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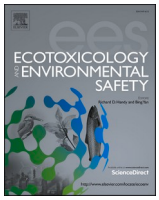

Resilient and sustainable production of peanut (*Arachis hypogaea*) in phosphorus-limited environment by using exogenous gamma-aminobutyric acid to sustain photosynthesis

Zhiyu Sun [a,1], Chunming Bai [b,c,1], Yifei Liu [a,c,d,e,*], Mingzhu Ma [a], Siwei Zhang [a], Huan Liu [a], Rui Bai [a], Xiaori Han [a], Jean Wan Hong Yong [d,f,**]

[a] College of Land and Environment, National Engineering Research Center for Efficient Utilization of Soil and Fertilizer Resources, Northeast China Plant Nutrition and Fertilization Scientific Observation and Research Center for Ministry of Agriculture and Rural Affairs, Key Laboratory of Protected Horticulture of Education Ministry and Liaoning Province, Shenyang Agricultural University, Shenyang, China
[b] Liaoning Academy of Agricultural Sciences, Shenyang, China
[c] The UWA Institute of Agriculture, The University of Western Australia, Perth, WA, Australia
[d] School of Biological Sciences, The University of Western Australia, Perth, WA, Australia
[e] School of Agriculture and Environment, The University of Western Australia, Perth, WA, Australia
[f] Department of Biosystems and Technology, Swedish University of Agricultural Sciences, Alnarp, Sweden

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ABSTRACT

Globally, many low to medium yielding peanut fields have the potential for further yield improvement. Low phosphorus (P) limitation is one of the significant factors curtailing *Arachis hypogaea* productivity in many regions. In order to demonstrate the effects of gamma-aminobutyric acid (GABA) on peanuts growing under P deficiency, we used a pot-based experiment to examine the effects of exogenous GABA on alleviating P deficiency-induced physiological changes and growth inhibition in peanuts. The key physiological parameters examined were foliar gas exchange, photochemical efficiency, proton motive force, reactive oxygen species (ROS), and adenosine triphosphate (ATP) synthase activity of peanuts under cultivation with low P (LP, 0.5 mM P) and control conditions. During low P, the cyclic electron flow (CEF) maintained the high proton gradient (ΔpH) induced by low ATP synthetic activity. Applying GABA during low P conditions stimulated CEF and reduced the concomitant ROS generation and thereby protecting the foliar photosystem II (PSII) from photoinhibition. Specifically, GABA enhanced the rate of electronic transmission of PSII (ETRII) by pausing the photoprotection mechanisms including non-photochemical quenching (NPQ) and ΔpH regulation. Thus, GABA was shown to be effective in restoring peanut growth when encountering P deficiency. Exogenous GABA alleviated two symptoms (increased root-shoot ratio and photoinhibition) of P-deficient peanuts. This is possibly the first report of using exogenous GABA to restore photosynthesis and growth under low P availability. Therefore, foliar applications of GABA could be a simple, safe and effective approach to overcome low yield imposed by limited P resources (low P in soils or P-fertilizers are unavailable) for sustainable peanut cultivation and especially in low to medium yielding fields.

1. Introduction

Peanut (*Arachis hypogaea* L.) is among the major oil and food legumes, and it is also a principal source of edible oil and protein in most developing countries (Zhuang et al., 2019; He et al., 2021; Li et al., 2022). As a leguminous crop, peanuts have high requirements for P and are sensitive to P bioavailability (Nikkhah et al., 2015; Cong et al., 2020; Shi et al., 2020; Dokwal et al., 2021). Many peanut-producing areas are located in developing countries and have low to medium-yielding fields, which implied that the soils in these countries are lacking in various

* Correspondence to: College of Land and Environment, Shenyang Agricultural University.
** Corresponding author at: School of Biological Sciences, The University of Western Australia, Perth, WA, Australia.
E-mail addresses: liuyifei@syau.edu.cn, yifeiliu6@hotmail.com (Y. Liu), jean.yong@slu.se (J.W.H. Yong).
[1] Zhiyu Sun and Chunming Bai contributed equally to this work.

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mineral nutrients and especially plant-available P (Kochian, 2012; Shi et al., 2020).

Phosphorus deficiency generally limits crop primary production and multiple factors contribute to the limitation of P bioavailability in soils (Zhu, 2016; Sharma et al., 2021; Wang et al., 2021). P is highly immobilized in soils due to chemical fixation by oxides and hydroxides of Fe^{3+} and Al^{3+} and the transformation of P to an organic state requires compatible microbial activities (López-Arredondo et al., 2014; Roy et al., 2016; Bindraban et al., 2020). Many of the world's farmlands are in regions with soil-bound P thereby leading to low P bioavailability for crop utilization (Kochian, 2012; Lambers, 2021). Many growers generally apply very high P-fertilization input in order to increase the P bioavailability. In due process, the high P-fertilization also increases the potential for P leaching through farmland drainage and surface runoff causing severe environmental pollution such as water eutrophication (Roy et al., 2016; Schneider et al., 2019; Bindraban et al., 2020; Janes-Bassett et al., 2022; Zhong et al., 2022). Most of the phosphate fertilizer is derived from phosphate rock, which is essentially a non-renewable resource (Fixen and Johnston, 2012). Based on the current production of P fertilizers globally, the naturally occurring phosphate rock resources will be exhausted in 50–100 years (Johnston et al., 2014; Lambers, 2021). It is noteworthy that the area of poor-yielding fields for peanut cultivation in China is large, and that the plant-available P in these soils is mostly in a state of deficiency (Shi et al., 2020). Thus, there is considerable interest to develop an effective nutritional regime to enhance P use efficiency where peanuts can grow normally to deliver high yield while using less P fertilizer.

P is an essential element for living organisms (Lambers and Oliveira, 2019; Wang et al., 2021). P is also an important component of nucleic acids, proteins, phospholipids, ATP, ADP, and various P-containing enzymes in plants (Lambers and Oliveira, 2019; Shi et al., 2020; Brown et al., 2022). P deficiency severely inhibits plant growth and development (Tiziani et al., 2020). For plants, the P deficiency has two typical characteristics: (a) the increased root-shoot ratio; (ii) severe photoinhibition (Liu, 2021). In general, P deficiency inhibits the growth of plant shoots, inhibits the growth of roots slightly, and even enhances the growth of roots (Fredeen et al., 1989). Plants under P deficiency allocate more photosynthetic carbon to roots. Growth depends on carbon gain through photosynthetic processes, and the photosynthetic processes are essentially dependent on P compounds (Veneklaas et al., 2012). Under a low P supply, the P contents of peanut leaves also decrease correspondingly (Shi et al., 2020). The low P levels in leaves are associated with low photosynthetic rates (Veneklaas et al., 2012). The internal phosphorus in barley leaves decreases as the P supply decreases (Lambers et al., 2011). Studies have shown that P deficiency can reduce Pi in chloroplasts, resulting in a decrease in ATP synthesis rate and ATP synthase activity (Carstensen et al., 2018). P deficiency is usually accompanied by oxidative stress (Zhang et al., 2017). The oxidative damage of the photosynthetic apparatus is caused by the imbalance between ROS scavenging and generation. The decrease in photosystems leads to photoinhibition (Khorobrykh et al., 2020). Furthermore, studies have shown that insufficient P supply leads to the thylakoid lumen acidification that alters the energy dissipation of PSII by triggering the energy-dependent quenching (qE) of NPQ (Yamori et al., 2016; Carstensen et al., 2018; Shi et al., 2020).

Interestingly, the non-proteinogenic amino acid γ-aminobutyric acid (GABA) was first isolated in 1949 from potatoes (Steward et al., 1949), prior to its discovery in animal extracts (Roberts and Frankel, 1950). With further research over the decades, it was suggested GABA might act as an inhibitory neurotransmitter in animals and humans (Owens and Kriegstein, 2002; Hellier, 2014; Hepsomali et al., 2020). With further intensive plant-related research, GABA is considered to be ubiquitous in plants (Steward et al., 1949; Li et al., 2021a). In recent years, GABA has been gradually applied to regulate the growth and development of plants under various biotic and abiotic stress (Bown and Shelp, 2016; Liu et al., 2021). The role of GABA in plants as a signaling molecule is beginning to emerge with many studies (Bown and Shelp, 2016; Fromm, 2020; Li et al., 2021a). GABA plays a crucial role in regulating plant growth and development (Uzma Jalil et al., 2019; Du et al., 2020; Khan et al., 2021). Exogenous GABA is assimilated and quickly metabolized to succinic acid that enters into the tricarboxylic acid cycle (TCA) cycle (Hijaz and Killiny, 2019). Exogenous GABA activates the TCA cycle to generate more energy and also acts as a signal molecule and metabolite to regulate protein expression in various physiological processes such as ROS scavenging systems and photoreaction (Fan et al., 2015). GABA alleviates oxidative stress by inducing a series of antioxidative enzymatic activities (Nayyar et al., 2013; Wang et al., 2017). The application of GABA enhances the actual quantum yield of PSII [Y(II)] under salt stress, and alleviates the inhibition of PSII (Li et al., 2016a). The GABA shunt of tomatoes under P deficiency is reported to be completely activated and not activated in control plants (Li et al., 2020). At present, the mechanism by which exogenous GABA alleviates foliar photoinhibition in plants under P deficiency is unknown (Li et al., 2020; Khan et al., 2021).

Due to its unique pharmacological as a non-proteinogenic amino acid, and non-toxic properties, GABA is sold as a dietary supplement in many countries to deliver a calming effect (anti-anxiety effects, enhancing sleep) in humans (Owens and Kriegstein, 2002; Foster and Kemp, 2006; Hellier, 2014; Hepsomali et al., 2020). From a biochemistry perspective, the amino group in GABA is not attached to the alpha carbon; thus it is not incorporated into proteins like the regular alpha-amino acids (Hellier, 2014). It is reported recently that various microorganisms in the environment, including bacteria, fungi, and yeast, could secrete GABA (Sarasa et al., 2020; Heli et al., 2022). GABA is also produced through a bio-based green route from agricultural waste, such as waste gluten (Renes et al., 2017). Furthermore, due to its wide availability and safe property, GABA is often used as an additive in food and feed materials (Sarasa et al., 2020; Heli et al., 2022). Thus, GABA can be widely used in agricultural production without affecting human health and the environment. Moreover, GABA is a safe substance as any amino acid leachates will be decomposed rapidly in soil (within minutes to hours) (Enggrob et al., 2019; Hill and Jones, 2019).

It was shown earlier that exogenous GABA improved photosynthesis and plant growth under abiotic stress (Braga-Reis et al., 2021). The aspect of GABA-mediated physiological responses to low P stress in plants was first reported in 2020 (Li et al., 2020). However, the relationship between exogenous GABA and plant growth status during low nutrient conditions (i.e., P deficiency) remains unclear. There is also no information about the effects of GABA on various foliar gas exchange functionality (photosystems activities, photosynthetic electron transport, and ATP synthase activity) under conditions of P limitation (Shelp et al., 2021). Therefore, from the perspective of delivering safe and sustainable peanut production, it is vital to understand the mechanism (s) of improving P utilization and especially under low P conditions. The main objectives of this study are: 1) To evaluate the effects of GABA on growth and development of peanuts under low P condition; 2) To evaluate the effects of GABA on ROS of peanuts under sub-low P condition; 3) To examine the effects of GABA on PSI activity, PSII activity, chlorophyll ATP synthase, and proton gradient in thylakoid lumen in peanut under low P condition by fluorescence probe technique.

2. Materials and methods

The common peanut cultivar in China, "Liaoning Baisha" was used in 2020. Peanut seeds were germinated in a sterile tray in an incubator for two days at 28 °C, then peanut seeds of uniform size were selected and transplanted into 50 pots (114 mm height, 136 mm diameter, 1 seeding per pot) of an equal amount of sand. The pots were placed in a controlled climate chamber (Converon, Winnipeg, Canada), with a daytime temperature of 28 °C and a nocturnal temperature of 23 °C at a relative humidity (RH) of 60 ± 5 %. The Hoagland's nutrient solution was supplied every two days. On day 14, 1.5 L ultrapure water was used in

each pot to remove any localized nutrient salt patches through flushing. Then the pots with peanut seedings of uniform size were divided into four treatment groups with the same quantity: (1) control plants (CK) (normal P level+ foliar spray of type 1 ultrapure water), (2) GABA (normal P level + foliar spray of 10 mM GABA), (3) LP (low P stress + foliar spray of type 1 ultrapure water), (4) LP + GABA (low P stress + foliar spray of 10 mM GABA). The nutrient solution is configured according to the published article of our previous study (Shi et al., 2020).

Based on preliminary experiments carried out for this specific peanut cultivar, two P levels in the form of KH_2PO_4 were used (Shi et al., 2020): the normal P level for CK (1 mM), and the low P level for LP (0.5 mM) in peanut cultivation. All nutrients were prepared with type 1 ultrapure water (Milli-Q Element, Millipore, Burling, USA). The pH of the solution was adjusted to 6 ± 0.3 with ultrapure HCl. To maintain the appropriate treatment conditions in the rhizosphere, 25 mL of the solution for each treatment was supplied every two days.

The optimum concentration of GABA (10 mM) was established in our previous experiments. The seedling leaves of the peanut were sprayed until dripping with ultrapure water. For the GABA and LP + GABA treatments, 10 mM GABA was evenly applied twice a day (at 8:00 and 16:00) for 3 days [6, 7, and 8 days of LP treatment (DoL)]. For the CK and LP treatments, ultrapure water was evenly applied twice a day (at 8:00 and 16:00) for 3 days [6, 7, and 8 days of LP treatment (DoL)].

At 9 DoL, three seedlings of each treatment were selected for measurements of biomass, plant height, leaf area, leaf relative chlorophyll concentration, leaf gas exchange, superoxide radical ($O_2^{\cdot-}$) generation rate, and leaf hydrogen peroxide (H_2O_2) concentration. Relative chlorophyll concentration was determined non-destructively on the third youngest fully expanded leaf of the main stem using a hand-held chlorophyll meter (SPAD-502 plus, Japan) at 10 DoL; the measurements were carried out on leaf lamina and avoiding the leaf veins (Ding et al., 2018b). For consistency, the net photosynthetic rate (Pn), Stomatal conductance (g_s), transpiration rate (Tr), and intercellular CO_2 conduction (C_i) were monitored on the third youngest fully expanded leaf with an open gas exchange system (GFS 3000; Heinz Walz, Effeltrich, Germany) at 9 DoL. All gas exchange measurements were taken at a PPFD of 1000 μmol photons $m^{-2}{\cdot}s^{-1}$, a constant airflow rate of 750 $\mu mol{\cdot}s^{-1}$. The concentration of CO_2, leaf cuvette relative humidity, and temperature were set to 400 μmol CO_2 mol^{-1}, 60 %, and 25 ℃, respectively (Shi et al., 2020). For authentication, the various gas exchange readings obtained using the GFS 3000 Walz system were checked using readings obtained using another open gas exchange system Li-6400 (Licor Inc, Lincoln, Nebraska, US) on peanuts and other species (Yong et al., 2010).

Chlorophyll fluorescence and P700 parameters were measured on the third youngest fully expanded leaf using the Dual-PAM 100 (Heinz Walz, Effeltrich, Germany) as described elsewhere (Wu et al., 2020a). The fluorescence slow kinetics were measured after fully dark adjustment at 9 DoL. The rapid light curves (RLCs) were determined at 10 DoL. The RLCs were determined at the light intensity of 0, 22, 30, 48, 106, 184, 226, 342, 513, 771, and 1190 μmol $quanta{\cdot}m^{-2}{\cdot}s^{-1}$. Y(I) is the actual quantum yield of PSI. The value of CEF = ETR(I) – ERT(II) and the ratio of the quantum yield of CEF to Y(II) [Y(CEF)/Y(II)] = [Y(I)-Y(II)]/YII were used to determine cyclic electron flow (Wu et al., 2020a). Chlorophyll fluorescence images were determined with an imaging-pulse-amplitude-modulated (PAM) chlorophyll fluorometer (Heinz Walz, GmbH, Effeltrich, Germany) as described elsewhere (Wu et al., 2020a). Electrochromic shift (ECS) single was monitored simultaneously using Dual-PAM 100 with a P515/535 module (Wu et al., 2020a). Three independent peanut seedlings per treatment were selected at 10 DoL for the determination of the following indicators. After 90 min of dark adaptation, P515 signal changes induced by a single turnover flash were measured. After 10 min of light at 1000 μmol $quanta{\cdot}m^{-2}{\cdot}s^{-1}$, dark adaptation 4 min, P 515 signal changes induced by saturating single turnover flashes were measured. The dark-light-dark induction curve reflects the change in membrane potential. Actinic light (AL; 600 μmol $quanta{\cdot}m^{-2}{\cdot}s^{-1}$) was turned on at 5 s and off at 125 s. The ΔpH and transmembrane potential (ΔΨ) across the thylakoid membranes were analyzed by using ECS signal changes.

As an index of lipid peroxidation, malondialdehyde (MDA) was assayed according to the method (Heath and Packer, 1968; Gupta et al., 2017). The samples (0.2 g) of peanut leaves were homogenized with trichloroacetic acid and centrifuged. The supernatant was measured according to the instructions. MDA content was calculated according to the following formula·H_2O_2 was assayed according to the previous method (Yu et al., 2003). Peanut leaf samples (0.5 g) were homogenized with phosphate buffer and centrifuged. The supernatant was measured according to the instructions. The H_2O_2 content (μmol /L) was calculated using the spectrophotometric readings measured at an absorbance of 415 nm. The generation rate of $O_2^{\cdot-}$ in peanut leaves was assayed according to the method (Elstner and Heupel, 1976; Nguyen et al., 2021). Peanut leaf samples (0.2 g) were homogenized with phosphate buffer and centrifuged at 10000 r/min for 20 min at 4 °C. Supernatant was measured according to the instructions. The $O_2^{\cdot-}$ content (μmol /L) was calculated based on a comparison of absorbance with a standard curve (using sodium nitrite as the standard) at 530 nm. The O_2- generation rate was expressed as:

$$MDA \quad content(\mu mol/L) = 6.45\ (\ OD_{532} - OD_{600}\ ) - 0.56OD_{450}$$

$$O_2^- \quad \text{generation rate}[\mu mol/(g{\cdot}h)] = \frac{2N\times m_1}{V_2\times m\times t}$$

Peanut leaves were ground to a uniform particle for leaf P analysis by digestion with H_2SO_4–H_2O_2 and quantification using the molybdenum blue method. P contents were obtained by P concentrations and dry weight (DW) values (Li et al., 2021b). Peanut leaves simple (0.1 g DW) were digested by microwave system (Milestone Ethos one, Italy) with 5 mL HNO_3 (65 %, guarantee reagent) 1 mL H_2O_2 (30 %, guarantee reagent), and 2 mL H_2O (type1 ultrapure water, Milli-Q Element, Millipore, Burlington, USA). The microwave digestion program was 10 min at 100 ℃, 10 min at 170 ℃, and 20 min at 180 ℃. The digest was diluted to 50 mL in water (type 1 ultrapure water) and filtered through a quantitative filter paper. The leaf concentration of calcium (Ca) was determined by flame atomic absorption spectrometry (280FSAA, Agilent Technologies, America). Ca contents were obtained by metal concentrations and DW Values.

The experiment adopted a random block design. The results presented here are the mean values and standard errors of three biological replicates of a final experiment, following earlier unpublished baseline studies. Statistical analysis was carried out using one-way ANOVA in Origin 2022. The results were presented as mean values and standard errors of three biological replicates. Fisher LSD tests at P = 0.05 were performed to highlight the differences among the three treatments. Indicate significant differences among treatments at $P < 0.05$.

3. Results

3.1. Plant growth, leaf P and Ca concentration

The relative growth and development parameters were measured to examine the effects of exogenous GABA on peanut growth under low P stress (Fig. 1). The low phosphorus (LP) treatment decreased plant height in peanuts. The LP + GABA treatment produced taller plants than the LP treatment (Fig. 1A). The LP treatment reduced dry matter accumulation in peanuts (Fig. 1B). Compared with LP, the LP + GABA treatment enhanced the dry weight of peanuts. And LP treatment reduced the leaf area in peanuts compared with CK (Fig. 1C). No significant differences in leaf area were observed between LP and LP + GABA treatments. The CK and GABA had the highest relative chlorophyll concentration (SPAD), followed by LP + GABA, with the lowest in LP (Fig. 1D). Compared to other treatments, the LP treatment reduced leaf P and Ca concentrations. And the GABA and LP + GABA enhanced them into the level of CK (Fig. 1E, F).

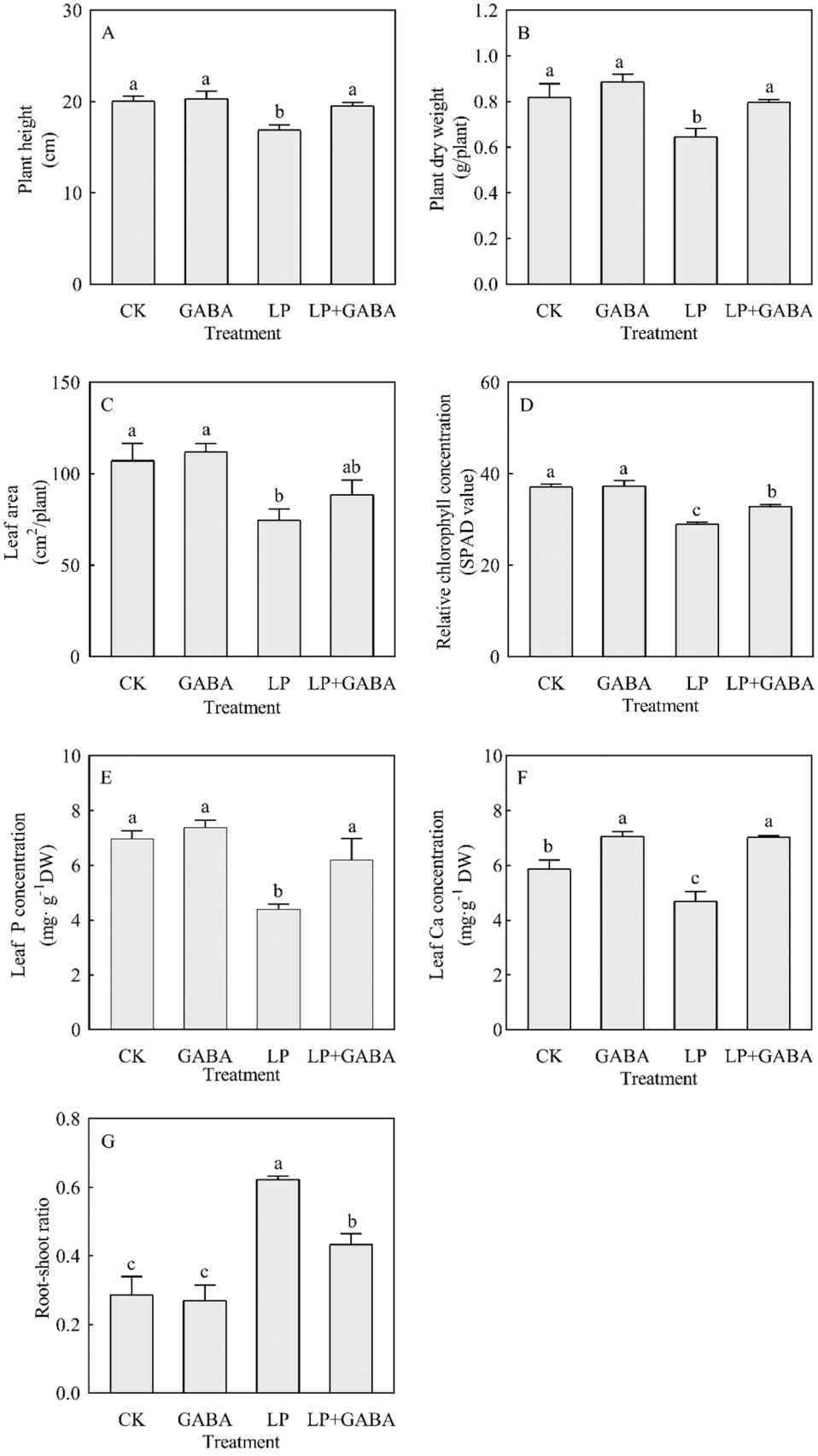

Fig. 1. Effects of exogenous gamma-aminobutyric acid (GABA) on (A) plant height, (B) total dry weight, (C) leaf area, (D) relative chlorophyll concentration (SPAD values), (E) leaf P concentration, (F) leaf calcium (Ca) concentration, and (G) root-shoot radio in peanut leaves under low P stress. Values are means of three biological replicates ± SE (n = 3). Different letters indicate significant differences among treatments at $P < 0.05$. Note: CK refers to the control plants and LP refers to plants receiving low phosphorus.

3.2. Superoxide anion generation rate, hydrogen peroxide, and lipid peroxidation

The peanut plants receiving low phosphorus (LP) treatment had a higher leaf $O_2^{\cdot-}$ generation rate and H_2O_2 content than the control plants (CK). And the combination of LP + GABA reduced them significantly (Fig. 2A, B). Additionally, exogenous GABA-induced alleviation of oxidative damage in peanut leaves under LP is evident in reduced MDA content (Fig. 2C).

3.3. Leaf gas exchange

To investigate the effects of exogenous GABA on the gas exchange capacity of peanuts under low P stress, we carried out measurements consistently on the third fully expanded true leaf (Fig. 3). No significant differences in C_i and Tr were observed among the different treatments. Compared with CK, LP reduced Pn and g_s; in contrast, LP + GABA enhanced Pn and g_s in peanuts leaves (Fig. 3).

3.4. Foliar photosystem activity

The leaves in LP treatment had a lower maximal quantum yield of PSII (F_v/F_m) than CK, while LP + GABA had similar values to CK (Fig. 4A). The leaves in the LP treatment had higher NPQ than that of CK; LP + GABA had lower NPQ than LP (Fig. 4B).

The LP treatment decreased the Y(II) relative to CK (Fig. 4C). The Y (II) of LP treatments declined and dissipated excess energy by increasing the regulatory quantum yield of PSII [Y(NPQ)] (Fig. 4E). The rise of non-regulatory quantum yield [Y(NO)] to a higher level indicates that the heat dissipation was not sufficient to dissipate the excess excitation energy of PSII in LP treatment. Compared to LP treatment, LP + GABA treatment enhanced Y(II) by reducing Y(NPQ) and Y(NO) (Fig. 4D, E).

The LP treatment reduced Y(I) compared with other treatments (Fig. 4F). The quantum yield of PSI nonphotochemical energy dissipation due to acceptor-side limitation [Y(NA)] had no significant differences among all treatments (Fig. 4G). Compared with other treatments, LP enhanced the quantum yield of PSI nonphotochemical energy dissipation due to donor-side limitation [Y(ND)] significantly (Fig. 4H).

3.5. Photosynthetic electron transport

The electron transport rate of PSII and PSI in peanut leaves increased with the intensification of light intensity. The LP treatment reduced both ETR(II) and ETR(I) than those of CK. The combined LP + GABA treatment enhanced both ETR(II) and ETR(I) under high-light conditions relative to LP (Fig. 5A, B). The LP + GABA treatment gave the highest CEF rate in all treatments beyond 186 μmol quanta m^{-2} s^{-1} of light intensity (Fig. 5C). The LP treatment increased the ratios of the quantum yield of CEF to Y(II) compared to CK, and LP + GABA enhanced it more than that of LP from 226 μmol quanta m^{-2} s^{-1} onward. And GABA treatment also enhanced the ratios of the quantum yield of CEF to Y(II) relative to CK (Fig. 5D).

3.6. Thylakoid membrane integrity, ATP synthase activity, and the proton motive force

The higher activity of ATP synthase, the faster the proton efflux and the faster the ESC signal will decay (Fig. 6B). LP treatment reduced the membrane integrity and ATP-Synthase activity of thylakoid based on P515 signaling (Fig. 6A). Compared with LP treatment, LP + GABA treatment enhanced the membrane integrity and ATP-synthase activity of thylakoids (Fig. 6A, B). GABA treatment enhanced the membrane integrity and ATP-synthase activity of thylakoid, relative to CK (Fig. 6A, B). Meanwhile, LP treatment also reduced the transmembrane potential

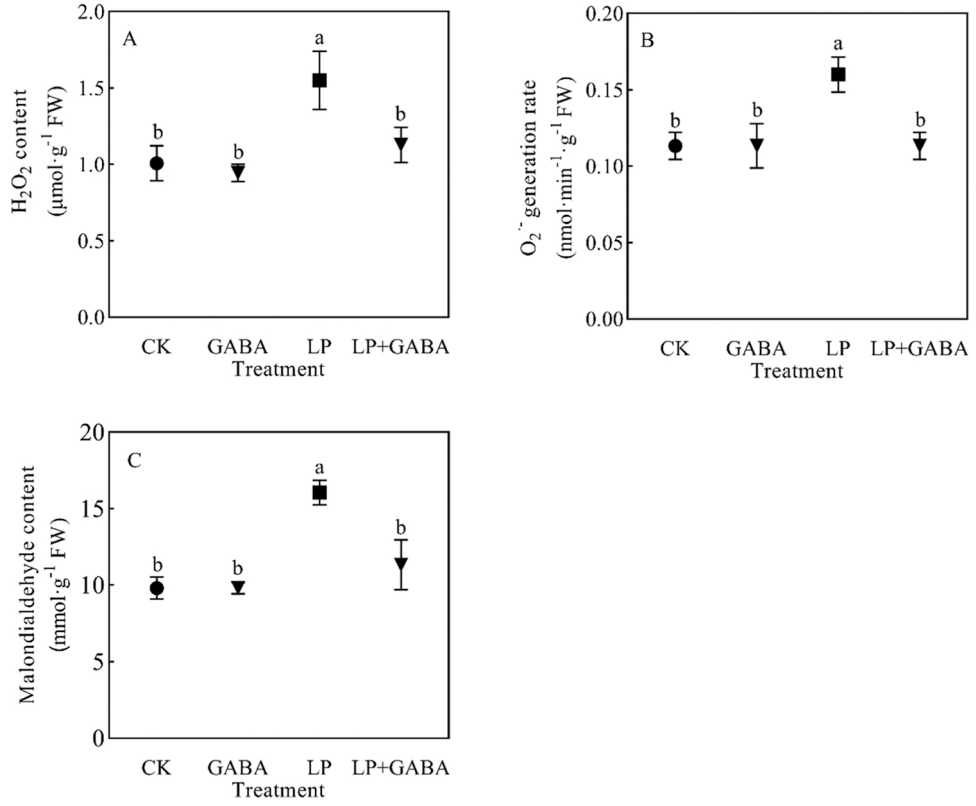

Fig. 2. Effects of exogenous gamma-aminobutyric acid (GABA) on (A) hydrogen peroxide (H_2O_2) content, (B) $O_2^{\cdot-}$generation rate, and (C) malondialdehyde (MDA) content in peanut leaves under low P stress. Values are means of three biological replicates ± SE (n = 3). Different letters indicate significant differences among treatments at $P < 0.05$. FW: fresh weight.

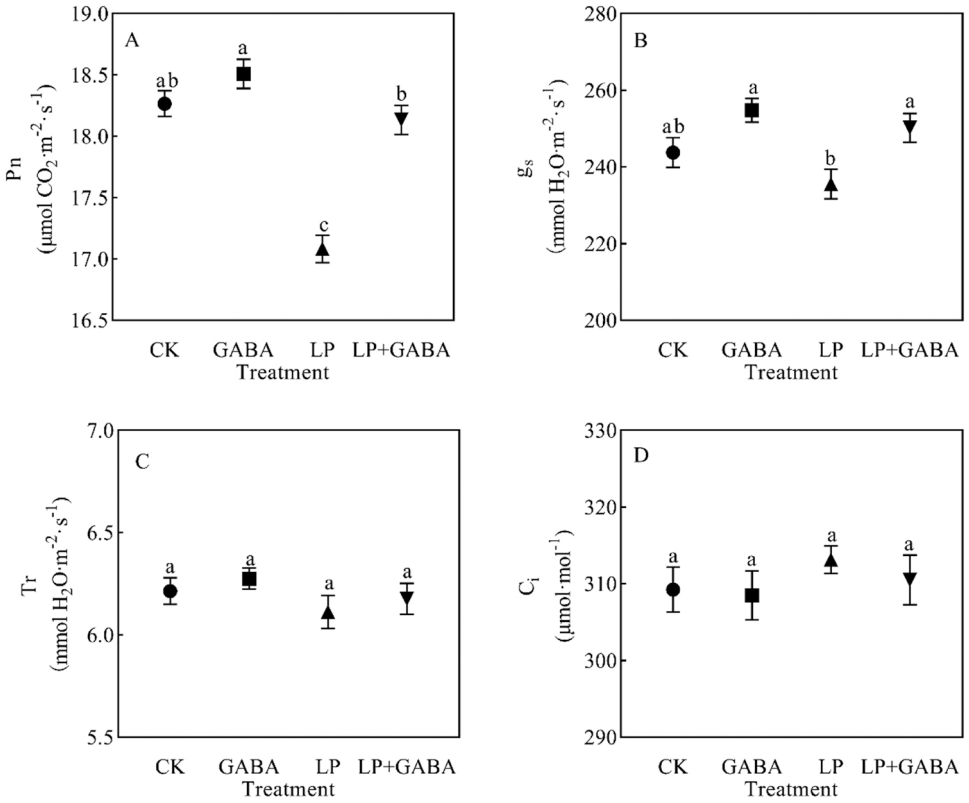

Fig. 3. Effects of exogenous gamma-aminobutyric acid (GABA) on (A) net photosynthetic rate (Pn), (B) stomatal conductance (g_s), (C) transpiration rate (Tr), and (D) intercellular CO_2 concentration (C_i) in peanut leaves under low P stress. Values are means of three biological replicates ± SE (n = 3). Different letters indicate significant differences among treatments at $P < 0.05$.

ΔΨ and increased the proton gradient ΔpH of the thylakoid lumen (Fig. 6D). Compared with LP treatment, LP + GABA treatment enhanced ΔΨ and decreased ΔpH. No significant differences were observed in proton motive force (PMF) among all treatments (Fig. 6E).

4. Discussion

4.1. Effects of low P stress on peanut photosynthetic capacity and growth

Phosphorus (P) has a crucial role in plant growth and development (Johnston et al., 2014; López-Arredondo et al., 2014; Ren et al., 2018; Lambers and Oliveira, 2019), and low P stress inhibited the development of peanuts (Figure1A-D), which is consistent with earlier findings (Shi et al., 2020). Under low P stress, the light-capturing ability was limited by smaller leaf area and lower leaf chlorophyll concentration (Fig. 1C, D). The results showed that the root-shoot ratio increased under low P stress (Fig. 1G). One of the typical responses to P deficiency is an increase in the root-shoot ratio (Liu, 2021). Earlier studies on melon, *Medicago truncatula*, *lupinus* species, and other crops showed that P deficiency inhibited plant growth and development (Ding et al., 2018a; Li et al., 2018; Wang et al., 2020; Dokwal et al., 2021; Pang et al., 2023).

Low P stress inhibited the net photosynthetic rate of peanut leaves (Fig. 3A). We demonstrated that the leaf P concentration of peanuts under low P stress was decreased (Fig. 1E), which is consistent with the leaf P concentration corresponding with a suboptimal P range (Shi et al., 2020). Concentrations of P and Ca^{2+} in peanut leaves decreased even under low phosphorus stress. Previous studies have shown that Ca^{2+} and P concentrations in lettuce leaves decreased simultaneously as the P supply was lowered (Hayes et al., 2019). The decrease of P in leaves will affect photosynthetic carbon assimilation and other metabolic processes in plants (Pieters et al., 2001; Carstensen et al., 2018). P deficiency lowered the photosynthetic rates due to its direct effect on the Pi level in the chloroplasts (Singh and Reddy, 2015). The dark reaction of photosynthesis is the main factor limiting the net photosynthetic rates of plants under P deficiency (Fleisher et al., 2012). Low P levels decreased photosynthetic rates and increased the proportion of starch in the product (Heldt et al., 1977). Due to low Pi and high sugars in plants under low P levels, ADP glucose pyrophosphorylase transcription increased, which caused starch accumulation in the chloroplasts of these plants (Zhang et al., 2014). The accumulation of starch induced by P deficiency can indirectly inhibit the activity of Calvin cycle enzymes, thereby reducing the photosynthetic rates (Zhang et al., 2014; Yan et al., 2015; Carstensen et al., 2018).

Based on our analyses, the $O_2^{\cdot-}$ generation rate (Fig. 2B), H_2O_2 content (Fig. 2A), MDA content (Fig. 2C), and the integrity of the thylakoid membrane (Fig. 6A) demonstrated that low P stress-induced photooxidation stress in peanut leaves. Phosphorus deficiency increased the accumulation of H_2O_2 in leaves when undergoing LP stress(Fig. 2), which is consistent with earlier findings in cucumbers (Wang et al., 2022). P deficiency caused an imbalance in ROS metabolism, causing a dramatic increase in ROS in chloroplasts (Foyer and Hanke, 2022; Wang et al., 2022). Even the leaves of cucumbers under moderately low phosphorus supply also produced excessive $O_2^{\cdot-}$ and H_2O_2, resulting in significant oxidative damage (Wang et al., 2022). ROS is the main cause of leaf membrane lipid peroxidation and chlorophyll reduction during photo-oxidation stress (Kim and Apel, 2013; Agnihotri et al., 2018; Gupta and Seth, 2022). Cellular membranes or organelle membranes are especially susceptible to ROS damage, thus increasing the MDA content (Singh et al., 2017; Su et al., 2019; Kumar and Seth, 2021). Some studies suggest that the decrease in antioxidant enzyme activity is the main cause of ROS metabolism imbalance (Wang et al., 2022). Antioxidant enzymes themselves are not sufficient to remove these ROS under stress (Kapoor et al., 2019; Kumar et al., 2023). When the downstream electron acceptor of the photosynthetic electron transport chain is limited,

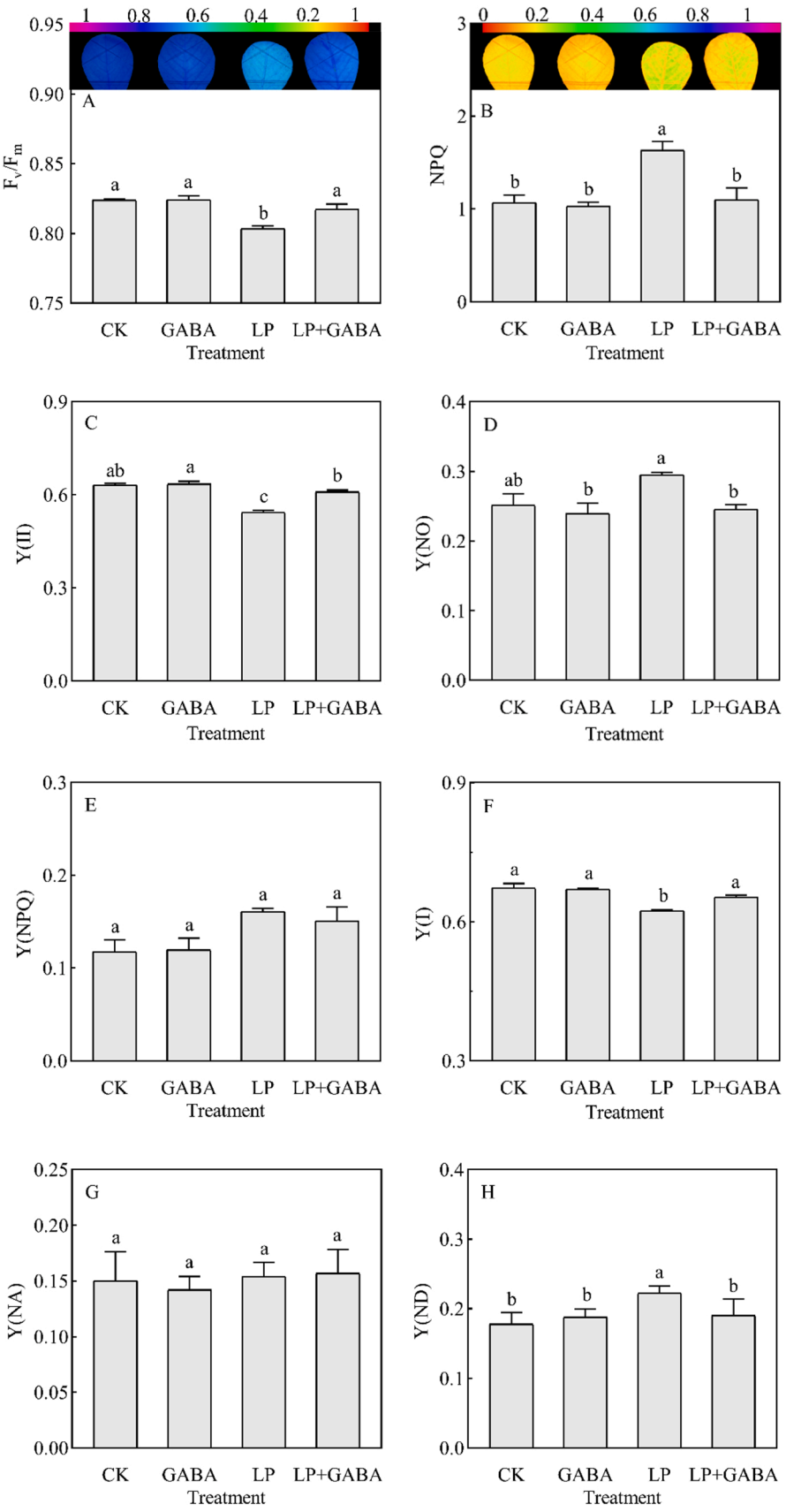

Fig. 4. Effects of exogenous gamma-aminobutyric acid (GABA) on (A) F_v/F_m, (B) NPQ, (C) Y(II), (D) Y(NO), (E) Y(NPQ), (F) Y(I), (G) Y(NA), and (H) Y(ND) in peanut leaves under low P stress. The color photos of A and B are foliar chlorophyll fluorescence images. Values are means of three biological replicates ± SE (n = 3). Different letters indicate significant differences among treatments at $P < 0.05$.

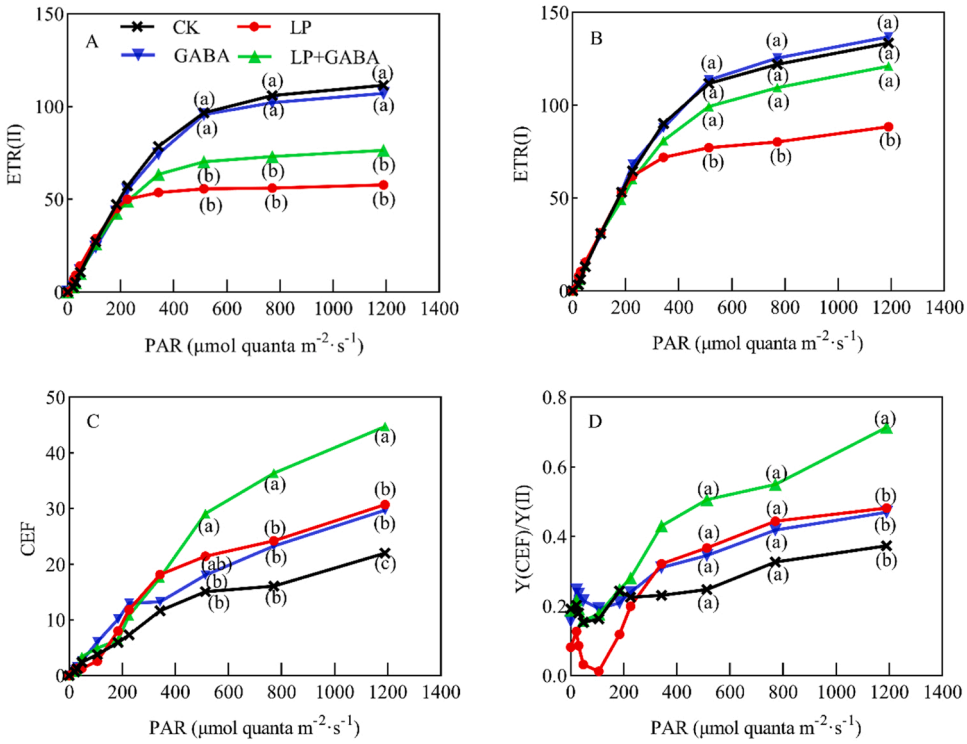

Fig. 5. Effects of exogenous gamma-aminobutyric acid (GABA) on (A) ETR(II), (B) ETR(I), (C) CEF, and (D) Y(CEF)/Y(II) in peanut leaves under low P stress. The value of CEF = ETR(I) – ERT(II) and the ratio of the quantum yield of CEF to Y(II) [Y(CEF)/Y(II)] = [Y(I)-Y(II)]/Y(II) were used to determine cyclic electron flow. Values are means of three biological replicates ± SE (n = 3). Different letters indicate significant differences among treatments at $P < 0.05$.

electrons are transferred to O_2, which makes chloroplasts known as the main production site of ROS (Foyer and Hanke, 2022).

When the various activities of the photosystems are poorly coordinated, the photosystems would encounter varying degrees of photoinhibition (Tikkanen et al., 2014). ROS are produced when excess electron flow from PSII to PSI and causes damage to PSI (Tikkanen et al., 2014). However, we found that PSII suffered more visible photoinhibition than PSI (Fig. 4). PSII and PSI have evolved opposing strategies to deal with excess electrons (Tikkanen et al., 2014). In contrast to PSII, the recovery from PSI is extremely slow (Tikkanen et al., 2014). Photoinhibition of PSII is one of the mechanisms for protecting PSI from photoinhibition (Tikkanen et al., 2014). The ΔpH and cycle electron flow plays a critical role in photoprotection for PSI through the regulation of the redox state of PSI (Wu et al., 2020a). We found that the ΔpH of peanut leaves increased under P deficiency, which is consistent with earlier findings in maize (Carstensen et al., 2018). In addition, our results found that the ΔpH of thylakoid was increased under the P deficiency (Fig. 6D), which means thylakoid lumen acidification. A high pH of the lumen caused by the stress defect disturbs the downregulation of the Cyt b_6f complex (Yamamoto and Shikanai, 2019; Arshad et al., 2022). Our results demonstrated that the CEF rate of peanut leaves was increased under the P deficiency. The CEF around PSI is thought to be essential for the photoprotection of PSI, which can protect PSI from the damage caused by excess electrons from the acceptor side of PSI (Wu et al., 2020a; Moustakas et al., 2022). PSI is very tolerant to excess light but sensitive to excess electrons (Tiwari et al., 2016). Studies have shown that the main biological role of CEF is to regulate rather than enhance ETR, especially to protect PSI from damage (Storti et al., 2020). Cyclic electron transport can also contribute to forming a major ΔpH under stress. The CEF provides extra ATP to accelerate the repair of PSII which is dependent on ATP synthesis (Allakhverdiev et al., 2005). Non-photochemical quenching (NPQ) represents the capacity of plants to dissipate excess excitation energy and resist photo-oxidation stress in the photosystems (Horton et al., 1994; Farooq et al., 2018; Arshad et al., 2022). The increase of NPQ (Fig. 4B) is also the adaptive mechanism of peanut PSII to excess light energy. Previous studies have confirmed the relationship between NPQ and thylakoid lumen acidification induced by P deficiency (Ruban, 2016; Arshad et al., 2022). Acidification of the thylakoid lumen activates the qE component of NPQ of PSII and reduces energy transfer efficiency from LHCII to the photosynthetic electron transport chain (Niyogi, 1999; Shikanai and Yamamoto, 2017). Although this caused Y(NPQ) to increase and Y(II) to decrease, which is manifested as PSII photoinhibition (Fig. 4A, C). Plant photosynthetic complexes have evolved to control and minimize electron transport to O_2 (Foyer and Hanke, 2022).

4.2. *GABA restored peanuts photosynthetic capacity and growth during P Deficiency*

Exogenous GABA both enhanced plant dry matter, plant height, and leaf chlorophyll concentration of peanuts under P deficiency (Fig. 1). P deficiency usually interferes with various metabolic processes, thereby limiting crop growth and development (Lambers and Oliveira, 2019). GABA is currently recognized as a new plant growth regulator (PGR) involved in the response to various adversities (Zhou et al., 2021). GABA was reported to be involved in facilitating plant resilience to various environmental stresses, such as heat (Li et al., 2016b), cold (Mazzucotelli et al., 2006), drought (Li et al., 2017b), low nitrogen (Chen et al., 2020) and NaCl (Wu et al., 2020b). The mechanism of GABA absorbed by plant leaves is the same as that of endogenous GABA (Hijaz and Killiny, 2019). Therefore, foliar spraying of GABA can enhance the level of GABA in plants (Li et al., 2016b; Cekic, 2018). GABA acts as a signal molecule or metabolite in regulating plant growth and various developmental events (Deng et al., 2020; Zhou et al., 2021). GABA may also be a regulator of plant ion transport (Kinnersley and Lin, 2000). Later studies further proved that GABA directly regulates the activity of plant-specific anion transporters (Ramesh et al., 2015; Ramesh et al., 2018). Our results showed that GABA reduced the root-shoot ratio under

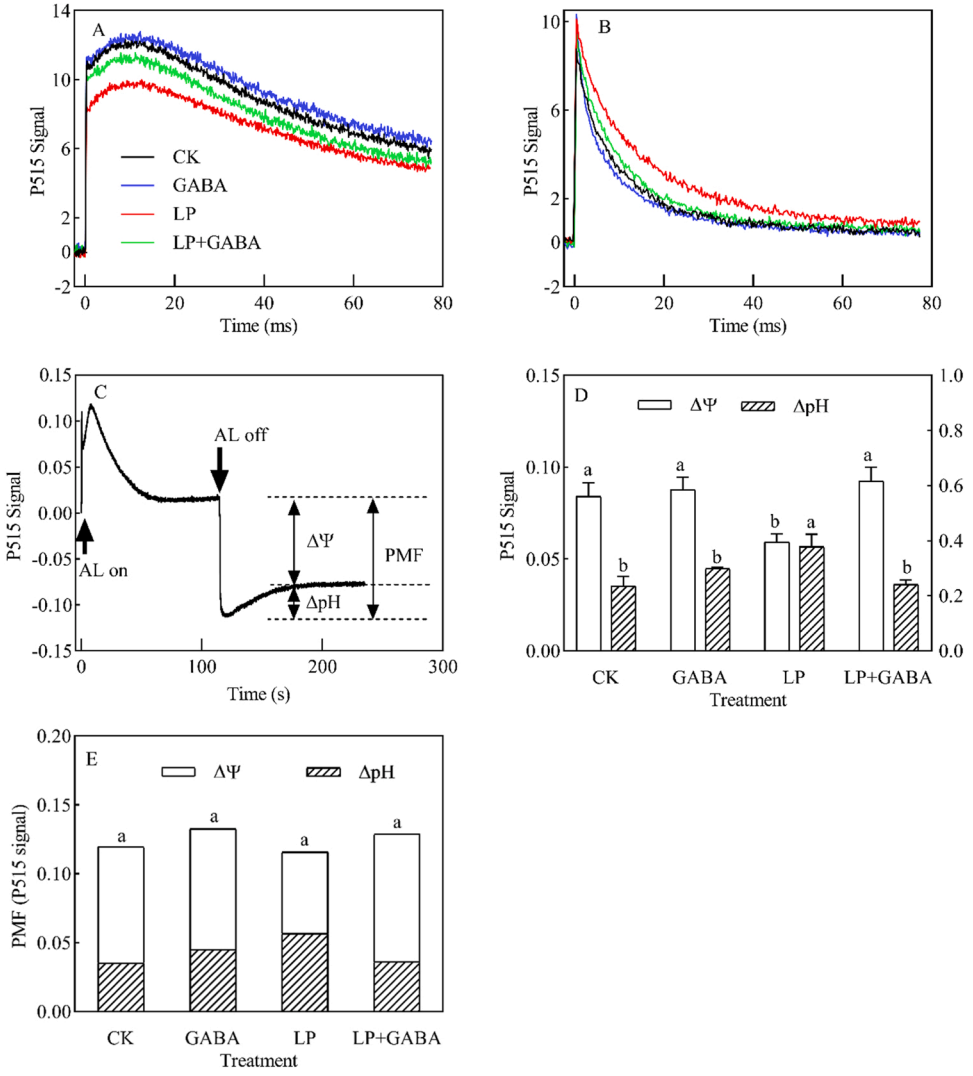

Fig. 6. Effects of exogenous gamma-aminobutyric acid (GABA) on rapid and slow P515 kinetics in peanut leaves under low P stress. (A) Rapid kinetics of darkness for 1 h; (B) Rapid kinetics of light for 10 min at 1000 μmol photons·m^{-2}·s^{-1} and darkness for 4 min; (C) ΔpH, Δψ, and PMF by using the slow 'dark–light–dark' signal induction transients of 515 nm signal after 12 h darkness; (D) ΔpH and Δψ from slow P515 kinetics curves; (E) Components of PMF. Values are means of three biological replicates ± SE (n = 3). Different letters indicate significant differences among treatments at $P < 0.05$.

the P deficiency (Fig. 1G). P deficiency disrupted the C allocation of plants (Hermans et al., 2006). The inhibition of P deficiency on stem growth is greater than that of the root, which forms a typical characteristic of P deficiency in plants with an increased root-shoot ratio. GABA altered C metabolic flux by modulating hormone homeostasis and transcriptional modification in hormonal signaling pathways (Xie et al., 2020).

Based on our combined analyses of the following: the CEF (Fig. 5), ROS (Fig. 2A, B), MDA concentration (Fig. 2C), the integrity of thylakoid membrane (Fig. 6A), and the photosystem activity (Fig. 4); the exogenous GABA protected the thylakoid membrane and photosynthetic machinery by reducing reactive oxygen species (Fig. 7). Under abiotic stress conditions, excess electrons in the photosynthetic electron transport chain cause ROS generation, leading to oxidative stress in the photosynthetic apparatus. The antioxidant enzyme system is usually the first barrier against ROS (Kapoor et al., 2019). The increase of ROS during P deficiency conditions indicated that the photosynthetic apparatus was under oxidative stress, and further, the photosynthetic membranes might have been damaged (Fig. 2C; 6 A). GABA involved in up-regulating antioxidant enzyme activity has been widely studied. But antioxidant defense system consisting of superoxide dismutase (SOD) and ascorbate peroxidase (APX) is not sufficient to remove these ROS (Kanazawa et al., 2017). Chloroplasts are the main sites for ROS production, and various abiotic stresses can lead to higher ROS production (Zhu, 2016). It is rarely reported that GABA reduces ROS by regulating the excess electrons of the photosynthetic electron transport chain which is the source of ROS generation. In our study, GABA alleviated the photoinhibition of PSI and PSII, which contributed to reducing excess electrons and ROS generation (Fig. 2). Studies have shown that exogenous GABA alleviated the photoinhibition of pepper seedlings under low light stress by up-regulating the antioxidant enzyme defense system and maintaining high photochemical efficiency (Li et al., 2017a).

Based on our analyses, ATP-synthase activity (Fig. 6B), photosystems activities (Fig. 4), ΔpH (Fig. 6D), photosynthetic electron transport (Fig. 5), and leaf P concentration (Fig. 1E) demonstrated that exogenous GABA largely alleviates the inhibition of high ΔpH on photosynthetic electron transport by enhancing ATP synthase activity under low P stress (Fig. 7). Peanuts sprayed with GABA probably allocated more P into the leaves (Fig. 1E). The combined lowering of nucleic acid P and metabolic P was the main reason for the decrease in leaf P concentration under P deficiency (Zhang et al., 2018). P deficiency disrupts plant metabolism and Pi level in the chloroplasts (Singh and Reddy, 2015; Muhammad et al., 2021). ATP synthase is very sensitive to chloroplast Pi, and any fluctuation of Pi can also affect the activity of ATP synthase (Carstensen et al., 2018). The relative stability of PMF in peanut leaves under P deficiency also indicated that it was not the main reason for the decrease of chloroplast ATP synthase activity (Fig. 6B). Priority to the distribution of phosphorus to photosynthetic leaves enhanced the use efficiency of phosphorus (Stitt et al., 2010; Hayes et al., 2019). The current results showed that GABA enhanced the P and Ca^{2+} levels in peanut leaves

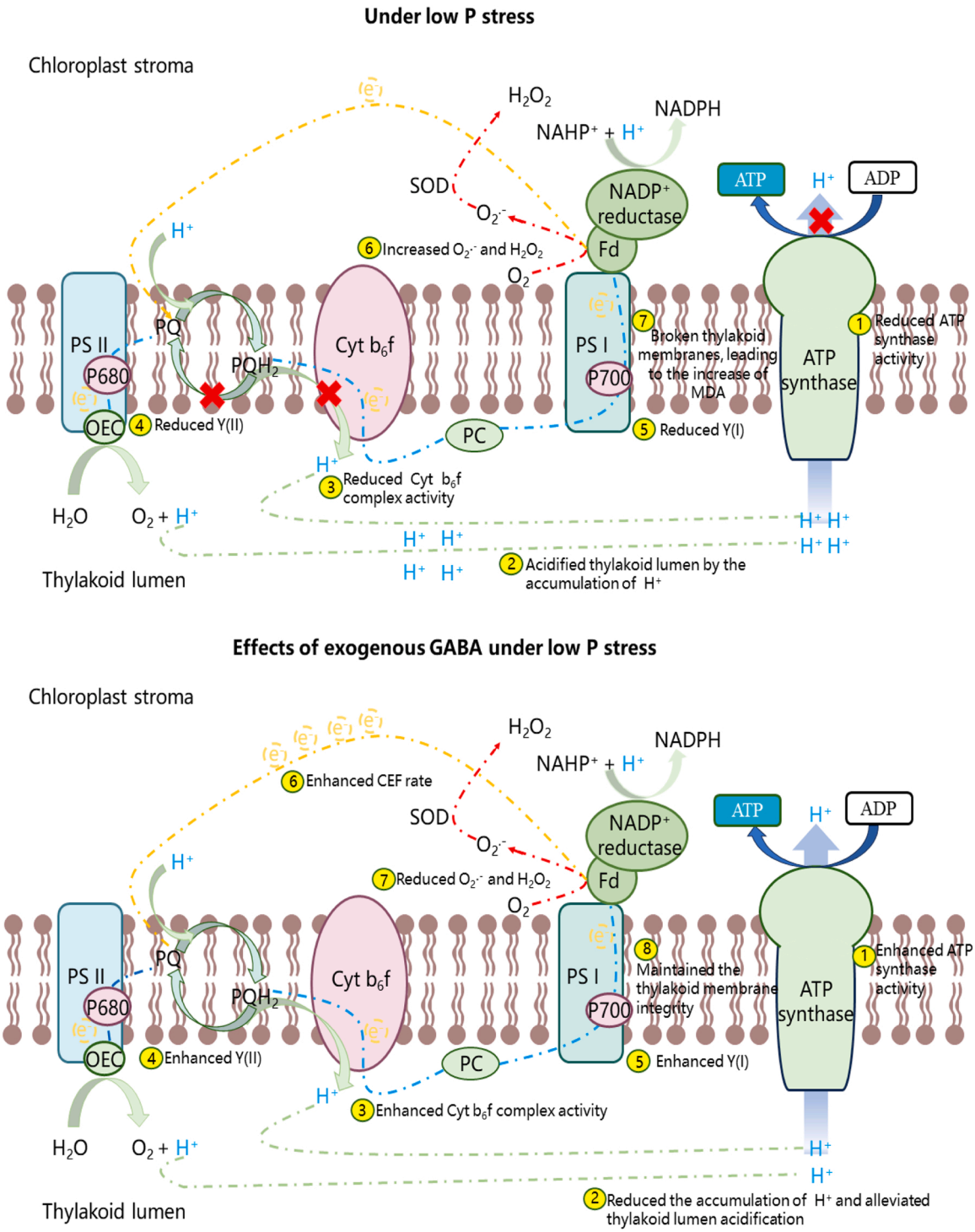

Fig. 7. Proposed model on the effects of exogenous gamma-aminobutyric acid (GABA) application on foliar photosynthetic electron transport in peanuts under low P stress. The figure of light reaction in the thylakoid membrane is adapted from the relative reference (Tikhonov and Vershubskii, 2017). Cyt: cytochromes; Fd: ferredoxin; OEC: Oxygen-evolving complex; PC: plastocyanin; PQ: Plastoquinone; P680: reaction center of PSII; P700: reaction center of PSI.

under P deficiency (Fig. 1E, F). Our findings are consistent with previous studies, which reported that the level of P and Ca^{2+} in lettuce leaves were proportional to P supply levels (Hayes et al., 2019). However, we also observed that GABA enhanced leaf P level under P deficiency because of the increased Ca^{2+} level, rather than the opposite. GABA may affect the allocation of P, although this effect may be indirect. Studies have shown that a high Ca^{2+} supply will enhance not only Ca concentration but also P concentration in leaves; this is attributed to the change in the P allocation pattern, rather than the uptake (Ding et al., 2018b). The uptake and distribution of Ca^{2+} in plants are regulated by exogenous GABA (Kinnersley and Lin, 2000). GABA has been proposed to also affect the flux of Ca^{2+} (Yu et al., 2014). Moreover, most Ca is located in mesophyll cells, while most P is located in epidermal cells, which avoids the deposition of Ca and P in the leaves (Ding et al., 2018b). The increase of Ca concentration in leaves induced by exogenous GABA under P deficiency and the role of Ca^{2+} in regulating the P allocation pattern further revealed that Ca^{2+} played an important role in modulating signal transduction in GABA-facilitated responses to P deficiency.

In our study, exogenous GABA reduced the ΔpH of the thylakoid, which means the acidity of the thylakoid lumen decreased (Fig. 6D). It is pointed out that the accumulation of H^+ in chloroplasts is mainly dependent on three factors: (i) H^+ coupled with LEF. (ii) H^+ coupled with CEF. (iii) H^+ outflow from the thylakoid lumen to chloroplast stroma, which is controlled by ATP synthase (Yang et al., 2018). Our results showed that GABA enhanced photosynthetic electron transport (Fig. 5), and ATP-synthase activity (Figs. 6B, 7) under P deficiency, indicating that the improvement of ATP synthase activity was the main reason for GABA to alleviated the acidification of thylakoid lumen in peanut leaves under P deficiency. The improvement of CEF and LEF undoubtedly delivers more H^+ to the thylakoid lumen. CEF generates a ΔpH gradient without PSII (Yamori et al., 2016). CEF around PSI can contribute a large ΔpH under stress in which LEF is limited (Yamori et al., 2016; Wu et al., 2020a). The reduction of ΔpH accelerated the oxidation of PQH_2 at Cyt b_6f, promoted the electron transfer to PSI, and contributed to the reduction of P680 (Suorsa et al., 2012; Tikkanen et al., 2015; Yang et al., 2018). Therefore, we concluded that exogenous GABA induced the ATP synthase, LEF and CEF to form a new proton gradient equilibrium under low P stress (Fig. 7).

In summary, we studied the NPQ (Fig. 4B), ΔpH (Fig. 6D), Y(NPQ) (Fig. 4E), and Y(NO) (Fig. 4D) to better understand the detailed photosynthetic processes in peanuts during optimal and deficient P nutrition conditions. We observed that various photoprotection mechanisms were engaged and these processes probably lowered the photosynthetic electron transport except for CEF under P deficiency. It is plausible that GABA removed the prevailing limitation imposed upon the photosynthetic electron transport through these photoprotection mechanisms (except for CEF) which included the photoprotection mechanism for quenching excess light energy (NPQ), slowing down the rate of oxidation/reduction at Cyt b_6f (photosynthetic-control), and the proton gradient regulation (Barbato et al., 2020). Interestingly, GABA could down-regulate the photoprotection mechanisms (except for CEF) to restore photosynthetic electron transport. The "turning off" of the photoprotective photosystem I was known to be an alternate way to alleviate foliar photoinhibition (Tikkanen and Grebe, 2018). This is also possibly the first report of using exogenous GABA to restore photosynthesis and growth during P deficiency (Fig. 7).

5. Conclusions

P deficiency inhibited various photosynthetic processes and the growth of peanuts. Specifically, the PSII encountered severe photoinhibition during P deficiency. The decrease of ATP synthase activity was the main reason to activate the photoprotection mechanism of proton gradient regulation of peanut leaves under low P stress.

Our study proved for the first time that exogenous and non-toxic GABA effectively restored the growth and development of peanuts when encountering P deficiency conditions. Specifically, the exogenous GABA enhanced the activity of chloroplast ATP synthase and slowed down the thylakoid lumen acidification. The exogenous GABA enhanced ETRII by pausing various photoprotection mechanisms including NPQ and proton gradient regulation. In addition, the exogenous GABA also stimulated cyclic electron transfer to deliver more electrons from PSII to PSI while concomitantly reducing ROS generation. Exogenous GABA application can effectively alleviate the two symptoms (increased root-shoot ratio and photoinhibition) of low-phosphorus stressed peanuts. These results indicated that the application of exogenous GABA could effectively improve the growth of peanuts during P deficiency. Interestingly, because of the signal amplification of GABA and Ca^{2+}, a new type of chelating fertilizer based on GABA and Ca^{2+} interaction may be a useful strategy in enhancing the low phosphorus adaptation in peanuts. It remains to be discovered whether GABA could improve P use efficiency in other crops and contribute globally to sustainable agricultural production in many regions with low P in the soils.

Patents

Not applicable.

Informed consent statement

Not applicable.

A novelty statement

We first demonstrated that gamma-aminobutyric acid (GABA), a non-proteinogenic amino acid, was effective in restoring peanut growth during P deficiency.

Supplementary materials

Not applicable.

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CRediT authorship contribution statement

Yifei Liu designed the experiment. **Zhiyu Sun, Chunming Bai,** and **Xiaori Han** experimented and collected data for preliminary analysis. **Di Wu, Zhiyu Sun, Mingzhu Ma, Huan Liu, Rui Bai**, and **Jean Wan Hong Yong** further analyzed the data and prepared the manuscript. **Zhiyu Sun, Chunming Bai, Yifei Liu, Mingzhu Ma**, and **Jean Wan Hong Yong** revised the manuscript. All authors contributed to the article and approved the submitted version.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Raw data used for this manuscript are available upon request to the corresponding author.

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