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RESEARCH

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Application of different foliar iron fertilizers for improving the photosynthesis and tuber quality of potato (*Solanum tuberosum* L.) and enhancing iron biofortification

Ruyan Zhang, Weina Zhang, Yichen Kang, Mingfu Shi, Xinyu Yang, Hong Li, Huifang Yu, Yong Wang and Shuhao Qin*

Abstract

Background: The potato (*Solanum tuberosum* L.) is an important food crop in the alkaline soil area of northwest China. It is abundant in ascorbic acid (vitamin C), which facilitates iron absorption in the body. The large consumption of potato makes it a good food source of iron absorption for human body. However, iron deficiency in alkaline soil regions reduced chlorophyll synthesis in the leaves, resulting in lower photosynthesis and less sugar supply to the plant's healthy organs, significantly restricted crop development and yield, and inflicted major economic losses.

Methods: In this study, a 2-year (2020–2021) field trial was designed. Under foliar application of five different iron fertilizers: ferric sulfate [($Fe_2(SO_4)_3$], T1; ferrous sulfate ($FeSO_4{\cdot}7H_2O$), T2; citric acid/ferric sulfate ($CA/Fe_2(SO_4)_3$), T3; citric acid/ferrous sulfate ($CA/FeSO_4{\cdot}7H_2O$), T4; ethylenediamine tetra acetic acid (EDTA-Fe·Na), T5, changes in potato plant photosynthesis were compared to no iron fertilization, CK conditions. The effects of various iron fertilizers on the yield, quality, and iron content of potato tubers, their correlations to chlorophyll levels, and the characteristics of photosynthetic fluorescence were studied.

Results: The results indicated that spraying iron fertilizers increased the yield, quality and Fe content of the tubers, which might be due to the improvement of the plants' photosynthetic pigment content, gas exchange parameters and chlorophyll fluorescence. In two consecutive years of cultivation, we found that potato tuber yield and Fe content increased in potatoes treated with five iron fertilizer sprays. Among them, tuber yield increased most significantly by T5 and T3 treatments compared to CK, while Fe content was significantly higher by the T5 treatment than by CK and other treatments. Tuber yield was increased by 33.28% and 18.85% in 2020 and 50.74% and 54.48% in 2021 by T5 and T3 treatments, respectively, compared to CK. Fe content was increased by 112.64% and 54.98% in 2020 and 2021 by T5 treatment, respectively, compared to CK.

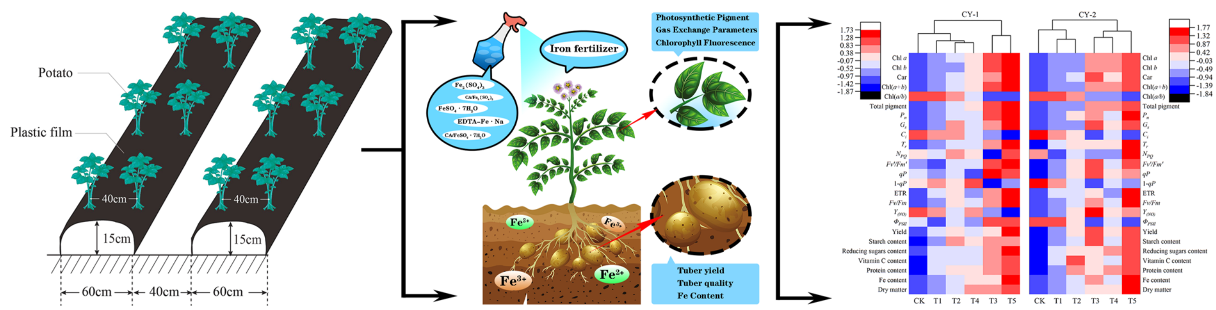
Conclusions: EDTA-Fe·Na and $CA/Fe_2(SO_4)_3$ excelled over the other iron fertilizers. The findings of this study are instructive for developing cost-effective iron fertilizer management systems to maximize the impact of iron biofortification on human health.

Keywords: Potato, Iron fertilizer, Gas exchange parameters, Chlorophyll fluorescence, Tuber quality

*Correspondence: qinsh@gsau.edu.cn

College of Horticulture, Gansu Agricultural University, Lanzhou 730070, China

Graphical Abstract



Background

The potato (*Solanum tuberosum* L.) is one of the world's most significant food crops. Based on statistics from the Food and Agriculture Organization of the United Nations, the total global potato production was 4.37×10^8 t in 2020 [1], with China, India, and Russia being the primary producers [2, 3]. Potatoes are an affordable food source. Its tubers are reached in minerals, vitamins C and B, amino acids, carbohydrates, and protein. Undoubtedly, it is highly regarded across the world [4].

Iron (Fe) is one of the necessary micronutrients for all living creatures. It is also an essential micronutrient with multiple cellular functions in normal human physiology [5]. Iron is a cofactor for around 140 enzymes in plants, which play a role in numerous physiological and biochemical processes [6]. It stimulates chlorophyll synthesis, facilitates respiration and photosynthesis [7], involves in nitrogen fixation in legume rhizomes, possesses redox activity, and is capable of electron transfer [8, 9]. Even though iron is abundant in soil, it can also be deficient in crops, because it is not in a plant-available form and is deficient for plants [10]. Iron absorption and transport across plant organs are greatly influenced by soil pH [11], resulting in iron shortage symptoms in crops [10]. Iron deficiency chlorosis (IDC) is a severe environmental issue affecting crops worldwide [12]. More than one-third of the world's land is covered by high pH alkaline or calcareous soils, and most frequently in semi-arid regions with calcareous soils are unsuitable for agricultural use [13, 14]. Approximately 80% of the iron in leaf chloroplasts is present. Therefore, an iron shortage reduces chlorophyll synthesis in leaves, resulting in less efficient photosynthesis in plants and less sugar transported to fruits or other organs, impeding crop development and yield and inflicting severe economic losses [15, 16]. It has been demonstrated that the ineffectiveness of iron in calcareous soils is one of the primary causes of the decline in wheat production [17].

Fertilizers are an essential source of soil nutrients that encourage plant development and boost yield [18]. When soluble fertilizers are introduced to soil, a sequence of events, including exchange/sorption, complexity, precipitation, and dissolution, occurs, and soil elements (such as iron oxides, clay, and calcium) influence their biological effectiveness [19, 20]. Complex natural slow-release fertilizers such as zinc, iron, and manganese have been developed. Although these compounds are insoluble in water, they are soluble in organic chelates, such as citrate and DTPA. Solubility in such chelates ensures the high availability of plant nutrients, i.e., high bioavailability [21]. However, excess iron is harmful to plants. Therefore, iron intake must be appropriately managed and corrected with the necessary techniques. Iron fertilization increases photosynthesis in plant leaves, and foliar spraying is the most popular and effective way to treat yellowing caused by iron deficiency in plants. Chelated iron fertilizers, inorganic iron fertilizers, and organic iron fertilizers are the three forms of iron fertilizers typically used in agriculture [22]. Some biological slow-release Zn, Fe, and Mn fertilizers have been created, and long-term integrated nutrient management boosts the efficacy of trace elements (Zn, Fe, Fe, Fe). Although these compounds are insoluble in water, they are soluble in organic chelates, such as citrate and DTPA, guaranteeing that plant nutrients are highly available or bioavailable [23]. Iron oxide foliar spray promoted nitrogen fixation and enhanced soybean production and nutritional quality [24].

However, these chelates' non-biodegradability and environmental buildup remain a concern [25, 26]. Biodegradable synthetic iron chelates are being developed and progressively implemented to solve this problem. Farmers use synthetic iron chelates to treat iron deficiency in cash crops. Despite the high cost, these fertilizers tend to dissolve, but chelates can prevent precipitation and increase heavy metal mobilization [27]. Various chelates are combined with an iron source (usually referred to as ferric sulfate) to create synthetic chelates. It has been demonstrated that certain iron chelates, such as Fe-EDDHA, have agronomic utility. Fe-EDDHA is very effective in improving the quality of grape berries [28]. The Fe-citrate fertilizer is superior for peanut iron nutrition, plant biomass, seed yield, and quality [29]. The application of EDTA-Na_2 Fe fertilizer can regulate the accumulation of Cd in rice seeds to maintain a high level of practical and ferrous iron in the soil [30]. Prior research on the effects of iron fertilization on crop productivity and quality focused on sweet potato [31], soybeans [32], wheat [33], rice [34], and other field crops. In the arid and semi-arid regions of northwest China, alkaline salt stress severely affects the quality of the soils. In this study, to achieve the purpose of potato iron biofortification and, at the same time, to screen out the more effective and practical iron fertilizer, we sprayed potato leaves with five iron fertilizers: $Fe_2(SO_4)_3$, $FeSO_4{\cdot}7H_2O$, CA/$Fe_2(SO_4)_3$, CA/$FeSO_4{\cdot}7H_2O$, and EDTA-Fe·Na. After the iron fertilization treatment, we measured the chlorophyll content of the leaves, photosynthetic and fluorescence characteristics, tuber yield, nutritional quality, and iron content. The purpose of the combined analysis of all measured indicators is to determine the response pattern of potatoes to various iron fertilization treatments to improve the potato fertilization system and, furthermore, to provide a scientific reference for achieving the potato iron fortification goal.

Materials and methods

Plant materials and treatments

Experimental site

The experiment was conducted from April 2020 to September 2021 at Gansu Agricultural University (52°12′59″N, 22°34′37″E) in Gansu Province, Northwest China. The area is located in a largely arid and semi-arid region typical of the Loess Plateau in China, with deep soils, high water retention, and soil consolidation capacity. The altitude of the site is 1300 m above sea level. The long-term annual precipitation at the site averages 391 mm, mainly from June to September. The average annual evaporation is 1531 mm, and the average annual temperature is 6.4 °C. The soils are alkaline and lack available iron. Table 1 shows the basic parameters of soil quality in the test site topsoil layer (0–30 cm depth).

Plant material and treatment methods

The experiment utilized a random group design, and the variety of potatoes tested was 'Longshu No.7', one of the main primary cultivars in Gansu Province, China. Potatoes were planted in 2020 (CY-1) and 2021 (CY-2) for two consecutive years. On-ridge complete black film mulching (thickness of film: 0.01 mm) (Fig. 1). The basal fertilizers (N, P, and K) were applied to the soil before planting. Nitrogen (urea), phosphorus (calcium superphosphate), and potassium (potassium sulfate) fertilizers were used at 326.1, 468.75, and 288.45 $kg{\cdot}ha^{-1}$, respectively. Each experimental plot had an area of 7.7 × 11.5 m in area. Each treatment comprised three experimental plots with biological replicates. On April 25 of each year, it was planted at a density of 5.25×10^4 $plants{\cdot}ha^{-1}$. There were six iron treatments: (i) no iron fertilization (CK); (ii) 9 mM $Fe_2(SO_4)_3$ fertilization (T1); (iii) 13.16 mM $FeSO_4{\cdot}7H_2O$ fertilization (T2); (iv) 7.81 mM CA/9 mM $Fe_2(SO_4)_3$ fertilization (T3); (v) 7.81 mM CA/13.16 mM $FeSO_4{\cdot}7H_2O$ fertilization (T4); (vi) 4.09 mM EDTA-Fe·Na fertilization (T5). We started foliar iron fertilizer treatments after all potatoes had emerged, spraying once in a 7-day cycle four times. Each spraying time is the same day before 11:00 am or after 5:00 pm. Spray from the bottom of the plant upward until the fertilizer on the plant's foliage falls in droplets.

Determinations

Determination of photosynthetic pigment contents

We used 80% acetone to extract photosynthetic pigments from plant leaves. A UV-1780 (Shimadzu, Japan) spectrophotometer was used to assess the extracts absorption values at various luminosities. The contents of Chl *a*, Chl *b*, Car, and Chl($a+b$) were measured according to the method of Lichtenthaler et al. [35]. The Chl *a/b*, and total pigment content were calculated with the following formulas: $\text{Chl}a/b = \text{Chl}a/Chlb$, $\text{Total pigment content }(mg\,g^{-1}\cdot F_W) = \text{Chl}a + \text{Chl}b + \text{Car}$.

Determination of gas exchange parameters and chlorophyll fluorescence

Using an LI-6400 XT Photosynthetic Fluorescence Measurement System [36], the net photosynthetic rate (P_n), stomatal conductance (G_s), transpiration rate (T_r),

Table 1 Chemical properties of the soil collected before treatment application in 2020 and 2021

Cultivation year	pH /	Soil capacity ($g{\cdot}cm^{-3}$)	Available N ($mg{\cdot}kg^{-1}$)	Available P	Available K	Available Fe	Organic matter
CY-1	8.30	1.17	17.90	25.90	1 116.70	2.71	12014.12
CY-2	8.40	1.65	20.10	23.20	2 057.00	2.63	14993.45

Available Fe content classification standard is low: < 2.5 $mg{\cdot}kg^{-1}$; medium: 2.5–4.5 $mg{\cdot}kg^{-1}$; rich: > 4.5 $mg{\cdot}kg^{-1}$, the critical value of iron deficiency is 2.5 $mg{\cdot}kg^{-1}$. CY-1 (2020), CY-2 (2021)

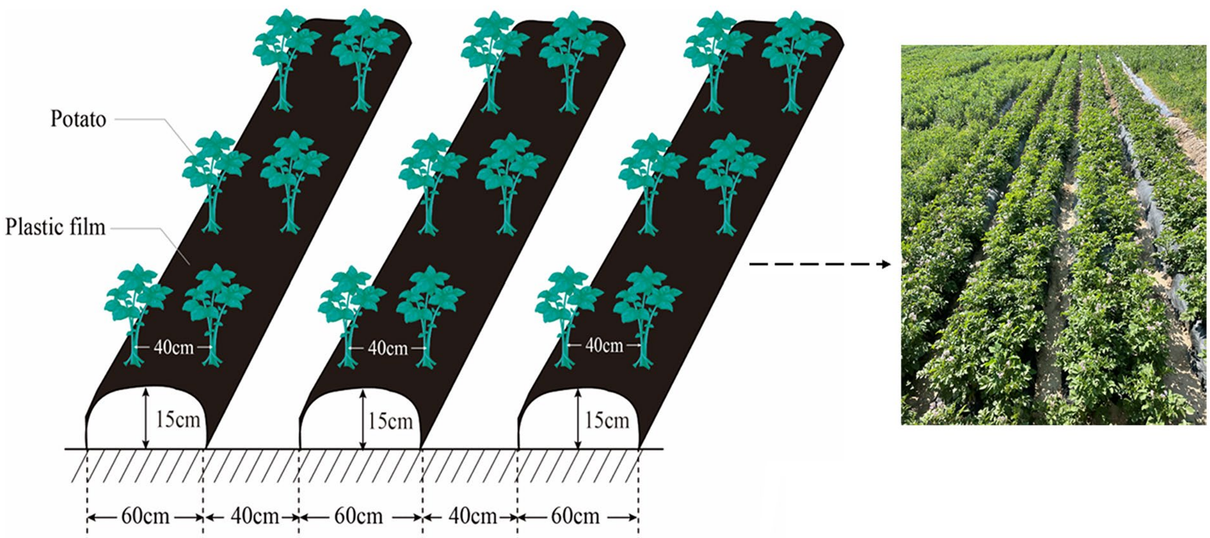


Fig. 1 Schematic diagram of black full-film ridge seeding coverage

and intercellular CO_2 concentration (C_i) of functioning potato leaves under each iron fertilizer treatment was determined. The parameters of the instrument were adjusted at an intensity of 1000 μmol·m^{-2} s^{-1} for the photosynthetic photon flux, a temperature of (26 ± 1) °C, an atmospheric CO_2 concentration of (380 ± 10) μmol·L^{-1} at room temperature, and relative humidity between 40% and 50%. The above measurements were conducted between 9:00, and 11:00 am on sunny days. Using the method of Liu et al. [36], chlorophyll fluorescence was measured on the marked leaves when photosynthesis was measured. The actual fluorescence (*Fs*) at a PAR level of 1000 μmol·m^{-2} s^{-1} was measured after 40 min of activation under natural light. For the complete dark adaptation of potato leaves, we used a night plant for dark adaptation. After measuring *F0* and *Fm* before dawn, we marked the leaves, opened the activated light to activate the same leaf (marked leaves), and then measured *Fs*, *Fm'*, and *F0'*. The ETR values of plant leaves activated under natural light for 30 min were determined. LI-6400XT will further complete the calculation of *Fv/Fm*, *Fv'/Fm'*, Φ_{PSII}, *qP*, *qN*, *qL*, $Y_{(NO)}$, and N_{PQ}. The calculation formula is as follows:

$$Fv/Fm = (Fm - F0)/Fm,$$

$$Fv'/Fm' = \left(Fm' - F0'\right)/Fm',$$

$$\phi_{PSII} = \left(Fm' - Fs\right)/Fm',$$

$$N_{PQ} = \left(Fm - Fm'\right)/Fm',$$

$$qN = \left(Fm' - Fs\right)/\left(Fm' - F0'\right),$$

$$qP = \left(Fm - Fm'\right)/\left(Fm - F0'\right),$$

$$qL = \left(Fm' - Fs\right)/\left(Fm' - F0'\right) \times F0'/Fs,$$

$$Y_{(NO)} = 1/\left[N_{PQ} + 1 + qL(Fm/F0 - 1)\right].$$

Determination of tuber yield

The tuber yield per hectare (kg ha^{-1}) was obtained by determining the tuber yield of each plot each year at harvest (8 October 2020, 11 October 2021). We randomly selected 10 tubers from five points in the east, west, north, south and center of the plot and calculated the average yield of each plant by measuring its fresh weight, then estimated the total number of plants in the plot based on the plant spacing, and then estimated the total yield of each plot based on the average yield of individual plants. We repeated the measurements three times for each plot [37].

Determination of tuber quality and dry matter and Fe content

A near-infrared mass spectrometry analyzer (FOSS, Denmark, NIRS DS 2500) was used to assess fresh potato starch, reducing sugars, vitamin C, protein, and iron content after harvest, according to the determination method of the Su et al. [38]. The samples were ring-cut using the method of cutting. The tubers were cut to a uniform thickness (≥ 0.5 cm) to cover the bottom of the sample cassette completely. Each indicator was set up with three biological replicates to guarantee that its mean value was accurately represented.

Statistical analysis

Every data set has been repeated an average of three times. The measured data were tallied using Microsoft Excel 2010. One-way ANOVA and Duncan's multiple extreme difference test ($P < 0.05$) were used to assess the separate effects of treatments on photosynthetic pigment contents, gas exchange parameters, chlorophyll fluorescence, and treatment on tuber yield and quality. Data are expressed as mean ± standard error. The relationship between each index was evaluated by Pearson correlation analysis. SPSS 25.0 (IBM 2017, Armonk, USA) was used for statistical analysis. Origin Lab 2022 (Origin Lab Corporation, Northampton, USA.) was used to prepare graphics.

Results

Response of photosynthetic pigment in the leaves of potato to iron fertilizer

Foliar spraying of different iron fertilizer treatments significantly increased the chlorophyll content of plant leaves. The iron fertilizer treatments leaf Chl *a*, Chl *b*, Car, Chl ($a+b$), and Total pigment levels were generally higher than the CK treatment (Fig. 2), with the most significant differences occurring in treatments T3 and T5 ($P < 0.05$). In 2020, the values of Chl *a/b* were significantly lower in T3 and T5 treatments than in the other treatments ($P < 0.05$), with the most significant difference being a 33.69% decrease in the T5 treatment. However, in 2021, the difference between the values of Chl *a/b* of each treatment and the control did not reach a significant level. The chord diagram (Fig. 3G, H) demonstrates the relationship between the various treatments and the levels of Chl *a*, Chl *b*, Car, Chl ($a+b$), and total pigments. The Chl *a* content accounted for the most significant proportion of the three pigments, followed by the Chl *b* content. The correlation between the six treatments and chlorophyll contents was as follows: T5 > T3 > T4 > T2 > T1 > CK. For 2 years of cultivation, the node arc lengths of the T5 and T3 treatments were 9.71 and 9.94, 9.16 and 9.30,

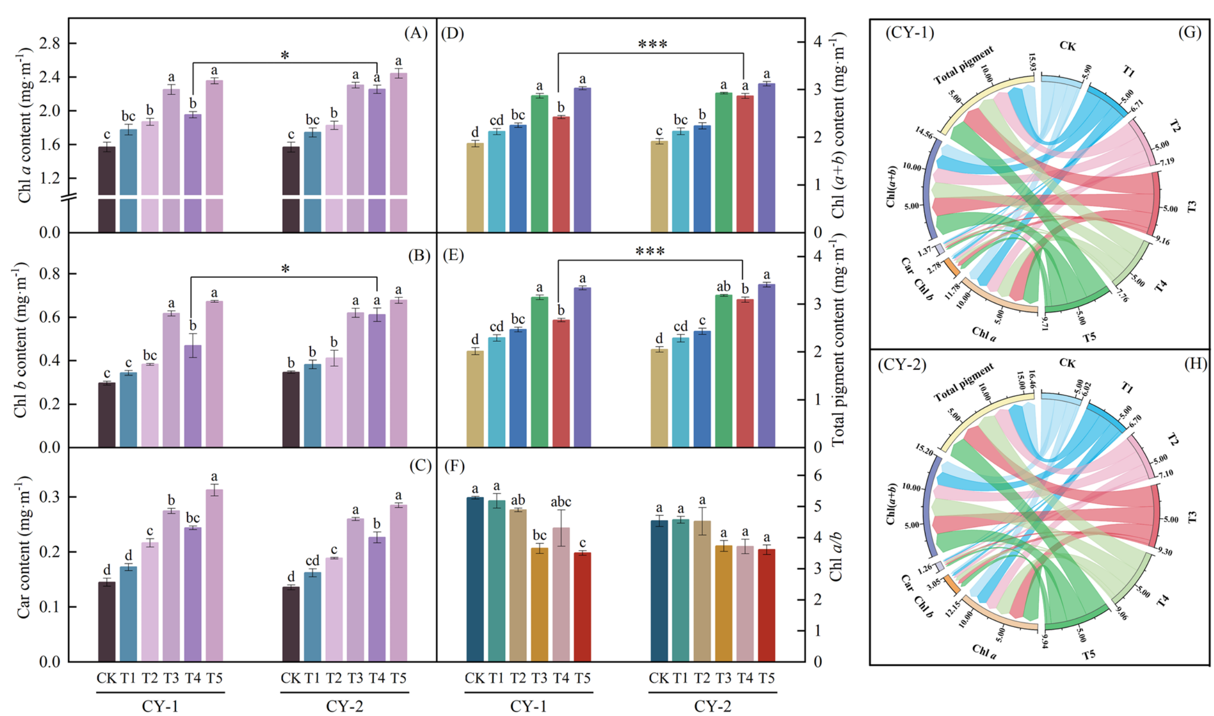


Fig. 2 Chl *a* (**A**), Chl *b* (**B**), Car (**C**), Chl ($a+b$) (**D**), Total pigment content (**E**), and Chl *a/b* (**F**) of potato as affected by different iron fertilizer treatments. Lowercase letters indicate significant differences among fertilization treatments ($p < 0.05$). Chord diagrams (**G**) and (**H**) represent the correlation of chlorophyll content under different treatments. CK-no iron fertilizer, T1-$Fe_2(SO_4)_3$ fertilizer, T2-$FeSO_4 \cdot 7H_2O$ fertilizer, T3-CA/$Fe_2(SO_4)_3$ fertilizer, T4-CA/$FeSO_4 \cdot 7H_2O$ fertilizer, T5- (EDTA-Fe·Na) fertilizer. CY-1 (2020), CY-2 (2021)

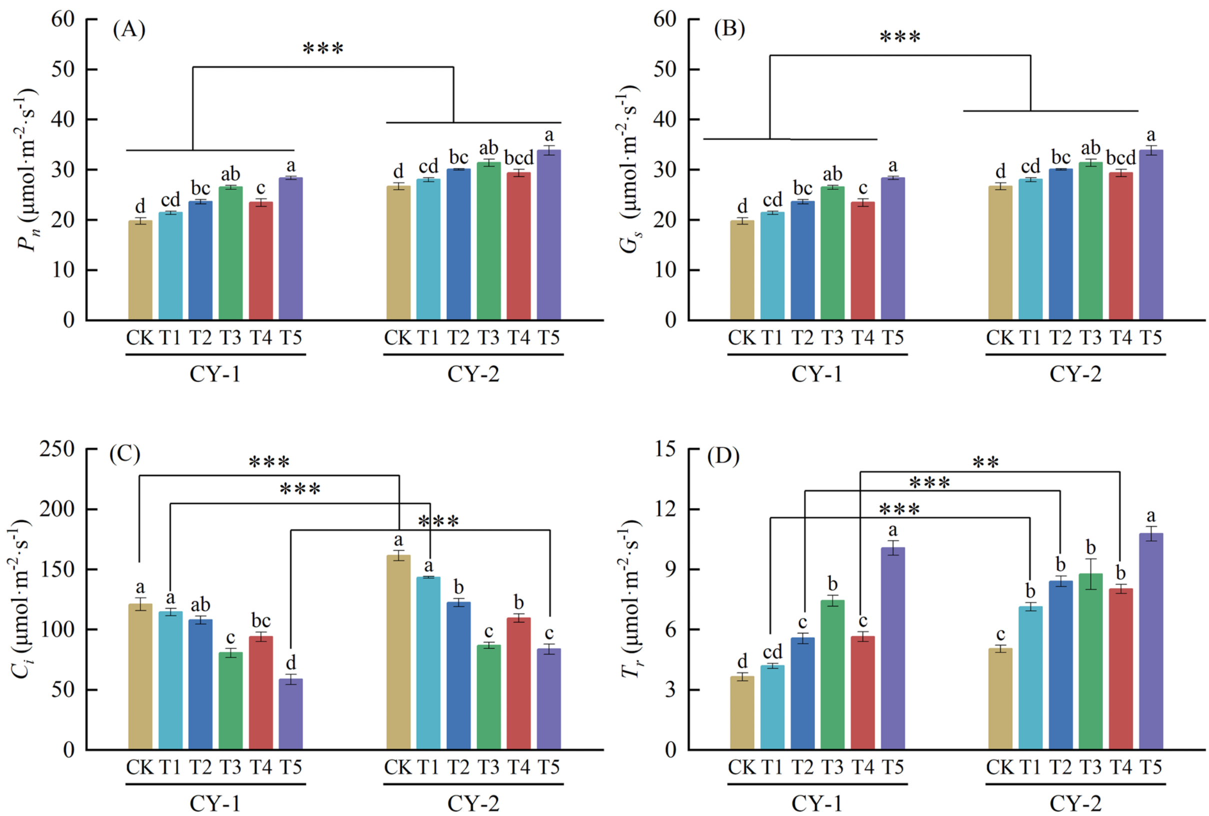


Fig. 3 P_n (**A**), G_s (**B**), T_r (**C**), C_i (**D**) of potato as affected by different fertilizer treatments. Lowercase letters indicate significant differences among fertilization treatments ($p < 0.05$). CK-no iron fertilizer, T1-$Fe_2(SO_4)_3$ fertilizer, T2-$FeSO_4{\cdot}7H_2O$ fertilizer, T3-CA/$Fe_2(SO_4)_3$ fertilizer, T4-CA/$FeSO_4{\cdot}7H_2O$ fertilizer, T5- (EDTA-Fe·Na) fertilizer

respectively. Pigment content was shown to be most correlated with T5 and T3.

Response of gas exchange parameters in the leaves of potato to iron fertilizer

Different iron fertilizer treatments impacted photosynthesis in leaves, and elicited distinct responses from photosynthetic traits. We found through 2 years of measurements in continuous field experiments that P_n of potato leaves was more significant in all five iron fertilizer treatments compared to the CK (Fig. 3A). The T5 therapy significantly increased P_n ($p < 0.05$), with 20% and 23% increase in 2020 and 2022, respectively. At the same time, Tr and G_s exhibited similar trends to P_n in 2 years of cultivation (Fig. 3B, D). Interestingly the results of the *Tr* assay found significant differences between the T3 and T5 treatments, and the T3 treatment was also significantly higher than the other treatments except for the T5 treatment. The response of C_i alterations was inverse to that of G_s, T_r, and P_n (Fig. 3C). In 2020, the T5 and T3 treatments were significantly lower than the other treatments, and T5 was also significantly lower than the T3 treatment. In addition, in 2021, we found that the differences between T5 and T3 treatments were not significant, although they were significantly lower than the other treatments.

Response of chlorophyll fluorescence in the leaves of potato to iron fertilizer

Response of Fv/Fm, Fv′/Fm′, ΦPSII, and Y(NO) in the leaves of potato to iron fertilizer

Changes in the chlorophyll fluorescence system of potato leaves were studied under different iron fertilization treatments (Table 2). We found that the values of *Fv/Fm* measured in 2020 (CY-1) differed significantly among treatments. The T5 treatment was significantly higher than the other treatments in both years of measurement ($P < 0.05$), but the differences in the values of *Fv′/Fm′*, Φ_{PSII}, and $Y_{(NO)}$ were not significant among treatments. In 2021 (CY-2), we found that except for the values of *Fv′/Fm′*, the values of *Fv/Fm*, Φ_{PSII}, and $Y_{(NO)}$ values reached significant levels under different treatments ($P < 0.05$). The value of *Fv/Fm* was significantly higher under the T5 treatment than under other treatments. The value of Φ_{PSII}

Table 2 *Fv/Fm*, *Fv′/Fm′*, Φ_{PSII}, $Y_{(NO)}$ of potato as affected by different iron fertilizer treatments

	Treatment	*Fv/Fm*	*Fv′/Fm′*	Φ_{PSII}	$Y_{(NO)}$
CY-1	CK	0.76 ± 0.0015d	0.69 ± 0.0085a	0.57 ± 0.0224a	0.26 ± 0.0138a
	T1	0.77 ± 0.0036c	0.68 ± 0.0111a	0.55 ± 0.0118a	0.26 ± 0.0028a
	T2	0.78 ± 0.0018bc	0.70 ± 0.0089a	0.57 ± 0.0137a	0.24 ± 0.0018a
	T3	0.80 ± 0.0001b	0.74 ± 0.0086a	0.64 ± 0.0103a	0.23 ± 0.0026a
	T4	0.78 ± 0.0001bc	0.70 ± 0.0192a	0.56 ± 0.0266a	0.26 ± 0.0032a
	T5	0.81 ± 0.0014a	0.75 ± 0.0050a	0.64 ± 0.0043a	0.22 ± 0.0042a
CY-2	CK	0.75 ± 0.0049e	0.66 ± 0.0495a	0.43 ± 0.0851c	0.32 ± 0.0103a
	T1	0.76 ± 0.0018de	0.69 ± 0.0113a	0.50 ± 0.0118bc	0.30 ± 0.0052ab
	T2	0.78 ± 0.0043c	0.71 ± 0.0207a	0.57 ± 0.0556abc	0.25 ± 0.0317b
	T3	0.80 ± 0.0018b	0.74 ± 0.0022a	0.68 ± 0.0184a	0.19 ± 0.0122c
	T4	0.78 ± 0.0019 cd	0.71 ± 0.0183a	0.59 ± 0.0242abc	0.24 ± 0.0054bc
	T5	0.82 ± 0.0027a	0.75 ± 0.0036a	0.65 ± 0.0052ab	0.19 ± 0.0009c

Data are mean ± standard error of the three replicates. Different lowercase letters in the same column indicate significant differences among treatments ($P < 0.05$). CK-No iron fertilizer, T1-$Fe_2(SO_4)_3$ fertilizer, T2-$FeSO_4 \cdot 7H_2O$ fertilizer, T3-CA/$Fe_2(SO_4)_3$ fertilizer, T4-CA/$FeSO_4 \cdot 7H_2O$ fertilizer, T5- (EDTA-Fe·Na) fertilizer. CY-1 (2020), CY-2 (2021)

was higher under T3 treatment than other treatments, increased by 58.08% compared to CK, and reached significant levels compared to CK and T1 treatments. The value of $Y_{(NO)}$ was significant under T3, and T5 treatments were significantly lower than those of CK, T1, and T2 treatments.

Response of NPQ, qP, ETR, and 1-qP in the leaves of potato to iron fertilizer

Conversely, N_{PQ} showed a downward trend with an excessively. In two consecutive years of the trial, none of the treatments significantly affected N_{PQ} (Table 3). In 2020, compared to CK, 1-*qP*, and *qP* were not significantly different under different treatments, while ETR was 27.11% higher than control under T5 treatment, the most significant difference ($P < 0.05$). In 2021, compared to CK, *qP* and 1-*qP* were most significantly different under T3 treatment, and ETR was most significantly different under T5 treatment.

Response of tuber yield of potato to iron fertilizer

Figure 4 shows the impact of iron fertilizer spraying on potato tuber yield and rate of growth. The results showed that the tuber yield of CK treatment in the second year (25036 kg ha^{-1}) was significantly reduced by 19.87% compared with that in the first year (30010 kg ha^{-1}). In 2020,

Table 3 N_{PQ}, *qP*, ETR, 1-*qP* of potato as affected by different iron fertilizer treatments

	Treatment	N_{PQ}	*qP*	ETR	1-*qP*
CY-1	CK	0.33 ± 0.0500a	0.83 ± 0.0259a	76.92 ± 3.1859b	0.17 ± 0.0259a
	T1	0.30 ± 0.0040a	0.81 ± 0.0054a	83.13 ± 0.3890b	0.19 ± 0.0054a
	T2	0.35 ± 0.0492a	0.83 ± 0.0115a	85.32 ± 0.4507ab	0.17 ± 0.0115a
	T3	0.23 ± 0.0261a	0.86 ± 0.0052a	88.35 ± 1.1659ab	0.14 ± 0.0052a
	T4	0.32 ± 0.0844a	0.80 ± 0.0165a	81.45 ± 0.1945b	0.20 ± 0.0165a
	T5	0.36 ± 0.0299a	0.85 ± 0.0024a	97.77 ± 6.9963a	0.15 ± 0.0024a
CY-2	CK	0.60 ± 0.1575a	0.63 ± 0.0879c	76.92 ± 2.9566b	0.37 ± 0.0879a
	T1	0.45 ± 0.0496a	0.72 ± 0.0092bc	82.80 ± 0.8351b	0.28 ± 0.0092ab
	T2	0.52 ± 0.0495a	0.80 ± 0.0654abc	84.99 ± 1.4417b	0.20 ± 0.0654abc
	T3	0.48 ± 0.0038a	0.92 ± 0.0253a	88.68 ± 2.0187ab	0.08 ± 0.0253c
	T4	0.48 ± 0.1105a	0.83 ± 0.0141ab	79.45 ± 0.6954b	0.17 ± 0.0141bc
	T5	0.67 ± 0.0476a	0.87 ± 0.0072ab	99.44 ± 1.1567a	0.13 ± 0.0072bc

Data are mean ± standard error of the three replicates. Different lowercase letters in the same column indicate significant differences among treatments ($p < 0.05$). CK-no iron fertilizer, T1-$Fe_2(SO_4)_3$ fertilizer, T2-$FeSO_4 \cdot 7H_2O$ fertilizer, T3-CA/$Fe_2(SO_4)_3$ fertilizer, T4-CA/$FeSO_4 \cdot 7H_2O$ fertilizer, T5- (EDTA-Fe·Na) fertilizer. CY-1 (2020), CY-2 (2021)

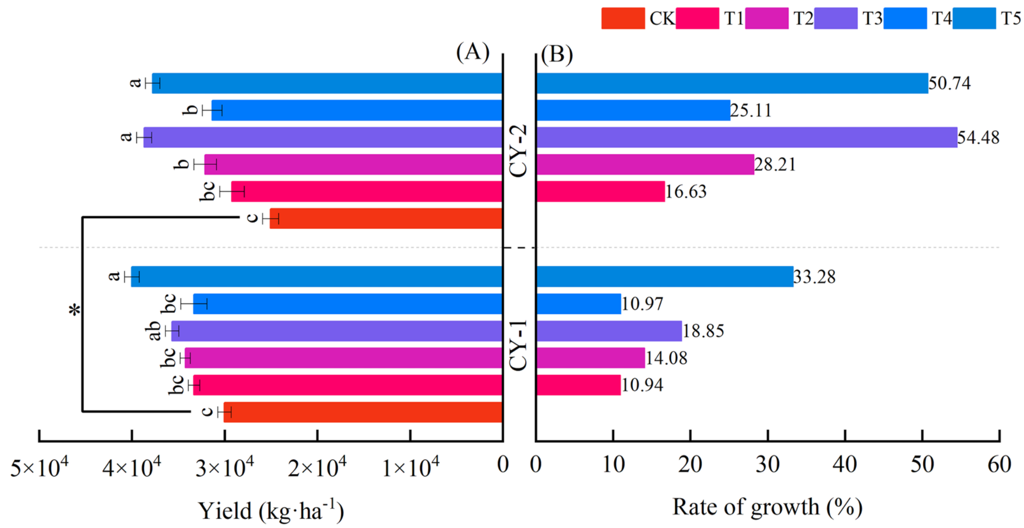


Fig. 4 Tuber yield (**A**) and rate of growth (**B**) of potato tuber as affected by different iron fertilizer treatments. Lowercase letters indicate significant differences among fertilization treatments (*p* < *0.05*). CK-No iron fertilizer, T1-$Fe_2(SO_4)_3$ fertilizer, T2-$FeSO_4 \cdot 7H_2O$ fertilizer, T3-CA/$Fe_2(SO_4)_3$ fertilizer, T4-CA/$FeSO_4 \cdot 7H_2O$ fertilizer, T5- (EDTA-Fe·Na) fertilizer. CY-1 (2020), CY-2 (2021)

the iron fertilizer treatment increased the yield by up to 33.28% (T5). By 2021, the yield increase was up to 54.48% (T3), significantly higher than that of CK ($P < 0.05$). In both consecutive years of the trial, tuber yield was most significantly increased by the T5 and T3 treatments. Among them, T3 and T5 treatments had the most significant increases in tuber yield, with increases of 18.5% (35666 kg ha^{-1}) and 33.28% (39996 kg ha^{-1}) in 2020 and 54.48% (29199 kg ha^{-1}) and 50.74% (37739 kg ha^{-1}) in 2021, respectively.

Response of tuber quality, dry matter, and Fe content of potato to iron fertilizer

Figure 5 shows the effects of iron fertilizer application on the quality of potato tubers. The results of the 2-year experiment showed that all iron fertilization treatments resulted in a significant general increase in starch (Fig. 5A), reducing sugar (Fig. 5B) and protein content (Fig. 5D) of potato tubers mentioned compared to CK. However, Vitamin C varied non-significantly except for T5 (Fig. 5C). In 2 years of consecutive experiments, we found that tuber Fe and dry matter content were also enhanced by Fe fertilization treatment. The order of effects of different iron fertilizer treatments on dry matter content of potato tubers was T5 > T3 > T2 > T4 > T1 > CK (Fig. 5E), and the order of tuber iron content was T5 > T3 > T2 > T4 > T1 > CK (Fig. 5F). In 2020, the dry matter content of tubers under T3, T4, and T5 treatments were significantly higher than that of CK, which was 32.17%, 33.69%, and 42.63%, respectively, the Fe content of the T5 treatment was 112.64% higher than CK. In 2021, the dry matter content of tubers under T3 and T5 treatments were significantly higher (30.79% and 31.7%) than that of CK. Fe content of tubers in T5 treatment was 54.98% higher than CK. The results showed that five types of iron fertilizer could effectively increase the iron content of the tuber, and the T5 treatment had the best effect.

Analysis of the difference in potato variables under different iron fertilization treatments

Multicollinearity among numerous factors is addressed via principal component analysis (PCA). The number of retained principal components (PCs) should be determined using the eigenvalues. The findings demonstrate reciprocal aggregation between the sample points corresponding to the samples of the six treatments in the picture, demonstrating the high degree of similarity between the treatments. The consistency of the response variable's arrow direction with the axis direction (negative to positive) can be used to determine whether there is a positive or negative correlation between the response variable and the PC. As indicative factors indicating the variations, tuber production, quality, and Fe content loaded substantially on the first and second significant components, respectively (Fig. 6A, B). All variables are positively associated with PC1. The total of PC1 and PC2 in 2020 was 81.4%, with PC1 and PC2 accounting for 72.4% and 9.0%, respectively, of the total variance. PC1 and PC2 accounted for 86.3% of the variance in 2021, with PC1 accounting for 73.5% and PC2 for 12.8%.

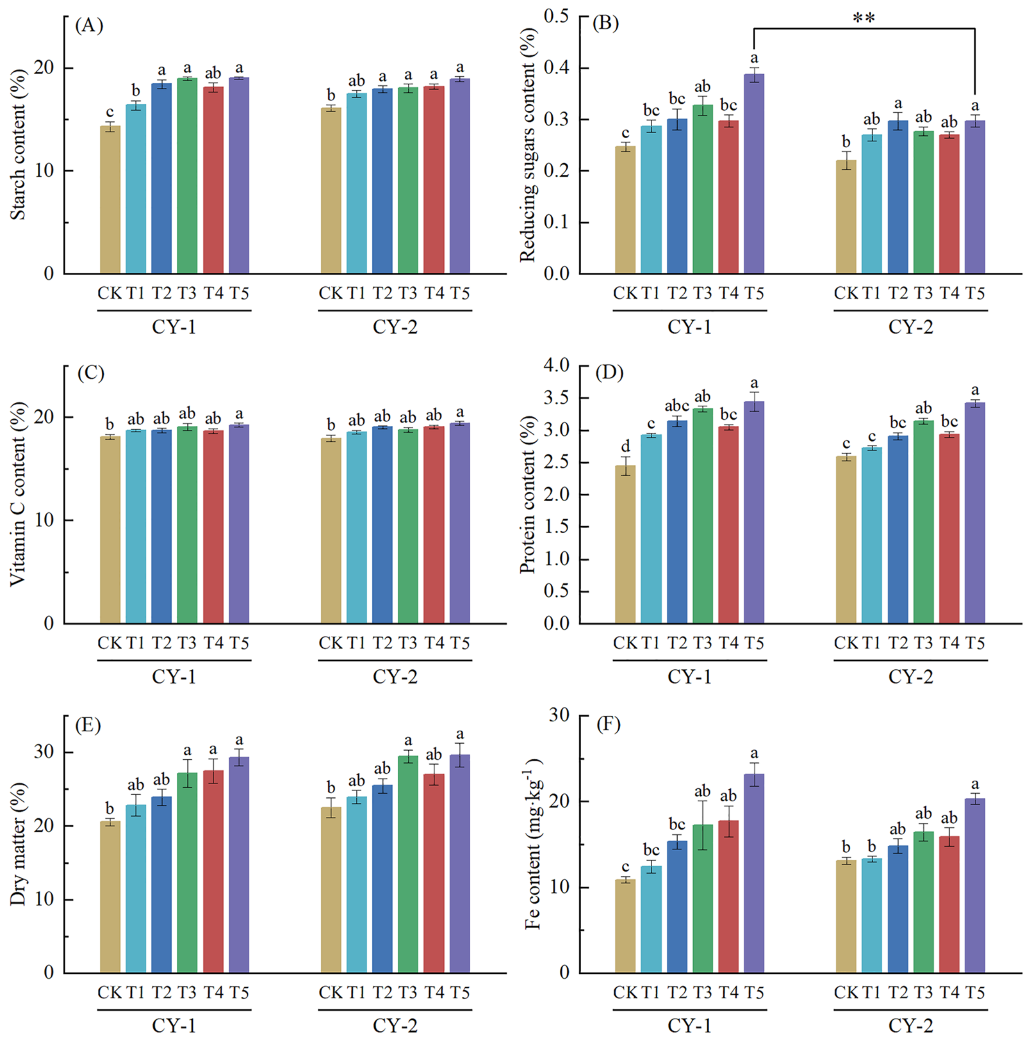


Fig. 5 Starch content (**A**), reducing sugars content (**B**), vitamin C content (**C**), protein content (**D**), dry matter (**E**), and Fe content (**F**) of potato tuber as affected by different iron fertilizer treatments. Lowercase letters indicate significant differences among fertilization treatments ($p < 0.05$). CK-No iron fertilizer, T1-$Fe_2(SO_4)_3$ fertilizer, T2-$FeSO_4{\cdot}7H_2O$ fertilizer, T3-CA/$Fe_2(SO_4)_3$ fertilizer, T4-CA/$FeSO_4{\cdot}7H_2O$ fertilizer, T5- (EDTA-Fe·Na) fertilizer. CY-1 (2020), CY-2 (2021)

Photosynthetic traits of potato leaves also varied among iron fertilizer treatments (Fig. 6C, D). The sum of PC1 and PC2 was 86% in 2020, with PC1 accounting for 71.4% of the whole variance and PC2 for 14.6% of the overall variance. The difference between PC1 and PC2 in 2021 is 86%, with PC1 accounting for 71.9% of the total variance and PC2 for 14.1%. P_n, G_s, T_r, chl *a*, chl *b*, car, Chl($a+b$), Total pigment content, *Fv/Fm*, *Fv'/Fm'*, Φ_{PSII}, *qP*, and ETR were situated in the first and fourth quadrants and exhibited positive associations with PC1. On the other hand, PC1 negatively correlated with 1-*qP*, Chl *a/b*, C_i, and $Y_{(NO)}$.

Correlation analysis of yield and quality of potato tuber with photosynthetic gas exchange characteristics and chlorophyll fluorescence.

Following dimensionless data processing for all variables, we evaluated the correlation between the variables (Fig. 7). In addition to the earlier experimental results, we conducted a correlation analysis between the photosynthetic indexes of potato plants treated with iron fertilizer and factors linked to tuber yield and quality. Tuber quality parameters such as dry matter, Fe content, starch, protein, reducing sugars content, and tuber yield were positively correlated with photosynthetic

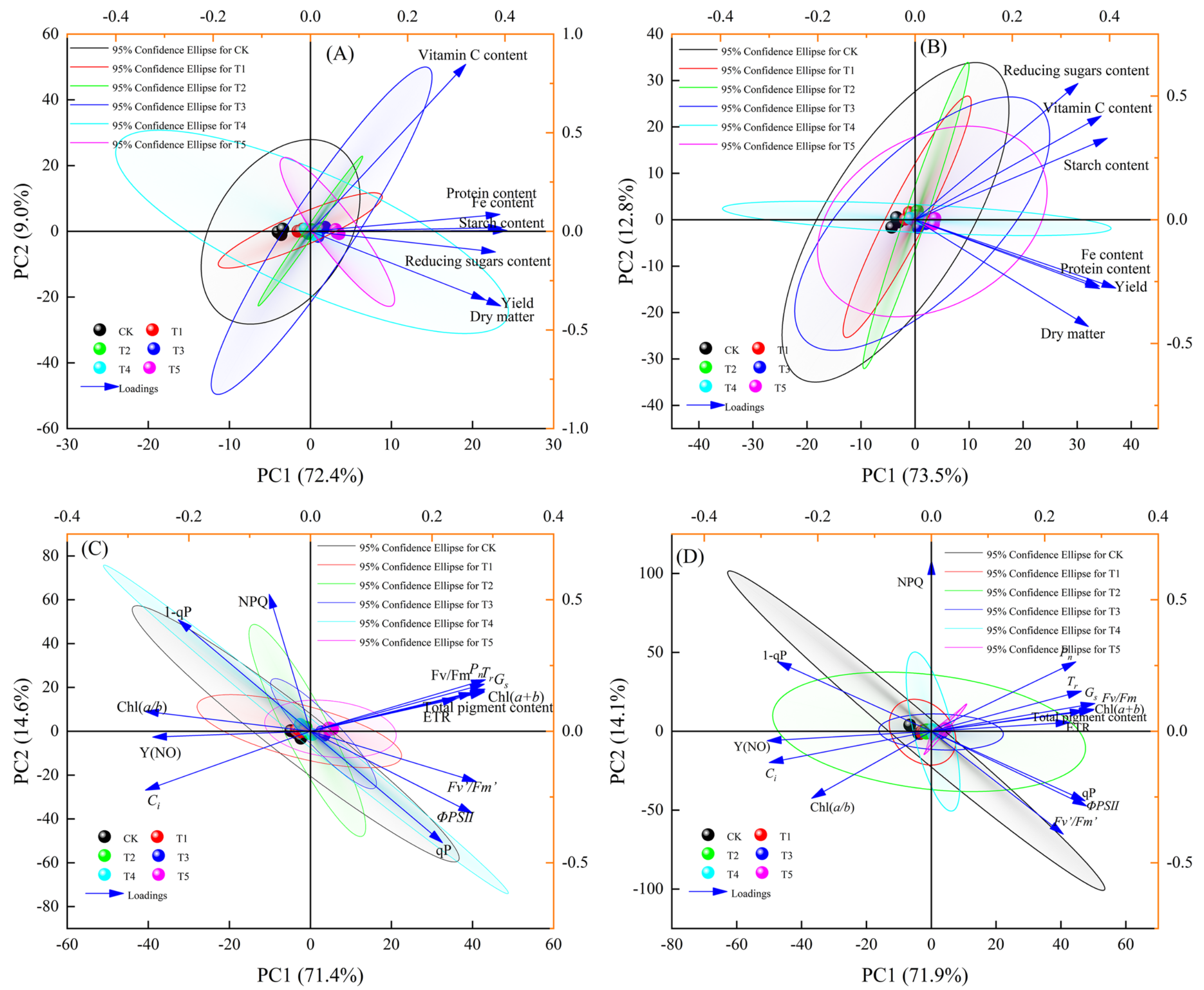


Fig. 6 Different iron fertilizer treatments photosynthetic properties, chlorophyll fluorescence metrics, and tuber production were examined using principal component analysis (PCA), which included score scatter plots and loading plots

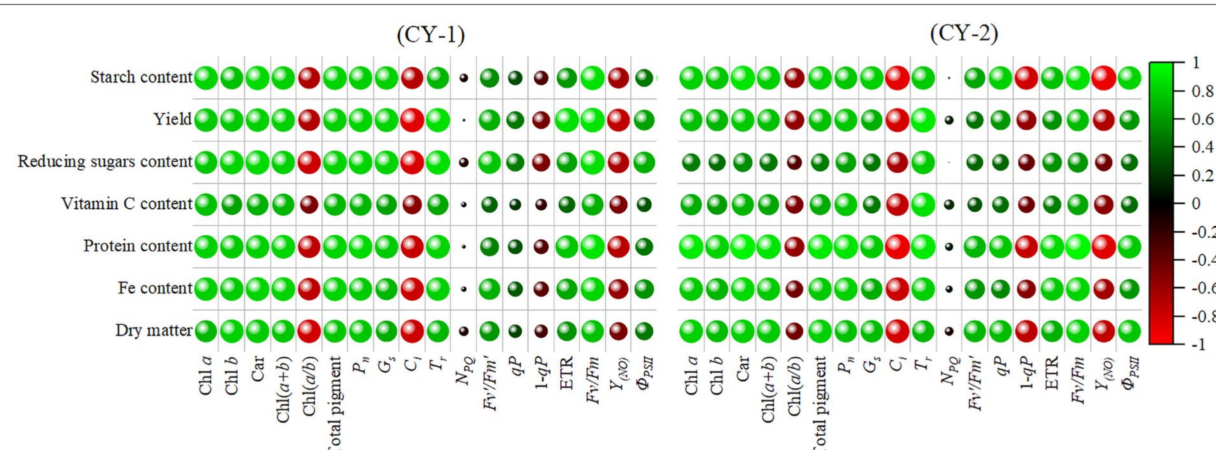


Fig. 7 Heat map of Pearson correlation coefficient matrix between photosynthetic parameters and tuber yield, quality, and iron content. Heat map of Pearson correlation coefficient matrix produced by Origin Pro 2022b 64Bit. CY-1 (2020), CY-2 (2021)

pigment (Chl *a*, Chl *b*, Car, Chl ($a+b$), and total pigments) content and gas exchange parameters (*Pn*, *Gs*, and *Tr*) of potato leaves and were more correlated and less correlated with chlorophyll fluorescence (*Fv/Fm*, *Fv'/Fm'*, Φ_{PSII}, N_{PQ}, *qP*, and ETR). There was a negative correlation with Chl*a/b*, *Ci*, 1-*qP*. The findings demonstrated that plant photosynthesis can significantly affect the features of tuber yield and quality and is also necessary for the biofortification of tuber iron.

Clustering relationship among different iron fertilizer treatments

The levels and trends of variables following iron fertilization treatment were represented in the heat map after the data were dimensionless (Fig. 8). The image showed the cluster analysis using 25 variables from six treatments. From the effects of clustering, T5 and T3 treatments were clustered into one category in 2020. In 2021, T5, T3, and T4 treatments were clustered into one category. It shows a more substantial similarity between T5 treatment and T3 treatment.

Discussion

Iron (Fe) is essential for plant growth and development and plays a vital role in plant photosynthetic physiology and biological processes. Agronomic biofortification may be easy, cost-effective, efficient, and applicable to most crops. Agronomic practices for potato biofortification include tuber application, foliar application, and soil application [39]. Studies have shown that seed initiation with different micronutrients can increase the micronutrient content of crop plants. In our study, Fe-fertilizer treatment improved chlorophyll content and photosynthesis of the "Longshu No.7" potato. Plants can use this to escape from iron overload and iron deficiency by reducing their growth rate, yield, chlorophyll synthesis, and photosynthesis efficiency [40]. Crop biofortification is now considered a sustainable, economical, and easy-to-implement strategy. Iron biofortification must be achieved through the widespread use of efficient iron fertilizer types and optimal fertilizer management practices [41]. Therefore, studying the response of potato plants to iron fertilizer will help us understand the mechanism of iron plantations in potatoes. Iron fertilizer treatment boosted the chlorophyll and carotenoid content of potato

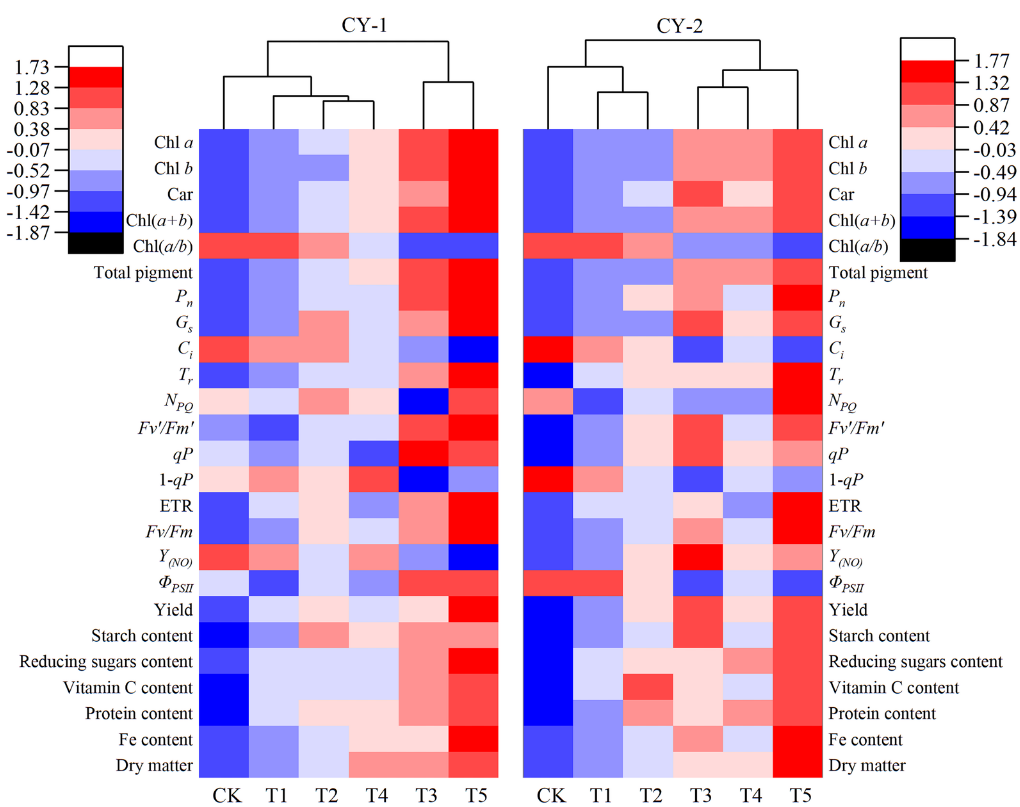


Fig. 8 Heat maps with a tree diagram between different iron fertilization treatments plotted with Origin Pro 2022b 64Bit. The legend in the figure indicates normalized parameters

leaves in this study (Fig. 3). Previous research demonstrated that foliar application of iron fertilizer considerably raised the chlorophyll content of *Mentha* [6] and *Vitis vinifera* [42], which is consistent with this study's conclusion. It showed that increasing iron fertilizer effectively promoted the increase of chlorophyll and other photosynthetic pigments. In this study, iron fertilizer significantly increased P_n, G_s, and T_r and decreased C_i. The results were consistent with the results of Dey et al. [43] that iron fertilizer treatment could increase the photosynthetic rate of rice. *Fv/Fm* and PSII were evaluated to quantify the photosynthetic ability of potato leaves during dark and light adaptation. *Fv/Fm* and PSII increased with the iron fertilizer treatment (Table 2), indicating that iron fertilizer reduced the photoinhibition of potato plants and promoted photochemical efficiency. Early studies showed that iron-deficient leaves protected leaves from high light irradiation by reducing leaf absorbance and increasing N_{PQ} and heat dissipation of light absorbed by PSII [44]. In our investigation, iron fertilizer treatment boosted N_{PQ}, *qP*, and ETR while decreased PSII excitation pressure (1-*qP*) (Table 3); this is likely owing to the improvement of N_{PQ} level and photosynthetic electron transport, which has reduced excess excitation energy in the reaction center [45, 46]. Further evidence showed that iron fertilizer can reduce the harmful effects of iron deficiency on the photosynthetic ability of potato plants by controlling the heat dissipation route. Previous studies suggested that an appropriate amount of Fe was conducive to improving the adverse effects of electrons and energy on the transport characteristics of the plant photosynthetic system [47]. Under iron deficiency conditions, the application of iron fertilizer can improve pigment content, quantum yield, and functional PSII reaction center performance in rice [43]. It shows that iron deficiency destroys energy transmission in electron and optical systems. Iron fertilizer treatment can repair the photosynthetic electron transport chain, improve electron transport efficiency, and promote energy distribution balance.

Successful fertilizer management practices typically use iron fertilizer to address iron deficiency symptoms in plants and increase crop yield. Spraying iron on leaves through different iron fertilizer treatments had positive effects on potato tuber yield parameters, namely, total yield per hectare and yield increase rate (Fig. 4). Between T3 and T5, there was no significant difference in tuber yield, but was significantly higher than other treatments. The observed variation trend of yield parameters was consistent with the effects of various treatments on plants photosynthetic and fluorescence parameters. Some studies have reported the importance of iron fertilizer in obtaining high-yield crops. The potato (*Chandramukhi*) tuber yields of Chatterjee C et al. had positive effects [48]. In addition, Bandyopadhyay et al. reported that compared with conventional trace elements, the use of slow-release fertilizer also significantly increased potato (*Jyoti variety*) yield and vitamin C content in tubers [49], which was consistent with tour conclusions. Iron deficiency is a global health problem for children and women. Ascorbic acid in potatoes promotes iron absorption, and increasing the iron content in potatoes may help alleviate iron deficiency in humans [50]. In addition, Itoh et al. emphasized the importance of iron fertilization in obtaining high-yield tubers [51]. In addition, Rubio et al. revealed that 100 g of tubers deliver 6% of the body's iron [52]. Dry matter accumulates photosynthetic components and ingested nutrients in plants; it influences crop yield production [53]. We discovered that the dry matter content of the tubers in the T3, T4, and T5 treatments were significantly higher than CK (Fig. 5) and the quality of the tubers could be substantially enhanced. Today, the most common micronutrient shortage in the world is iron deficiency anemia. Low iron content in crops often causes iron deficiency anemia. Given that the population consumes many potatoes, a food with a medium range of iron, potatoes are a good source of iron [54]. Ascorbic acid (vitamin C) is abundant in potatoes and aids in iron absorption. The bioavailability of iron greatly affects the amount of iron consumed in the diet. Compared to wheat, beans, and other crops, potatoes contain a much higher amount of iron accessible for intestinal absorption, between 63% and 79%, according to research [55]. As a result, potato tubers are a great source of mineral iron for the human body to absorb due to their high iron bioavailability. Iron fertilizer spraying increased tuber nutritional quality (Fig. 5). This study revealed a considerable increase in the Fe content of tubers, which was an exciting dietary discovery.

The combined analysis of the data from the measured indicators in the paper (Figs. 6, 7, 8) revealed that the various iron fertilizer treatments impacted tuber yield, quality, and photosynthesis. Under this impact, the factors have a synergistic or antagonistic relationship. It also showed the complexity of the link between photosynthesis and yield, quality, and iron content of potato tubers under iron fertilization regimens. PCA (Fig. 6) showed differences in photosynthetic characteristics and nutritional responses of tubers to different iron fertilization treatments, again indicating different effects of varying iron fertilization treatments. Correlation coefficients (Fig. 7) were used to assess the correlation between photosynthetic characteristics and tuber yield, quality, and iron content of potatoes under iron fertilization. Correlation coefficients were used to evaluate further the association between potato photosynthetic parameters and tuber characteristics (Fig. 7). Data on photosynthetic and tuber nutrient parameters and the iron content of potatoes under each treatment were subjected to cluster analysis (Fig. 8) to classify the treatments. Further testing proved that the T5 treatment was the best fertilizer, with the T3 treatment coming in second.

Conclusions

In summary, Iron fertilizer application methods (foliar sprays) and fertilizer types (inorganic iron, iron citrate, and chelated iron) not only have a significant impact on crop development and yield, but also critically on the quality of the finished potato. The combined data analysis (Figs. 6, 7, 8) revealed differences in the fertilization efficacy of the various iron fertilizer treatments. Photosynthesis in the leaves of the plant was responsible for the increase in tuber nutrition and iron content after treatment. This study's fertilization system was based on the assumption that all soil base fertilizers (N–P–K) were effectively fertilized by iron fertilizer spray. Moreover, because the pH value of the local soil is high and the plant Fe utilization efficiency is low, this study did not implement a Fe deficiency stress treatment but rather a direct Fe fertilizer supplementation. The following fertilization recommendations were so derived: (i) if potatoes are grown in soils with a high pH, inorganic iron fertilizer sprayed directly on the leaves (T1 and T2 treatments) can meet the plant's iron needs while saving money. (ii) When economic circumstances were met, Citric acid–inorganic iron fertilizer [CA/$Fe_2(SO_4)_3$ treatment] and chelated iron (EDTA-Fe·Na treatment) sprinkled on the leaves had a more significant effect on iron biofortification in potatoes. (iii) Foliar spray treatments are desirable and can mitigate the soil environment damage caused by the chemicals.

Abbreviations

CA: Citric acid; Car: Carotenoid content; Chl: Chlorophyll; Chl *a*: Chlorophyll a; Chl *b*: Chlorophyll b; Chl ($a+b$): Total chlorophyll content; Chl a/b: Chlorophyll *a/b*; C_i: Intercellular CO_2 concentration; EDTA-Fe·Na: Ethylenediamine tetra acetic acid; ETR: Electron transport rate; *F0*: Minimal fluorescence yield of the dark-adapted state; *F0/Fm*: Thermal dissipation quantum ratio; Fe: Iron; $Fe_2(SO_4)_3$: Ferric sulfate; $FeSO_4 \cdot 7H_2O$: Ferrous sulfate; *Fm*: Maximal fluorescence yield of the dark-adapted state; *Fv*: Variable fluorescence; *Fv/Fm*: Maximal quantum yield of PSII photochemistry; *Fv′/Fm′*: Effective photochemical quantum yield; G_s: Stomatal conductance; N_{PQ}: Non-photochemical quenching of PS II; PAR: Photosynthetically active radiation; P_n: Net photosynthetic rate; PSII: Photosystem II; *qN*: Coefficient of non-photochemical quenching; *qP*: Photochemical quenching of PS II; T_r: Transpiration rate; $Y_{(NO)}$: The quantum yield of non-regulated energy dissipation in PSII; 1-*qP*: Excitation pressure of PSΠ; Φ_{PSII}: The effective photosystem II quantum yield.

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Author contributions

RZ: conceptualization, software, methodology, and writing—original draft preparation. WZ: methodology. YK: investigation. MS: data curation. XY: visualization. HL: data curation. HY: resources. YW: formal analysis. SQ: funding acquisition, project administration. All authors have read and agreed to the submitted version of the manuscript. All authors read approved the final manuscript.

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Availability of data and materials

All data obtained from the current study are available from the corresponding author on a reasonable request.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

All co-authors have seen and agreed on the contents of the manuscript, and there is no financial interest to report.

Competing interests

The authors declare there are no conflicts of interests.

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