Effects of Microbial Fertilizer on the Growth, Physiology, and Chlorophyll Fluorescence Response of Spinach Seedlings

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Abstract

We investigated the effects of different types of bacillus on the growth, physiological characteristics, and chlorophyll fluorescence of spinach seedlings in the soil environment of a Qinxi demonstration garden in Taibai County. Five different fertilization treatments were conducted in the growing room: no fertilizer (CK), addition of *Bacillus subtilis* (*Bs*, T1), addition of *Bs* and *Bacillus mucilaginosus* (*Bm*) (T2), addition of *Bs* and *Bacillus amyloliquefaciens* (*Ba*) (T3), and addition of *Bs* and *Ba* (T4). There were significant differences in the plant height (PH), leaf length (LL), and leaf weight (LW) of the spinach seedlings (F=54.37, 13.30, and 46.03, respectively; P<0.01). The growth and physiological characteristics of the spinach seedlings attained a maximum under the *Bs* and *Bm* treatments. There were differences in the activities of the PSII reaction centers between the five treatments. Under the *Bs* and *Bm* treatments, the ABS/RC, TR_0/RC, and ET_0/RC increased significantly, while the DI_0/RC decreased. The OJIP curve increased under different types of fertilization, and the growth trends under the *Bs* and *Bm* treatments were the largest. The leaf light response curve (LC) increased significantly under the *Bs* and *Bm* treatments. The plant growth characteristics (LL, LW, PH) were positively correlated with the J-I-P test chlorophyll fluorescence parameters (PI_{ABS}, φP_0, φE_0, ψ_0, TR_0/RC, and ET_0/RC), and negatively correlated with φD_0 and DI_0/RC. The leaf physiological characteristics (SP, SC, Chla, Chlb, Chla+b, Chla/b, and WP) were positively correlated with the J-I-P test chlorophyll fluorescence parameters (PI_{ABS}, φP_0, φE_0, ψ_0, ABS/RC, TR_0/RC, and ET_0/RC), and negatively correlated with φD_0 and DI_0/RC. The leaf MDA was significantly positively correlated with φD_0 and DI_0/RC, and positively correlated with other J-I-P test chlorophyll fluorescence parameters. The *Bs* and *Bm* treatments promoted the growth of the spinach seedlings and improved the adaptability of the crops to soil by enhancing the effective phosphorus utilization rate.

1. Introduction

In recent years, with the rapid development of the vegetable industry in China, fertilization has become the main strategy for improving yields. With the cultivation of vegetables gradually increasing over the years, the excessive application of chemical fertilizers has led to progressively serious soil problems. Therefore, microbial fertilizers have gradually replaced chemical fertilizers as a good method to improve soil quality, while increasing vegetable yields. Microbial fertilizers are also referred to as bacterial fertilizer, biological fertilizer, and microbial inoculant (Shen et al., 2011), which contain beneficial microorganisms that provide fertilization for crops through their activities. The metabolic activities of microorganisms can improve soil fertility and crop quality, while increasing soil microorganism populations, reduce plant diseases, and enhance plant root activities. Cao et al. (2011) and Manjurul et al. (2012) showed that biological fertilizers rich in Trichoderma could increase mustard and tomato yields, while reducing the need for the excessive use of N, P, and K in crop cultivation.

Bacillus is a type of growth-promoting bacteria that can produce phytase at the rhizosphere of plants. It has strong resistance to ultraviolet light, high salt, high acid, high heat, and radiation, with the capacity to inhibit bacteria, prevent disease, and increase production (Cavalcanti et al., 2018; Guo et al., 2009). *Bacillus subtilis* can improve the stress resistance of plants (Pliego and Ramos., 2010) and the availability of nitrogen and phosphorus in soil (Idriss and Borriss., 2002). *Bacillus mucilaginosus* converts the unusable phosphorus in soil to available phosphorus for plants. Simultaneously, *Bm* can secrete substances that promote plant growth and development, such as growth hormones, gibberellin, and more (Liu et al., 2016; Zhao et al., 2009). *Bacillus amyloliquefaciens* has a certain antagonistic effect on bacterial diseases. Arrebola et al. (2009) showed that Bacillus amylolyticus PPCB004 could inhibit the mycelial extensions of Penicillium fungi.

Photosynthesis is a process through which plants convert captured light energy into biochemical energy. Chlorophyll fluorescence can reflect the processes of light reactions in plants (Goltsev et al., 2009). The chlorophyll fluorescence technique can accurately analyze the distribution of light energy without harming the leaves of plants, and is considered a rapid and nondestructive probe for measuring leaf photosynthesis. Through the analyses of chlorophyll fluorescence parameters, a further elucidation of the light energy absorption, utilization, transfer, and dissipation of plant chloroplast PSI and PSII processes can be obtained (Li et al., 2005).

The chlorophyll fluorescence technique has also been widely investigated for the detection of physiological changes in plants that are caused by bacterial and fungal infections. Cen et al. (2016) and Chen et al. (2012) revealed that there was a good correlation between chlorophyll fluorescence parameters and the degree of Verticillium wilt infection in cotton leaves. Tung et al. (2013) quantitatively analyzed the degree of infected tobacco (infected, unmanifested infection, healthy) according to Fv/Fm images, which indicated that chlorophyll fluorescence imaging technologies can detect the degree of infected tobacco. This revealed that the growth and physiological status of plants under different stresses can be monitored by chlorophyll fluorescence.

The purpose of this study was to explore the effects of different types of Bacillus on the growth, physiology, and chlorophyll fluorescence characteristics of spinach seedlings, and subsequently, to make reasonable plans for improving the quality and yields of spinach.

2. Materials And Methods

2.1 Experimental materials

These experiments employed the soil of a greenhouse at the Qinxi demonstration garden in Taibai County, Baoji County, Shaanxi Province as the test soil. The spinach (*Spinacia oleracea* L.) variety was big-leaf spinach, which belongs to the spinach genus Chenopodiaceae (annual herbaceous plant), and the seeds were provided by the demonstration garden. The *Bacillus subtilis*, *Bacillus mucilaginosus*, and *Bacillus amyloliquefaciens* selected the original strains produced by Lvlong Biotecnology Co., Ltd., which contained > 200 million live bacteria per gram of fertilizer.The fertilizer components are all the *Bacillus* and its metabolites, and the rate of miscellaneous bacteria < 3%.

2.2 Growth conditions and experiment design

The experiments were carried out in growth chambers at the College of Geography and Environment of Baoji University of Arts and Sciences under a 12 h day/night photoperiod at temperatures of 25/15 °C, respectively, a photosynthetically active radiation (PAR) of 300 μmol/(m^2·s), and humidity of 60%. The spinach seeds were soaked in deionized water for 12 h and placed in a 4 °C refrigerator for 24 h to promote germination. The germinated seeds were placed on a seedling tray, covered with nutritious soil, and germinated in the dark in a 25 °C incubator. Following seven days of treatment, the seedlings were transferred to pot (Ø25 cm x 20 cm high) filled with greenhouse soil 18 cm deep, and 10 seedlings were planted in each pot. The soil nutrients were comprised of 33.35 g/kg organic matter, 2.79 g/kg total nitrogen, 1.51 g/kg total phosphorus, and 10.75 mg/kg available phosphorus, and a pH of 5.2. For this experiment, 90 kg/hm^2 of phosphate fertilizer (potassium dihydrogen phosphate) was used as base fertilizer with microbial fertilizer 0.5 kg/hm^2. There were four treatments and three repeats (Table 1).

Table 1
combined application scheme of microbial fertilizer

Treatments	microbial fertilizer
Ck	No added
T1	*Bs*
T2	*Bs* + *Bm*
T3	*Bs* + *Ba*
T4	*Bm* + *Ba*
Bs: *Bacillus subtilis*, *Ba*:*Bacillus amyloliquefaciens*, *Bm*: *Bacillus mucilaginosus*	

2.3 Measurement of growth characteristics

For this experiment, all of the characteristics of the spinach leaves were measured 7 d after treatments. The plant height (PH), leaf length (LL) and leaf weight (LW) were measured with a scale.

2.4 Measurement of physiological characteristics

The leaf soluble protein (SP), soluble carbohydrate (SC), and water potential (WP) were closely related to plant metabolism. Chlorophyll (Chl) is an important substance in plant photosynthesis, and malondialdehyde (MDA) can reflect the degree of membrane lipid peroxidation. Within seven to 10 days of fertilization, the SP was determined by the Coomassie brilliant blue (CBB) method at 595 nm using a DR6000 spectrophotometer (Bradford, 1976), whereas the SC was determined using an anthrone method at 620 nm (Jermyn, 1975). The leaf Chla and Chlb concentrations were determined by Litchtenthaler (1983) at 645 and 663 nm, whereas the leaf MDA was determined by the thiobarbituric acid method (Draper et al., 1993). The WP (ψ) of the leaves was measured by dew point potentiometer (WP4, Decagon Devices, Pullman, USA).

2.5 Chlorophyll fluorescence measurements

Prior to the fluorometer measurements, the leaves were dark-adapted for 20 min and then measured using a FluorPen FP 100 Max hand fluorescence meter (Photon Systems Instruments, Brno,Czech Republic), and calculated according to the JIP-test algorithm proposed by Strasser et al. (2010) (Table 2).

Table 2
Analysis of chlorophyll fluorescence

Parameters and Formula	Meaning
F_0	minimum fluorescence
Fm	maximum fluorescence
F_J	Ft at Time 2 ms
F_I	Ft at Time 30 ms
F_t	The relative fluorescence intensity at Time t
$Fv = Fm-F_0$	variable fluorescence
$nFv = Fv/F_0$	variable to initial fluorescence ratio
$V_I = (F_J - F_0)/(Fm - F_0)$	Relative variable fluorescence at the I-step
$V_J = (F_J - F_0)/(Fm - F_0)$	Relative variable fluorescence at the J-step
M_0	Approximated initial slope (in ms – 1) of the fluorescence transient V = f(t)
PI_{ABS}	Performance index (potential) for energy conservation from exciton to the reduction of intersystem electron acceptors
ABS/RC	Absorption flux (of antenna Chls) per RC
$TR_0/RC = M_0(1/V_J)$	Trapping flux (leading to QAreduction) per RC
$ET_0/RC = M_0(1/V_J)\Psi o$	Electron transport flux (further than QA^-) per RC
$DI_0/RC = (ABS/RC – TR_0/RC)$	Dissipated energy flux per RC (at t = 0)
$\varphi P_0 = 1 – F_0/Fm$	Maximum quantum yield of primary photochemistry (at t = 0)
$\varphi E_0 = (1 – F_0/Fm)(1 – V_J)$	Quantum yield of electron transport (at t = 0)
$\Psi_0 = ET_0/Tr_0 = 1-V_J$	The probability that a trapped exciton moved an electron in electron transport chain further than QA^- (t = 0)
$\varphi D_0 = F_0/Fm$	Quantum yield (at t = 0) of energy dissipation

To compare the OJIP curve with the normalized PF transient curve between OJ and OK, the following formula was used:

$Vt = (F_t - F_0)/(Fm - F_0)$ (1)

$\Delta Vt = Vt^{TR} - Vt^{CK}$ (2)

$W_{OJ} = (F_t - F_0)/(F_j - F_0)$ (3)

$\Delta W_{OJ} = W_{OJ}^{TR} - W_{OJ}^{CK}$ (4)

$W_{OK} = (F_t - F_0)/(F_K - F_0)$ (5)

$\Delta W_{OK}=W_{OK}{}^{TR}-W_{OK}{}^{CK}$ (6)

The leaf light-response curve (LC) measurement, based on pulse modulated fluorometry (PAM) was designed using seven photosynthetic photon flux densities (PPFD) (10,20、50、100、300、500, and 1000 μmol/(m^2·s)) to acquire chlorophyll fluorescence parameter changing curves (Ft, QY) relating the rate of photosynthesis.

2.6 Statistical analysis of data

All collected data were subjected to one-way ANOVA analysis using SPSS (SPSS software version 22.0, Chicago, Illinois, USA). Differences between means were compared by Tukey's HSD test at $P < 0.05$. The correlations between parameters were determined using Pearson's simple correlation test function in SPSS.

3. Results

3.1 Effects of microbial fertilizer on growth and physiological characteristics of spinach seedlings

There were significant differences in the plant height (PH), leaf length (LL), leaf weight (LW), and other growth parameters of the spinach seedlings under the five different treatments (F = 54.37, 13.30, and 46.03, respectively; P < 0.01), where the growth characteristics attained a maximum under the T2 treatment (Table 3). Except for Chla + b and Chla/b, there were significant differences in the physiological characteristics of the leaves. Among them, the T2 treatment had the greatest impact on the physiological leaf responses. Compared with the F value, the difference in soluble sugar (SC) was the highest (F = 118.35; P < 0.01).

Treatments	Growth characters		
	Plant height (PH, cm)	Leaf length (LL, cm)	Leaf weight (LW, g/10 plants)
CK	3.53 ± 0.15 b	2.62 ± 0.26 b	0.95 ± 0.01 d
T1	5.23 ± 0.09 a	4.08 ± 0.05 a	2.08 ± 0.02 b
T2	5.27 ± 0.09 a	4.15 ± 0.09 a	2.69 ± 0.02 a
T3	5.05 ± 0.03 a	3.73 ± 0.18 a	1.47 ± 0.02c
T4	5.07 ± 0.03 a	3.89 ± 0.19 a	1.74 ± 0.01 c
F value	54.37**	13.30**	46.03**

Treatments	Physiological leaf characteristics							
	SP (mg/gFW)	SC (mg/gFW)	MDA (mg/gFW)	Chl a (mg/gFW)	Chl b (mg/gFW)	Chl a + b (mg/gFW)	Chl a/b (mg/gFW)	WP(Ψ,Mpa)
CK	1.83 ± 0.26c	0.73 ± 0.05d	5.49 ± 0.51a	1.16 ± 0.03e	0.36 ± 0.03c	1.52 ± 0.03e	3.25 ± 0.30b	-6.57 ± 0.25d
T1	3.31 ± 0.20b	2.26 ± 0.06b	1.80 ± 0.13b	2.73 ± 0.22b	0.58 ± 0.06ab	3.30 ± 0.15b	4.91 ± 0.83a	-4.23 ± 0.12b
T2	4.14 ± 0.04a	3.59 ± 0.13a	1.25 ± 0.14b	3.23 ± 0.04a	0.60 ± 0.03b	3.83 ± 0.07a	5.36 ± 0.25a	-2.79 ± 0.22a
T3	2.37 ± 0.08c	1.59 ± 0.11b	4.82 ± 0.31a	1.70 ± 0.07d	0.40 ± 0.01c	2.11 ± 0.07d	4.21 ± 0.09ab	-5.53 ± 0.26c
T4	3.28 ± 0.22b	2.23 ± 0.11c	2.09 ± 0.15b	2.29 ± 0.11c	0.47 ± 0.02bc	2.75 ± 0.09c	4.91 ± 0.43a	-4.79 ± 0.12b
F value	24.48**	118.35**	44.95**	50.63**	8.80**	0.12	3.34	48.72**

Values presented in each column of table have a mean ± standard deviation. The last portion of table refers to the F value. * P < 0.05, ** P < 0.01. SP: Soluble protein concentrations, SC: Soluble carbohydrate concentrations, MDA: malonaldehyde concentrations, Chl: chlorophyll, WP: Water potential.

3.2 Differential analysis of chlorophyll fluorescence parameters of spinach seedlings by adding microbial fertilizer

According to the values of the basic fluorescence parameters (Table 4), the F_0 value of the T2 treatment was the highest, which was 41.29% higher than that of CK. Further, the Fm value of the T2 treatment was 17751 later than that of CK, and there was a significant difference in F_V value under the five treatments. The relative variable fluorescence value (V_I) at 30 ms was highest under the T2 treatment. In terms of the nF_V value, the T2 treatment remained the largest. Compared with the F value, the difference in the F_0 was the largest (F = 112.57; P < 0.01).

Table 4

difference analysis of chlorophyll fluorescence parameters of microbial fertilizer added to five different treatments

Treatments	Leaf chlrophyll fluorescence intensity (relative unit)					
	F_0	Fm	Fv	V_J	V_I	Fv/F_0(nFv)
CK	7087 ± 188b	28967 ± 1440c	21880 ± 1628c	0.46 ± 0.06a	0.76 ± 0.01bc	4.07 ± 0.03b
T1	9233 ± 568ab	43142 ± 1342ab	33909 ± 1286ab	0.54 ± 0.03ab	0.82 ± 0.01ab	3.67 ± 0.11a
T2	10013 ± 10ab	46718 ± 1126a	36705 ± 1136a	0.47 ± 0.00ab	0.85 ± 0.02a	4.08 ± 0.11a
T3	9071 ± 108ab	31861 ± 131bc	22790 ± 23bc	0.47 ± 0.01b	0.76 ± 0.00c	2.51 ± 0.41b
T4	9786 ± 38a	41549 ± 1544ab	31763 ± 1506abc	0.46 ± 0.00ab	0.77 ± 0.01bc	3.25 ± 0.24a
F value	112.57**	31.13**	24.01**	2.48	3.85*	8.3**
Treatments	J-I-P test parameters (relative unit)					
	M_0	PI_{ABS}	ABS/RC	TR_0/RC	ET_0/RC	DI_0/RC
CK	0.91 ± 0.03ab	0.38 ± 0.29c	2.20 ± 0.08ab	1.37 ± 0.06ab	0.46 ± 0.04b	0.82 ± 0.06a
T1	1.08 ± 0.07a	1.24 ± 0.21ab	2.57 ± 0.03a	2.02 ± 0.01ab	0.94 ± 0.03a	0.55 ± 0.02c
T2	1.00 ± 0.02a	1.70 ± 0.23a	2.59 ± 0.03a	2.08 ± 0.04a	1.07 ± 0.01a	0.51 ± 0.01b
T3	0.67 ± 0.01ab	1.41 ± 0.01bc	2.01 ± 0.00b	1.43 ± 0.02b	0.76 ± 0.01ab	0.57 ± 0.01ab
T4	0.83 ± 001b	1.61 ± 0.03bc	2.35 ± 0.08ab	1.80 ± 0.01ab	0.96 ± 0.01ab	0.55 ± 0.00bc
F value	7.66**	4.09*	2.88	3.97*	2.04	8.16**
Treatments	Yield and efficiency					
	φP_0(Fv/Fm)	φE_0	Ψ_0	φD_0		
CK	0.62 ± 0.01c	0.22 ± 0.01ab	0.34 ± 0.01ab	0.37 ± 0.01ab		
T1	0.78 ± 0.01ab	0.37 ± 0.01a	0.47 ± 0.01a	0.21 ± 0.00c		
T2	0.80 ± 0.00a	0.41 ± 0.06b	0.52 ± 0.05a	0.19 ± 0.00bc		
T3	0.72 ± 0.0bc	0.38 ± 0.03ab	0.53 ± 0.02b	0.29 ± 0.03a		
T4	0.76 ± 0.01ab	0.41 ± 0.00ab	0.54 ± 0.00ab	0.24 ± 0.01bc		
F value	6.03**	2.48	2.71	6.02**		

The values presented in each column of table have a mean ± standard deviation. The last part of table refers to the F value. * $P < 0.05$, ** $P < 0.01$.

There were significant differences between the M_0 and PI_{ABS} values under the five different treatments (F = 7.66 and 4.09, respectively; $P < 0.05$). The T2 treatment had the greatest effect on the specific activity of each reaction center (RC), and there was a significant difference between the TR_0/RC and DI_0/RC. However, there was no significant difference in the ABS/RC and

ET_0/RC between the treatments. The quantum yield (φP_0), efficiency (φE_0), and ψ_0 values were highest under the T2 treatment, and with the exception of ψ_0, there were significant differences ($P < 0.01$). On the contrary, the value of φD_0 was lowest under the T2 treatment.

3.3 Transient analysis of prompt fluorescence OJIP of spinach seedlings by adding microbial fertilizer

By measuring the transient curve of OJIP as shown in Fig. 1, the OJIP curves of the five treatments were similar to those reported by Strasser et al. (2004). As can be seen from Fig. 1(A), compared with CK, the fluorescence trend of the other treatments gradually increased, among which the fluorescence value of the T2 treatment was the largest, which signified that it had a greater effect on the photosynthetic chemical rate of the leaves. As shown in Fig. 1(B), the difference of relative variable fluorescence (Vt) at point J (2 ms) was the largest. This is better illustrated in Fig. 1(C) ΔVt. Over time the difference between the treatments increased initially and then decreased, and the difference in the initial slope of the O-J stage curve was maximized. Oukarroum et al. (2007) regarded the double normalization of the OJIP transient between the peak value of 0.05 to 2 ms, and the difference of double normalization between the treatment and the control group as the K-band. It can be seen from Figs. 1D,E that there is an obvious positive K band under the T1 and T2 treatments, and a negative growth trend under the T3 and T4 treatments. The transient double normalization of OJIP between Fo and F_K and the difference of double normalization between the treatments and the control group were regarded as the L band (Oukarroum et al., 2007). As shown in Figure. 1F,G the W_{OK} value of the T2 treatment was higher, and there was a significantly higher positive L band.

3.4 Analysis of the difference of the Light response Curve (LC) of the spinach seedlings by adding microbial fertilizer

Compared with the five treatments, the quantum yield (QY) of leaves under the T2 treatment attained a maximum at ~ 20 μmol/(m^2·s) PPFD, which indicated that the light compensation point (LCP) was ~ 20 μmol/(m^2·s) PPFD. The maximum value of the other treatments was before 10 μmol/(m^2·s) PPFD, indicating that the LCP was earlier than 10 μmol/(m^2·s) PPFD.

3.5 Correlations between growth, physiological characteristics and leaf chlorophyll

fluorescence

Through correlation analyses the characteristics of the growth, physiology, and chlorophyll fluorescence of leaves were quantified. Plant growth characteristics (LL, LW, PH) were positively correlated with chlorophyll fluorescence parameters (PI_{ABS}, φP_0, φE_0, ψ_0, TR_0/RC, ET_0/RC) using a J-I-P test, and negatively correlated with φD_0 and DI_0/RC. The physiological characteristics of the leaves (SP, SC, Chla, Chlb, Chla + b, Chla/b, and WP) were positively correlated with PIABS, φP_0, φE_0, ψ_0, ABS/RC, TR_0/RC and ET_0/RC, and negatively correlated with φD_0 and DI_0/RC. The leaf MDA was significantly positively correlated with φD_0 and DI_0/RC, and positively correlated with other chlorophyll fluorescence parameters tested by J-I-P (Table 5).

Table 5
Correlations between leaf physiological characteristics and chlorophyll fluorescence parameters.

Index	LW	LL	PH	SP	SC	Chla	Chlb	Chla + b	Chla/b	MDA	WP
PI_{ABS}	0.75**	0.84**	0.92**	0.76**	0.81**	0.73**	0.55*	0.73**	0.68**	-0.71**	0.75**
φP_0	0.90**	0.89**	0.91**	0.89**	0.90**	0.93**	0.80**	0.93**	0.74**	-0.91**	0.90**
φE_0	0.58*	0.78**	0.89**	0.60*	0.65**	0.56*	0.38	0.55*	0.58*	-0.55*	0.58*
Ψ_0	0.74**	0.86**	0.95**	0.75**	0.79**	0.74**	0.56*	0.74**	0.68**	-0.72**	0.74**
φD_0	-0.90**	-0.89**	-0.91**	-0.89**	-0.90**	-0.93**	-0.80**	-0.93**	-0.74**	0.91**	-0.90**
ABS/RC	0.77**	0.51	0.40	0.78**	0.73**	0.83**	0.80**	0.84**	0.54*	-0.85**	0.78**
TR_0/RC	0.90**	0.74**	0.69**	0.90**	0.88**	0.94**	0.86**	0.96**	0.68**	-0.95**	0.90**
ET_0/RC	0.89**	0.88**	0.90**	0.91**	0.92**	0.91**	0.76**	0.92**	0.75**	-0.91**	0.90**
DI_0/RC	-0.80**	-0.90**	-0.97**	-0.78**	-0.82**	-0.80**	-0.65**	-0.81**	-0.70**	0.76**	-0.79**

* $P < 0.05$** $P < 0.01$. LL: Leaf length, LW: Leaf weight, PH: Plant height, SP: Soluble protein concentrations, SC: Soluble carbohydrate concentrations, MDA: malonaldehyde concentrations, Chl: chlorophyll, WP: Water potential.

4. Discussion

4.1 *Physiological characteristics of growth* and *leaves*

Spinach is a type of Chenopodiaceae plant that is extensively planted in facility agriculture, which has strong environmental adaptability (Chen et al., 2016; Long et al., 2013). In terms of plant phenology and biological characteristics, plant growth, leaf chlorophyll content, and other physiological characteristics are critical influencing factors (Bennie et al., 2016). The results of Yan et al. (2017) revealed that the application of microbial fertilizer could effectively improve the plant height of pakchoi. In this study, the addition of microbial fertilizer effectively improved plant height, leaf length, and leaf weight, among which the growth status of plants with the addition of *Bs* and *Bm* was more dominant. This was similar to the results of Samia et al. (2014).

In this research, microbial fertilizer was found to significantly enhanced the physiological characteristics of spinach seedling leaves (SP, SC, Chla, Chlb, Chla + b, Chla/b, and WP). Wang et al. (2018) found that the application of microbial fertilizer could effectively increase the chlorophyll content of winter wheat at the overwintering, jointing, and booting stages. The application of phosphate fertilizer can promote the synthesis of chlorophyll in leaves (Li et al., 2002).

For this study, the chlorophyll content of leaves treated with *Bs* and *Bm* was at a higher level. It may be possible that the *Bs* and *Bm* convert ineffective phosphorus into available phosphorus in the soil, which improves the availability of phosphorus and enhances the ability of plants to absorb it, thus promoting the synthesis of ATP and NADPH in leaves. When plants are under stress, the MDA content is typically an important indicator of membrane lipid peroxidation, which reflects the harmful effects of stress on plant cells and tissues (Han et al., 2013). In this study, the concentration of MDA in leaves treated with microbial fertilizer decreased.

4.2 Leaf chlorophyll fluorescence

In this research, the values of F_0 and Fm were enhanced, which indicated that the *Bs* and *Bm* promoted an increase in the size of leaf PSII antenna and a decrease in the non-radiative dissipation of chlorophyll in PSII antenna, thus increasing the capacity of leaves to capture light energy (Oukarroum et al., 2016). We observed higher Fv and Fv/F_0 (nFv) values under the *Bs* and *Bm*

treatments, which signified an increase in the efficiency of supplying electrons to PSII RCS and the photosynthetic quantum conversion rate of PSII RCS. This translated to less energy being used for non-photochemical dissipation in the dark adaptation process (Spoustova et al., 2013).

In this experiment, the ABS/RC values of the leaves increased, indicating that the *Bs* and *Bm* promoted the increased size of active RCS, which led to a higher number of active PSII reaction centers and enhanced dark accumulation (Pooja et al., 2010). Under the action of the *Bs* and *Bm*, the TR_0/RC value increased, which reflected the higher electron capture rate of RC, where more QA was converted to QA^-. This resulted in an increase in the electron transfer energy (ET_0/RC), thus reducing the energy dissipated by non-photochemical activities (DI_0/RC) (Yiotis and Manetas, 2010; Tang et al., 2017).

The φP_0 (Fv/Fm) represents the maximum quantum yield of PS Ⅱ, and the ψ_0 value reflects the electron transfer efficiency, from QA^- to QB, whereas φE_0 reflects the quantum yield of electron transport. The increase of φP_0, ψ_0, and φE_0 indicated that the *Bs* and *Bm* promoted the redox reaction following QA, which resulted in an increase of the electron transfer rate between QA^- and QB (Lebkuecher et al., 1999).

The change of the chlorophyll fluorescence curve is closely related to the physiological morphology of plants (Malaspina et al., 2015). Changes in the O-J segment are related to an increased number of inactive reaction centers, or the energy transfer from the LHCII to PSII reaction center (Tomar and Jajoo, 2013). The K and L bands reflect the connection between the S state of the PSII oxygen evolution complex (OEC) and PSII unit, as well as the energy connection between the PSII units (DaBrowski et al., 2016; Paunov et al., 2018).

An increase in the J-I segment can reflect a decrease in the relative number of active PQ molecules that is reduced by each active RC of PSII ((Paunov et al., 2018). Further, changes in the I-P segment are closely related to the pool of electron receptors (ferredoxin and NADPH) at the end of the PSI, signifying the kinetic flow rate to the electron receptors at the end of the PSI (Tsimilli Michael and Strasser, 2013). In this experiment, the OJIP transient curves of the spinach seedlings were affected by microbial fertilizer (Fig. 1).

Under the treatment of *Bs* and *Bm*, the relative fluorescence intensity of chlorophyll in the O-J segment exhibited a larger value, indicating that the population of active PSII centers decreased, whereas the QA^- accumulated massively. Figures 1E G show that there were obvious K bands and positive L bands under the *Bs* and *Bm* treatment, which indicated that the PSII had an inhibitory effect on the OEC. This resulted in a weakening of the connectivity between PSII and OEC and a decreased energy connection between the PSII units.

This may have been due to the variable light and ventilation that was present at different locations in the incubator, which may have led to PSI receptor side damage and chlorophyll protein denaturation in some plant leaves (Bertamini and Nedunchezhian, 2003). In addition, the fluorescence of J-I and I-P segments remained large under the *Bs* and *Bm* treatment, which indicated that the relative number of PQ decreased, while there was an increase in the pool of electron receptors (ferredoxin and NADPH) at the end of PSI, which led to a higher kinetic flow rate to the electron receptors at the end of PSI.

Combined with PI_{ABS}, the higher PI_{ABS} values under the *Bs* and *Bm* treatment indicated increases in the density of active PSII centers, the efficacy of photoreactions, and efficiency of biochemical dark redox reactions, as well as the production and utilization of NADPH (Habib et al., 2016).

5. Conclusions

The main results of our study are as follows: Bacillus promoted the growth of spinach seedlings and improved the adaptability of crops to soil by improving the effective phosphorus utilization rate. Simultaneously, the chlorophyll content, soluble protein and other physiological characteristics, chlorophyll fluorescence, and PSII photochemical activity were improved to a certain extent.

The results revealed that under the *Bs* and *Bm* treatment, the growth conditions for the spinach seedlings were best, and the physiological index was higher. According to this study, it is suggested that inorganic fertilizer and Bacillus may be used together for soil fertilization in greenhouses. It can not only promote the utilization of mineral elements in the soil by plants to improve crop quality, but also prevent soil acidification caused by the excessive accumulation of mineral elements. Future research should endeavor to control the fertilization concentrations of microorganisms, so as to provide an improved theoretical basis for agricultural fertilization.

Declarations

Declarations

The research passed the ethics approval and it was consent to participate. All the authors declare no competing interests and consent for publication. J. X. W. and Y.Y prepared the materials. B. B. Z designed the study. H. Z. did the data analysis. B. B. Z and Q. J wrote the main manuscript text. All authors discussed the results and commented on the paper.

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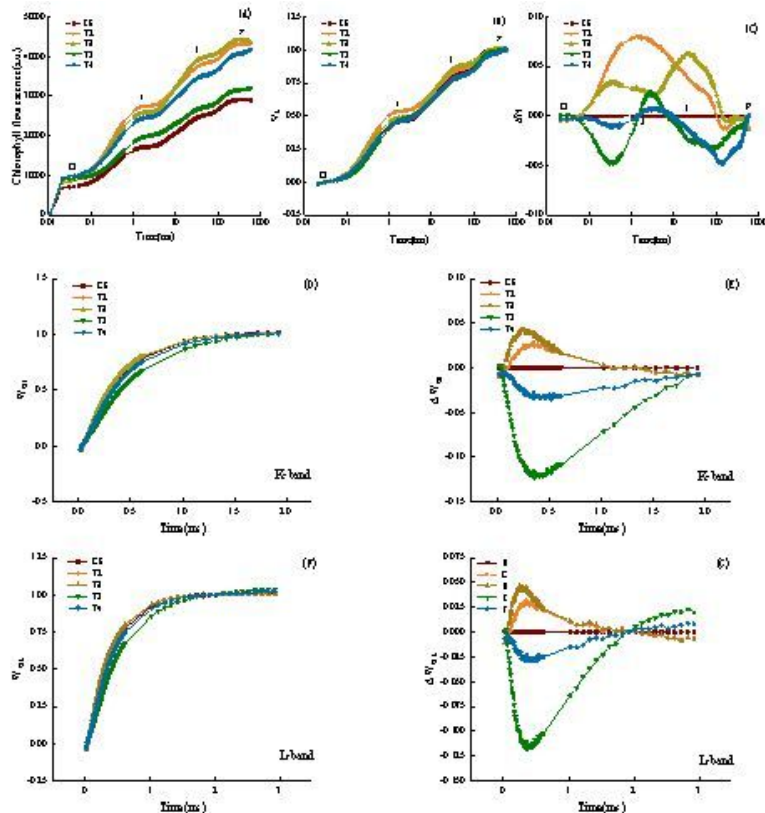
Figures

Figure 1

Shape change of fluorescence transient curve measured through then addition of microbial fertilizer in the five different treatments. Each curve represents the average of three independent measurements.
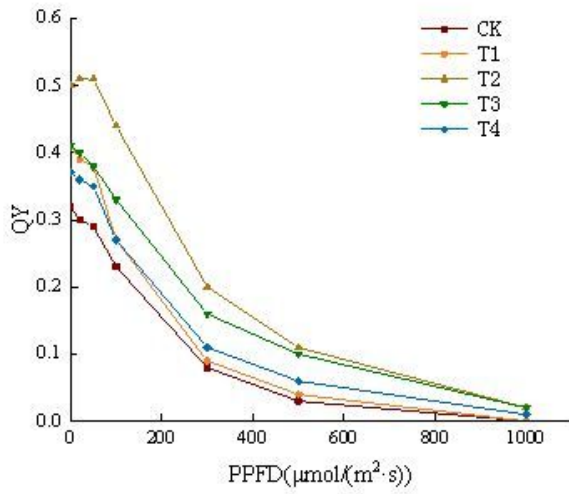

Figure 2

Light curve (LC) of quantum yield (QY) under the five different treatments. Data are the means of three experiments (±SD).