

## Effects of plant growth-promoting rhizobacteria on uptake and utilization of phosphorus and root architecture in apple seedlings under water limited regimes

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### Abstract

The aim of this research was to examine the relationships among *Pseudomonas fluorescens* (YX2) plant growth promoting rhizobacteria (PGPR), phosphorus (P) absorption by plants, and root system architecture in apple seedlings exposed to mild, moderate or severe drought stresses. All the treatments were divided into two groups: 1) inoculated with a plant rhizobacterial strain (YX2), and 2) the non-inoculated control. Under drought stress, the YX2 inoculation improved root growth, root activity by 6%, and uptake of P, thereby promoting apple seedling growth along with the dry weight of above-ground plant parts in the mild and moderate water stress regimes. Furthermore, the inoculation also promoted total P contents in plants under both mild and moderate drought stresses. Overall, application of *Pseudomonas fluorescens* (YX2) is a promising approach to enhance apple production in agricultural production systems.

### HANDLING EDITOR

Muhammad Ashraf

### ARTICLE HISTORY

Received: 4 Oct 2021

Accepted: 7 Dec 2021

Published: 2 Jan 2022

### KEYWORDS

Drought stress;

Apple;

Plant growth promoting

rhizosphere bacteria;

P uptake and utilization

## Introduction

Water shortage has a marked impact on agriculture worldwide (Wang et al., 2012). Changes in root architecture and low accumulation of dry weight are contemplated as the major responses of crops to water deficiency, which could be ascribed to impaired uptake of many of the essential inorganic nutrients (Zhu et al., 2002; Kunert et al., 2016; Ojuederie et al., 2019). Absorption of water and nutrients which maintains photosynthesis in plants could be boosted by bringing modification in root architecture and development (Comas et al., 2013). In fact, soil nutrients are taken up by plant roots via the rhizosphere, which is the key zone of interaction between plants and soils (Shen et al., 2013). The availability of water and nutrients to plant roots decreases under water limited conditions due to reduced mineralization in dried soils (Schimel et al., 2007) as well as the impaired nutrient and mass flow in soil (Singh et al., 2006; Silva et al., 2017).

Apple (*Malus pumila* Mill.) is an economically important crop in China, which has taken a predominant position in the world apple industry (FAO, 2016). In 2013, China contributed 49% and 46% of the total production and cultivated area of the world, respectively (FAO, 2016). However, the scarcity of good quality water is one of the major reasons of low apple productivity in the Loess plateau region (Li et al., 2017).

Plant growth promoting rhizobacteria (PGPR), directly associated with plant roots or colonized

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**TO CITE THIS ARTICLE:** Li, B., Zhang, C., Qi, M., Zheng, X., Mustafad, N.S., Ahmed, N., Anees, M., Ahanger, M.A., Zhang, L. (2022). Effects of plant growth-promoting rhizobacteria on uptake and utilization of phosphorus and root architecture in apple seedlings under water limited regimes. *International Journal of Applied and Experimental Biology* 1(1):1-8

within plant roots, are beneficial microorganisms for crop growth, which can effectively increase crop yield and prevent the disease occurrence (Dimkpa et al., 2010; Verhagen et al., 2010; Leontidou et al., 2020). In addition, some PGPR could improve the development and productivity of various crops in response to abiotic stresses such as wheat (Barnawal et al., 2017), potato (Gururani et al., 2013), French bean (Chauhan et al., 2015), etc. Drought resistance of plants can also be significantly enhanced by inoculation of PGPR, especially those that secrete ACC (1-aminocyclopropane-l-carboxylate) deaminase to modulate plant ethylene levels (Glick et al., 2007). Kasim (2013) showed that priming with PGPR significantly alleviated the damage caused by the drought stress induced reactive oxygen species (ROS) in wheat. It has also been shown that PGPR could promote root development and alter root architecture leading to an enhanced nutrient uptake by plants (Mantelin et al., 2004). However, little is known about the possible role of PGPR in regulating the uptake of phosphorus (P) by apple plants under drought conditions, although in other crops a positive role of PGPR in promoting growth has been reported, e.g., in bread wheat (Rana et al., 2012;), durum wheat (Saia et al., 2015), tomato (Calvo-Polanco et al., 2016), maize (Huang et al., 2017), and mung bean (Sarma and Saikia, 2014). Many studies have reported that low availability of P to plants growing in drought affected soils could be due to reduced P diffusion thereby resulting in poor uptake by roots (Singh et al., 2006; Suriyagoda et al., 2014).

Therefore, the aim of this research was to examine the relationships among PGPR, P absorption by plants, and root system architecture in apple seedlings exposed to light, moderate or severe drought stresses.

## Material and Methods

### Plant material

The PGPR strain 'YX2' of *Pseudomonas fluorescens* (NCBI accession number: KJ465990) used in this study was obtained from the College of Life Science, Northwest A & F University (Zheng et al., 2014). This strain was previously isolated from the soil of dryland orchards based on its phosphate solubilization ability. Previously, the strain has been reported to secrete ACC deaminase (Zheng et al., 2014).

### Greenhouse bioassays

The experiments were conducted in the greenhouses located in the Northwest A&F University, Yangling, China. The apple seeds (var. Mains micromalus Makino) were soaked in 0.2% (v/v) potassium permanganate solution for 5 min, followed by several rinses with tap water. The seeds were sown in wet washed river sand. When the seeds germinated (about 40 days after sowing), they were transferred to the seedling boxes (70 cm×40 cm×20 cm) containing the seedling culture substrate. The boxes were placed in the greenhouse conditions for two months. When the seedlings had 10 true leaves and attained a plant height of about 15 cm, they were transplanted into pots for further experimentation. Each pot contained 2.5 kg of potting material, 0.67 g urea, 1.96 g calcium superphosphate and 0.60 g potassium sulfate. The potting material consisted of a mixture of wheat field ploughed loam soil (Yangling, Shaanxi) and river sand in 2:1 ratio. Prior to use, the potting material was sterilized at 121°C for 30 min. The physico-chemical properties of the material were determined including pH (7.64), total nitrogen (1.02 g kg<sup>-1</sup>), available nitrogen (67.33 mg kg<sup>-1</sup>), total P (0.98 g kg<sup>-1</sup>), available P (21.10 mg kg<sup>-1</sup>), total potassium (15.75 g kg<sup>-1</sup>), available potassium (138.42 mg kg<sup>-1</sup>), and field capacity (34.15%).

### Treatments

Seedling roots were inoculated with *P. fluorescens* strain YX2 using the dipping method. The roots were dipped in the bacterial suspensions for about 20 min before planting. The residual bacterial suspension (1×10<sup>9</sup> cfu·fu<sup>-1</sup>) was injected into the root zones as well. Each treatment was watered every evening to maintain the soil moisture content at 70%-80% of field capacity. The control plants consisted of no-inoculated seedlings. After 20 days of seedling growth, the drought stress treatments were initiated. The drought treatments consisted of 1) the normal watering treatment, 70%-80% of field capacity (CK); 2) the mild drought treatment, 55%-65% of field capacity (LD); 3) the moderate drought treatment, 40%-50% of field capacity (MD); and 4) severe drought treatment, 25%-35% of field capacity (SD). Soil moisture contents were determined by the TDR-300 portable soil moisture tester at 1800 hours every day to maintain soil moisture contents within the range of the specific drought treatment. After 120 days, plants and the potting material samples were collected for analyses. The experiments were performed with 8 replicates.

### Determination of plant dry weight

The whole apple seedlings were uprooted and divided into two parts, i.e., the above-ground parts (shoot) and the underground parts (root). The plant samples were washed with tap water, dried at

105 °C for 30 min at first and then dried to constant weight at 80 °C. The dry weight of all parts of the plant was recorded and the root: shoot ratio calculated.

### **Determination of P contents in plant and soil**

The fresh seedling samples were collected from the pots and cleaned with distilled water. Total P concentration of plants was determined by the Vanadium Molybdenum yellow colorimetry method (Olsen et al., 1954). The soil total P was digested with hydrofluoric and perchloric acids, and the soil available P was extracted with sodium bicarbonate solution.

### **Determination of acid phosphatase activity in root system and alkaline phosphatase activity in soil**

Acid phosphatase activity in the root system was determined using standard analytical techniques (Dodd et al., 1987; Ciereszko et al., 2002). One gram root tissue was ground in 2 mL of 50 mmol L<sup>-1</sup> sodium acetate buffer with pH of 5.8. The total volume of acetate buffer used to grind and transfer the tissues into the eppendorf tubes was 10 mL. The root extracts were centrifuged at 9000×g for 20 min at 4 °C and 0.2 mL supernatant added to 5 mL p-nitrophenyl phosphate disodium (PNP, 5 mmol L<sup>-1</sup>). The reaction mixture was incubated at 30 °C. After 30 min, 1.0 mL NaOH (1 mol L<sup>-1</sup>) was immediately added to terminate the reaction and develop the color. An alkaline solution of PNP was used as a standard. The soil alkaline phosphatase (ALP) activity was assessed using standard analytical techniques (Hu et al., 2015) by incubation at 37 °C with borate buffer (pH 9).

### **Determination of root activity**

Each root sample was cleaned and cut into 1 cm pieces. The sample (0.5 g) was placed in each of 25 mL tubes along with 5 mL of 1% triphenyl tetrazolium chloride (TTC) and 5 mL of 0.1 M phosphate buffer. The tubes were capped and shaken gently in an oscillating shaker for 1 h at 37 °C. Then an aliquot of 2 mL of 1 mol L<sup>-1</sup> H<sub>2</sub>SO<sub>4</sub> was added to each tube. The roots were retrieved from the tubes and ground to get the leachate mixture. The red leachate was filtered into a volumetric flask, and added ethyl acetate to bring the volume up to 10 mL. The TTC reduction strength was determined by spectrophotometry at 485 nm; TTC reducing ability was calculated by dividing TTC reduction amount (g) on the product of root weight (g) and time (h) {TTC reducing ability = TTC reduction amount (g) / [root weight (g) × time (h)]}.

### **Root morphology determination**

The WinRHIZO (version v2013e pro) root scanner was used to analyze the changes in root morphology under different treatments.

### **Statistical analyses**

All data were recorded and graphs were prepared using the Excel 2003 (Microsoft, USA), and results presented as mean±standard error. Statistical analyses were performed with the SPSS version 20 software.

## **Results**

### **Plant dry matter**

Different drought treatments affected the dry matter accumulation in the above- and under-ground parts of the apple seedlings (**Figure 1**). With increase in drought intensity, the dry weight of the above- and under-ground parts of apple seedlings decreased gradually with or without inoculation of the strain YX2. Compared with the control (CK), the dry weights of above-ground and under-ground parts of apple seedlings inoculated with YX2 decreased by 33% and 15%, respectively, while they decreased by 33% and 16% in the non-inoculated treatment under the severe drought stress. Inoculation with the strain YX2 increased total dry matter of apple seedlings to varying degrees irrespective of the moisture conditions. There was no significant effect of inoculation with the strain YX2 on the weight of dry matter of the underground parts of apple seedlings exposed to varying drought treatments, however, the dry weight of the above-ground parts increased significantly at the MD treatment.

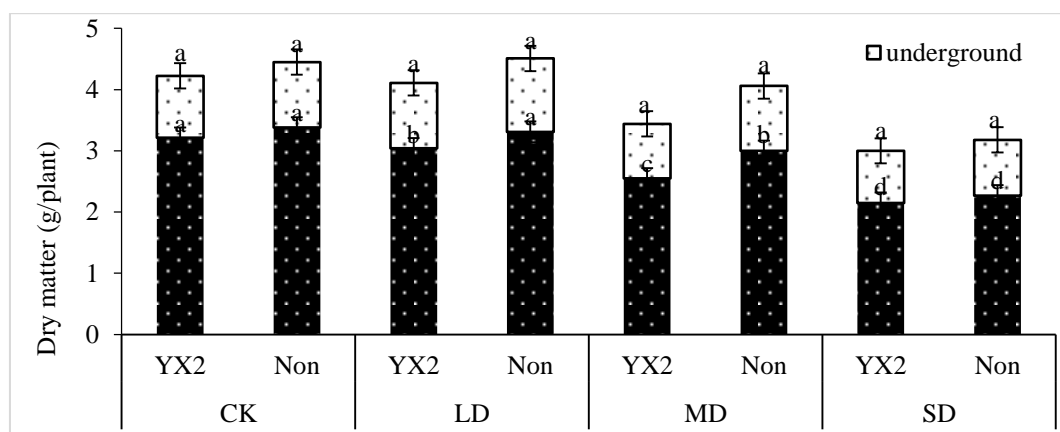
### **Plant and soil P contents and phosphatase activity**

Regardless of inoculation of the strain YX2, the total P content of the seedlings, root acid phosphatase activity, available P contents and soil alkaline phosphatase activity decreased gradually with increase in drought intensity, while the total P contents of the soil increased significantly (**Table 1**). In the LD and MD treatments, the contents of available P in soil increased by 16% and 39%, respectively, due to the inoculation with YX2. The bacterial inoculation significantly increased soil alkaline phosphatase

**Table 1. Effect of PGPR inoculation on plant total phosphorus, soil total phosphorus, soil available phosphorus, root acid phosphatase and soil alkaline phosphatase in apple seedlings**

Drought treatment	Inoculation treatment	Plant total phosphorus (g kg <sup>-1</sup> )	Soil total phosphorus (g kg <sup>-1</sup> )	Soil available phosphorus (mg kg <sup>-1</sup> )	Root acid phosphatase (nmol·g <sup>-1</sup> min <sup>-1</sup> )	Soil alkaline phosphatase (phenol, mg g <sup>-1</sup> d <sup>-1</sup> )
CK	NI	4.73±0.38 a	0.68±0.03 de	29.84±0.24 b	356.70±19.78 b	13.43±0.48 a
	I	5.27±0.42 a	0.65±0.06 de	31.95±1.33 b	444.17±24.47 a	13.85±1.27 a
LD	NI	5.03±0.33 a	0.69±0.03 de	31.82±1.63 b	258.30±28.81 de	12.15±0.75 a
	I	5.26±0.28 a	0.63±0.05 e	36.90±0.55 a	331.03±18.05 c	13.24±1.01 a
MD	NI	4.37±0.34 a	0.84±0.08 bc	22.94±1.86 c	219.57±7.18 ef	8.77±0.58 c
	I	5.02±0.53 a	0.74±0.08 cd	31.89±2.97 b	294.60±13.52 cd	10.59±0.66 b
SD	NI	4.31±0.11 a	0.95±0.04 a	19.09±1.92 c	192.63±7.92 f	7.18±0.69 c
	I	4.49±0.29 a	0.93±0.04 ab	21.71±0.50 c	222.27±13.19 ef	8.25±1.12 c

Data presented is mean of three replicates and different letters show significant difference at  $P \leq 0.05$ . CK, Control; LD, Light drought; MD, Moderate drought; SD, Severe drought; NI, Not inoculated; I, Inoculated

**Figure 1. Effects of different drought and PGPR treatments on dry biomass of seedlings**

CK, Control; LD, Light drought; MD, Moderate drought; SD, Severe drought

activity in the MD treatment, being 21% higher than that in the non-inoculated controls. In CK, LD and MD treatments, the acid phosphatase activity of apple roots increased significantly after inoculation with the strain YX2, but there was no significant effect on the SD treatment. Inoculation with YX2 promoted the uptake of phosphorus in apple roots and increased the total P content in apple plants.

### Plant P uptake and PGPR P contribution rate analysis

Inoculation with YX2 increased P uptake in apple seedlings (Table 2). Inoculation with YX2 increased P uptake in PGPR (YX2). The PGPR P uptake contribution also increased because of the inoculation with YX2. With increase in drought intensity, the P uptake and P uptake contribution rate of PGPR tended to first increase and then decreased. In the MD treatment, PGPR P uptake contribution was highest (26.25%). The inoculation with YX2 significantly promoted plant uptake of P in soil particularly under non-severe drought conditions.

### Correlations among P uptake and key enzyme activities

The correlations among the different parameters showed that the PGPR P uptake contribution rate was positively correlated with root acid phosphatase activity, soil available P content and soil alkaline phosphatase activity, but negatively correlated with total P content of the soil (Table 3). Root acid phosphatase was also negatively correlated with soil total P. In soil, total P and available P contents showed a negative correlation with alkaline phosphatase activity, however, the available P content and alkaline phosphatase were correlated positively.

### Root activity

With increase in drought intensity, the root activity of apple seedlings increased first and then decreased (Figure 2). The root activity increased to a varying extent upon inoculation with YX2. Compared with the non-inoculated treatments, the bacterial inoculation increased the root activity by 6%, 13%, 26% and 21% in the CK, LD, MD and SD treatments, respectively. There was a significant difference between inoculated and non-inoculated treatments in LD and MD.

**Table 2. Effect of PGPR inoculation on plant phosphorus uptake, PGPR phosphorus uptake, and GPR phosphorus uptake contribution rate in seedlings**

Indicator	CK		LD		MD		SD	
	NI	I	NI	I	NI	I	NI	I
Plant phosphorus uptake ( $\text{g} \times 10^{-3}$ )	19.98	23.45	20.67	23.72	15.03	20.38	12.93	14.28
PGPR phosphorus uptake ( $\text{g} \times 10^{-3}$ )	3.47		3.05		5.35		1.35	
PGPR phosphorus uptake contribution rate (%)	14.80		12.86		26.25		9.45	

Data presented is mean of three replicates and different letters show significant difference at  $P \leq 0.05$ .

**Table 3. Correlation coefficients of PGPR contribution rate of phosphorus uptake, leaf acid phosphatase, soil total phosphorus, soil available phosphorus and soil alkaline phosphatase**

Indicator	YX2 phosphorus uptake contribution rate	Root acid phosphatase	Soil total phosphorus	Soil available phosphorus	Soil alkaline phosphatase
YX2 phosphorus uptake contribution rate	1	-	-	-	-
Root acid phosphatase	0.120	1	-	-	-
Soil total phosphorus	-0.266	-0.813	1	-	-
Soil available phosphorus	0.374	0.605	-0.953*	1	-
Soil alkaline phosphatase	0.086	0.910	-0.967*	0.848	1

Data presented is mean of three replicates and different letters show significant difference at  $P \leq 0.05$ .

### Root growth parameters

The total root length, mean diameter, surface area and the volume of apple seedlings underwent changes under different treatments (Table 4). With increase in drought intensity, the total length of root system increased, the mean diameter of the root system decreased, and the surface area and volume of the root system decreased under non-inoculated conditions. Total root length, average diameter, surface area and volume of the root system increased to some extent when inoculated with YX2 in each treatment. In the LD treatment, the total root length reached the highest value (503 cm), which was significantly higher than that in the other treatments.

### Discussion

The present studies showed that the drought stress significantly inhibited the accumulation of dry matter in apple seedlings. It is well known that under the conditions of high soil moisture contents, seedlings can accumulate more biomass and chlorophyll, thereby distributing more biomass in the above-ground parts to subsequently increase the rate of photosynthesis (Guo et al., 2007). However, in contrast, under the drought stress, the biomass allocation of seedlings changes significantly (Gautam et al., 2003). Under drought stress, most plants reduce the biomass allocation for above-ground parts, and allocate more resources to the underground parts, so that they can gain more water and nutrients and accrue more underground biomass (Greco et al., 2003). Here in this study, the inoculation with the PGPR strain YX2 increased the dry weight of above- and under-ground parts of apple seedlings, and promoted the growth of seedling roots. This may be attributed to the fact that the strain YX2 might have increased the amount of organic acids secreted by the root system, which enhanced the dissolution of nutrients in the soil, and then the roots took up more nutrients resulting in better root growth compared with those of non-inoculated treatment, thereby improving the dry matter accumulation of the above-ground parts of the apple seedlings. Under drought stress, a high amount of ethylene produced by plants might cause harm to the plant itself, while ACC deaminase can reduce ethylene levels, protecting plants from the drought-induced growth inhibition (Mayak et al., 2004). The PGPR strain YX2 used in this study might have produced ACC deaminase which improved the ability of plants to resist drought stress. Huadonglai (2013) investigated the effects of PGPR bacteria on the growth of tomato roots at different PEG-6000 levels. The results showed that the roots of PGPR-treated tomato plants were more densely developed. Phosphorus as one of the three major essential nutrients for plant growth and development, accounts for 0.2% of the dry weight of plant cells (Smith et al., 2011). It is indispensable for plant growth and metabolism such as signal transduction, energy conversion, photosynthesis and respiration, and biological macromolecules' synthesis (Schachtman et al., 1998; Heuer et al., 2017). Therefore, improving P uptake in plants plays an important role in promoting plant growth, protecting biodiversity, and maintaining ecosystem productivity. In this study, drought inhibited the uptake of P in soil containing



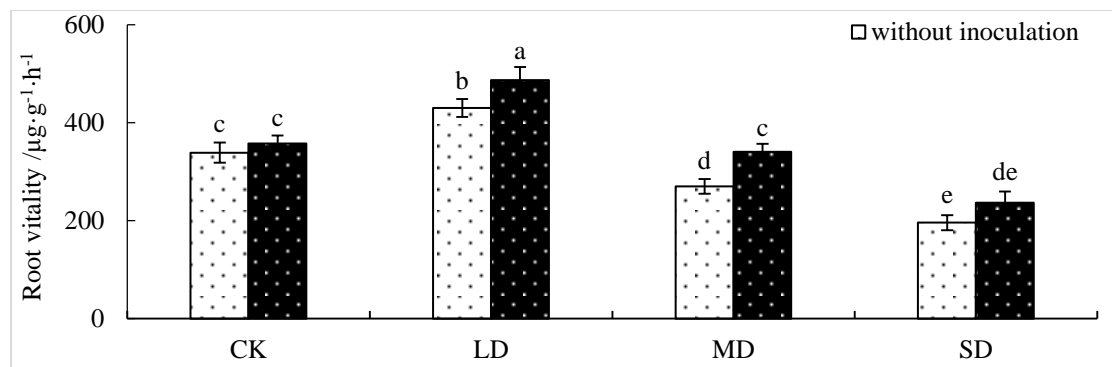


Figure 2. Effects of different drought and PGPR treatments on root activity of seedlings

Table 4. Effect of PGPR inoculation on root total length, root average diameter, root surface area and root volume of seedlings

Drought treatment	Inoculation treatment	Root total length (cm)	Root average diameter (mm)	Root surface area (cm <sup>2</sup> )	Root volume (cm <sup>3</sup> )
CK	Non-inoculation	401±43 bc	0.66±0.09 a	60.04±7.02 abc	0.56±0.04 bc
	Inoculation	413±28 b	0.71±0.11 a	60.82±4.08 abc	0.59±0.05 bc
LD	Non-inoculation	406±24 bc	0.62±0.03 a	65.33±7.77 ab	0.63±0.05 ab
	Inoculation	503±37 a	0.64±0.06 a	69.97±7.55 a	0.70±0.07 a
MD	Non-inoculation	352±36 cd	0.62±0.04 a	55.20±5.96 bc	0.62±0.06 abc
	Inoculation	412±33 b	0.66±0.06 a	57.11±5.66 bc	0.65±0.04 ab
SD	Non-inoculation	347±38 d	0.61±0.04 a	53.83±4.14 c	0.54±0.05 c
	Inoculation	406±37 bc	0.62±0.03 a	58.11±8.51 bc	0.59±0.07 bc

Data presented is mean of three replicates and different letters show significant difference at  $P \leq 0.05$ .

apple seedlings, but after inoculation with the strain YX2, the contents of the available P in soil and the accumulation of P in plants increased under different drought conditions. This may have been due to the reason that inoculation with the strain YX2 significantly promoted the root activity of apple seedlings resulting in increased amounts of organic acids secreted by the roots. Organic acids can dissolve the insoluble phosphate effectively increasing the available P contents in the rhizosphere soil and facilitating the uptake of P by plant roots. At the same time, the total root length and root biomass of apple seedlings increased significantly, and the root vigor also increased after inoculation with the strain YX2, which may have promoted the uptake of nutrients. This is coherent with a previous study on the effects of PGPR on plant nutrient uptake (Egamberdiyeva et al., 2007). Our results also showed that under moderate drought conditions the effect of the strain YX2 on root growth, root activity and P uptake of apple seedlings was superior to those of the other drought treatments, indicating that YX2 had certain selectivity to water stress regimes.

Acid phosphatase is considered as a key enzyme involved in the activation of insoluble phosphates by mycorrhizal symbionts (Shibata et al., 2003). Acid phosphatase activity and P uptake contribution rate of YX2, available P content, and soil alkaline phosphatase were positively correlated, which indicated that YX2 might have induced the apple seedling roots to exude more acid phosphatase and activate insoluble phosphates increasing soil available P contents and alkaline phosphatase activity.

## Conclusion

Inoculation with the strain YX2 of *Pseudomonas fluorescens* under drought stress improved root growth of apple seedlings, root activity and uptake of P in the soil thereby promoting apple seedling growth. The microorganism agents obtained from the rhizosphere soil of apple orchard in dryland and inoculated into apple rhizosphere soil under controlled conditions had positive effects on improving the ecological environment of apple rhizosphere soil. At the same time, the efficiency of P taken up by the roots was improved; the fixation ability of soil rhizosphere to nutrient ions was enhanced and the root system activity was also enhanced. Therefore, the application of specific microbial products in agricultural production system is meaningful in research pursuits aiming at the use of potential microbial agents.

## Acknowledgement

This study was funded by the National Key Research and Development Program of China [grant number 2017YFE0114000], Shaanxi Science and Technology Major Project [No. 2020zdzx03-02-01]

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