

RESEARCH ARTICLE

Enhancing quinoa growth under severe saline-alkali stress by phosphate solubilizing microorganism *Penicillium funiculosum* P1

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Abstract

Promoting the growth of plants and improving plant stress-resistance by plant growth-promoting microorganism increasingly become a hotpot. While, most researchers focus on their supply role of nutrition or plant hormone. In this study, a novel mechanism that phosphate solubilizing microorganisms promoted plant growth under saline-alkali stress through secretion of organic acids, was proposed. The effects of desulfurization gypsum, humic acid, organic fertilizer and phosphate-solubilizing microorganism *Penicillium funiculosum* P1 (KX400570) on the growth of quinoa (*Chenopodium quinoa* cv. Longli 1), showed that the survival rate, stem length and dry weight of quinoa treated with P1 were 2.5, 1.5, 1 and 1.5 times higher than those of sterile water (CK) under severe saline-alkali stress. The growth-promoting effect of P1 on quinoa was much better than that of other treatment groups. In addition, P1 promoted the growth of quinoa because the organic acids (malic acid, citric acid, succinic acid, etc.) from P1 stimulated the antioxidant system and promote the photosynthesis of quinoa, further promote quinoa growth.

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1. Introduction

The area of saline-alkali land is about one billion hm^2 and increases every year [1, 2]. Innutrition [3] and lower diversity or abundance of microorganisms [4] are major characters of saline-alkali soil [5]. Therefore, severe saline-alkali stress endangers plant growth [3] by disturbing photosynthesis [6] and water-uptake [7].

To reduce the stress of saline-alkali soil for plant, the researchers presented lots of methods, including physical [8], chemical [9] and biological methods [10]. Among of them, physical methods, especially salt washing method, worked best [11]. While, its complex operation and high cost hindered their large-scale application [11]. Comparatively, chemical methods were the most common methods for improving saline-alkali soil because of their simple operation and low cost. However, chemical methods such as desulfurization gypsum [12] and humic acid [13], did not work in a short-term [12–14]. The biological methods, including plant of pioneer crops [15], application of microorganism agent [16–18] and organic fertilizer, etc.

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[19–21], worked better to improve saline-alkali soil in a short term. However, biological methods commonly worked on lighter level saline-alkali soil, such as *Chenopodium quinoa*. As a halophyte, *Chenopodium quinoa* grows well and improves soil properties under moderate salt stress (100–200 mM of NaCl) [22]. However, the survival rate is low under high saline-alkali stress. While, many microorganisms with stronger vitality and saline-alkali tolerance can be used to improve higher saline-alkali land. In addition, biological methods are sustainable. Recently, more and more researchers focus on this topic.

Many saline-alkali resistance microorganisms [23] not only reduced soil salinity [24], but also had the functions of nitrogen fixation [25, 26], potassium [27] and phosphate solubilization [10, 28]. Because of their functions, these microorganisms have great application potential on improving the nutrition of saline-alkali soil. Thus, these microorganisms may be used to improve saline-alkali soil [10, 23, 24]. Moreover, many microorganisms secrete bioactive substances such as IAA [28–30], ACC deaminase [31, 32], gibberellin [30], exopolysaccharides [33, 34] and polyamine [35] etc., which activate the antioxidant system of plants and promote plant growth. Therefore, salt-tolerant multifunctional growth-promoting microorganisms have been attracted more attention in recent years [36, 37].

Studies showed that microorganism agent would improve saline-alkali soil properties. Under field experiment, *Trichoderma asperellum* [38] inoculated into maize decreased the pH of maize rhizosphere soil by 0.25 and increased the contents of organic matter, available nitrogen, phosphorus and potassium in soil as well. Then, the yield of maize increased by 12.41%. Similarly, Jiangbao Xia et al. showed that the microorganisms reduced soil salt content and increased soil available phosphorus and potassium content under field experiment as well [39]. Further, the microorganisms promoted the uptake of nutrients by *Sesbania cannabina* and improved the total biomass of *Sesbania cannabina* by 1.4 times under field experiment. Besides, microorganisms improved the saturated hydraulic conductivity better than gypsum and helped water transport more smoothly in saline-alkali soil [40].

Microorganism agent not only affect soil properties, but also help plants resist saline-alkali stress by regulating physiological and biochemical reactions in plants. Under saline-alkali stress, *Bacillus licheniformis* activated the pathways related to abiotic stress resistance and iron acquisition in chrysanthemum, and improved the survival rate, photosynthesis and biomass of chrysanthemum [41]. Jiali Liu et al. [42] isolated two plant growth promoting strains under saline-alkali stress. After inoculating into alfalfa rhizosphere, the antioxidant system of alfalfa was activated and the activities of antioxidant enzymes were increased. The stem height, shoot dry weight and root dry weight of alfalfa were increased by about 20%, 33% and 100%, respectively. Similarly, Haiyun Li et al. [43] inoculated composite microorganism agents into the rhizosphere of oat seedlings. The antioxidant enzymes activities of oat were increased, and the dry weight, plant height and root length of the oat seedlings were increased by about 200%, 100% and 30%, respectively. Interestingly, Chintan Kapadia et al. [44] inoculated tomatoes with microbial consortia under salt stress. Compared with the control, the treatment inoculated with microbial consortia did not change soil properties, but increased tomato chlorophyll content and mineral uptake. However, so far, few strains are available for severe saline-alkali soil. P1 has the ability of phosphate solubilization and secreting organic acids in severe saline-alkali soil [45]. Therefore, P1 may benefit for improving saline-alkali soil properties and promoting plant growth. Moreover, helping quinoa adapt to severe saline alkali stress and then improving saline-alkali soil property by microorganisms are still research gap.

Herein, we studied the performance of phosphate-solubilizing fungi P1 to improve severe saline-alkali soil and promote the growth of quinoa. Hence, we illustrated the reason why P1 promoted the growth of quinoa under severe saline-alkali stress through the comparison of desulfurization gypsum, humic acid and organic fertilizer treatments.

2. Materials and methods

2.1 Materials source

Phosphate-solubilizing fungi *Penicillium funiculosum* P1 (KX400570) was preserved in our lab, and the organic acids secreted by P1 were determined qualitatively and quantitatively by liquid chromatograph Ultimate3000 [45]. The organic fertilizer was also prepared ourselves [46]. Quinoa used in this paper was *Chenopodium quinoa* cv. Longli 1, which was bred by Pasture and Green Agriculture Institute of Gansu Academy of Agricultural Sciences. And the quinoa seeds were bought from Shanxi Jinnongyuan Land Products Co., Ltd.. Desulfurized gypsum and humic acid were purchased from Liyuan Environmental Protection Materials Co., Ltd. and Shenzhen Dugao New Biological Technology Co., Ltd., respectively.

2.2 Plant growth conditions and treatment

Pot experiments. The saline-alkali soil used in pot experiments was collected from the surface 0–20 cm depth of Liujia saline-alkali soil (44°13'59.559'E, 125°6'41.349'N) in Nong'an County, Changchun City, Jilin Province, China. The soil was dried and separated by a 0.9 mm sieve, and then put into no-hole pots. The maximum, minimum diameter and height of the pots are 18.5 cm, 10 cm and 11 cm, respectively. After that, different treatments were applied to the soil, including sterile water (CK), desulfurized gypsum (FGD), commercial amendments (MA, humic acid content \geq 70%, 3% of the soil), organic fertilizer (OF, 3% of the soil) and phosphate-solubilizing fungi P1 (P1, 20 mL bacterial solution per 1 kg soil after PDA culture), respectively. Quinoa seeds sterilized were planted in pots (1 kg soil per pot, 35 seeds per pot, 3 replicates). The plants were placed in a greenhouse with a room temperature of 25°C, and the light / dark cycle was 16 h / 8 h. Samples were tested after 30 days. And each test was repeated triple.

Soilless culture experiment. Quinoa seeds sterilized were seeded in a 200 mL container with vermiculite. Each container was input suitable Na_2CO_3 - NaHCO_3 buffer solution (0.1 M Na_2CO_3 : 0.1 M Na_2HCO_3 = 5: 5) and Hoagland solution. Then five exogenous organic acids were added to the system, which were oxalic acid, acetic acid, succinic acid, citric acid and malic acid. The type and concentration of these organic acids were determined by the secretion of P1. And then 10 quinoa seeds were planted in each container. Each treatment has three parallel samples.

2.3 Biochemical analysis

Chlorophyll content of quinoa was determined by portable chlorophyll meter (TYS-4N, Beijing Zhongkeweihe Instrument Company, China). Three mature leaves of three quinoa plants were selected, and the average value of each leaf was recorded three times in vivo.

Proline content was tested by the Acidic Ninhydrin Method [47]. 0.5 g of plant leaves were added into 5 mL 3% sulfosalicylic acid solution, and then heated in boiling water for 10 minutes. After that, 2 mL centrifuged supernatant was mixed with 2 mL acetic acid and 2 mL acidic ninhydrin reagent. Then, it was heated in boiling water bath for 30 min. Then, the solution was cooled, then mixed with 4 mL toluene and stood. Finally, the upper solution was taken and tested at 520 nm wavelength by L5S BMS (INESA Analytical Instrument Co., Ltd), and proline content was got from a standard curve prepared using analytical grade proline and expressed as mg/g FW.

Malondialdehyde (MDA) content was tested by thiobarbituric acid (TBA) method. 0.1 g plant samples were added to 5 mL 5% trichloroacetic acid (TCA) solution, and then ground, homogenized and centrifuged. After that, 2 mL supernatant was taken and mixed with 2 mL

0.67% thiobarbituric acid (TBA). Then, the mixing solution was heated in boiling water bath for 30 min, and following with the measurement of absorbance values at 450 nm, 532 nm and 660 nm by L5S BMS (INESA Analytical Instrument Co., Ltd). MDA content was expressed on a nmol/g FW basis [48].

Antioxidant enzyme (POD, SOD) activity was detected by Zhou W *et al.* [49] method. In the SOD experiments, 0.1 g plant samples were grinded with liquid nitrogen, and then washed with 5 mL phosphate buffer (pH 7.8). After centrifugation, the crude enzyme solution was got. Then, 0.05 g crude enzyme solution reacted with SOD reaction solution, under 4000lx light intensity for 20 min. And then, the absorbance was measured at 560 nm by L5S BMS (INESA Analytical Instrument Co., Ltd). SOD reaction solution was prepared by 0.05 mol/L phosphate buffer (pH 7.8), 130 mmol/L methionine solution, 750 μ mol/L nitro-blue tetrazolium solution, 100 μ mol/L EDTA- Na_2 solution and 20 μ mol/L riboflavin solution, according to the ratio of 5: 1: 1: 1: 1. In the POD experiment, 0.1 g plant samples were grinded with liquid nitrogen, and then washed with 5 mL phosphate buffer (pH 5.5). After centrifugation, the crude enzyme solution was got. Then, 0.1 mL crude enzyme solution was added to POD reaction solution, and then the absorbance was measured at 470 nm by L5S BMS (INESA Analytical Instrument Co., Ltd). POD reaction solution was prepared with 2.9 mL phosphate buffer (pH 5.5), 1.0 mL 2% hydrogen peroxide and 1.0 mL 0.05 mol/L guaiacol, according to the ratio of 5: 1: 1: 1: 1. Each test was repeated triple.

2.4 Soil physical and chemical properties

Soil physical and chemical properties include pH, electrical conductivity, HCO_3^- content, organic matter, available nitrogen, available phosphorus and available potassium. Soil pH and electrical conductivity were measured after mixing the soil with deionized water at a ratio of 1: 5 by pH meter (SevenDirect SD20 HA Kit, METTLER TOLEDO company) and conductivity meter (DDS-11A, Shanghai INESA Scientific Instrument Co, Ltd.), respectively [24].

Soil organic matter were tested by $\text{K}_2\text{Cr}_2\text{O}_7\text{-H}_2\text{SO}_4$ digestion method [50]. 0.5 g soil sample was mixed with 1 mol/L $\text{K}_2\text{Cr}_2\text{O}_7$ solution. Then, concentrated sulfuric acid and o-phenanthroline indicator was added into the reaction system. And then, the solution was titrated with 0.5 mol/L FeSO_4 till brick red. Finally, record the consumption of FeSO_4 and calculate the content of organic matter through the consumption.

Available nitrogen was tested by alkali-diffusion method [51]. 2.0 g soil sample was evenly mixed with 10 mL 1 mol/L NaOH in the external chamber of the diffusion dish, and then 2 mL boric acid indicator was put into the internal chamber. Then, the reaction was done at 40°C for 24 h. After that, the reaction solution was titrated with 0.01 mol/L H_2SO_4 till yellow. Finally, record the consumption of H_2SO_4 and calculate the content of organic matter through the consumption.

Available phosphorus was tested by molybdenum antimony colorimetric method [52]. 2.5 g soil samples were added in 0.5 mol/L NaHCO_3 to extract available phosphorus, and then the filtrate was reacted with molybdenum antimony reagent for 30 min, and read absorbance at 880 nm by L5S BMS (INESA Analytical Instrument Co., Ltd). Then, available phosphorus concentration was determined according to the standard curve.

Available potassium was tested by flame photometry [53]. Available potassium was extracted from 5.0 g soil sample by neutral NH_4OAc solution. Then, the soil filtrate and a series of potassium standard solutions were determined by 6400A flame photometer (INESA Analytical Instrument Co., Ltd. And then, standard curve was established, and potassium concentration in filtrate was determined according to standard curve. Each test was repeated triple.

Table 1. Growth indicators of quinoa under different treatments.

Treatment	Survival rate (%)	Fresh weight(mg/plant)	Dry weight(mg/plant)	Root length(cm)	Stem length(cm)
CK	10.48±1.65b	90.7	7.6	4.02±0.38d	4.99±0.99b
FGD	16.19±1.65b	97.7	8.6	4.64±0.39c	5.65±0.72b
MA	13.33±3.30b	147.5	13.2	4.66±0.38c	5.53±0.67b
OF	29.52±4.36a	171.7	17.1	7.44±0.57b	7.96±0.65a
P1	34.29±5.71a	187.4	19.1	10.12±0.45a	7.76±0.68a

CK: sterile water, FGD: desulfurized gypsum, MA: commercial amendments (humic acid content \geq 70%, 3% of the soil), OF: organic fertilizer (3% of the soil), P1: phosphate-solubilizing fungi P1(20 mL bacterial solution per 1 kg soil after PDA culture). Means \pm standard deviations for three replicates. Different letters within a column indicate significant differences at $P < 0.05$.

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2.5 Statistical analysis

One-way ANOVA were determined using SPSS Statistics 25 (IBM, New York, USA). The PCoA was used to express the impact of different treatment methods on soil physical and chemical properties and plants by principal coordinates using a Bray-Curtis dissimilarity matrix calculated from the taxonomic abundance matrices. The PCoA plots were generated using the vegan package [54] in R studio (version 4.1.1).

3. Results and discussion

Salinity-alkali stress seriously inhibits the growth of plants [5, 55]. Generally, amendments are applied to improve saline-alkali soil under saline-alkali stress [9, 13, 39, 56]. However, chemical amendments work little on plant growth in a short term under saline-alkali stress [9, 14]. From Table 1, although the MDA content (Fig 1A) and proline content (Fig 1B) were decreased after the application of desulfurization gypsum and humic acid, the growth of quinoa seedlings were not significantly improved. The results have the same tendency with that of Yonggan Zha *et al.* [12] and Hu, Y. W. *et al.* [13]. While, organic fertilizer works better on

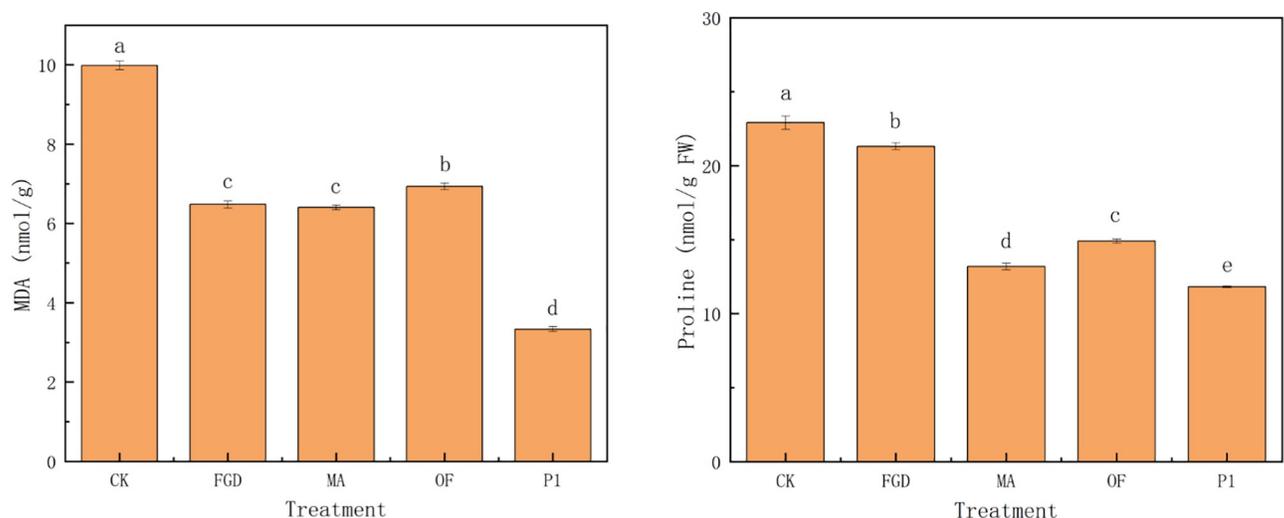


Fig 1. Treatments on MDA and proline of quinoa. CK: sterile water, FGD: desulfurized gypsum, MA: commercial amendments (humic acid content \geq 70%, 3% of the soil), OF: organic fertilizer (3% of the soil), P1: phosphate-solubilizing fungi P1(20 mL bacterial solution per 1 kg soil after PDA culture). Error bars represent the standard deviation of the replicates ($n = 3$). Different letters within a column indicate significant differences at $P < 0.05$.

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Table 2. Physical and chemical properties of saline-alkali soil under different treatments.

Treatment	pH	EC (mS/cm)	HCO ₃ ⁻ (mg/kg)	Available-N (mg/kg)	Available-P (mg/kg)	Available-K (mg/kg)	Organic carbon (g/kg)
CK	9.12±0.02ab	0.96±0.02d	48.80±3.05a	130.67±5.35b	14.39±0.30e	268.92±27.56b	7.85±0.83b
FGD	9.15±0.02a	1.02±0.01cd	47.78±1.76a	120.17±5.35b	16.98±0.36c	179.28±11.02c	5.59±0.08c
MA	8.51±0.02d	1.23±0.01b	28.47±1.76c	75.83±5.35c	27.63±0.28b	268.9±25.51b	5.72±0.61c
OF	9.06±0.04b	1.55±0.03a	41.68±1.76b	175.00±9.26a	151.32±0.36a	494.97±16.54a	20.48±1.51a
P1	8.77±0.08c	1.08±0.0c	40.67±1.76b	166.83±2.02a	15.71±0.36d	183.18±5.51c	9.71±0.83b

CK: sterile water, FGD: desulfurized gypsum, MA: commercial amendments (humic acid content \geq 70%, 3% of the soil), OF: organic fertilizer (3% of the soil), P1: phosphate-solubilizing fungi P1 (20 mL bacterial solution per 1 kg soil after PDA culture). Means \pm standard deviations for three replicates. Different letters within a column indicate significant differences at $P < 0.05$.

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quinoa's growth. After organic fertilizer treatment, quinoa survival rate, root and stem length, fresh and dry weight, were increased by about 2 times, 60% and 1 times compared with CK, respectively (Table 1). Moreover, the content of both MDA (Fig 1A) and proline (Fig 1B) decreased by about 65%. This indicated that organic fertilizer treatment not only alleviated saline-alkali stress, but also effectively promoted quinoa growth. Why did organic fertilizer improve quinoa growth?

Recent studies provided possible answers: organic fertilizers were beneficial for optimizing saline-alkali soil microbial community [20, 38]. Organic fertilizer provided nutrients for microorganisms and then increased the abundance of beneficial microorganisms [20, 57]. And then, thanks to these beneficial microorganisms, organic fertilizer plays a role on assisting plants to resist saline-alkali stress [19, 58]. In another words, beneficial microorganisms were able to help plants resist saline-alkali stress [34]. Then, will it be better if we apply beneficial microorganisms directly to the saline-alkali soil?

To solve the problem, we studied the effect of phosphate-solubilizing microorganism P1 on assisting quinoa to resist saline-alkali stress and its mechanism. As shown in Table 1, the treatment inoculated with P1 better promote the growth of quinoa under severe saline-alkali stress than organic fertilizer. The survival rate of quinoa increased by about 20% (Table 1). Both fresh weight and dry weight of quinoa increased by about 10% (Table 1). Furthermore, compared with organic fertilizer, MDA (Fig 1A) and proline (Fig 1B) content of quinoa decreased by 52% and 21%, respectively. Thus, beneficial microorganisms were more favorable for promoting plant growth.

In summary, soil amendments were beneficial for promoting the growth of quinoa under saline-alkali stress. Among them, microbial agent P1 worked best. P1 increased the survival rate, root and stem length, fresh and dry weight per plant by 2.5 times, 1.5 times, 55%, 1 time and 1.5 times, respectively, under severe saline-alkali stress. The experimental results were consistent with those of Lei Kong *et al.* [59] and Mehrnoush Eskandari Torbaghan *et al.* [60]. Lei Kong *et al.* [59] inoculated AMF fungi into saline-alkali soil. And the stem length of tomato was increased by 39.32%. But, P1 increased the stem length of quinoa by 55%. Mehrnoush Eskandari Torbagha *et al.* [60] inoculated *Alkalibacillus haloalkaliphilus* in saline-alkali soil. And the fresh weight of wheat was increased by about 70%. But, P1 doubled the fresh weight of quinoa. What caused the remarkable effect of phosphate-solubilizing microorganisms P1 on quinoa?

Previous researches illustrated that phosphate-solubilizing microorganisms can improve soil properties and provide suitable growth environment for plant growth [61–63]. Fungi P1 had similar function as well. P1 secreted organic acids during their growth process. These organic acids decreased soil pH and bicarbonate concentration (Table 2). But improvement of soil properties was impossible for the reason of quinoa growth. From the statistical analysis,

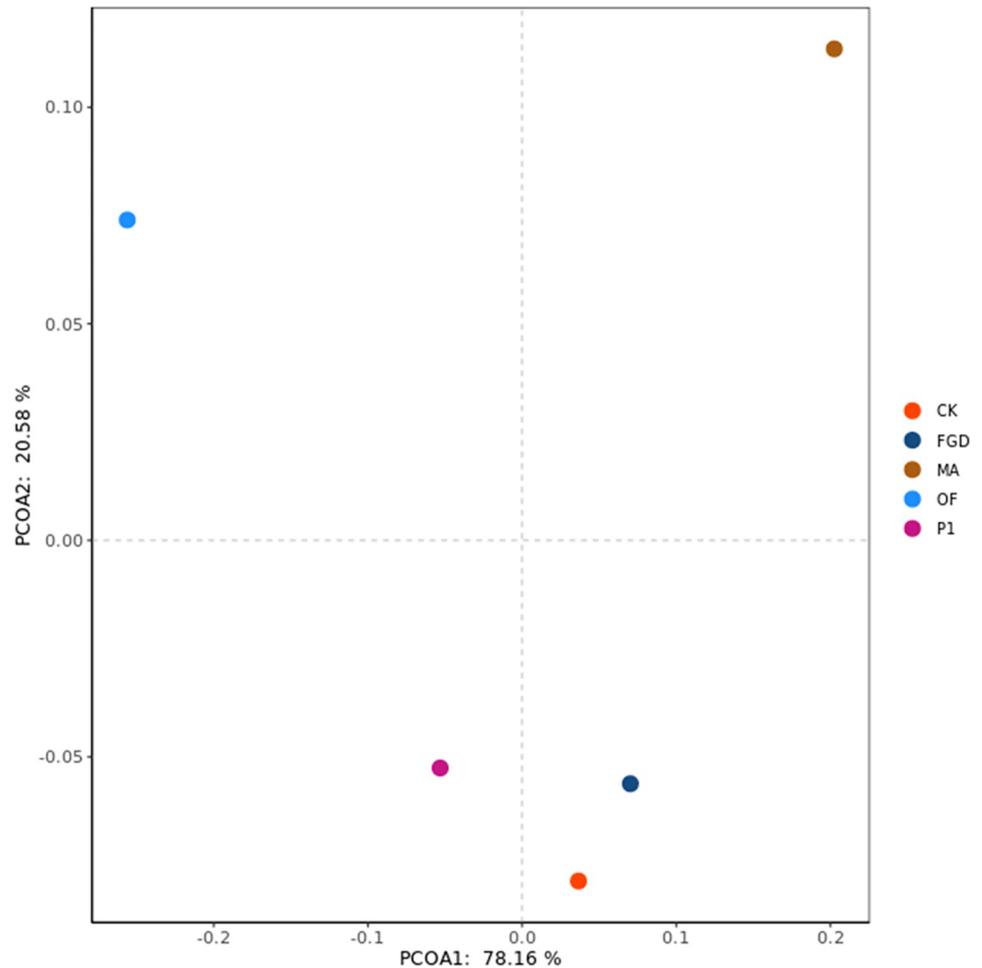


Fig 2. Difference visualization of saline-alkali soil physical and chemical properties under different treatments. CK: sterile water, FGD: desulfurized gypsum, MA: commercial amendments (humic acid content $\geq 70\%$, 3% of the soil), OF: organic fertilizer (3% of the soil), P1: phosphate-solubilizing fungi P1 (20 mL bacterial solution per 1 kg soil after PDA culture). The data comes from all the data about the physical and chemical properties of soil mentioned in this paper. Principal coordinates use a Bray-Curtis dissimilarity matrix calculated from the taxonomic abundance matrices.

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organic fertilizer treatment performed best on improving saline-alkali soil properties, followed by humic acid, as shown in Fig 2. However, the treatment with P1 works the best on the growth of quinoa (Fig 3). This phenomenon indicated that some important factors were ignored. Then, whether organic acids secreted by P1 not only reduced the saline-alkali soil pH, but also promoted plant growth?

Some exogenous organic acids promoted the growth of plants. Some exogenous organic acids such as succinic acid increased organic acids (succinic acid, citric acid, malic acid and oxalic acid, *etc.*) in plant cytoplasm [64]. The increase of intracellular organic acid content was beneficial for plants to resist saline-alkali stress especially oxalic acid [65], and then resulted in promoting plant growth [66, 67]. Does P1 secrete similar organic acids? In order to solve this problem, we detected the organic acids secreted by P1. As shown in Table 3, P1 secretes a variety of organic acids, among which oxalic acid, acetic acid, citric acid, malic acid and succinic acid are abundant. Therefore, we studied the effects of exogenous oxalic acid, acetic acid, citric acid, malic acid and succinic acid on the growth of quinoa, respectively (Fig 4). The results

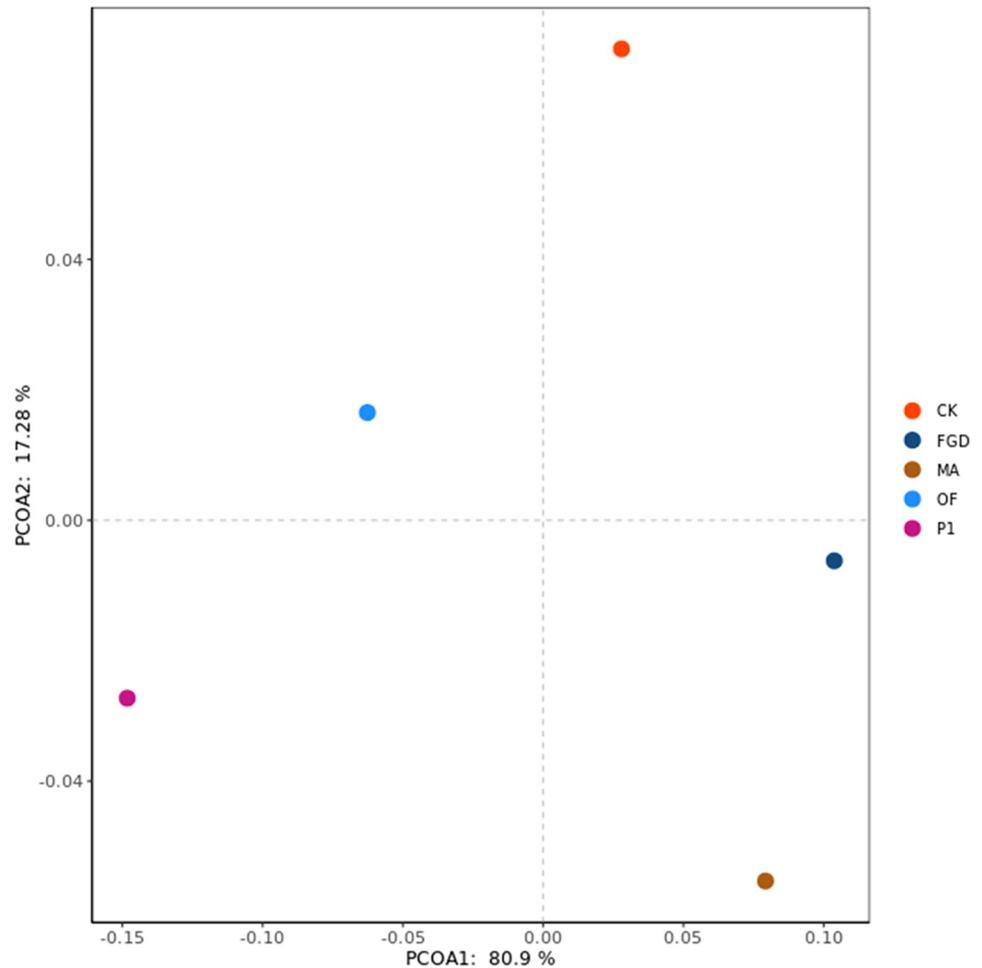


Fig 3. Difference visualization of quinoa growth under different treatments. CK: sterile water, FGD: desulfurized gypsum, MA: commercial amendments (humic acid content $\geq 70\%$, 3% of the soil), OF: organic fertilizer (3% of the soil), P1: phosphate-solubilizing fungi P1 (20 mL bacterial solution per 1 kg soil after PDA culture). The data comes from all the data about the quinoa mentioned in this paper. Principal coordinates use a Bray-Curtis dissimilarity matrix calculated from the taxonomic abundance matrices.

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Table 3. Organic acids produced by P1.

Organic acid	Concentration (mg/L)	Molarity (mM/L)
Oxalic Acid	763	8.4741
Acetic Acid	154.6795	2.5758
Succinic Acid	209.1741	1.7713
Citric Acid	247.1276	1.2863
Lactic acid	69.0017	0.7660
Formic acid	12.4190	0.2698
Propionic acid	19.8204	0.2676
Malic acid	20.0531	0.1495
VC	6.4761	0.0367689
Tartaric acid	5.4689	0.0364375

Qualitative and quantitative analysis of organic acids were used by high performance liquid chromatography.

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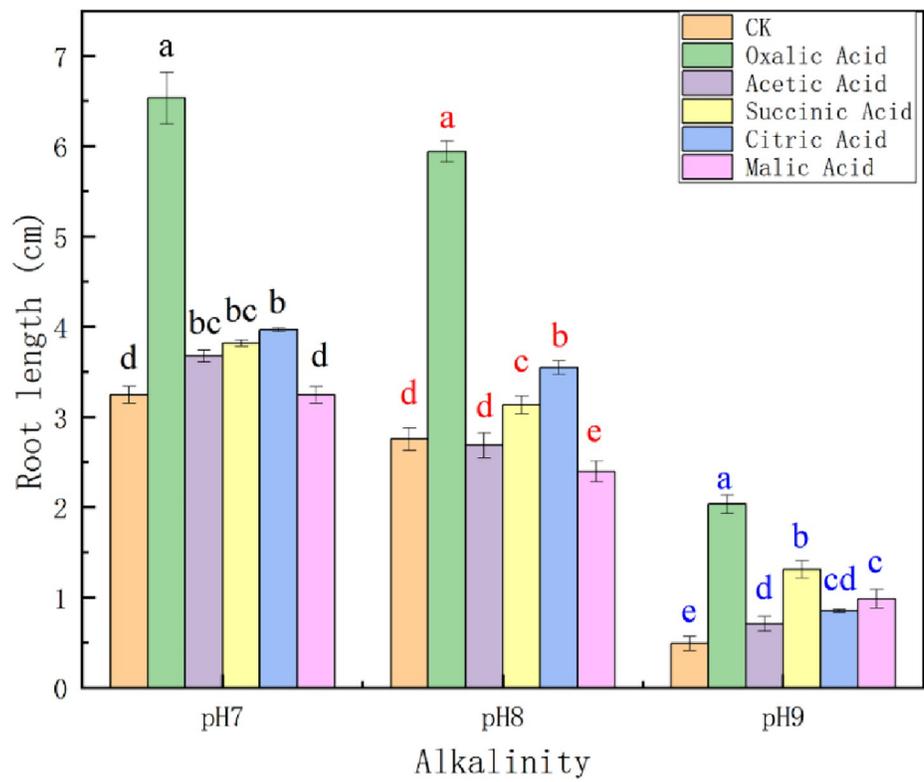
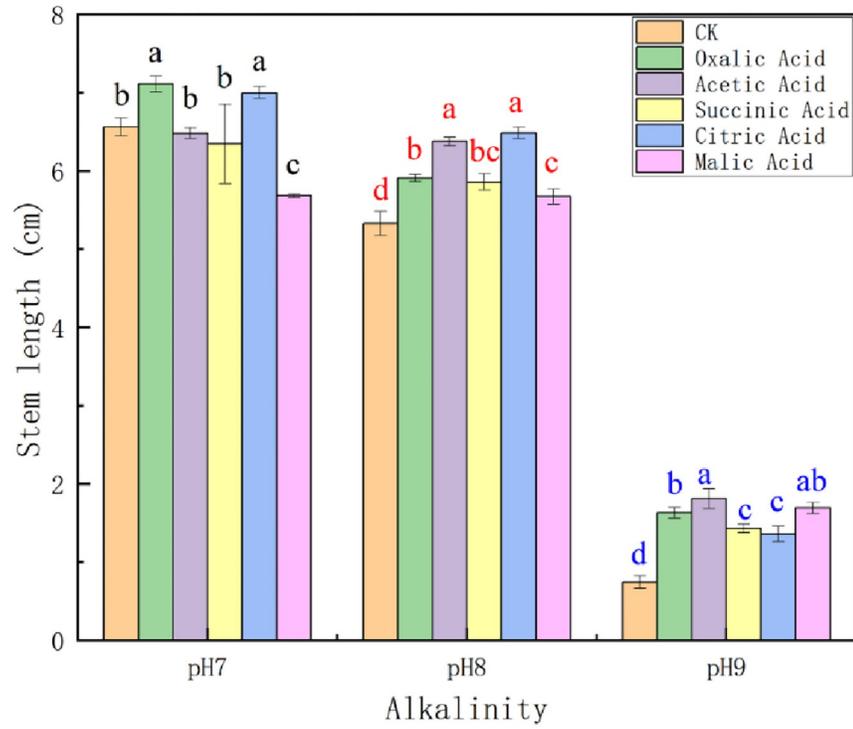


Fig 4. Effects of different organic acids on stem length (a) and root length (b) of quinoa. CK: Hoagland solution, Oxalic Acid: Hoagland solution and oxalic acid, Acetic Acid: Hoagland solution and acetic acid, Succinic Acid: Hoagland solution and succinic acid, Citric Acid: Hoagland solution and citric acid, Malic acid: Hoagland solution and malic acid. Error bars represent the standard deviation of the replicates (n = 3). Different letters within a column indicate significant differences at $P < 0.05$. The color of letters represents the significant difference that they belong to the same group.

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showed that all these acids had obvious growth-promoting effects on quinoa under different pH buffer conditions. Moreover, the growth-promoting effect of organic acids on plants increased with the increase of pH. Among them, oxalic acid, acetic acid and malic acid had better growth-promoting effect on quinoa. In pH 9 buffer solution, the stem length and root length of quinoa in oxalic acid treatment group increased by 1.5 and 3 times, respectively. Both stem and root length of quinoa in the acetic acid and malic acid treatment groups both increased by about 1.5 times.

Organic acids promoted plant growth may due to the following two reasons. Firstly, organic acids activated the antioxidant system of plants under stress, and improved the tolerance of plants to abiotic stress. Thus, plant growth was improved. Succinic acid induced the accumulation of succinic acid, citric acid, malic acid and oxalic acid in plants, up-regulated the gene expression of malate dehydrogenase (MDH) and phosphoenolpyruvate carboxylase (PEPC) in plant roots, and effectively alleviated aluminum stress and promote the growth of plants [64]. Oxalic acid and citric acid improved the SOD and POD activities of *Larix olgensis* and activated the antioxidant system of the plants under lead stress and improved the growth of *Larix olgensis* [68]. Citric acid and acetic acid improved the SOD and POD activities of *Melilotus officinalis* under copper stress, and improved the growth of *Melilotus officinalis* [69]. From here we see that the activation of antioxidant system can effectively assist plants to resist stress and promote plant growth. Similar results were obtained in this experiment. The organic acids secreted by P1 also activated the antioxidant system, and increased SOD (Fig 5A) and POD (Fig 5B) in quinoa by 30% and 16%, respectively. Secondly, organic acids promoted photosynthesis of plants. Malic acid enhanced photosynthesis by increasing chlorophyll content [70] or inducing stomatal opening [71]. Acetic acid also enhanced photosynthesis by increasing chlorophyll content [72]. Citric acid enhanced photosynthesis by increasing carotenoid content and the number and volume of chloroplasts in mesophyll cells [70]. Similar phenomenon was observed in our experiment. The chlorophyll content in quinoa treated with P1 was significantly higher than other treatments. (Fig 6) Therefore, organic acids promoting photosynthesis of quinoa is another reason why these organic acids promote quinoa growth. It can be found that the organic acids secreted by P1 played an important role on assisting quinoa to resist saline-alkali stress and promoting its growth.

In summary, the main reason why phosphate-solubilizing microorganisms promote plant growth is that they secrete organic acids. On the one hand, these organic acids reduced soil pH and provided a better growth environment for plants. On the other hand, they affect the metabolic system of plants, improve the resistance capability of plants and promote photosynthesis.

4. Conclusion

Different methods of alleviating saline alkali stress have great differences in promoting plant growth, due to different mechanisms. Although the improvement effect on soil was obvious after the application of chemical amendments, the growth of quinoa was only improved little. However, biological methods not only worked on improving saline-alkali soil properties, but also promoted quinoa growth. Both organic fertilizer and P1 microbial agent activated the antioxidant system of quinoa. And the organic acids secreted by P1 not only played a role on soil properties improvement, but also were used as bioactive substances to promote quinoa.

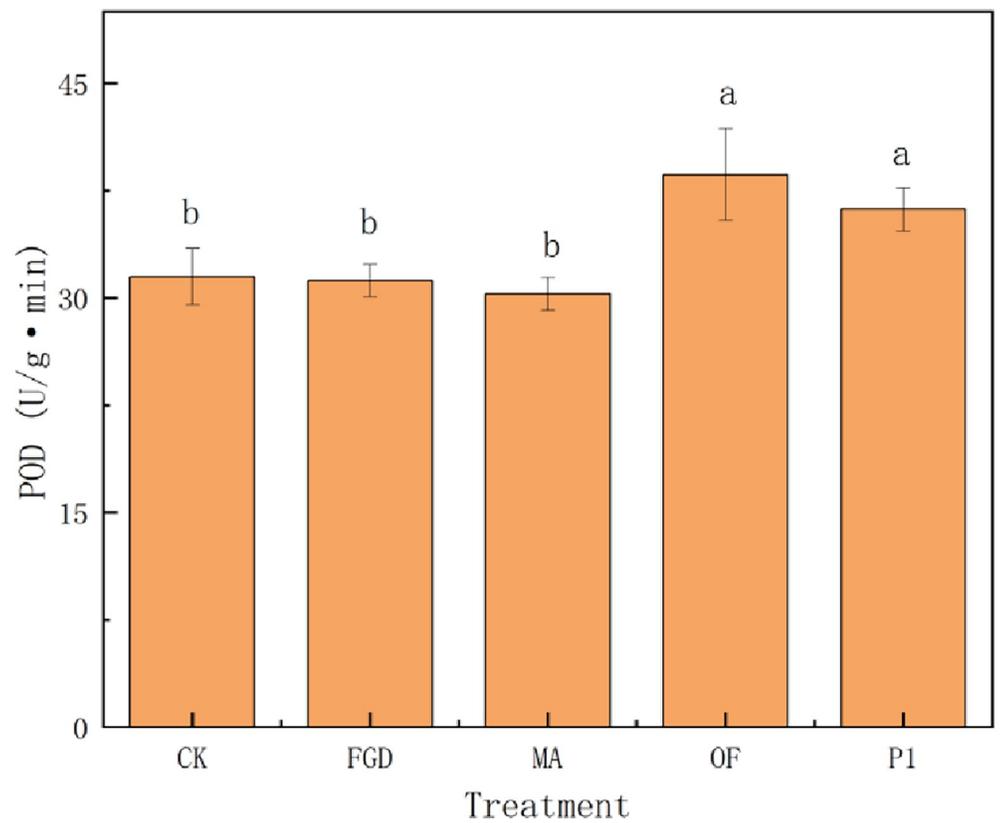
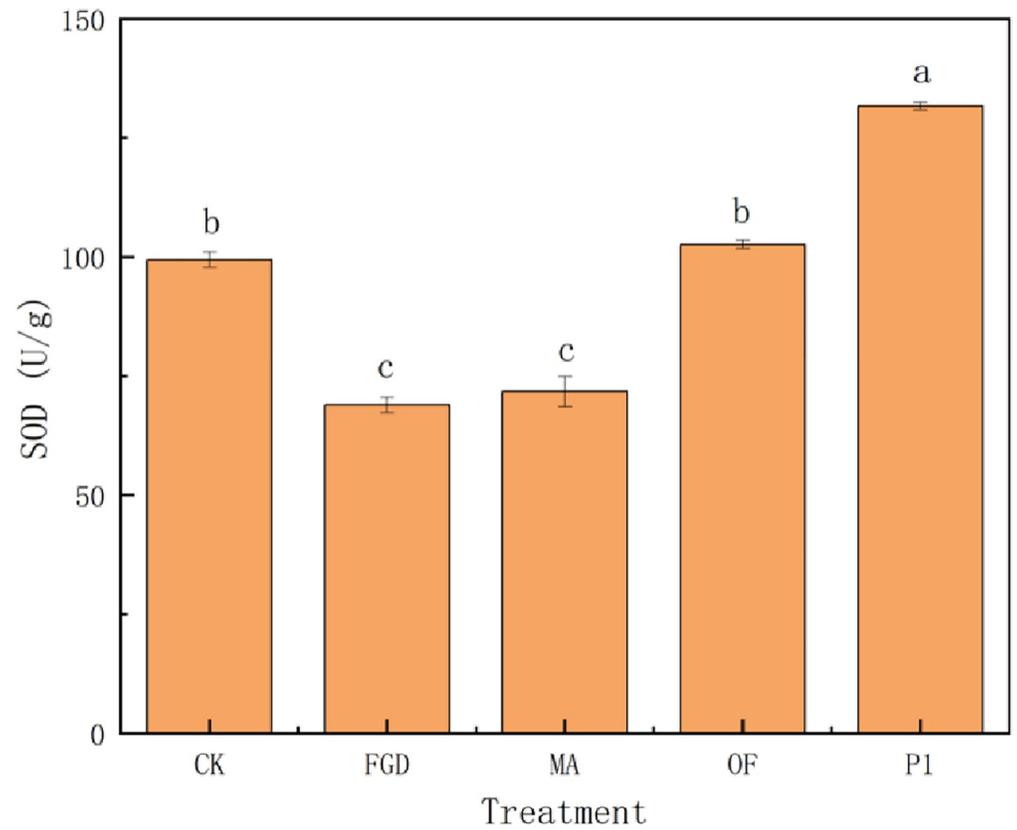


Fig 5. Effects of different treatments on antioxidant enzymes in quinoa. CK: sterile water, FGD: desulfurized gypsum, MA: commercial amendments (humic acid content $\geq 70\%$, 3% of the soil), OF: organic fertilizer (3% of the soil), P1: phosphate-solubilizing fungi P1 (20 mL bacterial solution per 1 kg soil after PDA culture). Error bars represent the standard deviation of the replicates ($n = 3$). Different letters within a column indicate significant differences at $P < 0.05$.

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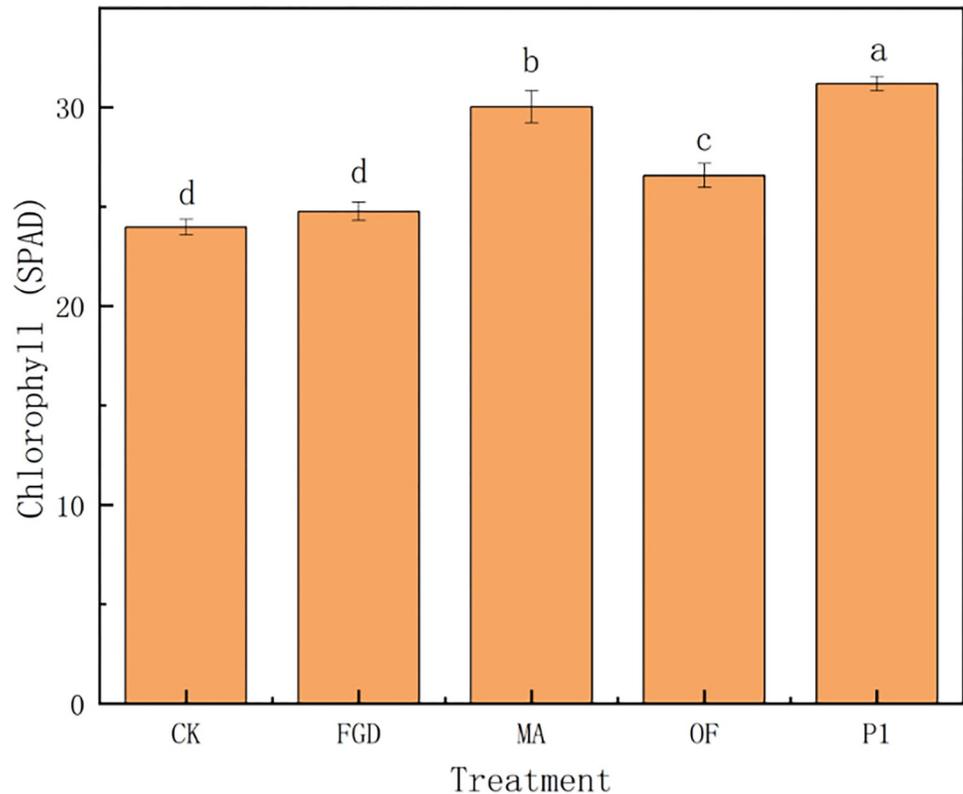


Fig 6. Effects of different treatments on chlorophyll content in quinoa. CK: sterile water, FGD: desulfurized gypsum, MA: commercial amendments (humic acid content $\geq 70\%$, 3% of the soil), OF: organic fertilizer (3% of the soil), P1: phosphate-solubilizing fungi P1 (20 mL bacterial solution per 1 kg soil after PDA culture). Error bars represent the standard deviation of the replicates ($n = 3$). Different letters within a column indicate significant differences at $P < 0.05$.

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We believe that small molecular organic acids which have biological activity secreted by microorganisms play a very important role on plant resistance to stress. In the future, P1 agent is going to be applied in open field experiments and the effect of P1 on other plants, such as rice, wheat, corn and so on, will be studied as well. Furthermore, the effect of P1 on soil microorganism community and saline-alkali stress response gene expression of plant are going to be studied.

Supporting information

S1 Appendix.
(XLSX)

Author Contributions

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Investigation: Qilin Hu.

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Supervision: Jianfeng Zhang.

Validation: Qilin Hu.

Visualization: Qilin Hu.

Writing – original draft: Fengyuan Jin.

Writing – review & editing: Yingxu Zhao, Xiaoyu Lin.

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