024 (Supplementary Table 1). The interannual variation trends of SOD and POD activities were relatively stable, with similar increases across different phosphorus and potassium levels, whereas CAT activity showed a more pronounced increase under high potassium treatments. With increasing phosphorus and potassium application, the activities of all three enzymes generally exhibited a significant upward trend. At a given potassium level, enzyme activity increased with phosphorus application, reaching a plateau after P3, which was significantly higher than P1 and P2. At each phosphorus level, enzyme activity continued to increase with potassium application, with K3 and K4 significantly higher than K1 and K2. Combined phosphorus and potassium application showed a clear synergistic effect. At the same potassium level, P3 treatment significantly enhanced enzyme activity compared to P1 and P2, while at the same phosphorus level, high potassium

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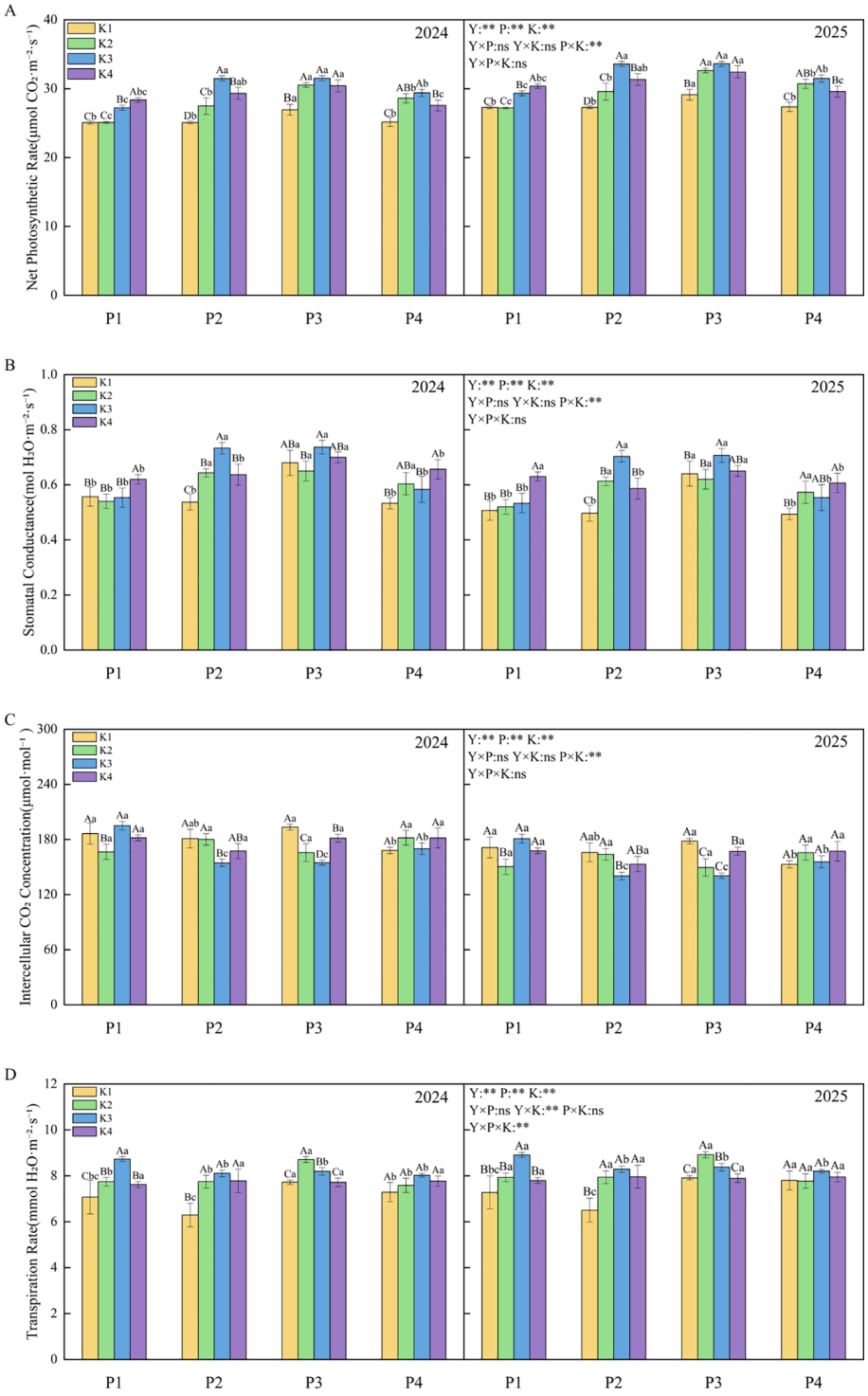


FIGURE 3
Effects of phosphorus and potassium fertilization on photosynthesis of *Echium vulgare* L. **(A)** Net photosynthetic rate (Pn), **(B)** Stomatal conductance (Gs), **(C)** Intercellular CO concentration (Ci), **(D)** Transpiration rate (Tr). Mean comparisons were performed using In the figure, 2024 and 2025 represent two independent experimental years. P1, P2, P3, and P4 represent phosphorus application rates of 0 kg ha^{-1}, 80 kg ha^{-1}, 120 kg ha^{-1}, and 160 kg ha^{-1}, respectively; K1, K2, K3, and K4 represent potassium application rates of 0 kg ha^{-1}, 60 kg ha^{-1}, 90 kg ha^{-1}, and 120 kg ha^{-1}, respectively. The vertical bars represent the standard error (SE). Different lowercase letters above the error bars indicate significant differences among different phosphorus treatments at the same potassium level ($P < 0.05$), and different uppercase letters above the error bars indicate significant differences among different potassium treatments at the same phosphorus level ($P < 0.05$). Y, C, and T represent year, cultivar, and treatment, respectively. "*" indicates a significant difference at the 0.05 probability level, and "**" indicates a significant difference at the 0.01 probability level.

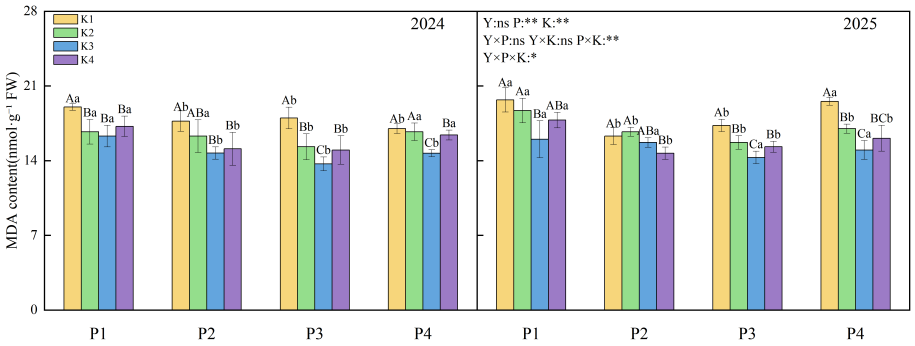


FIGURE 4
Effects of phosphorus and potassium fertilization on malondialdehyde content of *Echium vulgare* L. In the figure, 2024 and 2025 represent two independent experimental years. P1, P2, P3, and P4 represent phosphorus application rates of 0 kg ha^{-1}, 80 kg ha^{-1}, 120 kg ha^{-1}, and 160 kg ha^{-1}, respectively; K1, K2, K3, and K4 represent potassium application rates of 0 kg ha^{-1}, 60 kg ha^{-1}, 90 kg ha^{-1}, and 120 kg ha^{-1}, respectively. The vertical bars represent the standard error (SE). Different lowercase letters above the error bars indicate significant differences among different phosphorus treatments at the same potassium level ($P < 0.05$), and different uppercase letters above the error bars indicate significant differences among different potassium treatments at the same phosphorus level ($P < 0.05$). Y, C, and T represent year, cultivar, and treatment, respectively. "*" indicates a significant difference at the 0.05 probability level, and "**" indicates a significant difference at the 0.01 probability level.

treatments under P3 exhibited the most pronounced effect. The lowest enzyme activities were consistently observed in the control without phosphorus or potassium (P1K1), whereas P3K3 and P3K4 treatments maintained the highest activities across both years, significantly exceeding other combinations. Analysis of variance indicated that year, phosphorus, and potassium had highly significant main effects on the activities of all three enzymes. The interaction between phosphorus and potassium was highly significant. Interactions between year and phosphorus were not significant for any enzyme, and interactions between year and potassium were not significant for POD activity. No significant three-way interactions were observed, suggesting that the effect of phosphorus was stable across years, the regulation of SOD and CAT by potassium was influenced by interannual environmental conditions, and the synergistic regulation of phosphorus and potassium was consistent across years.

3.5 Effects of phosphorus and potassium fertilization on thousand-grain weight and grain yield of *Echium vulgare* L.

Both thousand-grain weight (TGW) and grain yield exhibited significant interannual differences (Supplementary Table 2). In 2025, TGW was generally slightly higher than in 2024, while the interannual response of grain yield was significantly influenced by phosphorus and potassium levels. With increasing phosphorus and potassium application rates, TGW and grain yield initially increased and then declined, with the highest values observed under P3 and K3 levels; further increases to P4 and K4 resulted in reduced or plateaued gains. A clear synergistic effect of phosphorus and potassium fertilization was observed: P1K1 consistently showed the lowest TGW and grain yield across both years, whereas optimal combinations such as P3K3 and P3K4 achieved the highest values, exhibiting the most pronounced yield enhancement. Analysis of variance indicated that year, phosphorus, and potassium had highly significant main effects on TGW and grain yield, and the interaction between phosphorus and potassium was also highly significant. The interaction between year and phosphorus significantly affected grain yield, and the interaction between year and potassium significantly affected TGW. The three-way interaction among year, phosphorus, and potassium significantly affected grain yield, indicating that potassium effects were relatively stable across years, whereas phosphorus regulation of grain yield was influenced by annual environmental conditions. Overall, the synergistic effect of phosphorus and potassium on yield remained consistent across different years.

3.6 Pearson correlations among parameters

The correlation matrix among chlorophyll content (CC), photosynthetic parameters (Pn, Gs, Tr), antioxidant enzyme activities (SOD, POD, CAT), MDA, thousand-grain weight (TGW), and grain yield (GY) is presented in Figure 5. CC, Pn, Gs, Tr, SOD, and CAT were strongly positively correlated with each other ($r > 0.71$, $P < 0.01$) and were strongly negatively correlated with MDA ($r < -0.62$, $P < 0.01$). TGW and GY were positively correlated with most photosynthetic and antioxidant indices and also showed a very strong positive correlation with each other ($r = 0.98$, $P < 0.01$).

4 Discussion

Chlorophyll is essential for plant photosynthesis (Janse et al., 2018). In this study, chlorophyll content in Echium vulgare leaves reached its maximum under the P3K3 treatment, which was significantly higher than under other phosphorus–potassium combinations. With increasing phosphorus and potassium application, chlorophyll content generally showed an increasing–decreasing trend, with a slight decline observed under the P4K4 treatment, indicating that excessive nutrient supply did not further enhance chlorophyll accumulation. At the physiological level, appropriate phosphorus and potassium supply may contribute to maintaining

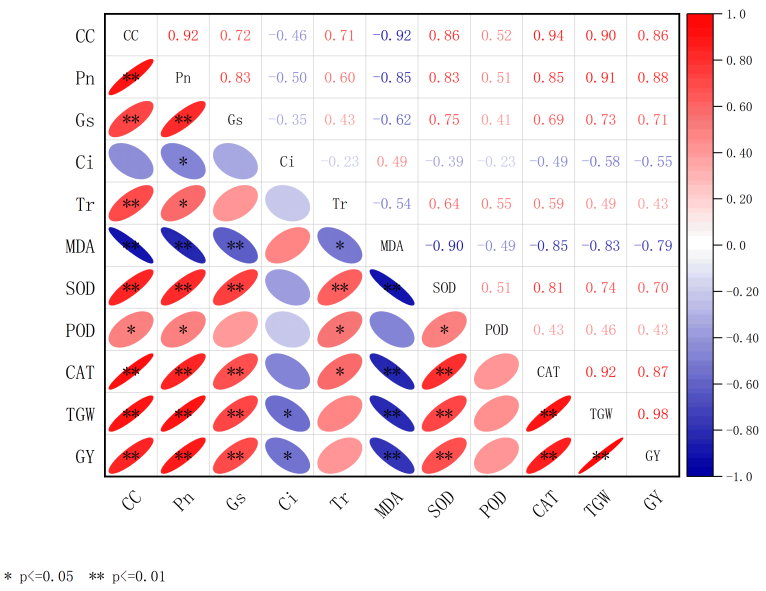


FIGURE 5
Correlation analysis of photosynthetic, antioxidant, and yield traits of *Echium vulgare* L. **Blue blocks represent negative correlations, and red blocks represent positive correlations; the larger the block, the weaker the correlation. Red lines indicate a correlation coefficient of 1. Values in upper triangular matrix represent Pearson correlation coefficients (r); * and ** are significantly correlation at P < 0.05 and P < 0.01, respectively CC, Chlorophyll content; Pn, Net photosynthetic rate; Gs, Stomatal conductance; Ci, Intercellular CO concentration; Tr, Transpiration rate; MDA, Malondialdehyde content; SOD, Superoxide dismutase activity; POD, Peroxidase activity; CAT, Catalase activity; TGW, 1000-grain weight; GY, Grain yield**.

chlorophyll content by supporting pigment metabolism, chloroplast function, and delayed senescence processes. However, the underlying molecular mechanisms remain unclear. It is speculated that P and K nutrition may be involved in the regulation of chlorophyll metabolism-related enzymes and stress-responsive signaling pathways, thereby indirectly affecting pigment stability (Tan et al., 2023). Both phosphorus and potassium had highly significant main effects on chlorophyll content, with a significant interaction between them. The interaction between year and phosphorus was also significant.

Photosynthesis is the fundamental basis for crop yield formation, and mineral nutrients regulate the photosynthetic process through multiple pathways (Lu et al., 2025). Among these, delaying crop senescence is a key approach, as senescence leads to leaf yellowing, shortened functional duration, and cessation of photosynthesis, thereby adversely affecting growth and final yield (Abeledo et al., 2020). Phosphorus and potassium can modulate the expression of genes involved in stomatal development and photosynthetic carbon fixation. For example, they influence the transcription of the Rubisco carboxylase gene, thereby affecting stomatal characteristics and carbon assimilation efficiency in leaves (Simões et al., 2020).Potassium deficiency has been reported to reduce stomatal conductance and increase mesophyll resistance to CO_2 diffusion, whereas phosphorus deficiency exerts a relatively smaller effect on gas exchange parameters (Javornik et al., 2023). Both phosphorus and potassium deficiencies can decrease stomatal aperture and transpiration; phosphorus deficiency tends to reduce stomatal density, while potassium deficiency may increase it (Nagarajah and Ratnasuriya, 1978). Maintaining an appropriate phosphorus–potassium ratio can help preserve turgor pressure in guard cells, enhance stomatal conductance (Gs), and reduce intercellular CO_2 concentration (Ci), thereby improving CO_2 assimilation efficiency (Simões et al., 2020). Consistent with these reports, in the present study, phosphorus or potassium deficiency generally led to a decline in photosynthetic performance, highlighting the importance of balanced P–K nutrition for optimal leaf function.

Under abiotic stress, excessive accumulation of reactive oxygen species (ROS) can oxidize and degrade carbohydrates, lipids, proteins, and nucleic acids within plant cells, ultimately causing severe damage to cellular structures and physiological functions (Gill and Tuteja, 2010). Accelerated plant senescence promotes lipid peroxidation, leading to increased malondialdehyde (MDA) content and enhanced cell membrane permeability (Chen et al., 2018). The activity of antioxidant enzymes in leaves influences the rate of senescence, with higher enzyme activity enhancing the plant's ability to scavenge harmful substances and thereby slowing the senescence process (Spanic et al., 2020). Consistently, antioxidant enzyme activities in this study were highly negatively correlated with MDA content (Figure 5). Phosphorus and potassium fertilization can influence crop stress resistance and senescence by regulating the expression of key antioxidant genes (Liu et al., 2022). An appropriate P–K ratio can significantly upregulate SOD, POD, and CAT gene expression, strengthen ROS scavenging, inhibit membrane lipid peroxidation, and reduce MDA accumulation. In contrast, nutrient imbalance can suppress antioxidant gene expression, disrupt ROS metabolism, and accelerate leaf senescence. With increasing phosphorus application, SOD, POD, and CAT activities initially increased and then plateaued, while MDA content continuously declined, consistent with the findings of Zheng et al. (2013). Increasing potassium supply progressively enhanced antioxidant enzyme activities and reduced MDA content, in agreement with Tang et al. (2024).

Phosphorus and potassium are essential macronutrients for plant growth and development, and their appropriate application is critical for crop yield formation. In this study, both insufficient and excessive P and K application reduced yield in Echium vulgare. At low to moderate fertilization levels, a synergistic interaction between phosphorus and potassium was observed, whereas excessive application resulted in antagonistic effects. This may be associated with improved physiological activity, delayed leaf senescence, extended photosynthetic duration, enhanced photosynthetic efficiency, and increased translocation of photosynthates to seeds, ultimately promoting yield formation (Wang, 2011).

Notably, when phosphorus was applied at 80 kg ha^{-1} without potassium fertilization, and when potassium was applied alone, seed yields of *E. vulgare* were 207.84 and 209.28 kg ha^{-1}, respectively, which were not significantly different from the unfertilized control (207.30 kg ha^{-1}). This indicates that single nutrient application at low rates was insufficient to improve dry matter accumulation, highlighting the importance of balanced P–K nutrition for yield enhancement. Compared with previous studies reporting a maximum yield of 361.49 kg ha^{-1} under combined fertilization (P_2O_5 120 kg ha^{-1} and K_2O 60 kg ha^{-1}), the present study achieved comparable yields with a higher potassium input (K_2O 90–120 kg ha^{-1}), suggesting potential optimization of fertilization strategy and cost efficiency. Overall, appropriate P–K combined application increased yield by approximately 100–150 kg ha^{-1} compared with single or no fertilization, indicating that balanced fertilization is more effective than individual nutrient application in improving productivity of *E. vulgare*. The optimal ranges of P_2O_5 (90–120 kg ha^{-1}) and K_2O (90–120 kg ha^{-1}) may serve as a reference for field management, subject to local soil fertility conditions.

5 Conclusions

The results demonstrated that phosphorus and potassium fertilization simultaneously optimized photosynthetic performance, enhanced antioxidant capacity, and increased thousand-grain weight and yield. Phosphorus primarily regulated the plant's fundamental physiological homeostasis; with increasing application, photosynthetic performance and antioxidant enzyme activities initially increased and then declined, while malondialdehyde (MDA) content first decreased and subsequently increased. Excessive phosphorus disrupted cellular metabolic balance, accelerating chlorophyll degradation and functional decline. In contrast, potassium mainly strengthened the plant's antioxidant defense system and stomatal regulation; increasing potassium supply steadily enhanced antioxidant enzyme activities and continuously reduced MDA content, with excessive potassium only slightly attenuating the positive regulatory effect. The combined application of phosphorus and potassium outperformed no fertilization or single-element application. Among all treatments, P3K3 exhibited the best performance, significantly increasing leaf chlorophyll content and gas exchange capacity, enhancing SOD, POD, and CAT activities, reducing MDA accumulation, mitigating membrane lipid peroxidation, delaying leaf senescence, and ultimately improving the yield of *Echium vulgare*.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding author.

Author contributions

XL: Data curation, Investigation, Methodology, Writing – original draft, Writing – review & editing. JY: Formal analysis, Investigation, Methodology, Writing – review & editing. AY: Conceptualization, Supervision, Writing – review & editing. HX: Conceptualization, Funding acquisition, Supervision, Writing – review & editing.

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Conflict of interest

The author(s) declared that this work was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fagro.2026.1877413/full#supplementary-material

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