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Journal of Chemical and Pharmaceutical Research, 2013, 5(12):520-524

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Effects of *Piriformospora indica* on Chinese cabbage production and resistance to waterlogged stress

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ABSTRACT

As one of the important natural disasters, waterlogging has alwalys had serious effects on the yield and quality of Chinese cabbage in the middle and lower region of the Yangtze. After the use of solid fertilizer, liquid fertilizer and no fertilizer of Piriformospora indica, the influence of waterlogging on growth and related physiological index of the Chinese cabbage were analysed at 7 d of waterlogging treatment. The results showed that the use of solid fertilizer and liquid fertilizer of Piriformospora indica could significantly reduce the waterlogging damage, enhance the yield of Chinese cabbage, and improve the content of protein, vitamin C, soluble sugar, chlorophyll, CAT and SOD activity, and promote root growth.

Key words: *Piriformospora indica*, Chinese cabbage, Waterlogged Stress, Yield, Quality, Resistance

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Piriformospora indica is is a kind of endophytic fungi of plant roots with wide hosts [1]. It could improve the growth, flowering in advance, and production of plant[2]. In addition, *Piriformospora indica* may promote the absoption of plant to nutrients, and enhance plant resistance to biotic stresses and abiotic stresses. For example, *Piriformospora indica* could increase rape resistane to drought[3]. Waterlogging is a climatic disaster and secondary to drought in importance. Waterlogging takes place frequently in the the middle and lower regions of the Yangtze River in summer. As a result, the crop production decrease seriously, and even no seeds are gathered[4]. For example, when cotton plants were waterlogged, the lighter disaster lead to the decrease by 10-20% of crop production, and the serious cause to the drop by 50% of production [5]. At the same time, cotto quality is also influenced [6]. For many years, many scholars at home or abroad have focused on a lots of researches on the physiological change of crops under waterlogged disaster, but the study on the prevention of waterlogged disaster and restore after waterlogged disaster have been little carried out.

EXPERIMENTAL SECTION

Chinese cabbage (variety: yellow seedling for four seasons) was sown in the square plastic trays full of sands on March 27, 2012, and growed in light growth incubator. When the seedling growed with four leaves and 1 heart-leaf, we transplanted the consistent growing seedlings into the filelds. the space of them is 20 cm × 25 cm, and there is 14000 plants/667m^2. There were three treatments in our experiment: treatment 1 as the control group, only waterlogged processing(0 days); 2 with *Piriformospora indica* solid manure used as as base fertilizer and waterlogged processing(7 days); 3 with *Piriformospora indica* liquid fertilizer as base fertilizer and waterlogged processing(7 days). Each treatment were repeated 3 times and experimental plots were arranged randomly.The fertilizing amount of *Piriformospora indica* manure is 20 Kg/ 667 m^2 without any organic fertilizer and chemical fertilizer. When Chinese cabbage growed with seven leaves and 1 heart leaf, plants were waterlogged for 7 days. The irrigated depth was in consistant with the heart leaf of Chinese cabbage.The leaves at 0 d and 7 d after waterlogged processing were selected to determine the related indexes. The content of vitamin C was determined using titration method [7]; The soluble sugar content was measured by anthrone method [8]; The nitrate content

was determined by UV spectrophotometry, and Guaiacol-peroxidase activity determination method refere to Zhu etc[9]. SOD, AsA-POD activity determination method refere to Giannopolitis etc[10]，The determination of malondialdehyde content refered to Cakmak, etc[11]，The Content of proline (Pro) refered to Tang etc[12]，Chlorophyll content determination refer to Lü[13]. Each indexes were measured three times, then taken the average.

RESULTS

2.1 Effects of *Piriformospora indica* on growth and developmen of waterlogged Chinese cabbage

Piriformospora indica could influence the growth and development of waterlogged Chinese cabbage. After 7 days of waterlogged disaster, the solid and liquid manure of *Piriformospora indica* may significantly improve plant height and leaf area of Chinese cabbage in comparision with only waterlogged Chinese cabbage (CK). Thereinto, the plant height of Chinese cabbage was 15.3 times higher than that of CK and the leaf area was 3 tims larger than that of CK using the liquid manure of *Piriformospora indica* (Fig. 1).

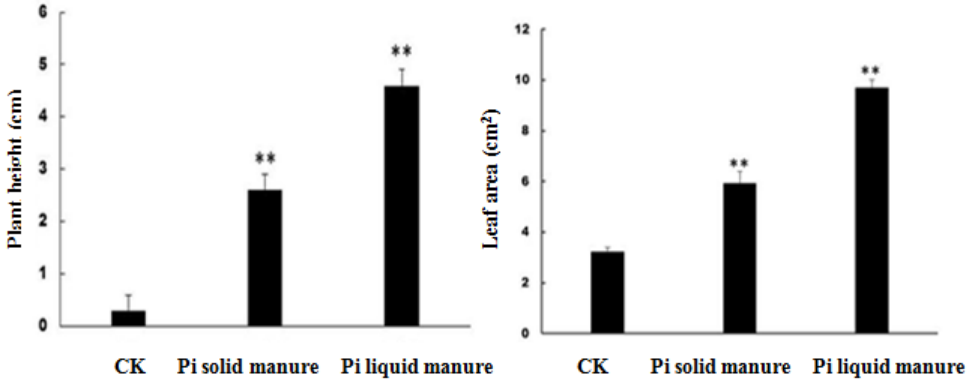

Fig. 1 Effects of *Piriformospora indica* on plant height and leaf area of waterlogged Chinese cabbage

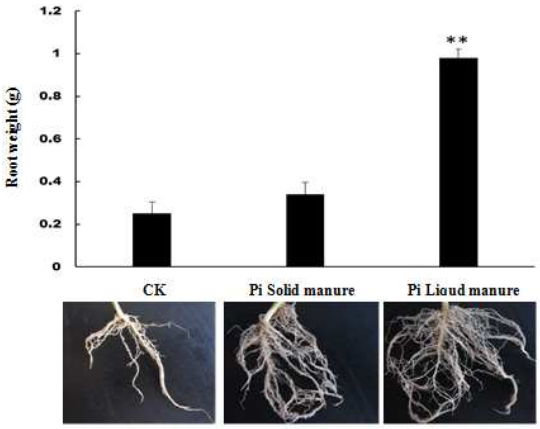

Fig. 2 Effects of *Piriformospora indica* on root developmen of waterlogged Chinese cabbage

As shown in Fig. 2, After 7 days of waterlogged disaster, the solid and liquid manure of *Piriformospora indica* may improve the root weight of Chinese cabbage in comparision with only waterlogged Chinese cabbage (CK) and their latreal and fibrous root of the former is more than the latter. After liquid manure fertilization praticularly, the root weight of Chinese cabbage was 3 time larger than that of CK..

2.2 Influences of *Piriformospora indica* on yield and economic efficiency of waterlogged Chinese cabbage

The average yield of Chinese cabbage per 667 m^2 after fertilizing the liquid or solid manure of *Piriformospora indica* was all higher than CK, and their yield-increase rate was 21.05% and 10.53 respectively. If the price of Chinese cabbage was 2 ¥ per kg, the average economic efficiency of the solid manure was 3,066.82 ¥ /667m^2, and their production was 533.47 ¥ /667m^2. The average economic effenciency of liquid manure was 2800.14 ¥ , and their economic production was 533.47 ¥ /667m^2 (Tab. 1).

Tab.1 Influences of *Piriformospora indica* on yield and economic efficiency of waterlogged Chinese cabbage

treatment	Major heavy（kg/667m^2）	yield-increase rate（%）	economic efficiency（¥/667m^2）	Economic production（¥ /667m^2）
CK	1266.73	--	2533.35	--
Pi Solid manure	1400.07	+10.53	2800.14	266.79
Pi Liquid manure	1533.41	+21.05	3066.82	533.47

Note：The economic efficiency of Chinese cabbage was counted 2 ¥ per kg; Pi: Piriformospora indica

2.3 Effects of *Piriformospora indica* on quality of waterlogged Chinese cabbage

As seen in Table 2, *Piriformospora indica* manure could obviously improve the total soluble sugar content and Reduced Vc content of waterlogged Chinese cabbage, and their content were respectively 4.66% and 3.66% higer using solid manure, or 6.95% and 3.89% higer using liquid manure than CK content. At the same time, *Piriformospora indica* manure could decrease the content of nitrate.

Tab. 2 Influences of *Piriformospora indica* on quality of waterlogged Chinese cabbage

treatment	Reduced Vc (mg·kg^{-1})	growth rate (%)	total soluble sugar (mg•kg^{-1})	growth rate（%）	nitrate (mg·kg-1)	growth rate (%)
CK	923.37	---	10.08	---	628.23	---
Pi Solid manure	966.46	+4.66	10.81	3.66	616.58	-1.85
Pi Liquid manure	987.58	+6.95	31.49	3.89	605.46	-3.62

Note: Pi: Piriformospora indica

2.4 The mechanism research of P. Indica resistance to waterlogging

2.4.1 The determination of *p. Indica* colonizing the roots

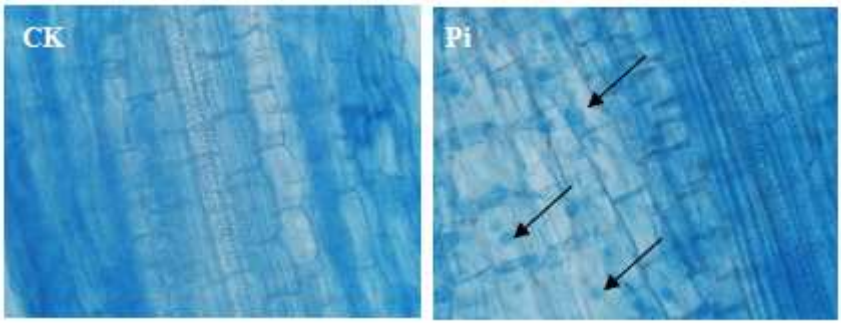

Fig. 3 The determination of *p. Indica* colonizing the roots by Phenolic medan staining method

Fig. 3 showed that *P. Indica* could colonize the root of Chinese cabbage and forme the mycorrhiza. Some research had showed that *P. Indica* could promote plant growth[14], accelerate the absorption of nitrogen and phosphorus [15]when then colonizing the root of plants [16] , improve the tolerance to adversity stress of crops, induced the plants producing the system resistance[1]. Therefore, we concluded that the improving of resistance to water disaste rwas related with *P. Indica* colonizing in Chinese cabbage roots (Fig. 3).

2.4.2 The influence of *P.indica* on the number and groups of rhizosphere microorganisms with Chinese cabbage suffering from water disaster

Tab. 3 The influence of *P. Indica* on the number and groups of rhizosphere microorganisms with the Chinese cabbage suffering from water disaster

treatments	Waterlogging(0d)			Waterlogging(7d)		
	microorganism species（species）	the number of bacteria（10×^{4}）	the number of fungi（10×^{3}）	microorganism species（species）	the number of bacteria（10×^{4}）	the number of fungi（10×^{3}）
CK	6	35	66	6	32	21
Pi Solid manure	7	42	74	7	40	68
Pi Liquid manure	8	43	79	8	43	76

Note: Pi: Piriformospora indica

FromTab. 3 we saw that the quantity of rhizosphere bacteria and fungi had improved greatly compared with CK at the stage of waterlogging 0d. The microbial population reduced little under the waterlogging, but it decreased very

obviously in CK.. Some researches had showed that rhizosphere microorganisms played an important role in formation of the soil fertility and transforming the plant nutrition. so it was the important reason that Chinese cabbage resistance to waterlogging was due to the existence of a large number of rhizosphere microorganisms.

2.4.3 The influence of *P. Indica* on enzyme activity of the Chinese cabbage

Tab. 4 The influence of *P. Indica* on chlorophyll content of the Chinese cabbage suffered from waterlogging

chlorophyll content ($mg \cdot kg^{-1}$)	Ca content		Cb content		CT content	
	waterlogging 0d	waterlogging 7d	waterlogging 0d	waterlogging 7d	waterlogging 0d	waterlogging 7d
CK	13.46	6.04	4.62	2.37	18.03	8.39
Pi Solid manure	9.62	9.68	3.33	3.66	12.97	13.29
Pi Liquid manure	9.96	10.12	3.79	3.89	13.71	13.97

Note: Pi: Piriformospora indica

From Tab.4 we can see that Ca, Cb, and CT reduced significantly in CK, While they were increased by using *P. Indica* fertilizer under the waterlogging. And chlorophyll a, chlorophyll b and total chlorophyll content increased by 67.55% , 64.13% and 66.62% compared to CK by using *P. Indica* liquid fertilizer respectively.

Tab. 5 The influence of *P. Indica* on SOD content of the Chinese cabbage suffered from waterlogging

SOD content ($mmol \cdot g^{-1} \cdot min^{-1}$)	waterlogging 0d	waterlogging 7d
CK	5.42	5.11
Pi Solid manure	5.46	7.16
Pi Liquid manure	5.40	7.35

Note: Pi: Piriformospora indica

From Tab.5 we can see that SOD decreased in CK, but it was increased by using *P. Indica* Fertilizer under waterlogging.This indicated that *P. Indica* could induce the expression of SOD activity, make it reaction to waterlogging stress signal, eliminate excess free radicals in the body of plant positively, and reduce the harm from reactive oxygen species in cabbage damage ultimately.

Tab. 6 The influence of *P. Indica* on MDA content of the Chinese cabbage suffered from waterlogging

MDA content ($umol \cdot g^{-1}$)	Waterlogging (0d)	Waterlogging (7d)
CK	1.42	1.68
Pi Solid manure	1.65	1.16
Pi Liquid manure	1.39	0.77

Tab. 6 showed that the MDA concentration increased in CK， but it showed a decline trend by using the *P. Indica* fertilizer, and MDA concentrations declined more obviously by using the *P. Indica* liquid fertilizer under waterlogging.This indicated that *P. Indica* could alleviate the damage to cell membrane caused by waterlogging of cabbage.

Tab.7 The influence of *P. Indica* on CAT activity of the Chinese cabbage suffered from waterlogging

CAT activity ($U \cdot g^{-1} \cdot min^{-1}$)	waterlogging 0d	waterlogging 7d
CK	20.30	38.49
Pi Solid manure	26.44	71.30
Pi Liquid manure	29.51	98.82

Tab. 7 showed that the CAT activity increased little in CK， but it increased largely by using the *P. Indica* fertilizer, and CAT activity increased more obviously by using the *P. Indica* liquid fertilizer.This indicated that *P. Indica* could improve the plants resistantance to waterlogging by inducing CAT expression under the Waterlogging conditions.

Tab. 8 The influence of *P. Indica* on POD activity of the Chinese cabbage suffered from waterlogging

POD activity ($U \cdot g^{-1} \cdot min^{-1}$)	waterlogging 0d	waterlogging 7d
CK	29.73	21.46
Pi Solid manure	15.89	11.94
Pi Liquid manure	5.91	1.86

Tab. 8 showed that the POD activity reduced little in CK，but it reduced largely by using the *P. Indica* fertilizer, and POD activity reduced more obviously by using the *P. Indica* liquid fertilizer. This suggested that *P. Indica* could

improve the plants resistace to waterlogging by closing POD expression under the Waterlogging conditions.

CONCLUSION

In this study, *P. Indica* could reduce the harm to waterlogging, promote the growth, increase Vc content, protein content, soluble sugar content, and reduce the content of nitrate in Chinese cabbage suffered from waterlogging. But its molecular mechanism of resistance to waterlogging and improving the quality need to be studied further.

P. Indica was a plant endophytic fungi separated from India desert. It was easy to artificial cultivate, and its host range was very wide, It could improve the system disease resistance and stress resistance when it was colonized in the plants .It was friendly to environment ,and had very good commercial development and industrialization prospect of application if it was developed as bio-bacterial manure fertilizer.

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