



**Full Length Article**

## Interspecific Root Interactions Enhance Disease Resistance of Peanut by Intercropped Millet

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Received 23 December 2019; Accepted 07 January 2020; Published 02 April 2020

### Abstract

Intercropping is commonly practiced worldwide because of its yield advantages, resource use efficiency and disease resistance. However, the knowledge of disease resistance via belowground root interactions in cereal/legume intercropping systems remains quite limited. Pot experiments were conducted to quantify the effect of root interactions by intercropped millet on peanut disease under conditions of full, partial and no root interactions based on TMT (tandem mass tag) labelling. Disease incidence and disease index were assessed at flowering stage and antioxidant enzymes in peanut leaves were analyzed. The results showed that root rot disease and leaf spot disease of peanut were alleviated by intercropped millet through root interspecific interactions from below-ground in the cereal/legume intercropping. The contents of chlorophyll and the nutrient contents of peanut were increased in peanut by root interactions, which indicated that the growth of peanut were improved. The number of bacteria, fungi and actinomycetes was increased by 57.3, 133 and 49.7% in peanut rhizosphere of full root interactions compared to the treatment without root interactions, respectively. There were 10 differentially expressed protein spots in intercropped peanut roots, which were involved in response to disease. The results showed that stress-response proteins displayed a differential expression levels in intercropped peanut, the disease resistance was improved via up-regulated proteins including class III acidic endochitinase, and down-regulated pectinesterase, and lipoxygenase etc. The results indicated that the intercropping root intermingling could contribute to the resistance to disease of intercropped species via improvement of nutrient acquisition and differential expressed stress-response proteins. The present study provides a new insight into plant disease control in cereal and legume intercropping by root interspecific interactions. © 2020 Friends Science Publishers

**Key words:** Disease; Intercropping; Peanut; Millet; Stress resistance

### Introduction

Interspecific facilitation of intercropping was demonstrated and well-documented as one plant species has a positive effect on the growth and productivity when intercropping with another species (Zuo *et al.* 2015; 2018). The facilitation includes multiple benefits to higher productivity and to multiple ecosystem processes (Bedoussac *et al.* 2015; Zou *et al.* 2019a). Over-yielding of intercropping is often associated with more efficient capture and utilization of water and nutrient acquisition (Latati *et al.* 2016), with better control of pests (Agboton *et al.* 2013), weeds (Silva *et al.* 2013) and diseases (Zhu *et al.* 2000).

Significant yield advantage has been observed in various intercropping systems worldwide, especially in cereal and legume combinations (Bedoussac *et al.* 2015). Intercropped with neighbor plant can potentially reduce the diseases which were often regarded as one of the most determinant factors influencing crop production. Crops in the mixture are less damaged by pest and disease than those

in the monoculture, which is an additional mechanism of over-yielding. Disease suppression has been found in intercropping systems, with 73% of documented studies (among 206 studies) reporting reduced disease incidence in intercrops, commonly in the range of 30–40%, but up to 80% in some systems compared with crop monocultures (Boudreau 2013), such as powdery mildew on wheat which was decreased by 49% in wheat and faba bean intercropping and *Ascochyta* blight on faba bean was reduced by 82% in triticale-faba bean intercropping. The severity of maize leaf blight was decreased by 17.0 to 19.7% in the intercropping compared with that in monoculture (Li *et al.* 2009). In particular, legumes are prone to yield loss since they are more sensitive to stress and are known to be weak competitors against pathogen attack (Brooker *et al.* 2015). The broad bean chocolate spot disease in the intercropping was decreased by 31.7 to 33.8% compared to that in the monoculture in a 2-year field study (Li *et al.* 2009). The peanut bud necrosis disease incidence was reduced by 60.3 to 69.1% in the intercrops compared with those in

monoculture (Gururaj *et al.* 2005). As a result of effective control of disease, an increase yield of pot was achieved 7.36 to 15.48% in various treatments. Disease infection showed to be seriously limited the yield and production of grain legumes. The reduction of disease serves as a vital factor to over-yielding by the facilitation of intercropped species from interspecific root interactions (Bedoussac *et al.* 2015). As much as 20% of yield loss is caused by leaf spot disease each year. It has been recognized that intercropping can show better disease control thus to be considered as a promising solution against disease. However, little attention was paid to disease suppression from below-ground in current cereal/legume intercropping. Therefore, it is essential to investigate the effects from below-ground via root interspecific interactions on the disease incidence of legume by intercropped cereal.

Previous studies showed that millet (*Setaria italica*) and peanut (*Arachis hypogaea*) intercropping system was a successful cropping system and widely practiced by small household farmers in semi-arid area of Northeastern China due to not only the more annual revenue caused by overyielding, but also ecological service functioning (Feng 2013; Zou *et al.* 2019b). Peanut is a major oilseed crop, with the cultivated area for peanut was 4700 k hectare in China (Li *et al.* 2014), and has become one of the primary cash crops because of its annual income for farmers in semi-arid area. However, there are serious problems with sole cropped peanut in the areas, such as a continuous decline in yield and an increased incidence of disease (Feng 2013, which was called “replanting disease” or “consecutive monoculture problems”). It was reported that disease incidence is up to 70% in long-term monoculture fields for peanut loss. Intercropping was considered as an effective solution to consecutive replanting problems for disease management (Zhu *et al.* 2000). Millet has been widely grown by local farmers because of their draught resistance and adaption to the stress of nutrient deficiency due to a relative deep root distribution and the retained residue in the field over the winter (Feng 2013). Thus, it came to be a key issue of how to make good use of millet and peanut as a solution to replanting disease of peanut. In cereal/legume intercropping, there are few published data on effects of intercropped cereal on disease resistance of legume by root interspecific interactions.

Intercropping partially served as a successful ecological strategy to control plant disease (Gao *et al.* 2014), while there is little knowledge to the mechanism underlying the resistance to diseases from below-ground root interactions at a molecular level. Here we address this question by performing proteomic analyses, which can provide a powerful tool to study complex biological processes at the molecular level (Gravatt 2007). This technical tool has been successfully used to quantify the effects of interspecific root interactions in cereal/legume intercropping systems (Xiong *et al.* 2013; Zou *et al.* 2019b). To understand the mechanisms behind disease resistance of

millet/peanut intercropping, it allows the determination of whether the disease resistance-response could be alleviated by interspecific root interactions between intercropped cereal and legume crops.

The objectives of this study were 1) to evaluate the disease incidence and disease index of legume in the intercropping systems and 2) to explore the mechanisms underlying the disease resistance of legume intercropped with cereal through the identification of functional proteins involved in biological metabolism in the intercropping.

## Materials and Methods

### Experimental design

Pot experiments were conducted in 2015 at Fuxin Long Term Observation and Experimental Station of Liaoning Academy of Agricultural Sciences at Shenyang, northeast China. Foxtail millet and peanut plants were intercropped under three different root manipulation treatments, which affected root systems in different ways (as shown in Fig. 1). Treatments included: (1) a solid root barrier with no root or water contact or nutrient exchange between intercropped species (no root interactions); (2) a pore size mesh barrier (30  $\mu\text{m}$ ) with no root contact but allowed water and nutrients exchange (partial root interactions), and (3) no barrier with full root contact and exchange of water and nutrients (full root interactions). Pots were conducted in a complete randomized block design with 12 replicates of each treatment. Each experimental pot (32 cm in height and 36 cm in diameter) was filled with 15 kg of air-dried soil. Neither interspecific root rhizosphere nor spatial effects did exist in the solid barrier treatments between two species. Mesh barrier stand for the root rhizosphere but without spatial complementarity effects between two species. There are both root rhizosphere and spatial effects as well between two species in no barrier treatment. For these three barrier patterns, there are 4 millets and 2 peanuts in the same space of soil. The same crop densities in the same volume of soil.

Soils were collected from the Fuxin Long-term Observation and Experimental Station, Liaoning, China, where millet and peanut intercropping is commonly practiced. Soils are sandy with a total N content of 1.21, 0.87, 18.3 g N kg<sup>-1</sup> soil total N, P, K and a water content of 2.8%. Soils were sieved to pass through a mesh size (2 mm) and then used to fill each of the experimental pots. The soil came from a field which has been planted peanut solely for more than 10 years before the soil was collected in April 2015. Higher incidence of peanut leave spot and root rot happened in the field crops thus caused lower production compared with that discontinuous monocultured field in the last 10 years. The incidence of peanut leaf spot was up to 100% in the sole peanut field in 2014.

### Sampling and measurement

Chlorophyll content was measured by the methods given by

Feng (2013). The first fully expanded leaf from the top of the canopy was used for the measurements in peanut. Each leaf sample was analyzed three times to minimize instrumental error.

The number of soil cultural bacteria, fungi, and antinomycetes were counted at the harvest time of peanut. Soil in the rhizosphere of peanut was collected and removed to the lab. The incubation method and colony forming unit counting were followed by previous methods (Zhang *et al.* 2013).

### Assessment of disease incidence of peanut

The incidence of peanut leaf spot caused by *Cercospora arachidicola* in peanut occurred naturally and was investigated at 60 days after sowing in 2015. The grade of peanut leaf spot was estimated as follows: 0 = peanut leaves without brown spot (no infection); 1 = 1–25% leaf infection; 2 = 26–50% leaf infection; 3 = 51–75% leaf infection; 4 = 76–100% brown spot in peanut leaves. The incidence of root rot disease of peanut caused by soil-borne fungi was also investigated at the same time. The symptom of peanut root rot showed as brown lesions in the taproot and on the side roots as early symptoms. The total rot of peanut roots along with the brown of the entire plant would be occurred when it progressed seriously (Li *et al.* 2014). The roots were classified depending on the root rot symptoms on a scale of as following: 0 = no lesions; 1 = small root lesions; 2 = central root lesions; 3 = root lesions; 4 = dead peanut. The investigation of diseases was conducted on each peanut in the experiment (24 plants more than 1000 leaves per treatment for leaf spot).

### Sample preparation for proteomic analysis

The methods of protein extraction in peanut root and digestion and TMT (tandem mass tag) labeling were followed previous study (Li *et al.* 2016; Zou *et al.* 2019a).

### Statistical analysis

Disease incidence and index were calculated following (Li *et al.* 2014): Disease incidence (%) = (number of diseased leaves or plants / total number of leaves or plants examined) 100%; and Disease index =  $[\sum(\text{number of diseased leaves or plants} \times \text{disease grade})] / [(\text{total number of leaves or plants} \times \text{highest disease grade})] \times 100$ .

ANOVA analysis was done by using analysis of variance tests in SAS (V8.2) software package. Tukey Post-Hoc tests were performed to examine the statistical significance of differences ( $P < 0.05$ ) between means.

## Results

### Effects of intercropped millet on disease resistance of peanut

Root interspecific interactions significantly reduced the

disease index of peanut leaf spot and root rot. Disease incidence of peanut leaf spot and root rot in full root interspecific interactions was decreased by 18.6 and 46.1% compared to the crops without root interspecific interactions treatment (Fig. 2). Disease index was decreased by 55.5 and 62.5%, respectively.

### Effects of intercropped millet on chlorophyll contents of peanut leaves

There was no significant difference of chlorophyll contents in peanut leaves under the three root interactions treatments, while both chlorophyll a and chlorophyll b was increased significantly under the full root interactions treatment compared to those without root interactions treatment at flowering and maturity stage (Table 1). The chlorophyll contents were increased along with the plant growing and decreased before harvest. The contents of chlorophyll a and chlorophyll b in peanut leaves at flower stage were increased by 61.7 and 55.6% in full root interspecific interactions treatment compared with those without root interactions, respectively.

### Mineral element acquisition of peanut in the intercropping

The nutrient acquisition of peanut was increased by root interspecific interactions (Table 2). Nitrogen and K uptake in peanut seeds was increased by 69.8 and 68.3% in full root interactions compared to those without root interactions. Phosphorus acquisition in peanut shoot and root was increased by 57.6 and 92.8% in full root interactions compared to those without root interactions, respectively. The Fe acquisition in peanut shoots in complete root interactions was 11.2 and 4.04 times compared with that without root interactions. The Zn and Se acquisition were also significantly enhanced by root interactions. Calcium acquisition in shoot and root of peanut was increased by 315 and 84.2% in full root interactions compared to those without root interactions, respectively. While the ratio of K/Ca was decreased significantly in peanut leaves in complete root interactions compared to the crops without root interactions.

### Effects of intercropped millet on antioxidant enzymes of peanut

Superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD) activities were increased in peanut leaves by root interactions while malondialdehyde (MDA) content was declined (Fig. 3). The activity of SOD, POD and CAT in peanut leaves was increased by 18.9, 100.1 and 101.2% in full root interactions compared to those in no root interactions treatment, respectively. The activity of MDA was decreased by 19.9 and 65.0% in partial and full root interactions treatment compared with that in no root interactions treatment, respectively.

**Table 1:** Effects of different root barrier treatments on the contents of chlorophyll in intercropped peanut leaves

Growth stages	No root interactions		Partial root interactions		Full root interactions	
	Chlorophyll-a	Chlorophyll-b	Chlorophyll-a	Chlorophyll-b	Chlorophyll-a	Chlorophyll-b
Seedling	1.31±0.17 a	0.22±0.01 a	1.43±0.07 c	0.27±0.02 c	1.07±1.72 c	0.14±0.00 c
Flowering	1.36±0.13 a	0.23±0.02 a	2.07±0.13 b	0.33±0.02 b	1.55±2.33 b	0.22±0.01 b
maturity	1.36±0.06 a	0.25±0.01 a	2.39±0.09 a	0.42±0.03 a	1.94±0.05 a	0.28±0.01 a

Small letters if different indicate significant difference at P = 0.05

**Table 2:** Nutrient acquisition of peanut leaves under three root interactions patterns

Peanut Organs	Root separation patterns	N (g pot <sup>-1</sup> )	P (g pot <sup>-1</sup> )	K (g pot <sup>-1</sup> )	Ca (g pot <sup>-1</sup> )	Fe (mg pot <sup>-1</sup> )	Zn (mg pot <sup>-1</sup> )	Se (mg pot <sup>-1</sup> )
Shoot	No RI-shoot	140±13.6 a	32.8±3.29 b	121±7.68 b	13.6±0.56 b	66.3±8.70 c	29.3±2.31 b	20.8±1.71 b
	Partial RI-shoot	116±21.4 a	29.5±4.67 b	154±20.3 a	16.7±2.11 b	148±13.7 b	33.7±2.54 b	25.5±1.89 b
	Full RI-shoot	162±16.9 a	51.7±3.54 a	156±14.2 a	56.5±2.01 a	742±33.8 a	55.1±2.58 a	52.3±3.24 a
Seed	No RI-seed	56.9±6.01 c	14.5±2.08 b	39.9±4.6 b	0.40±0.01 c	31.7±2.46 c	6.42±0.83 c	3.63±1.14 c
	Partial RI-seed	78.7±2.82 b	17.3±1.76 ab	41.0±5.33 b	1.10±0.11 b	71.8±10.7 b	11.9±1.04 b	15.3±1.51 b
	Full RI-seed	96.6±8.69 a	24.4±2.94 a	67.7±6.30 a	1.66±0.28 a	128±20.2 a	19.5±1.36 a	20.3±2.06 a
Root	No RI-root	21.5±6.08 b	4.75±1.03 b	13.1±4.02 a	1.08±0.47 b	269±17.8 c	7.16±1.94 b	70.1±1.85 a
	Partial RI-root	32.1±4.34 a	8.16±1.01 a	12.0±1.03 a	1.90±0.12 a	330±20.9 b	10.2±1.02 a	80.9±6.96 a
	Full RI-root	34.6±4.80 a	9.16±0.25 a	14.8±1.06 a	1.99±0.25 a	405±11.7 a	9.77±0.33 a	74.1±4.45 a

No RI refers to no root interactions (solid barrier), Partial RI for partial root interactions (mesh barrier) and Full RI for full root interactions (no barrier)

Within peanut organs, the same small letter indicates no significant difference between three root separation patterns treatments at P = 0.05

### Effects of intercropped millet on differentially expressed proteins of peanut root

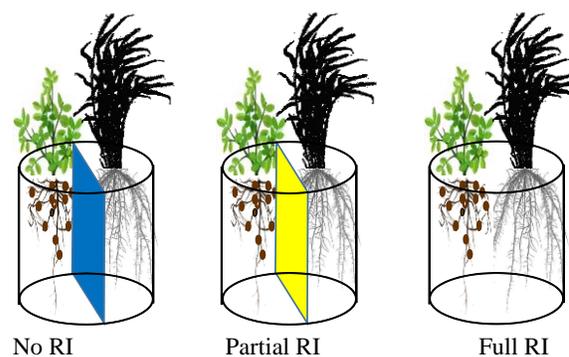
There were 3 up-regulated and 6 down-regulated proteins in full root interactions in peanut root, and 4 up-regulated and 5 down-regulated proteins in partial root interactions compared to that without root interactions, respectively (Table 3). Lipoxygenase (Q4JME7, Q42780, A0A0D4CI71) served as key enzyme in jasmonate (JA) biosynthesis, was decreased in abundance in peanut root of the full root interactions treatments compared with that without root interactions. Defensive proteins such as class III acidic ndochitinase (O48642) and pectinesterase (I1JAV9) was down-regulated in complete root interactions compared with that in no root interactions (Table 3).

### Microbial quantity in peanut rhizosphere

Root interspecific interactions had a significantly effect on the quantity of microbial in peanut rhizosphere (Table 4). The number of bacteria, fungi, actinomycetes was increased by 57.3, 133 and 49.7% in peanut rhizosphere of full root interactions compared to those in no root interactions, respectively.

### Discussion

The results in the present study showed that root inerspecific interactions decreased the disease index of peanut leaf spot and root rot, which were similar to the previous studies. The intercropping reduced the damping-off in seedling and root rot and leaf spot disease of peanut significantly when intercropped with *Atractylodes lancea* (Li et al. 2014). Minimum rust severity was found in field pea which was intercropped with mustard (Singh et al. 2014). Intercropping of peanut with bajra, sorghum, pigeon pea and maize



**Fig. 1:** Three root separation patterns: No RI means no root interactions-solid barrier, Partial RI means partial root interactions-mesh barrier and Full RI means full interactions-no barrier

significantly reduced the incidence of peanut bud necrosis disease (caused by peanut bud necrosis virus) in Karnataka, India, in 1995, 1996, 2003 and 2004 (Gururaj et al. 2005). In a 2 year of field experiments, intercropping of maize and soybean suppressed the occurrence of soybean red crown rot, a severe soil-borne disease caused by *Cylindrocladium parasiticum* (Gao et al. 2014). It indicates that resistance in soybean to red crown rot could be promoted when intercropped with maize. This supports the view that intercropping may be a successful ecological strategy to control soil-borne plant disease and should be incorporated in sustainable agricultural management practices (Gao et al. 2014). In our present study, Disease incidence of peanut leaf spot and root rot in full root interspecific interactions was decreased by 18.6 and 46.1% compared with those without root interspecific interactions treatment.

Disease suppression can derive from a variety of factors, including decreased pathogen source (host plant availability, effects on dispersal or infection phases in the life cycle of the causal agent, microclimatic effects on

**Table 3:** Differentially expressed proteins in peanut root under no barrier (NB), mesh barrier (MB) and solid barrier (SB) by tandem mass tag (TMT) technique

Accession No.	Protein name	Peptide matched Number	Accumulated level	
			MB/SB	NB/SB
C metabolic				
O48642	Class III acidic endochitinase	2	1.189	1.956
I1JAV9	Pectinesterase	8	0.599	0.646
Lipid metabolic				
Q4JME7	Lipoxygenase	2	0.635	0.931
Q42780	Lipoxygenase	2	0.662	0.828
A0A0D4CI71	Lipoxygenase	2	0.592	0.684
Response				
Q2PYP7	17.3 kDa class I heat shock protein	10	1.075	0.578
B4UW51	Class II small heat shock protein Le-HSP17.6	3	0.936	0.597
Q43374	Mannose/glucose-binding lectin	5	1.405	2.125
I1L0D8	Peroxidase	2	0.649	1.037
C6TBS7	Uncharacterized protein	3	1.557	1.387

**Table 4:** Effect of root interactions on microbial quantity in peanut rhizosphere

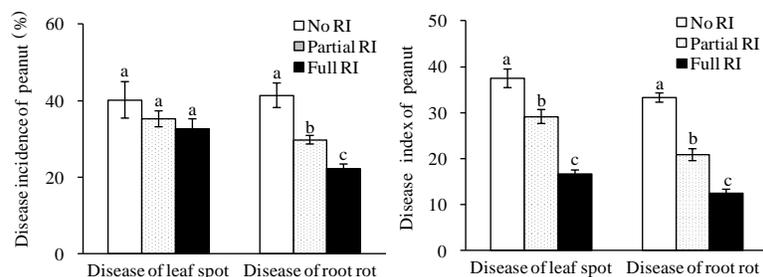
Root Interaction	Bacteria ( $\times 10^6$ CFU g <sup>-1</sup> dry soil)	Fungi ( $\times 10^4$ CFU g <sup>-1</sup> dry soil)	Actinomycetes ( $\times 10^5$ CFU g <sup>-1</sup> dry soil)
No RI	20.6 $\pm$ 4.11b	3.83 $\pm$ 1.28 b	7.75 $\pm$ 2.31 a
Partial RI	23.7 $\pm$ 3.88 ab	7.25 $\pm$ 2.19 a	9.14 $\pm$ 2.42 a
Full RI	32.4 $\pm$ 4.03 a	8.94 $\pm$ 2.82 a	11.6 $\pm$ 3.87 a

CFU means colony forming units. Small letters if different indicate significant difference at P = 0.05

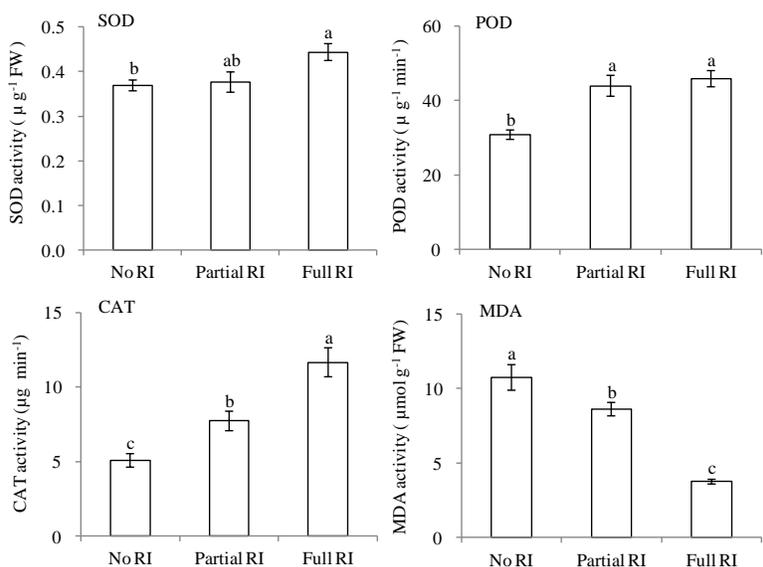
pathogen establishment) and the growth and development status of intercropped species (Li *et al.* 2014). In the present study, disease of legume was significantly alleviated by root interspecific interactions. To some extent, the disease resistance came from the growth and development of status of legumes. The P, K, Ca, Fe, Zn, Se acquisition was increased significantly in peanuts in full root interactions compared with that in no root interactions, which means the growth and development status of peanut was enhanced by root interactions. Potassium was reported to be a key element to the contribution of plant disease resistance, for example, potassium could decrease take-all of wheat (Huber and Haneklaus 2007). Zinc was higher in wheat tissues where *Rhizoctonia cerealis* was less severe compared with those spring blight was more severe (Huber and Graham 1999). Brooker *et al.* (2015) reported that intercropping stimulated the acquisition of Fe and Zn in maize shoot intercropped with faba bean, chickpea and soybean compared with those in the monoculture. Selenium was associated with the reactive oxygen species and was considered as a inhibitor to alleviate the damage of this reactive oxygen species. The results were similar to our present study. In present study, the Ca, Fe, Zn and Se acquisition was significantly enhanced by root interactions, which means the nutrient acquisition would contribute to peanut disease resistance when allow root interspecific interactions. Mineral nutrients such as Ca that suppress diseases through increasing resistance of the middle lamella and the structural integrity, cell wall components, and cell membranes to the extracellular macerating enzymes which was produced by the pathogens (Kelman *et al.* 1989). Calcium can prevent the invasion of pathogen via improving the stability of cell wall of plant and modifying

the structure of cell membrane. Calcium was also reported to increase the cohesion of the plant cell walls, thus enhancing the resistance to fungal pathogen penetration (Serrano *et al.* 2013). The Ca concentration was increased in legume leaves in present study; it is indicated that mineral concentration contributed to the improvement of resistance to disease of legume by root interspecific interactions. The contents of both chlorophyll a and chlorophyll b were increased by root interspecific interactions, and the photosynthetic rate was also increased (Zou *et al.* 2019b). The Fe acquisition was sharply increased (10.2 times) in peanut shoots in full root interactions compared with that in no root interactions. The results was similar to previous studies (Zuo *et al.* 2000), which suggested that the root rhizosphere interactions improved Fe nutrient and chlorophyll content of peanut intercropped with maize. In another study, the root interactions increased soybean chlorophyll content and photosynthetic rate which indicated the growth and development of legume was improved by this root interspecific interactions.

Soil microbial quantity has a strong influence on plant growth and productivity by under-ground root interactions via nutrient availability through mineralization. In this study, the results showed that the number of bacteria, fungi and actinomycetes in peanut rhizosphere were significantly increased by root interspecific interactions. The increase of the root activity of soybean can improve rhizosphere soil microbial environment, physical and chemical properties, and development status of species (Zhang *et al.* 2013). Peanut root rot caused by soil-borne fungi. In the case of soil-borne pathogens, the results in present study demonstrate that root interspecific interactions significantly reduce the incidence of peanut root rot.



**Fig. 2:** Effects of root interspecific interactions on disease of peanut intercropped with millet (A) Disease index of peanut (B) Incidence of peanut under solid barrier (No RI), mesh barrier (Partial RI), no barrier (Full RI) Bars: standard error of the mean  
The same small letter indicates no significant difference between three root interspecific interaction treatments for peanut disease at P = 0.05

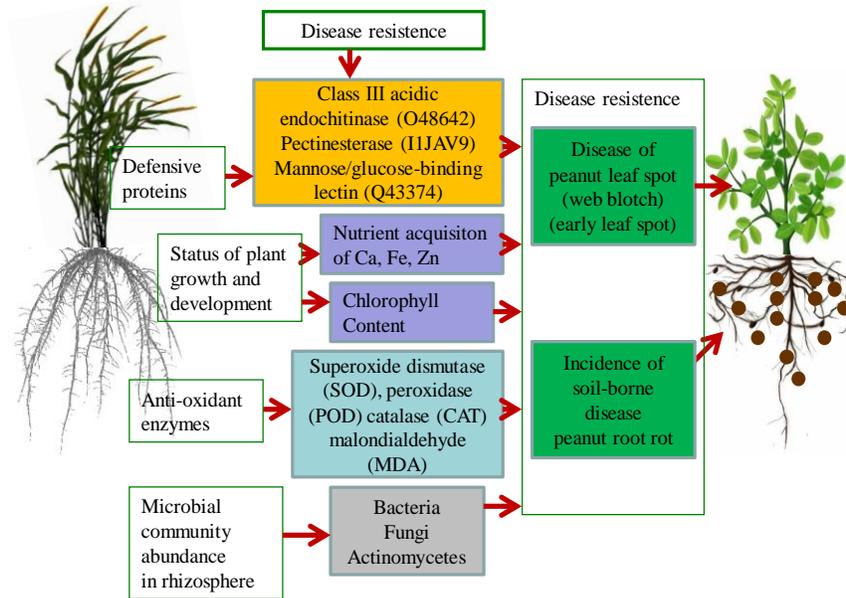


**Fig. 3:** Anti-oxidant enzyme in peanut leaves under different root interactions: solid barrier (No RI), mesh barrier (Partial RI), no barrier (Full RI). Bars: standard error of the mean  
The same small letter indicates no significant difference between three root interspecific interaction treatments for peanut disease at P = 0.05

STRING analysis results showed that the proteins associated with lipid metabolic were down-regulated in peanut root, including lipoxygenase (Q4JME7, Q42780, A0A0D4CI71). Lipoxygenase served as key enzyme in jasmonate (JA) biosynthesis, was decreased in abundance in peanut root of the full root interactions treatments compared with that without root interactions, which implying that JA content was less in the root interactions treatment. JA acts as an important signaling molecule, for crop growth and in response to biotic and abiotic stress (Greelman and Mullet 1995; Zou *et al.* 2019a). The decrease synthesis of JA indicated that the defense of diseases and stress conditions were eliminated in legumes when allow complete root interactions (Schaller and Stintzi 2009). Similar results was found in maize and peanut intercropping, two key enzymes in JA biosynthesis was increased in abundance in maize roots, which indicate that JA act as an important molecular signal in the intercropping rhizosphere interactions (Xiong *et al.* 2013).

The proteins associated with lipid metabolic in peanut roots were down-regulated in present study, indicating that the JA was decreased when allow full root interactions compared to those without root interactions. The down-regulation of JA in present study indicated that root interactions improved the defensive ability for crops against the pathogens. The decreased in abundance defensive proteins indicated that the potential disease risk of intercropped crops was decreased to a lower level by interspecific root interactions compared to the treatment in no root interspecific interactions in cereal/legume intercropping.

Endochitinase (O48642) and pectinesterase (I1JAV9) were defensive proteins, and play an important role on the resistance of pathogen attack (Karen *et al.* 1986). The down-regulation of the two proteins in peanut roots of complete root interactions indicated that the risk of disease to peanut was eliminated under root interactions compared to that without root interactions. The inhibition of rhizobia growth



**Fig. 4:** Mechanisms of disease resistance underlying the interspecific facilitation in cereal/legume intercropping

by fusarium root rot was found in peanut rhizosphere, intercropping decreased damping-off and root rot disease and increased seed yield significantly (Abdel-Monaim and Abo-Elyousr 2012). Previous study showed that acidic endochitinase and basic endochitinase could be induced by infection (Rainer *et al.* 1997). Mannose/glucose-binding lectin is capable of combination with monose to defend pathogens against diseases (van Eijsden *et al.* 1992). The down-regulation of defensive proteins indicated the growing conditions of rhizosphere for intercropped species were improved and the potential risk of pathogen attack was decreased by root interactions.

## Conclusion

The present results showed that plant growth, development status and stress-response differentially expressed proteins in intercropped peanut root from intercropping root intermingling could contribute to the resistance to pathogens infection conditions for legume in the intercropping. The disease resistance was improved via up-regulated proteins including class III acidic endochitinase and down-regulated pectinesterase, and lipoxygenase etc. The disease resistance to the plant could be alleviated by the root interactions from below-ground in the cereal/legume intercropping.

## Acknowledgements

This work was supported financially by the National Key Research and Development Program of China (Project No. 2016YFD0300202), National Natural Science Foundation of China (No.31461143025); LiaoNing Revitalization Talents Program (XLYC1807056, XLYC1907089).

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