 DOI: 10.19601/j.cnki.issn1943 – 9903.2018.5.019

Effects of Iron and Zinc Fertilizers on Antioxidant Activity of Pear-Jujube in Loess Hilly Region

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Abstract With eight-year-old pear-jujube trees with uniform and good growth as the research object, different concentrations of iron and zinc fertilizers were sprayed to the leaves, and the changes in the contents of vitamin C, total flavonoids, enzyme, as well as the removal rates of hydroxyl radicals, 1,1-diphenyl-2-trinitrophenylhydrazine (DPPH) and hydrogen peroxide by polyphenols in pear-jujube were studied, so as to explore the effects of iron and zinc fertilizers on antioxidant activity of pear-jujube in loess hilly region. The results showed that different treatments affected the content of vitamin C and significantly increased the content of total flavonoids in pear-jujube. In the treatment of 0.6% $FeSO_4$ + 0.3% $ZnSO_4$ (L3), the contents of vitamin C and total flavonoids were both highest, 2.86 mg/g and 3.02 mg/g, 21.8% and 105.4% higher than CK ($P < 0.05$). Different fertilization treatments effectively reduced the activities of ascorbate oxidase and polyphenol oxidase in pear-jujube. The activity of ascorbate oxidase was lowest in the treatment of 0.6% $FeSO_4$ + 0.3% $ZnSO_4$ (oxidized ascorbic acid 0.069 mg/(g · min) FW, 75.1% lower than CK); and the activity of polyphenol oxidase was lowest in the L3 treatment (oxidized ascorbic acid 0.146 mg/(g · min) FW, 42.0% lower than CK). Polyphenols of pear-jujube could effectively remove hydroxyl radicals, DPPH · and hydrogen peroxide. This was more significant in L3 treatment, of which the antioxidant activity was the best.

Key words Pear-jujube, Iron fertilizer, Zinc fertilizer, Polyphenols, Antioxidant properties

1 Introduction

Plants need a lot of nutrients during their growth, and the demands for nitrogen, phosphorus and potassium are relatively large. However, trace elements and rare earth elements are also indispensable for improving the quality of crops[1]. Although the demand for trace elements in plants is small, the effect of trace elements on the growth and development of plants is equally important compared with that of macro elements. Trace elements are necessary nutrient elements for animals and plants and directly participate in various life activities of the organism. Iron and zinc are the two most demanding trace elements[2-3]. They take part in important biochemical reactions involved in life activities and are also components of many functional proteins[4]. Studies[5-6] have shown the iron-containing chelates present in plants are mainly citric acid, niacinamide and mugineic acids.

The experiment was carried out in the experimental base of red dates in Mengcha Village, Mizhi County, Yulin City, Shaanxi Province. The experimental area belongs to a typical loess hilly-gully region, where soil erosion and land desertification are serious, vegetation is scarce, and the ecological environment is extremely fragile. Jujube tree has strong resistance and low environmental requirements, can be planted in different ecological areas where other fruit trees are difficult to grow, and can form tens of thousands of hectares of contiguous forest belts and forest areas. *Zizyphus jujuba* Mill. (Rhamnaceae: *Zizyphus*)[7] covers a lot of varieties, among which, pear-jujube belongs to fresh jujube, is rich in nutrition and has a health-care effect[8]. The medicinal value of jujube has been widely studied. Fresh jujube has strong palatability and rich nutrition. Studies have shown that the content of vitamin C in per 100 g of fresh jujube fruit reaches 3 000 – 8 000 mg[9]. Gou Qian *et al.*[10] found that the fruit of Lingwu long jujube is rich in quinic acid, soluble sugar, various mineral elements and cyclic nucleotides. The polysaccharides of jujube have the function of eliminating oxygen free radicals in the human body, and their activity size is positively correlated with the content of sugars[11]. Red dates have a central inhibition effect. The naringenin-C-glycosides in jujube have the effects of causing catalepsy. In addition, they also have functions of protecting liver, fighting bacteria, fighting metastasis and fighting tumors[12], which are closely related to the polyphenol compounds such as cyclic adenosine monophosphate and catechol[13-14]. Polyphenolic compounds refer to the general term for plant components that have numerous phenolic hydroxyl groups in the molecular structure, including phenolic acids and flavonoids. Polyphenolic compounds have a certain degree of antioxidant activity. Many products have been included in the category of natural antioxidants and are excellent hydrogen or electron donors. They have a clear elimination effect for radicals such as superoxide anions and hydroxyl radicals that can cause peroxidation and damage the structure and function. Some research[15] found that jujube contains different amounts of polyphenolic substances and total flavonoids in the skin, pulp and fruit nucleus. The antioxidant activity is positively correlated with total phenols content and total flavonoids content. Iron and zinc play an important role in the secondary metabolism of plants. Zinc participates in the activation of glutamate dehydro-

Received: March 9, 2018 Accepted: May 14, 2018
Supported by Natural Science Foundation of Shaanxi Province (2016KCT-23).
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genase, ethanol dehydrogenase, *etc*, and iron is a component of peroxidase, catalase, *etc*. These are all important materials for plants' secondary metabolism such as antioxidation. At present, dwarf and close-growing jujube trees have been planted on a large area in the hilly-gully region of the Loess Plateau. To achieve a high yield and quality, there is also a unique requirement for fertilization. Therefore, the test was conducted by spraying different concentrations of iron and zinc micronutrients to compare the antioxidant activity of pear-jujube among different treatments.

2 Materials and methods

2.1 Overview of experimental area The experiment was conducted at the experimental base of red dates in Mengcha Village, Mizhi County, Yulin City, Shaanxi Province. The average annual rainfall in the test site is 451.6 mm. The soil on the test site is dominated by loess soil and is relatively poor. The drip irrigation quota for the trial period was 135 m^3/ha. In 2012, irrigation was conducted twice on May 15 and June 10. The basic physical properties of the soil are shown in Table 1. The pH was 8.6; the bulk density was 1.21 g/cm^3; the content of available potassium (101.9 mg/kg) was medium; and the contents of alkali-hydrolyzable nitrogen (34.73 mg/kg), organic matter (2.1 g/kg) and available phosphorus (2.90 g/kg) were low. There was a loss of microelements in the soil[16]. In the test soil, the contents of available iron, available zinc, available manganese and available copper were 6.13, 0.13, 5.80 and 0.74 mg/kg, respectively.

Table 1 Basic physical characteristics of the experimental soil %

Soil layer//cm	Sand (1.000 - 0.050 mm)	Fine sand (0.050 - 0.001 mm)	Clay (<0.001 mm)	Physical clay (<0.010 mm)
0 - 20	27.67	68.98	2.51	17.70
20 - 40	29.17	68.19	2.44	16.46
40 - 60	31.22	65.75	2.46	16.07

2.2 Experimental materials The eight-year-old pear-jujube trees (*Z. jujuba* Mill. cv. 'Lizao') with uniform and good growth were selected as the research object.

2.3 Experimental methods Under drip irrigation conditions, N, P and K fertilizer were applied as basic fertilizers, and iron and zinc fertilizers of different concentrations were sprayed to the leaves. During the budding stage, half of the urea (237.5 g/plant), calcium superphosphate (2 273 g/plant) and potassium sulfate (245 g/plant) were applied to the roots; during the fruit enlargement period, the other one half of the urea was applied. During the budding period, iron and zinc fertilizers were applied according to Table 2 three times at an interval of 10 days. The amount of spraying was 4 L/plant. There were 5 repetitions for each treatment. Protective trees were arranged on both side of each test tree, and the area of each plot was 500 m^2.

Table 2 Fertilization treatments for pear-jujube trees %

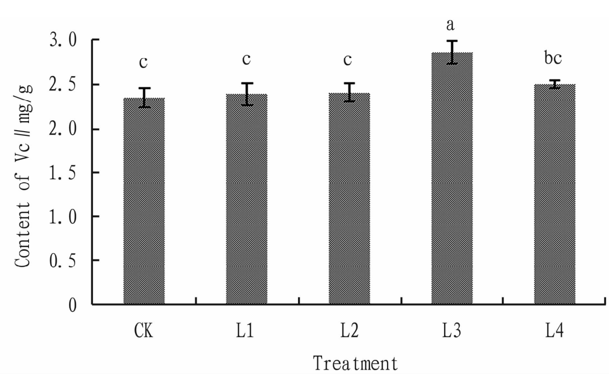
Treatment	CK	L1	L2	L3	L4
$FeSO_4$	0	0.3	0.3	0.6	0.6
$ZnSO_4$	0	0.3	0.6	0.3	0.6

2.4 Index measurement The activities of vitamin C oxidase and polyphenol oxidase were determined referring to the method of Gao Junfeng[17]. The samples were kept fresh and the entire process was performed at a low temperature while extracting enzymes. The total flavonoids content was determined using sodium nitrite-alumimum nitrate-sodium hydroxide coloration method[18-20]. The measurement procedures mainly included sample extraction (ultrasonic extraction), standard curve preparation and sample determination. Jujube was crushed, added with ethanol, extracted ultrasonically, centrifuged, and concentrated to a certain volume for determination of total polyphenols[21]. The total phenol content was determined using the Folin-Ciocalteu colorimetric method[22].

2.5 Data analysis Data processing and analysis was performed using Microsoft Excel 2010, and SPSS17.0 was used to conduct significance test.

3 Results and analysis

3.1 Effects of iron and zinc fertilizers on vitamin C content of pear-jujube Vitamin C is an important index for measuring the intrinsic quality of pear-jujube. Vitamin C is a very strong antioxidant. The antioxidant activity of vitamin C accounts for 52.62% of the antioxidant activity of red dates[23], and it is one of the main components of red dates in delaying aging and preventing cardiovascular and cerebrovascular diseases. As shown in Fig.1, different fertilization treatments increased the vitamin C content in the fruit of pear-jujube; under the condition of foliar spraying, high-concentration iron fertilizer contributed to the increase of vitamin C; and among different treatments, the vitamin C content in pear-jujube ranked as L3 > L4 > L2 > L1 > CK. Among the fertilization treatments, the vitamin C content of L3 reached the maximum, 2.86 mg/g, 21.8% higher than CK ($P<0.05$). The vitamin C contents in other fertilization treatments were all higher than that in the CK group ($P>0.05$).



Note: Different lowercase letters between treatments indicate significant differences at 5% level. The same below.

Fig.1 Effect of different fertilization treatment on vitamin C content of pear-jujube

3.2 Effects of iron and zinc fertilizers on total flavonoids content of pear-jujube Total flavonoids are a large class of natural products. Flavonoids can increase the role of vitamin C in humans and have important functions such as scavenging free radicals, preventing vascular sclerosis, enhancing blood vessel elasticity, delaying senility, preventing and treating cardiovascular and cerebrovascular disease, lowering blood pressure and lowering blood fat. Jujube contains a variety of health-care substances, and one of the important ones is rutin. As shown in Fig. 2, different fertilization treatments significantly increased the content of total flavonoids in pear-jujube; and the content of total flavonoids in different iron and zinc fertilization treatments was significantly different. Among them, the content of total flavonoids in L3 treatment was the highest, reaching 3.02 mg/g, which was 105.4% higher than that of CK ($P<0.05$). Among different fertilization treatments, the total flavonoids content in pear-jujube followed the descending order L3 > L4 > L2 > L1 > CK. On the whole, different fertilization treatments were effective to increase the total flavonoids content of pear-jujube; and among them, the effect of L3 treatment was the most significant.

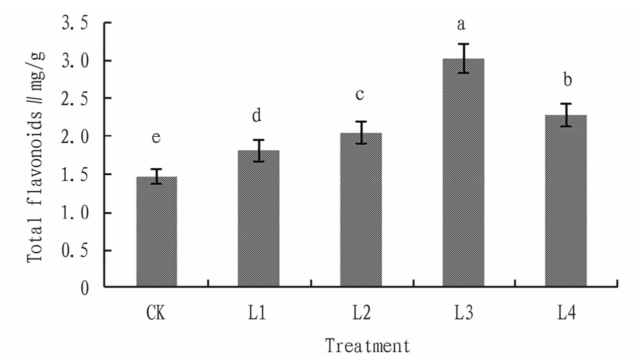


Fig. 2 Effect of different fertilization treatment on total flavonoids content of pear-jujube

3.3 Effects of iron and zinc fertilizers on enzymes content of pear-jujube As shown in Fig. 3, micro-fertilizer treatments had a significant effect on the activity of ascorbic acid oxidase and polyphenol oxidase in pear-jujube. Fertilization treatments could effectively reduce the activity of ascorbic acid oxidase and polyphenol oxidase in pear-jujube. However, there were some differences in the trends between the two. Among different fertilization treatments, the activity of ascorbic acid oxidase followed the descending order CK > L2 > L1 > L3 > L4. The activity of ascorbic acid oxidase in the L4 treatment was lowest, oxidized ascorbic acid 0.069 mg/(g · min) FW (75.1% lower than that of CK, $P<0.05$), followed by that in the L3 treatment, 0.141 mg/(g · min) FW (48.9% lower than that of CK, $P<0.05$). The activity of polyphenol oxidase of pear-jujube in different fertilization treatments was all reduced compared with that of CK. The L3 treatment showed the lowest polyphenol oxidase activity, oxidized ascorbic acid 0.146 mg/(g · min) FW (42.0% lower than that of CK, $P<0.05$), followed by L2 treatment, oxidized ascorbic acid 0.151 mg/(g · min) FW (40.0% lower than that of CK, $P<0.05$). No significant differences were found in the polyphenol oxidase activity among different fertilization treatments ($P>0.05$). Therefore, the treatment that could effectively reduce the ascorbic acid oxidase activity and polyphenol oxidase activity at the same time was L3, which had a significant difference with CK ($P<0.05$).

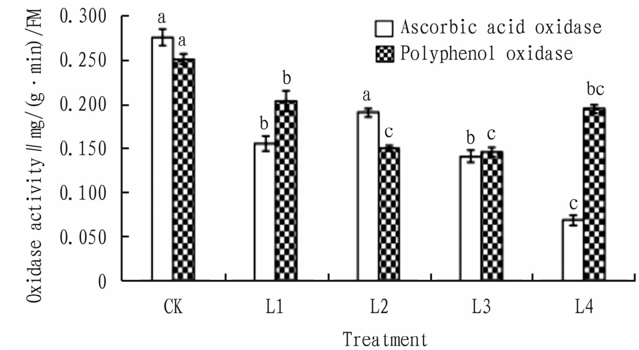


Fig. 3 Effect of different fertilization treatment on oxidase activity of pear-jujube

3.4 Antioxidant activity of polyphenols in pear-jujube

3.4.1 Scavenging capacity of polyphenols of pear-jujube for hydroxyl radicals. As shown in Fig. 4, the removal rate of polyphenols in pear-jujube for hydroxyl radicals increased with the increased concentration of polyphenols. When the concentration of polyphenols reached 1.0 mg/g, the removal rate of hydroxyl radicals tended to be stable in different fertilization treatments. In different fertilization treatments, the removal rate of hydroxyl radicals basically reached the maximum when the polyphenols concentration rose to 1.2 mg/g. The scavenging rate of hydroxyl radicals was highest in the L3 treatment. When the concentration of polyphenols rose to 1.2 mg/g, the scavenging rate of hydroxyl radicals reached 92.99%. In the L4 treatment, the scavenging rate of hydroxyl radicals was the second highest (85.36%). The scavenging rates of hydroxyl radicals in different fertilization treatments were all higher than that of CK.

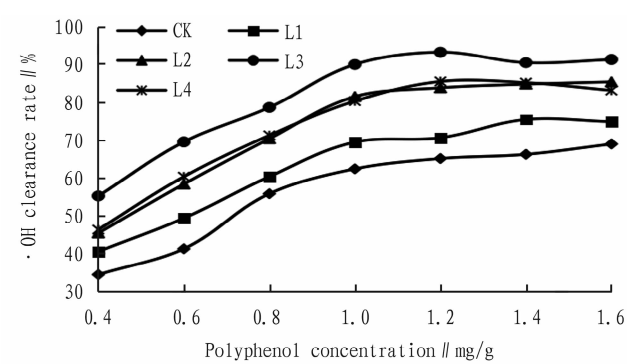


Fig. 4 Scavenging capacity of polyphenols in jujube for · OH

3.4.2 Scavenging capacity of polyphenols of pear-jujube for DPPH ·. As shown in Fig. 5, the scavenging rate of DPPH · increased with the increased concentration of polyphenols. There was a turning point at 0.8 mg/g. When the polyphenols concentration was below 0.8 mg/g, the scavenging rate of DPPH · showed a linear increase; and when the concentration was greater than 0.8 mg/g, the scavenging rate of DPPH · by polyphenols in pear-jujube gradually stabilized. Among different treatments, the scavenging rate of DPPH · ranked as L3 > L4 > L2 > L1 > CK. The scavenging rates of DPPH · in the fertilization treatments were all higher than that of CK ($P < 0.05$). The scavenging rates of DPPH · in the L1, L2 and L4 treatments were lower than that of the L3 treatment, and the differences among treatments were small. The polyphenols of pear-jujube in the L3 treatment showed the most significant scavenging effect for DPPH ·, and the scavenging rate reached 83.66%, 23.6% higher than that of CK.

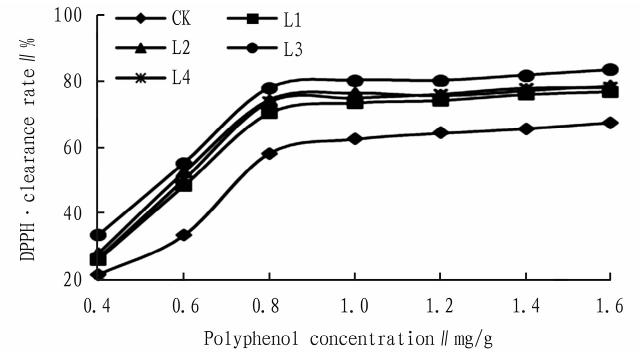


Fig. 5 Scavenging capacity of polyphenols in jujube for DPPH ·

3.4.3 Scavenging capacity of polyphenols of pear-jujube for hydrogen peroxide. In different treatments, the scavenging rate of hydrogen peroxide increased with the increased concentration of polyphenols (Fig. 6). When the concentration of polyphenols rose to 1.2 mg/g, the scavenging rate of hydrogen peroxide reached the maximum. In the L3 treatment, the scavenging rate of hydrogen peroxide by polyphenols in pear-jujube was the highest, 93.03%. The scavenging rates of hydrogen peroxide in different fertilization treatments were all higher than that of CK.

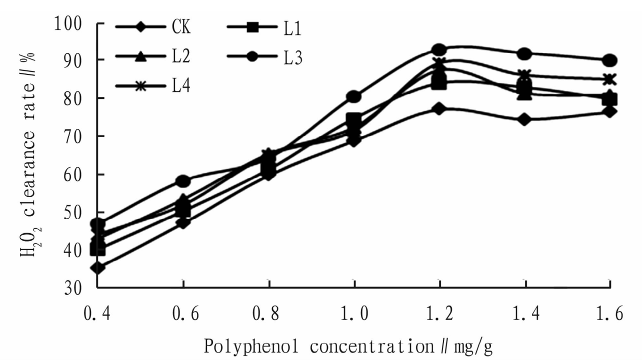


Fig. 6 Scavenging capacity of polyphenols in jujube for H_2O_2

4 Conclusions

Different concentrations of iron and zinc fertilizers were sprayed to the leaves of pear-jujube trees, and the changes in the contents of vitamin C, total flavonoids and enzymes, as well as the scavenging capacity of polyphenols in pear-jujube for hydroxyl radicals, DPPH · and hydrogen peroxide were studied. The results showed that when iron and zinc fertilizers were sprayed, the contents of vitamin C and total flavonoids in pear-jujube were increased, and the activity of ascorbic acid oxidase and polyphenols oxidase was reduced. The polyphenols of pear-jujube could effectively remove hydroxyl radicals, DPPH · and hydrogen peroxide. It suggests that iron and zinc fertilization treatments can effectively improve the anti-oxidation characteristics of pear-jujube. Among them, the anti-oxidant activity of (0.6% $FeSO_4$ + 0.3% $ZnSO_4$) was the highest.

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Table 4 The impact of Ca on peanut yield

Treatment	100-fruit weight//g	Number of peanut per kilogram	Ratio of shelled peanut//%	Yield//kg/ha	Increase rate//%
Ca0 (CK)	161.3	742	67.56	2 411.40	0.00
Ca1	175.1	730	69.24	2 729.10	13.18
Ca2	187.6	686	72.80	2 993.25	24.13
Ca3	168.1	714	68.72	2 607.45	8.13

4 Conclusions and discussions

The research shows that Ca application could inhibit vegetative growth of peanut and is favorable for preventing rapid growth and lodging. Under the condition of Ca deficiency, more assimilation products are stranded in the stem and leaves, there is overnutrition in aboveground part of peanut plant, and pod development is hindered. Under the condition of sufficient Ca, assimilation products of peanut could be normally transferred to pod for storage. With pod expands, stem and leaf gradually wither. The research results showed that the height of main stem, the length of side shoot, total branch number, number of fruiting branch, number of blighted fruit and number of rotten fruit all declined to a certain degree with Ca application amount increased. Both number and rate of full fruit increased with Ca application amount increased, in which the impact was the most obvious in the treatment Ca2, and biological yield was also the highest. Accumulation amount and distribution rate of dry matter in root, stem, leaf, petiole and fruit needle in each treatment showed declining trends with Ca application amount increased, which was the least when Ca application amount was 150 kg/ha, and they showed rising trends when increased Ca amount again.

The past research results showed that accumulation amount of dry matter showed positive correlation with yield, and pod yield increased with dry matter increased. That is to say, the more the dry matter accumulation, the higher the pod yield[7-8]. The research results showed that change trends of accumulation amount and distribution ratio of dry matter in pod were consistent with that of dry matter accumulation amount per plant with Ca application amount increased, and they all increased obviously. Yield increase extent was the maximum when Ca application amount was 150 kg/ha, which was 24.13%, followed by Ca1 (13.17%) and Ca3 (8.13%). Marine biological Ca fertilizer could increase effective fruit number and rate of full fruit, and decline fruit number per kilogram, to realize yield increase. Ca fertilizer not only provides rich Ca for crop but also could improve soil, anti-disease, anti-continuous cropping ability and anti-adversity of crop, yield and quality. Therefore, peanut plantation could properly use Ca fertilizer to compensate yield loss brought by nutrient deficiency, reaching the target of yield increase.

In this paper, Ca application could make dry matter accumulation of root decline, which was consistent with the conclusion of Zhang Juncheng. But many research reports prove that root system growth of plant is hindered under the condition of Ca deficiency, and the cause needs further test demonstration.

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