

Allelopathic effects of root exudates of Chinese onion on tomato growth and the pathogen *Fusarium oxysporum*(Sch1) f.sp. *lycopersici*

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ABSTRACT

Fusarium wilt [caused by *Fusarium oxysporum* (Sch1) f.sp. *lycopersici* (Sacc.) Snyder et Hansen] is a serious soil-borne disease of tomato worldwide, especially in continuous monocropping. Rotations with Chinese onion [*Allium cepa* L. var. *aggregatum* G. Don (syn. var. *multipilcans* Bailey)] improved the tomato growth and reduced the occurrence of Fusarium wilt. We investigated the mechanism underlying this phenomenon. Root exudates of 47 accessions of Chinese onion were assayed for effects on germination and seedling growth of tomatoes in the laboratory and field. Genetic similarity of accessions was determined using ISSR-PCR technique. Allelopathic potential of root exudates of onion different accessions were also assayed on *F. oxysporum* f.sp. *lycopersici*. The root exudates of most Chinese onion accessions stimulated the seedling growth of tomato. The allelopathic potential was accession dependent, and not correlated to DNA-fingerprinting. Two pairs of accessions, 'Sui Hua-Ning An Hongcheng' and 'Wu Chang Hongqishe-Qi Tai He II' with similar DNA-fingerprints but with different allelopathic potential were screened. Their root exudates inhibited the spore germination, mycelial growth, biomass and sporulation of *F. oxysporum* f.sp. *lycopersici*. Sui Hua and Wu Chang Hongqishe was more inhibitory to pathogen than Qi Tai He II and Ning An Hongcheng(except biomass) ($p < 0.05$). Our results suggested that in crop rotations, the root exudates of Chinese onion accessions promoted the tomato growth and decreased the incidence of Fusarium wilt. The accession Sui Hua and Wu Chang Hongqishe decreased the incidence of pathogen and thereby improve the tomato yield.

Key words: Chinese onion, root exudates, allelopathy, ISSR, *F. oxysporum* f.sp. *lycopersici*

INTRODUCTION

Tomato (*Lycopersicon esculentum* Mill.) is major vegetable crop and is often cultivated in greenhouses in China. Fusarium wilt [caused by *Fusarium oxysporum* (Sch1) f.sp. *lycopersici* (Sacc) Snyder et Hansen], is major soil-borne disease worldwide and is difficult to control. It drastically reduces the tomato yield in continuous monocropping (4,30). To overcome this problem, various cropping systems (crop rotation, intercropping, mixed cropping) are used in greenhouses to improve the tomato yield and to reduce the disease (3,24). Many vegetable crops are suitable for intercropping or crop rotation with

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tomato. For example, tomato rotation with alfalfa and soybean for 2 and 3 years increases the tomato yield and after 3-years rotations decreases the nitrogen dose of tomato due to biological nitrogen fixation than grown in continuous monoculture (19). Compared with the continuous tomato monocropping, rotation with garlic influences the tomato quality: (Decrease the nitrate and nitrite contents of tomatoes, increase the ratio of sugar to acid and increase the soluble proteins in tomato). Garlic root exudates have bacteriostatic effects on tomato bacterial wilt (7).

Chinese onion [*Allium. cepa* L. var. *aggregatum* G. Don (syn. var. *multiplcans* Bailey)] is important race of “aggregatum group” of *A. cepa*. Our research showed that cucumber rotation with Chinese onion increases the diversity and evenness indices of bacterial communities in cucumber rhizosphere soil and hence increases the yield of cucumber than its monocropping (12,28,36). The allelopathic potential of cucumber root exudates varied among the different onion accessions (32). Further study showed that rotation with Chinese onion significantly increased the tomato growth, and reduced or delayed the occurrence of *F. oxysporum* f.sp *lycopersici* and these effects were better than cucumber. The same accession of Chinese onion (Shuang Cheng and L04) has variable effects on tomato and cucumber.

This study aimed to investigate the mechanism of increased tomato yield and reduced the fusarium disease. Forty seven Chinese onion accessions were collected from different regions of Chinese provinces of Hei Longjiang and Ji Lin. The allelopathic potential of their root exudates was evaluated on the germination and seedling growth of tomato in laboratory and field experiments. The relationships between DNA-fingerprinting of accessions and their allelopathic potential on tomatoes were also analyzed. Using the results of laboratory and field experiments and employing inter-simple sequence repeat (ISSR) analysis, suitable cultivars with similar DNA-fingerprints and maximum allelopathic potential for rotation with tomatoes were screened. The effects of root exudates’ were also evaluated on spore germination, mycelial growth, biomass and sporulation of *F. oxysporum* f.sp *lycopersici*.

MATERIALS AND METHODS

This study was done from May to October, 2011 at the Horticultural Experimental Station and Vegetable Physiology and Ecology Research Lab, Northeast Agricultural University, Harbin, China. The 47 Chinese onion accessions were collected from Ji Lin and Hei Longjiang provinces in China (Table 1). Seeds of tomato cultivar ‘Moonlight’ were obtained from the Tomato Research Group of our University. Strains of *F. oxysporum* f.sp *lycopersici* were provided by Department of Plant Pathology of our University. The experimental soil was sandy loam black soil (Mollisol) containing 5.61% organic substance, 134.02 mg·kg⁻¹alkaline hydrolytic nitrogen, 47.56 mg·kg⁻¹available phosphorus, 146.19 mg·kg⁻¹available potassium, 0.516 mS·cm⁻¹ conductance rate, and pH 7.01 (1).

Chinese onion bulbs were planted in pots (8 cm × 8 cm × 10 cm). Each pot was filled with 150 g soil with one bulb per pot and 80 bulbs per Chinese onion accession.

There were three replications in completely randomized experimental design. All bulbs

Table 1. Names and source locations of 47 Chinese onion cultivars used in this study. Province abbreviations: JL = Ji Lin; HLJ = Hei Longjiang

No.	Accessions	No.	Accessions
	Origin: Ji Lin (JL)	24	Hei Longjiang
1	Bai Chengll	25	2 Hao
2	Si Liu Ban	26	Yi Chun
3	Du Tou	27	Shuang Yashan
4	Nong An I	28	Yi An II
5	Chang Chun	29	Hei He
6	Ban Zi Cong	30	Qing An
7	Ji Lin I	31	Ning An Hongcheng
8	Bai Cheng I	32	Qi Qi Har
9	Nei Meng	33	Wu Chang Hongqishe
10	Si Ping	34	Lan Xi
11	Ji Lin II	35	Sui Hua
	Origin: Hei Longjiang (HLJ)	36	Xue Jia
12	Xi Lin	37	Shuang Cheng II
13	Yan Jiang Village	38	Sui Ling
14	Bei An	39	Bin Xian I
15	Yi An I	40	Qi Tai He II
16	Wu Chang Lalin	41	3 Hao
17	La Lin	42	Qi Tai He I
18	Fu Jin	43	Wu Chang
19	A Cheng Nanjiao	44	6 Hao
20	Shuang Cheng I	45	Bin Xian II
21	Bai Quan	46	4 Hao
22	Mi Shan	47	Hui Nan
23	A Cheng		

were grown in greenhouse under natural light, with day/night temperatures of 28°C/15°C. After 10 to 15 days, samples of young leaves were collected and frozen at -80°C for DNA extraction for ISSR. Root exudates were collected after 30 days.

I. ALLELOPATHIC STUDIES

Collection and treatment of root exudates: Root exudates were collected as per suggested methods (34,27). Thirty days after planting, the onion plants were carefully uprooted from the pots to avoid root damage. Soil attached to the roots was washed off with running water, and then the roots were rinsed three times with distilled water and twice with deionized water. The plants were placed in hydroponic cultivation channels (60 cm× 40 cm× 15 cm), with 200 plants per channel. Then, 800 ml of deionized water was added. After 24 h, the culture solutions were collected, first filtered through a 3-layer

Buchner's filter and then through 0.45 μm pore size membrane. The filtered root exudates of each Chinese onion accession were diluted to 5 ml·plant⁻¹ and were stored at 4°C until use. Before the test, the root exudates were lyophilized for 12-16 h to yield the dry crude extract. Then, the dry residue of each root exudates was dissolved in water to prepare a concentration of 10 mg·ml⁻¹.

Effects of root exudates of Chinese onion on tomato

Lab Bioassay: Tomato seeds were sterilized in 70% ethanol for 30 s, rinsed four times in sterile water and sterilized again with 10% sodium hypochlorite for 30 min, then rinsed four times in sterile water (9). Twenty- five tomato seeds (receiver plant) were sown in Petri dishes (9 cm dia) lined with two layers of Whatman No.1 filter paper. Root exudates (5 ml) from 47 Chinese onion accessions (donor plant) were added to each Petri dish and sterile water (5 ml) was used as control. Three replicates of each treatment were used. The Petri dishes were placed in a growth chamber at 25 \pm 1 °C in the dark. Every two days, 2 ml root exudates or sterile water were added to each Petri dish. Seeds with radicle protrusion > 1 mm were considered germinated. After 36 h treatment, germination (%) was calculated.

Tomato seeds were placed on two layers of Whatman No. 1 filter paper glass Petri dishes (9 cm dia). Twenty- five tomato seeds were placed on top of filter paper layer. Then, Petri dishes were transferred to growth chamber [25 \pm 1/15 \pm 1 °C with 4000 Lux light for 14/10-hr light/dark photoperiod]. Every two days, 2 ml root exudates or sterile water were added to each Petri dish. The shoot height (SH, cm), root length (RL, cm), Seedling dry weight (SDW, g) were recorded 8 days after incubation.

Pot Culture: Tomato seeds were soaked in hot water (50-60°C) for 15 min, then these seeds were sown in pots (8 cm \times 8 cm \times 10 cm). Each pot was filled with 150 g soil and had one tomato seedling, each treatment comprised of three replicates in a completely randomized experimental design and the mean of 5-measurements per replicate was calculated. All pots were kept in greenhouse [natural light, day/night temperatures of 28°C/15°C]. After 5, 10, 15d tomato sowing, root exudates (15 ml) from 47 Chinese onion accessions (donor plant) were added to each pot and sterile water (15 ml) was used as control. The seedlings height (HS, cm) and dry weight (SDW, g) were recorded after 20 days. The sensitivity index (*RI*) (26) was calculated as under:

$$RI = 1 - C/T \quad (T \geq C) \quad \text{or} \quad RI = T/C - 1 \quad (T < C),$$

Where, *C*: control and *T*: treatment value. Positive *RI* values imply growth promotion and negative values imply growth inhibition. The synthesis effect, *SE*, (20) was calculated as the average of 5-test items.

II. ISSR analysis

Leaf samples, together with mortars and pestles, were frozen at -80°C for 30 min and then the plant material was ground into fine powder. Total DNA was extracted following the manufacturer's protocol using a plant genomic DNA kit (TIANGEN, Beijing, China) and quantity of the extracted DNA was checked on 1.2% agarose gels. The purified DNA was subsequently stored in 1.5 ml tubes at -20°C prior to PCR amplification.

A total of 45 random primers (University of British Columbia sequences, synthesized by Shanghai Sangon Biological Engineering Technology and Services, Shanghai, China) were screened with five test samples for polymorphism and reproducibility. Of these 45 primers, 17 were selected and used for PCR amplification. PCR amplifications were conducted in 25 µl final volumes containing 40 ng of template DNA, 10 µl of 10 mM Tris-HCl (pH 8.3), 2.0 µl of 50 mM KCl, 2.0 µl of 25 mM MgCl₂, 3.0 µL of 2.5 mM dNTPs, 3.0 µL of 100 pM primer, 3.0 µL of 10× PCR Buffer, and 0.5 µL (0.5 units) of *Taq* DNA polymerase (Takara Shuzo Shanghai, China). PCR amplifications were done on a Gradient Thermocycler (Biometra, Goettingen, Germany) under following conditions: initial denaturation at 94 °C for 5 min, followed by 45 cycles at 94°C for 30 s (denaturation), 52°C for 30 s (annealing), and 72°C for 90 s (extension), and a final extension step at 72°C for 10 min. Amplification products were resolved on 1.2% agarose gels in 0.5 × Tris-acetic acid-EDTA buffer, and stained with ethidium bromide (0.5 µg·ml⁻¹). Bands were visualized under UV light using the AlphaImager HP gel documentation system (AlphaImager H P., Alpha Innotech Corp), recorded on Polaroid 667 film, and scored. Product molecular sizes were determined by reference to 100 bp (GE Healthcare, USA) and 1k bp DNA mass ladders (MBI Fermentas, Shenzhen, China). PCR reactions were repeated at least twice.

III. Studies on *Fusarium*

(i). **Spore germination of *F. oxysporum* f.sp *lycopersici***: The concave glass slide method was used (22), a *F. oxysporum* f.sp *lycopersici* spore suspension was mixed with an identical volume of root exudates and two drops of mixture were placed on a concave slide. The concave glass slides with mixture were kept into porcelain plates with lids and grown at 25°C. After 12 h, the spore germination was determined by microscope. Each treatment was replicated six times in a completely randomized experimental design. Finally, the mean rate of spore germination was used for statistical analysis. Sterilized water was used as control.

$$\text{Rate of spore germination (\%)} = \frac{\text{No. germinated spores in control} - \text{No. germinated spores in treatment}}{\text{No. germinated spores in control}} \times 100$$

(ii). **Mycelium growth of *F. oxysporum* f.sp *lycopersici***: Effects of root exudates on mycelium growth were determined by method of Wu *et al* (13). 5 ml root exudates were added to 15 ml PDA culture medium (containing 200 g potatoes, 20 g agar and 20 g glucose per 1 L). The final concentration of root exudates in PDA medium was 25%. Sterilised water was used as control. Equal numbers of spores of *F. oxysporum* f.sp *lycopersici* with uniform age were inoculated in each culture medium. The culture plates were placed upside down and incubated at 28 °C. The plates were observed after 24, 48, 72, 96, and 120 h. The diameter of mycelia was measured with a Vernier Caliper. Each treatment comprised of three replicates and the mean of 5-measurements per replicate was calculated.

(iii). **Pathogen biomass of *F. oxysporum* f.sp *lycopersici*:** Effects of root exudates on pathogen biomass was determined by method of Yang *et al* (31). Five ml root exudates were added to 15 ml PDA culture medium (containing 200 g potatoes, 20 g agar and 20 g glucose per 1 L). The final concentration of root exudates in PDA medium was 25%. Sterilised water was used as control. Equal numbers of spores of *F. oxysporum* f.sp *lycopersici* with uniform age were inoculated in each culture medium. The culture plates were placed upside down and incubated at 28 °C. The microbial biomass was determined by dry weight colorimetry (620 nm) after 120 h. Each treatment comprised of three replicates and the mean of five measurements per replicate was calculated.

(iv). **Sporulation quantity of *Fusarium oxysporum*:** Effects of root exudates on Sporulation quantity of *F. oxysporum* f.sp *lycopersici* was determined by method of Yang *et al* (31). The methods used for pathogens biomass was also used in sporulation studies. Sporulation was observed with suspensions prepared with 15 mL diluents under inverted phase contrast microscope and quantity were counted with blood cell counting, three replicates and the mean of five measurements per replicate was calculated.

Statistical analysis

Data of all experiment results were analyzed by Microsoft Office Excel 2003 or ANOVA first. Mean of each experiment were compared by Least Significant Differences at the 5% and 1% level using SAS 8.2 statistical software. Only distinct and reproducible bands produced by ISSR primers were scored as “present” (1) or “absent” (0) for the potato onion cultivars included in this research. A binary data matrix was prepared and used to calculate Jaccard’s similarity coefficient (21) between all possible pairs of accessions. A dendrogram (cluster diagram) was constructed from the calculated similarity coefficient for ISSR, using the unweighted pair group method with mathematical averages (UPGMA) as implemented in NTSYS-pc v2.02 (18).

RESULTS

Lab Bioassay

Root exudates of all Chinese onion accessions increased the germination of tomato seeds (Table 2), except the accession 6 Hao. Root exudates of Shuang Yashan and Yi Chun caused maximum stimulation than others ($p < 0.01$). Root exudates of 38 accessions stimulated the tomato root elongation ($p < 0.01$). The root exudates of 7- accessions (2 Hao, Bai Quan, Nong An I, Fu Jin, Ban Zi Cong, Yan Jiang Village, Shuang Cheng I) inhibited the growth of tomato seedling shoots but 4-accessions (2 Hao, Nong An I, Bai Quan, Shuang Cheng I) were most inhibitory ($p < 0.01$). The root exudates of 33 accessions stimulated the shoot growth of tomato seedlings ($p < 0.01$). The dry weight per plant of tomato seedling was stimulated by root exudates of all accessions, except the Xi Lin and Mi Shan. Five accessions (Yan Jiang Village, Qing An, 4 Hao, A Cheng Nanjiao, Bai Cheng I) were more stimulatory than others ($p < 0.01$).

Table 2. Effects of Chinese onion root exudates on tomato seed germination and seedling growth in Lab bioassay

Chinese onion accession	Germination (%)	Seedling growth		
		Root length (cm)	Shoot length (cm)	Dry weight/plant (mg)
Control (DW)	20.0±1.4	5.0±0.3	4.6±0.6	2.6±0.7
Origin: Ji Lin (JL)				
Bai Cheng II	21.8±1.0*	7.3±0.6**	5.5±0.1**	3.4±0.7*
Si Liu Ban	22.8±1.3	7.8±0.37**	5.6±0.3**	2.7±0.2
Du Tou	21.8±1.0*	6.7±0.5**	5.3±0.2**	3.2±0.1*
Nong An I	23.0±1.6*	6.6±1.0*	4.1±0.3**	3.0±0.3
Chang Chun	21.8±1.0*	7.5±0.6**	5.4±0.1**	3.1±0.3
Ban Zi Cong	22.3±1.0*	5.8±1.2*	4.3±0.5**	3.4±0.6
Ji Lin I	22.0±0.8*	5.7±1.1	5.1±0.3**	3.0±0.3
Bai Cheng I	22.3±1.0*	7.4±0.6**	5.8±0.2**	3.5±0.5**
Nei Meng	22.0±0.8*	7.6±0.3**	6.2±0.4**	3.0±0.3
Si Ping	22.0±1.2*	7.7±0.6**	5.7±0.4**	3.4±0.4*
Ji Lin II	20.8±1.5	7.5±0.8**	6.0±0.1	3.1±0.2
Origin: Hei Longjiang (HLJ)				
Xi Lin	22.0±0.8*	6.6±0.7*	4.8±1.0*	2.6±0.4
Yan Jiang Village	23.0±0.8*	6.1±0.2*	4.3±0.4*	3.5±0.3**
Bei An	21.5±0.6*	7.9±0.5**	5.1±0.2**	3.4±0.5*
Yi An I	22.3±1.3*	8.6±0.6**	5.9±0.4**	3.1±0.6
Wu Chang Lalin	22.3±1.0*	7.8±0.6**	6.3±0.3**	3.1±0.5*
La Lin	23.3±1.0*	6.8±0.5**	4.9±0.2**	3.2±0.1*
Fu Jin	23.0±0.8*	6.0±1.1*	4.4±0.2*	2.8±0.4
A Cheng Nanjiao	21.2±1.0*	7.1±1.3**	5.2±0.4**	3.7±0.4**
Shuang Cheng I	22.5±0.6*	6.8±0.6**	4.4±0.2**	3.2±0.1*
Bai Quan	22.8±0.5*	6.5±0.5*	4.1±0.4**	2.9±0.1
Mi Shan	22.8±1.3*	6.3±0.1**	4.6±0.2**	2.6±0.3
A Cheng	21.5±0.6*	8.9±0.3**	5.9±0.1**	3.2±0.4*
Hei Longjiang	21.3±1.0*	8.0±0.4**	5.8±0.2**	3.3±0.6*
2 Hao	22.3±1.7*	7.2±0.5**	4.3±0.1**	2.9±0.3
Yi Chun	23.3±0.5**	8.1±0.8**	5.5±0.1**	3.0±0.1
Shuang Yashan	24.0±0.8**	5.9±0.9**	4.6±0.4*	2.9±0.4
Yi An II	21.5±1.3*	7.4±0.6**	5.3±0.2**	3.1±0.4
Hei He	21.5±1.3*	7.6±0.9**	6.1±0.4**	3.1±0.5
Qing An	21.3±2.1	8.4±1.4**	5.5±0.3**	3.5±0.7**
Ning An Hongcheng	21.5±0.6*	7.5±0.9**	5.2±0.2**	3.0±0.3
Qi Qi Har	21.0±0.0*	7.7±0.8**	5.9±0.3**	3.2±0.3
Wu Chang Hongqishe	22.3±1.0*	9.1±0.7**	6.1±0.5**	3.0±0.4
Lan Xi	21.8±1.0*	8.6±1.0**	5.0±0.2**	3.1±0.4
Sui Hua	21.8±0.5*	9.3±0.5**	5.9±0.3**	3.1±0.3
Xue Jia	22.5±1.0*	7.1±0.4**	5.9±0.4**	2.8±0.2
Shuang Cheng II	21.5±0.6*	8.2±0.5**	5.6±0.1**	3.1±0.3
Sui Ling	20.8±1.0*	8.2±1.4**	5.7±0.2**	3.2±0.0*
Bin Xian I	22.8±1.0*	7.8±0.5**	5.7±0.2**	2.8±0.2
Qi Tai He II	22.5±1.3*	7.8±0.4**	4.6±0.5**	2.9±0.2
3 Hao	22.0±1.2*	6.9±0.7**	5.5±0.4**	3.1±0.5
Qi Tai He I	22.0±1.8*	8.0±0.4**	5.8±0.3**	3.1±0.3
Wu Chang	21.0±0.8*	8.1±0.7**	5.8±0.1**	3.0±0.1*
6 Hao	20.0±0.8*	7.8±0.6**	5.7±0.3**	3.3±0.3*
Bin Xian II	22.0±1.4*	7.6±0.4**	5.4±0.5**	3.3±0.9**
4 Hao	20.3±1.0*	8.7±0.4**	5.9±0.3**	3.6±0.4**
Hui Nan	21.3±1.0	7.7±0.7**	5.2±0.2**	3.2±0.7

DW: Distilled water, *and **within a column differ significantly at 0.05 and 0.01 level over control, respectively.

The allelopathic effects reflect the allelopathic potential of a donor plant on an acceptor plant. At the test exudates concentration (10 mg·ml⁻¹) used in this experiment, the root exudates of all accessions stimulated the tomato seedling growth (*SE* values of 0.13-0.39, Table 3).

Table 3. Allelopathic potential of onion accessions root exudates on tomato seed germination and seedling growth in Lab bioassay

Chinese onion accession	Germination (%)	Root length (cm)	Shoot length (cm)	Seedling dry weight (mg)	Allelopathy synthesis effect (<i>SE</i>)
Control (DW)	0.00	0.00	0.00	0.00	0.00
Origin: Ji Lin (JL)					
Bai Cheng II	0.09	0.47	0.19	0.31	0.28
Si Liu Ban	0.14	0.57	0.23	0.04	0.27
Du Tou	0.09	0.34	0.14	0.23	0.23
Nong An I	0.12	0.38	0.01	0.30	0.20
Chang Chun	0.09	0.51	0.18	0.19	0.27
Ban Zi Cong	0.09	0.21	0.05	0.48	0.21
Ji Lin I	0.10	0.15	0.12	0.15	0.13
Bai Cheng I	0.11	0.49	0.26	0.35	0.33
Nei Meng	0.10	0.52	0.36	0.15	0.31
Si Ping	0.10	0.54	0.25	0.31	0.30
Ji Lin II	0.04	0.50	0.30	0.19	0.25
Origin: Hei Longjiang (HLJ)					
Xi Lin	0.07	0.38	0.17	0.13	0.19
Yan Jiang Village	0.12	0.28	0.07	0.52	0.24
Bei An I	0.08	0.58	0.10	0.31	0.27
Yi An I	0.11	0.73	0.30	0.19	0.36
Wu Chang Lalin	0.11	0.56	0.37	0.19	0.34
La Lin	0.13	0.42	0.21	0.39	0.28
Fu Jin	0.12	0.26	0.08	0.22	0.17
A Cheng Nanjiao	0.04	0.49	0.27	0.61	0.37
Shuang Cheng I	0.10	0.42	0.08	0.39	0.25
Bai Quan	0.11	0.36	0.02	0.26	0.18
Mi Shan	0.11	0.31	0.13	0.13	0.18
A Cheng	0.08	0.78	0.29	0.23	0.36
Hei Longjiang	0.06	0.60	0.26	0.27	0.32
2 Hao	0.09	0.50	0.04	0.26	0.24
Yi Chun	0.16	0.62	0.20	0.15	0.32
Shuang Yashan	0.17	0.23	0.14	0.26	0.20
Yi An II	0.08	0.48	0.15	0.19	0.25
Hei He	0.08	0.53	0.34	0.19	0.31
Qing An	0.06	0.69	0.19	0.35	0.28
Ning An Hongcheng	0.08	0.51	0.13	0.15	0.22
Qi Qi Har	0.05	0.55	0.28	0.23	0.30
Wu Chang Hongqishe	0.11	0.82	0.33	0.15	0.38
Lan Xi	0.09	0.73	0.09	0.19	0.28
Sui Hua	0.09	0.87	0.28	0.19	0.39
Xue Jia	0.13	0.42	0.28	0.08	0.21
Shuang Cheng II	0.08	0.65	0.22	0.19	0.29
Sui Ling	0.04	0.65	0.24	0.23	0.32
Bin Xian I	0.14	0.57	0.23	0.08	0.28
Qi Tai He II	0.13	0.57	0.00	0.12	0.20
3 Hao	0.10	0.37	0.19	0.19	0.24
Qi Tai He I	0.10	0.61	0.26	0.19	0.30
Wu Chang	0.05	0.62	0.26	0.15	0.29
6 Hao	0.00	0.55	0.24	0.27	0.29
Bin Xian II	0.10	0.53	0.17	0.27	0.27
4 Hao	0.01	0.74	0.28	0.38	0.37
Hui Nan	0.06	0.54	0.13	0.23	0.26

DW: Distilled water.

Pot Culture

The results of tomato seedling growth in pots were consistent with the laboratory experiments, namely, most root exudates of Chinese onion accessions increased the tomato growth (Table 4).

Table 4. Effect of Chinese onion root exudates on tomato in Pot experiment

Chinese onion accession	Seedling height (cm)	Seedling dry weight (mg/plant)
Control (DW)	13.0±0.9	5.2±0.6
Origin: Ji Lin (JL)		
Bai Cheng II	17.3±1.0*	6.7±0.1*
Si Liu Ban	18.2±1.1**	5.4±0.1
Du Tou	16.2±0.9*	6.3±0.3*
Nong An I	14.5±0.9	5.9±0.5
Chang Chun	17.6±0.9*	6.1±0.0
Ban Zi Cong	13.6±1.2	6.7±0.2*
Ji Lin I	14.7±1.0	5.9±0.5
Bai Cheng I	17.8±1.1*	6.9±0.5**
Nei Meng	18.6±0.9**	5.9±0.3*
Si Ping	18.1±0.5**	6.7±0.0*
Ji Lin II	18.2±1.0	6.1±0.5*
Origin: Hei Longjiang (HLJ)		
Xi Lin	15.3±3.08*	5.2±0.5
Yan Jiang Village	14.0±1.9	6.9±0.3**
Bei An	17.6±1.4*	6.7±0.3*
Yi An I	19.7±1.0**	6.9±0.6**
Wu Chang Lalin	19.0±1.0**	6.4±0.6*
La Lin	15.8±0.6	6.3±0.1
Fu Jin	14.0±0.5	5.5±0.1
A Cheng Nanjiao	16.6±0.6*	7.3±0.0**
Shuang Cheng I	15.1±0.8	6.3±0.0
Bai Quan	14.3±1.2	5.7±0.5
Mi Shan	14.7±1.9	5.2±0.5
A Cheng	20.0±1.1**	6.3±0.2*
Hei Longjiang	18.6±1.2**	6.5±0.2*
2 Hao	15.4±0.51*	5.7±0.6**
Yi Chun	18.4±1.2	6.9±0.1
Shuang Yashan	14.2±3.1	5.7±0.3
Yi An II	17.2±1.4*	6.1±0.5
Hei He	18.6±1.0**	6.1±0.2
Qing An	18.8±1.0**	6.9±0.5**
Ning An Hongcheng	17.2±1.3*	5.9±0.5
Qi Qi Har	18.4±1.0	6.3±0.1*
Wu Chang Hongqishe	20.5±1.2**	6.9±0.0*
Lan Xi	18.4±1.0**	6.1±0.5
Sui Hua	20.5±1.5**	7.0±0.5**
Xue Jia	17.6±1.9*	5.5±0.0
Shuang Cheng II	18.6±1.1**	6.1±0.6*
Sui Ling	18.9±1.0	6.3±0.0
Bin Xian I	18.2±0.9**	5.5±0.3
Qi Tai He II	16.7±0.8*	5.7±0.0
3 Hao	16.6±1.2**	6.1±0.0
Qi Tai He I	18.6±1.3**	6.1±0.0
Wu Chang	18.77±1.0**	5.9±0.3
6 Hao	18.1±1.1*	6.5±0.0*
Bin Xian II	17.6±1.3*	6.5±0.1*
4 Hao	19.7±0.7**	7.1±0.6**
Hui Nan	17.4±1.1	6.3±0.0

DW: Distilled water, * and ** within a column differ significantly at 0.05 and 0.01 level over control, respectively.

Root exudates of most accessions stimulated the tomato seedling height, and root exudates of 22 accessions were more stimulatory than others ($p < 0.01$). The dry weight per plant of tomato seedling was stimulated by root exudates of most accessions, while 2-accessions

Table 5. Allelopathic potential of Chinese onion accessions root exudates on tomato in pot culture

Chinese onion accession	Seedling height (cm)	Seedling dry weight (mg)	Allelopathy synthesis effect (SE)
Control (DW)	0.00	0.00	0.00
Origin: Ji Lin (JL)			
Bai Cheng II	0.25	0.23	0.23
Si Liu Ban	0.29	0.04	0.17
Du Tou	0.20	0.19	0.21
Nong An I	0.10	0.13	0.14
Chang Chun	0.26	0.16	0.22
Ban Zi Cong	0.05	0.23	0.19
Ji Lin I	0.12	0.13	0.12
Bai Cheng I	0.27	0.26	0.27
Nei Meng	0.30	0.13	0.22
Si Ping	0.28	0.23	0.23
Ji Lin II	0.29	0.16	0.17
Origin: Hei Longjiang (HLJ)			
Xi Lin	0.15	0.00	0.11
Yan Jiang Village	0.08	0.26	0.19
Bei An	0.26	0.23	0.23
Yi An I	0.34	0.16	0.25
Wu Chang Lalin	0.32	0.16	0.24
La Lin	0.18	0.19	0.20
Fu Jin	0.08	0.07	0.11
A Cheng Nanjiao	0.22	0.30	0.29
Shuang Cheng I	0.14	0.19	0.19
Bai Quan	0.09	0.10	0.12
Mi Shan	0.12	0.00	0.11
A Cheng	0.35	0.19	0.24
Hei Longjiang	0.30	0.21	0.25
2 Hao	0.16	0.10	0.17
Yi Chun	0.29	0.13	0.23
Shuang Yashan	0.09	0.10	0.13
Yi An II	0.24	0.16	0.21
Hei He	0.30	0.16	0.23
Qing An	0.31	0.26	0.20
Ning An Hongcheng	0.24	0.13	0.16
Qi Qi Har	0.29	0.19	0.24
Wu Chang Hongqishe	0.37	0.13	0.24
Lan Xi	0.29	0.16	0.20
Sui Hua	0.37	0.16	0.26
Xue Jia	0.26	0.07	0.12
Shuang Cheng II	0.30	0.16	0.20
Sui Ling	0.31	0.19	0.25
Bin Xian I	0.29	0.07	0.19
Qi Tai He II	0.23	0.10	0.14
3 Hao	0.22	0.16	0.20
Qi Tai He I	0.30	0.16	0.21
Wu Chang	0.31	0.13	0.21
6 Hao	0.28	0.21	0.24
Bin Xian II	0.26	0.21	0.21
4 Hao	0.34	0.28	0.28
Hui Nan	0.26	0.19	0.21

(Xi Lin and Mi Shan) were inhibitory ($p < 0.01$). The *SE* values of the 47 Chinese onion accessions ranged from 0.11 to 0.29 (Table 5).

Combining the laboratory and pot culture results, among the 47 onion cultivars the 10-accessions (SuiHua, Wu Chang Hongqishe, A Cheng Nanjiao, 2 Hao, Yi An I, A Cheng, Wu Chang Lalin, Bai Cheng I, Sui Ling and Hei Longjiang (arranged in Decending Order) had highest allelopathic potential. While, the 10 accessions with lowest allelopathic potential were Ji Lin I, Fu Jin, Mi Shan, Bai Quan, Xi Lin, Shuang Yashan, Nong An I, Qi Tai He II, Ban Zi Cong and Ning An Hongcheng (arranged in Decending Order).

Screening of different allelopathic potential Chinese onion accessions

The genetic diversity of 47 Chinese onion accessions was determined using ISSR maker as per Liu (15). The genetic similarity of 47 Chinese onion accessions varied from 0.4969 to 0.8616. The varieties were classified into six groups ($D= 0.72$) by cluster analysis of ISSR markers (Fig.1). Combining the results of 47 allelopathic potential determination and ISSR, there was no significant correlation between the allelopathic potential of root exudates of Chinese onion accessions and DNA-fingerprinting. To investigate the underlying mechanism of allelopathy and screen the suitable accessions for rotation with tomato, of the 47 accessions two pairs of accessions [Sui Hua-Ning An Hongcheng] and [Wu Chang Hongqishe-Qi Tai He II] which had similar DNA-fingerprinting and maximum differences in allelopathic effect were screened. This screening aimed to provide, for the first time, information on the genetic mechanism for the allelopathy, whilst keeping the similar genetic background.

Tomato Fusarium wilt

The exudates of all 4-accessions of Chinese onion (Sui Hua, Ning An Hongcheng, Wu Chang Hongqishe, Qi Tai He II) differing in allelopathic potential inhibited the *F. oxysporum* f.sp *lycopersici* spore germination during 120 h of cultivation and the rate of spore germination was 24.94%-43.7% (Fig.2). The accessions Sui Hua and Wu Chang Hongqishe were inhibitory to pathogen than Qi Tai He II and Ning An Hongcheng ($p < 0.05$) and control ($p < 0.05$). The degree of pathogen sporulation quantity was similar to spore germination. The accessions Sui Hua and Wu Chang Hongqishe were significantly inhibited the sporulation than Qi Tai He II and Ning An Hongcheng and control ($p < 0.05$, Fig.3). The root exudates of 4-accessions significantly inhibited the mycelium growth (Fig.4). After 24 h culture, Sui Hua was more inhibitory than control, whereas the other accessions were not significantly different from control ($p < 0.05$). While after 48 h culture, Sui Hua and Wu Chang Hongqishe were significantly inhibitory than control ($p < 0.05$) and after 96 h culture, Sui Hua and Wu Chang Hongqishe were significantly more inhibitory than Qi Tai He II and Ning An Hongcheng ($p < 0.05$) i.e. they all differed significantly from control ($p < 0.05$). After 120 h culture, all root exudates from the Chinese onions except Ning An Hongcheng root exudates significantly inhibited the pathogen biomass than control ($p < 0.05$, Fig. 5).

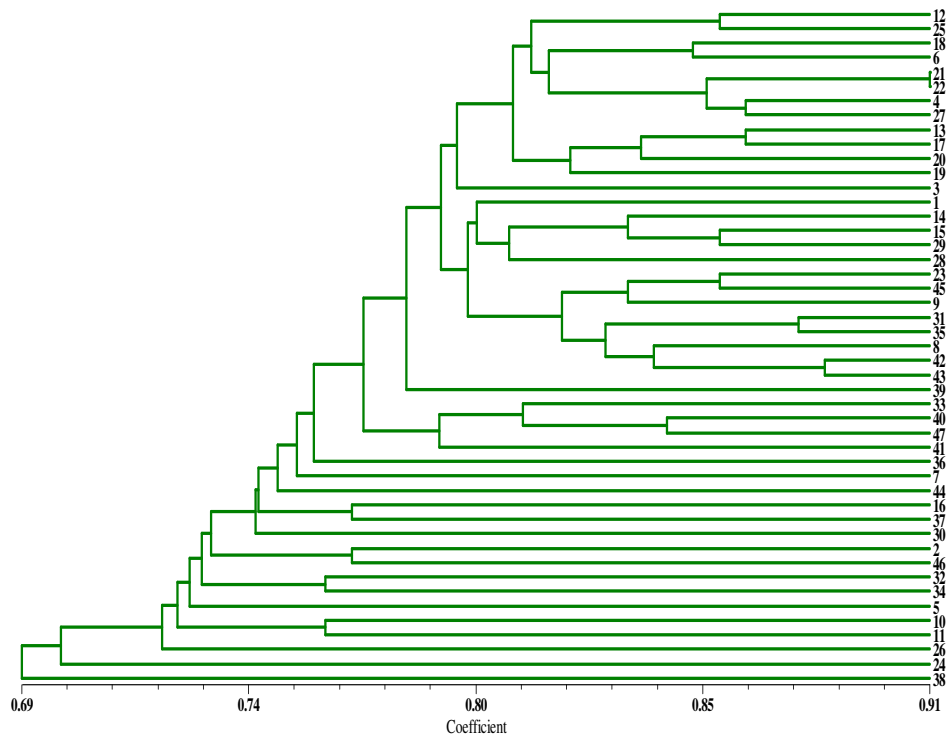


Figure 1. Dendrogram generated from UPGMA cluster analysis of ISSR data from 47 Chinese onion accessions. The accession numbers 1- 47 on the right side of diagram correspond to List in Table 1.

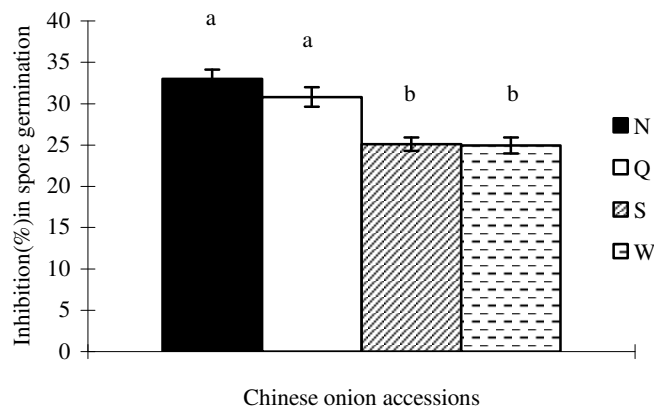


Figure 2. Inhibitory effects of 4-Chinese accessions on the spore germination of *Fusarium*. S: Sui Hua, N: Ning An Hongcheng, W: Wu Chang Hongqishe, Q: Qi Tai He II, Values followed by a different letter within a column differ significantly at the 0.05 level by LSD test.

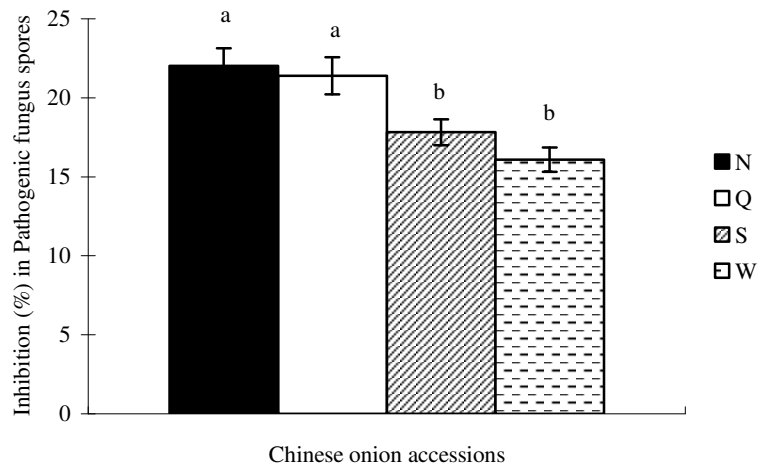


Figure 3. Inhibitory effects of 4-Chinese accessions on Pathogenic fungus spores of *Fusarium*. S: Sui Hua, N: Ning An Hongcheng, W: Wu Chang Hongqishe, Q: Qi Tai He II, Values followed by a different letter within a column differ significantly at the 0.05 level by LSD test.

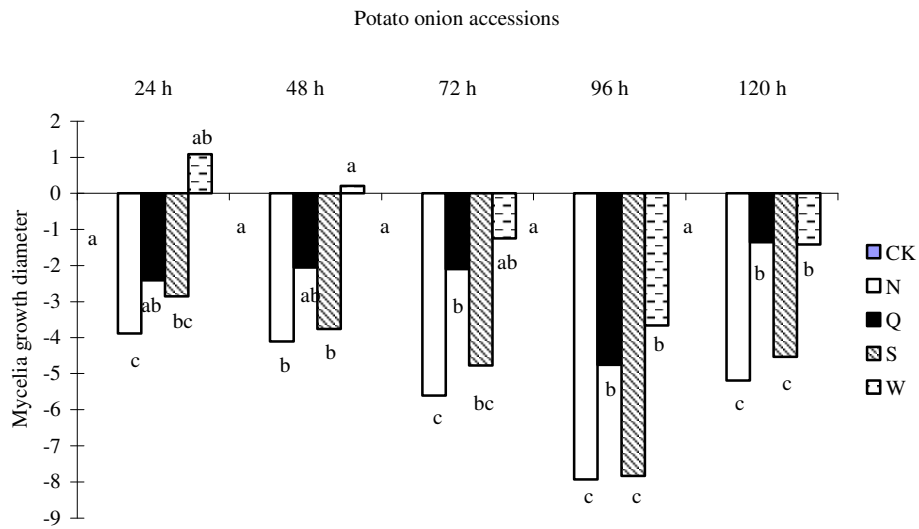


Figure 4. Effects of 4-Chinese accessions on *Mycelia* growth of *Fusarium* compared to control. S: Sui Hua, N: Ning An Hongcheng, W: Wu Chang Hongqishe, Q: Qi Tai He II, Values followed by a different letter within a column differ significantly at the 0.05 level by LSD test.

DISCUSSION

Allelopathic effects of root exudates on tomato

It is known that allelopathy in crops correlates significantly with the crop variety (16). The difference in the allelopathic potential among crop varieties results from the different types and concentrations of allelochemicals that different crop varieties produce and release (11). There are significant differences among different donor cultivars with the same concentration on the same acceptor plant (6,13,29). In this study, allelopathy effects of root exudates from Chinese onion accessions on seed germination and tomato growth were variable in different varieties at 10 mg·ml⁻¹ concentration. This is consistent with previous reports (13,29). Among the 47 onion accessions, the 10-accessions with highest allelopathic potential were: Sui Hua, Wu Chang Hongqishe, A Cheng Nanjiao, 2 Hao, Yi AnI, A Cheng, Wu Chang Lalin, Bai Cheng I, Sui Ling, and Hei Longjiang (arranged in Decending Order). While the 10 lowest allelopathic potential accessions were: Ji Lin I, Fu Jin, Mi Shan, Bai Quan, Xi Lin, Shuang Yashan, Nong An I, Qi Tai He II, and Ban Zi Cong (arranged in Decending Order).

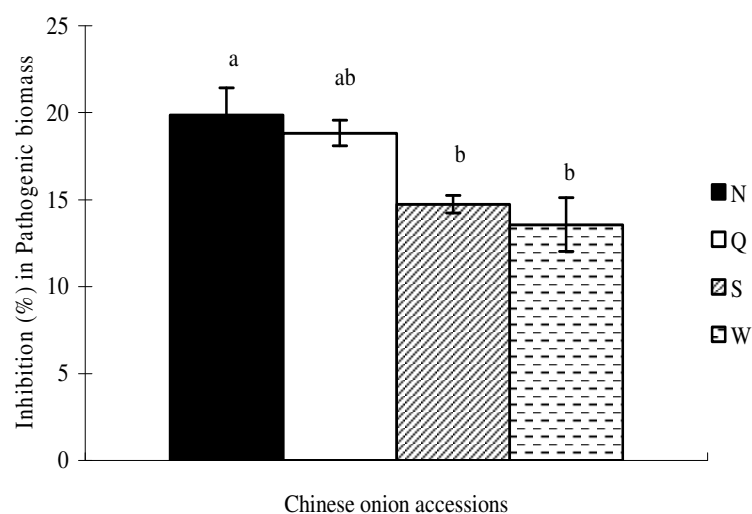


Figure 5. Inhibitory effects of 4-Chinese accessions on pathogenic biomass of *Fusarium*. S: Sui Hua, N: Ning An Hongcheng, W: Wu Chang Hongqishe, Q: Qi Tai He II, Values followed by a different letter within a column differ significantly at the 0.05 level by LSD test.

The correlation between genetic similarity and allelopathic potential

Recently much work has been done on correlation between the allelopathic activity of different donor plants on an acceptor plant and their genetic basis. Of 347 wheat accessions, 30 varieties with strong allelopathic potential were shown to have considerable variation in agronomic traits. Thus, there is weak genes chain between the allelopathy genes and other genes determined by agronomic traits (5). The similar results were found in rice and wheat. Some cultivars with high allelopathic potential, small genetic distance

and from similar geographical regions were clustered in one group, implying that the gene conferring allelopathy might be alleles. However, there were many cultivars in one group which had great variability in allelopathic potential, small genetic difference and similar geographical location (8,10,23). Thus there is no significantly close chain between the allelopathic and agronomic traits and it provides an important basis for genetic and breeding research of allelopathy. Some researchers believed the reason for specific resources with smaller proportions can be attributed to oriented selection for other traits in breeding programmes. So it is easy to find strongly allelopathic potential cultivars from local varieties or the ancestor wild plants (11,17).

In this study, the ISSR cluster was significantly associated with the genetic races and different geographical regions. There was no significant correlation between the DNA-fingerprinting and allelopathic potential. This is because there was a considerable allelopathic variation in potato germplasms, which had genetic similarity and at the same time, there was significant variation in genetic similarity of potato germplasms with the same allelopathic potential. However, some Chinese onion accessions with higher allelopathic potential (Sui Hua, Bai Cheng I, Wu Chang Lalin and Yi An I or Hei Longjiang, Yi Chun, Si Ping and Sui Ling) or lower allelopathic potential (Xi Lin, Fu Jin, Bai Quan, Nong An I, Mi Shan) were clustered together. It implied that the genes conferring the allelopathy may be alleles. Also some cultivars with different allelopathic potential could be clustered into the same group, performing lower level of genetic polymorphism, which is attributed to selection oriented for the other traits in breeding programmes. Genetic variation caused by alleles was determined by quantitative traits, but whether allelopathy in the Chinese onion was quantitative traits requires further study.

Effect of root exudates on tomato Fusarium wilt

The study showed that various cultivars exhibited different resistance to plant disease. This could be reflected in the root exudates (2). Wang postulated that root exudates from sand culture stimulated *Verrucillium wilt* spore germination and mycelium growth and root exudates from different cultivars showed stimulatory effects (25). The root exudates from disease-sensitive cucumber cultivars stimulated the cucumber Fusarium wilt, whereas, the effect of disease-resistant cultivars is not reported (13). The inhibitory effects of root exudates are related to their carbohydrate and amino acids content. The carbohydrate content in root exudates of disease-resistant cotton cultivars was significantly lower than in disease-sensitive cultivars (35). At 0.6 g·L⁻¹ or less concentration, methionine and phenylalanine inhibits the growth and development of *F. oxysporum* f.sp. *neviium* (14). In this study, root exudates of 4- Chinese onions accessions inhibited the spore germination, mycelium growth, sporulation quantity and pathogen biomass of Fusarium wilt. The inhibitory effects varied with accessions and the accessions with high allelopathic potential had stronger inhibitory effects on tomato Fusarium wilt than those with low allelopathic potential. The relationship between the composition of root exudates and their inhibitory effects will be important focus in future studies.

The application of plant allelopathy in cropping systems is mainly focussed on the cropping arrangement (33). Interplanting of cucumber with Chinese onion can improve the microecological environment of cucumber rhizosphere soil and thus increases the cucumber yield (37). In this study, we found that the allelochemicals released from root exudates of Chinese onion accessions were actively involved in the promotion of tomato

growth and lessen the incidence of Fusarium wilt during crop rotations. Thus crop rotation with Chinese onion (Sui Hua and Wu Chang Hongqishe) is essential to reduce the incidence of tomato Fusarium wilt and thereby to increase the tomato yield.

CONCLUSIONS

In crop rotations, the allelochemicals released from root exudates of Chinese onion accessions promoted the tomato growth and reduced the incidence of Fusarium wilt. Accessions Sui Hua and Wu Chang Hongqishe had allelopathic potential to minimize the incidence of pathogen and to improve tomato yields, hence were found suitable for tomato-Chinese onion rotations.

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REFERENCES

1. Bao, S.D. (2000). *Soil Assay on Properties of Agrochemistry*. China Agriculture Press, Beijing.
2. Buxton, E.W. (1962). Root exudates from banana and their relationship to strains of the Fusarium causing Panama wilt. *Annals of Applied Biology* **50**: 269-282.
3. Campiglia, Mancinelli, R., Radicetti, E. and Caporali, F. (2010). Effects of cover crops and mulches on weed control and nitrogen fertilization in tomato (*Lycopersicon esculentum* Mill.). *Crop Protection* **29**: 354-363.
4. Cuartero J., Bolarin M.C. and Asins M.J. (2006). Increasing salt tolerance in the tomato. *Journal of Experimental Botany* **57**: 1045-1058.
5. Dilday, R.H., Yan, W.G., Moldenhauer, K.A.K and Gravois, K.A. (1998). Allelopathic activity in rice for controlling major Aquatic weeds. In: *Allelopathy in Rice* (Ed., Olofsdotter) Pp. 7-26, International Rice Research Institute, Manila, Philippines.
6. Geng, G.D., Cheng, Z.H., Zhang, S.Q., Meng, H.W. and Jiao, H.R. (2005). Allelopathy of tomato varieties. *Chinese Agricultural Science Bulletin* **21**: 314-316. (Chinese).
7. Hao, L.X. (2010). *Study on Biological Effects of Rotation Tomato/Garlic Under Plastic Tunnel*. Master's Thesis, Northwest Agricultural and Forest University, Yangling, China.
8. He, H., Qin., Jia, X.L., Liang, Y.Y., Shen, L.H., Song, B.Q., Guo, Y.C., Liang, K.J. and Lin, W.X. (2004). Assesment of Genetic Diversity of Allelopathic Rice Germplasm Based on RAPD and ISSR. *Acta Genetica Sinica* **31**: 888-894. (Chinese).
9. Inderjit and Dakshini, KMM. (1995). On laboratory bioassays in allelopathy. *The Botanical Review* **61**: 28-44.
10. Joshi, C.P. and Nguyen, H.T. (1993). RAPD analysis based intervarietal genetic relationship among hexaploid wheat. *Plant Science* **93**: 95-103.
11. Kong, C.H., Hu, F., Chen, X.H., Chen, Y.P. and Huang, S.S. (2002). Assessment and utilization of allelopathic Crop varietal resources. *Scientia Agricultura Sinica* **35**:1159-1164. (Chinese).
12. Li, Q.H., Wu, F.Z. and Yang, Y. (2009). Effects of rotation and interplanting on soil bacterial communities and cucumber yield. *Acta Agriculturae Scandinavica Section B-Soil and Plant Science* **59**: 431-439.
13. Li, Z.H. and Shen, Y.X. (2006). A study on allelopathy of winter regrowth from ten varieties of *Medicago sativa*. *Acta Prataculturae Sinica* **4**: 36-42. (Chinese).

14. Liu, B., We, F.Z., Yang, Y. and Wang, X.Z. (2009). Amino acids in watermelon root exudates and their effect on growth of *Fusarium oxysporum* f.sp. *neivium*. *Allelopathy Journal* **23**: 23-29.
15. Liu, S.Q. and Wu, F.Z. (2013). Phenotype and genetic diversity in Chinese onion cultivars from three provinces of northeast China. *Scientia Horticulturae* **43**: 128-134.
16. Putnam, A.R. and Duke, W.B. (1974). Biological suppression of weeds: evidence for allelopathy in accessions of cucumber. *Science* **185**: 370- 372.
17. Putnam, A.R. and Duke, W.B. (1978). Allelopathy in agroecosystems. *Annual Review of Phytopathology* **16**: 431-451.
18. Rohlf F.J., NTSYS-pc N.T. (1998). *Numerical Taxonomy and Multivariate Analysis System, Version 2.02*. Exeter Software, Setauket. New York, USA
19. Seliga, J.P. and Shattuck, V.I. (1995). Crop rotation affects the yield and nitrogen fertilization response in processing tomatoes. *Scientia Horticulturae* **64**:159-166.
20. Shen, H.M., Guo, H.R. and Huang, G.B. (2005). Allelopathy of different plants on wheat, cucumber and radish seedlings. *Chinese Journal of Applied Ecology* **16**:740-743. (Chinese)
21. Sneath P.H.A. and Sokal R.R. (1973). *Numerical Taxonomy*. Freeman, San Francisco, CA
22. Song, L. (2006). *Study on the Effects of Garlic Bulb Crude Extracts on Inhibition of Fusarium Oxysporum f.sp. cucumerinum and F. oxysporum Schl.f.sp. nioeum (E.F. Smith) Snyder&Hansen and its Mechanism*. Master's Thesis, Northeast Agricultural University, Harbin.
23. Sun, H.Y. (2005). *Evaluation of Allelopathic Wheat Germplasm and its Genetic Diversity Study*. Master's Thesis, Fujian Agriculture and Forestry, Fuzhou. (Chinese).
24. Tsao, S. and Yin, M. (2001). *In vitro* antimicrobial sulphides occurring naturally in garlic and Chinese leek oil. *Journal of Medical Microbiology* **50**: 646-649.
25. Wang, F. (2003). *The Mechanism of Eggplant (Solanum melongena L.) Replanting Problem*. Ph.D. Thesis, China Agricultural University, Beijing. (Chinese).
26. Williamson, G.B. and Richardson, D. (1988). Bioassays for allelopathy: Measuring treatment responses with independent controls. *Journal of Chemical Ecology* **14**: 181-187.
27. Wu, F.Z., Meng, L.J. and Wen, J.Z. (2002). Effect of cucumber root exudation on mycelial growth of *Fusarium oxysporum* f. sp. *China Vegetables* **5**: 26- 27. (Chinese).
28. Wu, F.Z., Wang, S. and Yang, Y. (2008). Effects of rotation and intercropping on bacterial communities in rhizosphere soil of cucumber. *Chinese Journal of Applied Ecology* **19**: 2717- 2722. (Chinese).
29. Wu, H., Pratley, J., Lemerle, D. and Haig, T. (2000). Evaluation of seedling allelopathy in 453 wheat (*Triticum aestivum*) accessions against annual ryegrass (*Lolium rigidum*) by the equal-compartment-agar method. *Australian Journal of Agricultural Research* **51**: 937-944.
30. Xu, Y.H., Li, Y. and Xu, X.Y. (2008). Progress in research on Fusarium wilt of tomato. *Journal of Northeast Agricultural University* **39**(11): 128-134. (Chinese).
31. Yang, G. (2004). *Experimental Directory of Microbiology*. Science press, Beijing, China. Pp. 50-53 (Chinese).
32. Yang, Y., Wu, F.Z. and Liu, S.W. (2011). Allelopathic effects of root exudates of Chinese onion accessions on cucumber yield and *Fusarium oxysporum* f. sp. *Cucumerinum*. *Allelopathy Journal* **27**: 75-86.
33. You, H.X., Liang, Y.L., Lv, W., Chen, Z.J., Du, S.N. and Xu, F.L. (2006). Research on the allelopathy of root secretions of different crops on cucumber (*Cucumis sativus* L.). *Journal of Northwest Sci-Tech University of Agriculture and Forestry (Natural Science)* **34**: 101-105. (Chinese).
34. Yu, J.Q., Ye, S.F., Zhang, M.F. and Hu, W.H. (2003). Effects of root exudates, aqueous root extracts of cucumber (*Cucumis sativus* L.) and allelochemicals on photosynthesis and antioxidant enzymes in cucumber. *Biochemical Systematics and Ecology* **31**:129-139.
35. Yuan, H.X., Li, H.L., Wang, Y., Fang, W.P. and Wang, Z.Y. (2001). The root exudates of cotton cultivars with the different resistance and their effects on *Verticillium dahliae*. *Acta Phytopathologica Sinica* **32**:127-131. (Chinese).
36. Zhou, X.G., Yu, G.B. and Wu, F.Z. (2011). Effects of intercropping cucumber with onion or garlic on soil enzyme activities, microbial communities and cucumber yield. *European Journal of Soil Biology* **47**: 279-287.
37. Zhuang, Y., Wu, F.Z., Shang, Q.M. and Yang, Y. (2009). Effect of intercropping on cucumber soil microbial diversity and production. *Scientia Agricultura Sinica* **42**: 204-209. (Chinese).