Inter-cropping with Southern Corn Rust Resistance Maize Genotype Improved Maize (Zea mays L.) Defense Response

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Abstract: Intercropping is regarded as an effective and environment friendly cultivation practise in disease control. SCR infection severity and the differences of defense related enzymes activity under SCR inoculation were observed between sensitive and resistance genotypes and between mono- and inter-cropping cultivation manners of the same genotype. Compared to mono-cropping, the disease grade of SCR sensitive genotype inter-cropped with SCR resistance genotype decreased from 5 and 7 to 3 and 3 in 2006 and 2007, respectively. The defense related enzymes activities under SCR inoculation in SCR sensitive genotype were higher when inter-cropping with SCR resistance genotype than in mono-cropping. The result indicates that the disease resistance of plants is not only related to its hereditary but could also be affected by the genetic diversity of the cropping community. These results suggest that by intercropping the SCR susceptible genotype with the SCR resistant genotype can significantly enhance the defense related metabolism of the susceptible genotype under SCR inoculation and improve the resistance to SCR.
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Key words: southern corn rust (SCR); disease resistance; defence response; genetic diversity

1 Introduction

Southern corn rust (SCR), caused by *Puccinia polysora* Underw. is a serious disease in maize (*Zeamays* L.) production. The prevalent of SCR can cause severe losses in maize yield. Severe epidemics of SCR resulted yield loss was estimated up to 50% in 1950 in Liberia, Ivory Coast, Gold Coast, Dahomey and Nigeria (Rhind et al., 1952), and 30-50% yield loss in USA in 1972-1974(Futrell, 1975). Later, SCR was reported by Duan and He in Hainan Province in 1972(Duan and He, 1984), then spread and turned epidemic in the North China, which led to a serious yield loss of up to 20-30% in 1998(Liu and Wang, 1999).

Due to severe destructive effects of SCR on maize production, lots of studies were carried out to prevent SCR epidemic. Some scholars focus on searching for resistance genes/lines to breed highly resistant maize hybrids. And several SCR resistant germplasms (Futrell et al., 1975; Ye, 2000) and SCR resistance genes were identified (Chen et al., 2004; Jines et al., 2007; Scott and Zummo, 1989; Scott et al., 1984; Zhang et al., 2010; Zhou et al., 2007). However, in most cases, one resistance gene can only be resistant against one virulent strain. And high mutation rate of the pathogen could lead the dominant resistance gene loses resistance ability then result in a high risk of using cultivar with only one dominant resistance gene for disease control in the agricultural production system (Mew et al., 1992). Other scholars regard chemical control as an effective manner in preventing plant disease epidemics. But chemical control is always along with high risks of pesticide resistance and environmental pollution.

Within this context, genetic diversity in disease control draws more attention by the agronomists (Browning and Frey, 1969; Wolfe, 1985), and intercropping practice does have effects on disease control and reduced yield losses significantly (Boudreau, 1993; Sikirou and Wydra, 2008; Trenbath, 1993). However, due to inter-cropping with different kinds of crops planting is more difficult for agricultural machine operation; the application of inter-cropping is limited to the developing countries where cheap labour is abundant. To overcome the limitation of inter-specific intercropping in disease control, another inter-cropping model by using multiline cultivars was developed and has been proven effective (Mundt, 2002). For example, Zhu, et al (2000) reported that compared with monoculture, an 89% greater yield and 94% less severe was investigated for disease-susceptible rice varieties when they were planted in mixtures with resistant varieties. And using genetic diversity, a broad resistance to diseases and pests can be built, and sustainable crop production can be realized effectively (Leung et al., 2003). But the defense response of different cultivar in the inner-species inter-cropping system under pathogen

infection is not well-known. To gain better insight into the induced resistance to SCR by inner-species inter-cropping, here we investigated SCR severity and defense-related enzymes activity variation of SCR sensitive cultivar and SCR resistance cultivar in mono- and inter-cropping cultivation practice.

2 Materials and methods

2.1 Location, hybrids and plots design

Field trials were conducted in the scientific and educational park of Henan Agricultural University, Zhengzhou, Henan province, China in 2006 and 2007. Two hybrids (ZhengDan958 and LuDan981), which differ in their susceptibilities to *Puccinia polysora* Underw, were used in the experiment. The hybrid Zhengdan958 is sensitive to SCR according to Ren (2005). The other hybrid LuDan 981 has a strong ability to resistant to SCR (Ye, 2000). The experiment was a randomized complete block design with four replicates. Treatments were as follows: ZhengDan958 Mono-cropping (ZD958M), LuDan981 Mono-cropping (LD981M) and ZhengDan958 intercropped with LuDan981 (ZD958‖LD981) with 1:1 row ratio (Fig. 1). Each plot was 6m long, 6m wide, and consisted of ten rows of maize with rows spaced 0.6m apart and plants 0.24m apart within the row for a population density about 6.75plants·m^{-2}. Plots were separated by 1m on each side and inter-plots were left bare. Plots were sown in 8th June, 2006 and 1st June, 2007 and harvested in 28 September, 2006 and 25 September, 2007, respectively.

2.2 Evaluation of disease severity

Disease severity of each treatment was assessed on a 1-to-9 scale based on Wang (2005), with 1 indicating disease rate is below 5%, 3=6%-10%, 5=11%-30%, 7=31%-70%, and 9=71%-100%. . Rating was conducted at the end of August. The mean disease rating per plot was calculated based on the five ear leaves of 20 plants in the center of each plot.

2.3 Enzymological analysis

Enzymological analysis was conducted in 2007. Maize plants used for enzyme activity analysis were planted in 25th July with the same plot design. To avoid air-borne pathogens infection, two or four rows in the middle of each plot were covered by plastic rectangle cover in the size of 6m*1m*1m with an air conditioner to exchange the inner air with the outer air and keep an appropriate growth environment before emergence (Figure. 1). And sterilized absorbent cotton was placed at the inlet and outlet to filter the microorganisms and replaced every day at 3:00 pm. The air temperature and humidity in the plastic cover were shown in figure 2. The SCR pathogen, *P. polysora* Underw, was isolated from infected leaves of susceptible maize plants in the scientific and educational park of Henan Agricultural University, Zhengzhou, Henan province. Pathogen purification and inoculation were carried out according to Chen (2004). Inoculation was conducted carefully in the shortest time at the six-leaf stage (V6) on a windless evening. The inoculated leaves was harvested at 0, 12, 24, 48, 72, 120 and 168 hours after inoculation, then frozen in liquid nitrogen and stored at -70℃ until use for analysis of enzymatic.

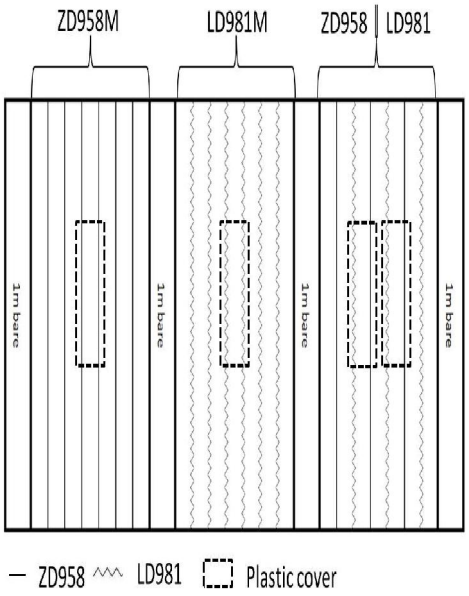

Figure 1 Field layout schematic diagram of the field trial of one replicate.

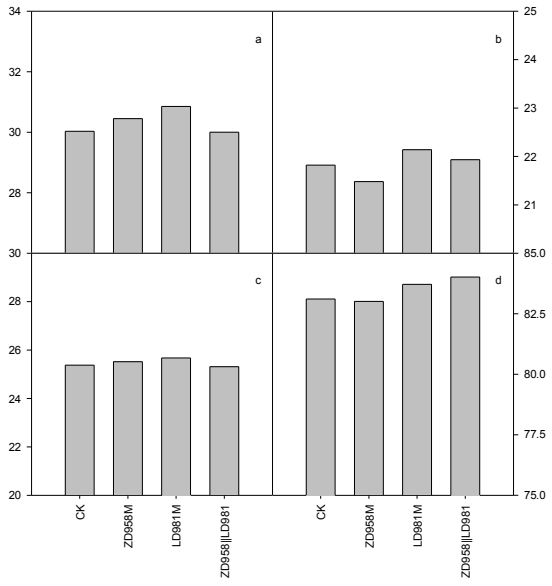

Figure 2 Temperature and relative humidity out of the plasitic cover (CK) and in the plasitic cover of different culivation manners (ZD958M, LD981M and ZD958‖LD981). a: daily maximum temperature (℃); b: daily minimum temperature (℃); c: daily average temperature (℃); d: relative humidity (%).

2.3.1 Lipoxygenase

Lipoxygenase (LOX) were analyzed according to (Romero and Barrett, 1997) with some modifications. In short, LOX were extracted by homogenizing with a pre-chilled mortar and pestle with 0.2M pre-chilled phosphate buffer of pH6.5 with 5mmol/L β-Mercaptoethanol and 1% poly vinyl pyrrolidone (PVP). The extract was kept on ice for 10 min, and then centrifuged at 10,000×g for 10 min at 4℃. The supernatant was used immediately for measuring LOX activity. The LOX activity was measured by monitoring the increase in absorbance at 234nm. One unit of enzyme activity was defined as that amount of enzyme that produced a change in absorbance of 1.0/min at 234 nm, under the assay conditions. The assay mixture (1 ml) contained 200 mmol/L sodium phosphate buffer (pH 7.0) and 2.5 mmol/L linoleic acid.

2.3.2 Phenylalanine ammonia lyase

Phenylalanine ammonia lyase (PAL) were analyzed by the method of (Okey et al., 1997). In short, PAL was extracted by homogenizing with a pre-chilled mortar and pestle with 0.03M pre-chilled borax buffer of pH8.8 with 5mmol/Lβ-Mercaptoethanol and 1% poly vinyl pyrrolidone (PVP). The extract was kept on ice for 10 min, and then centrifuged at 10,000×g for 10 min at 4℃. 0.5ml supernatant was added to a new tube with 0.5ml 0.02M L- Phenylalanine and 2ml borax buffer of pH8.8. The mixture was incubated at 30℃ for 30min. At the end of the reaction, 0.5ml 6M HCl was added to each tube to stop the reaction. The absorbance at 290nm was recorded. One unit of enzyme activity was defined as that amount of enzyme that produced a change in absorbance of 0.01/h at 290 nm.

2.3.3 Polyphenol oxidase

Polyphenol oxidase (PPO) was analyzed by histochemical method according to Fu (2003). Hand-sliced leaf sample was placed in pre-chilled 0.2M pre-chilled phosphate buffer of pH7.2 at 3℃ for 3min, then the sample was transferred to 1% catechol solution and incubated at 37℃ for 10 hours. PPO can catalysis catechol to brown components. The sample was placed on the slides, examined and photographed under optical microscope by a magnification of 375×. The parallelism control was boiled, and analyzed with the same procedure.

2.4 Statistical analysis

Independent-samples T-test was employed to test for significant difference between treatments at $P \leq 0.05$. All analyses were performed using SPSS 16.0 for Windows.

3 Results

3.1 Disease severity

The observation in the field indicated that, symptoms emerged at 63 days after sowing (DAS), and reached the peak at 77DAS. In the vertical profile, SCR symptoms first emerged at the ear leaf, the pustules were mostly on the abaxial of the leaf. Later, pustules were appeared in the leaves above the ear. In the severely infected plants, much larger pustules appeared in the lower leaf sheathes. The SCR was more severe in 2007 than in 2006, which is in accordance with more spores collected in 2007 than in 2006. In the inter-cropping population, the disease severity of the SCR sensitive hybrid ZD958 decreased significantly (Table 1). There were no pustules detected in the leaves of the SCR resistance hybrid LD981. Table1 The disease condition of southern corn rust in maize mono-/intercropping population

Year	2006		2007	
Treatment	Disease rate (%)	Disease grade	Disease rate (%)	Disease grade
ZD958M	32.4a	5	54.3a	7
ZD958I	12.5b	3	15.2b	3
LD981M	0.0c	1	0.0c	1
LD981I	0.0c	1	0.0c	1

Note: Different minuscule of the same line in the same year indicate different significantly at $P<0.05$

3.2 Lipoxygenase (LOX) Activity

The activity of LOX increased soon after SCR inoculation in both mono- and inter-cropping population leaves. And the activity of LOX with ZD958 reached the peak at 72h and 48h for the mono- and inter-cropping population leaves, respectively (Figure 3). Then the activity of LOX decreased gradually. The activity of LOX in the inter-cropped maize leaves of ZD958 was a little lower than in the mono-cropped plants but increased more rapidly after SCR inoculation. The highest activity of LOX after SCR inoculation were 61.90% and 100.74% higher than before SCR inoculation in both the mono- and inter-cropped ZD958's leaves, respectively. Activity of LOX in LD981 increased after SCR inoculation with a less significant. The activity peak of LOX appeared at 12h after SCR inoculation. The highest activity of LOX after SCR inoculation were 97.22% and 32.39% higher than before SCR inoculation in the mono- and inter-cropped LD981's leaves, respectively. The relatively high activity of LOX in mono- and inter-cropped leaves of LD981 lasted for 120h and 72h respectively.

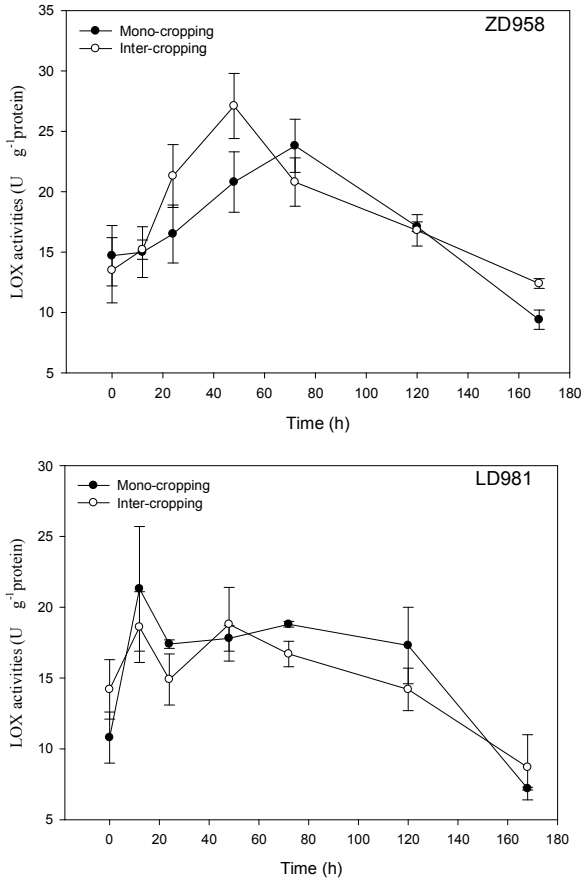

Figure 3：The LOX activities of maize leaves after SCR inoculation.

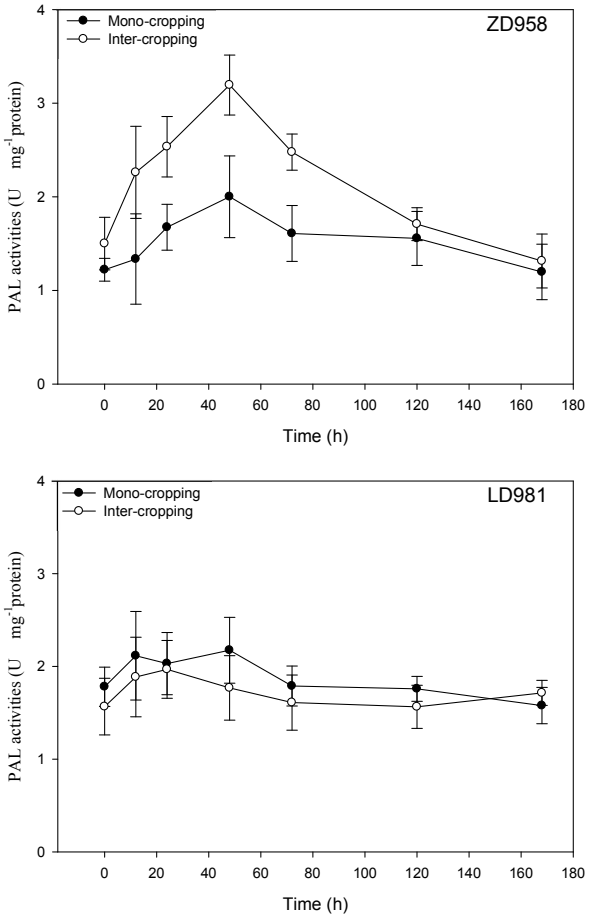

Figure 4 The PAL activities of maize leaves after SCR inoculation

3.3 Phenylalanine Ammonia-Lysae (PAL) Activity

The activity of PAL increased soon after SCR inoculation in both mono- and inter-cropping population leaves. And the activity of PAL with ZD958 reached the peak at 48h in both mono- and inter-cropping population leaves (Figure 4). Then the activity of PAL decreased gradually. The activity of PAL in the inter-cropped maize leaves of ZD958 was a little higher than in the mono-cropped plants and increased more rapidly after SCR inoculation. The highest activity of PAL after SCR inoculation were 63.91% and 112.87% higher than before SCR inoculation in the mono- and inter-cropped ZD958's leaves, respectively. However, activity of PAL in LD981 increased after SCR inoculation with a less significant. The highest activity of PAL after SCR inoculation were 22.13% and 25.64% higher than before SCR inoculation in the mono- and inter-cropped LD981's leaves, respectively.

3.4 Polyphenol oxidase (PPO) Activity

Before SCR inoculation, the activity of PPO in the guard cells of ZD958M was high, and then PPO activity decreased until 24h after SCR inoculation (Figure 5). Later, PPO activity increased gradually to 72h. At 168h after SCR inoculation, it is hard to detect the activity of PPO in the guard cells of ZD958M. Before SCR inoculation, PPO activity in guard cells of ZD958I was high, and then PPO activity decreased till 12h after SCR inoculation. Later, PPO activity increased gradually to 72h and kept relatively high till the latest sampling. In the first 120h after SCR inoculation, there were two peaks of PPO activity in guard cells of LD981M at 12h and 72h after SCR inoculation, respectively and one peak of PPO activity in the guard cells of LD981I. In both mono- and inter-cropped LD981, PPO activity was lowest at 120h after SCR inoculation, and then increased.

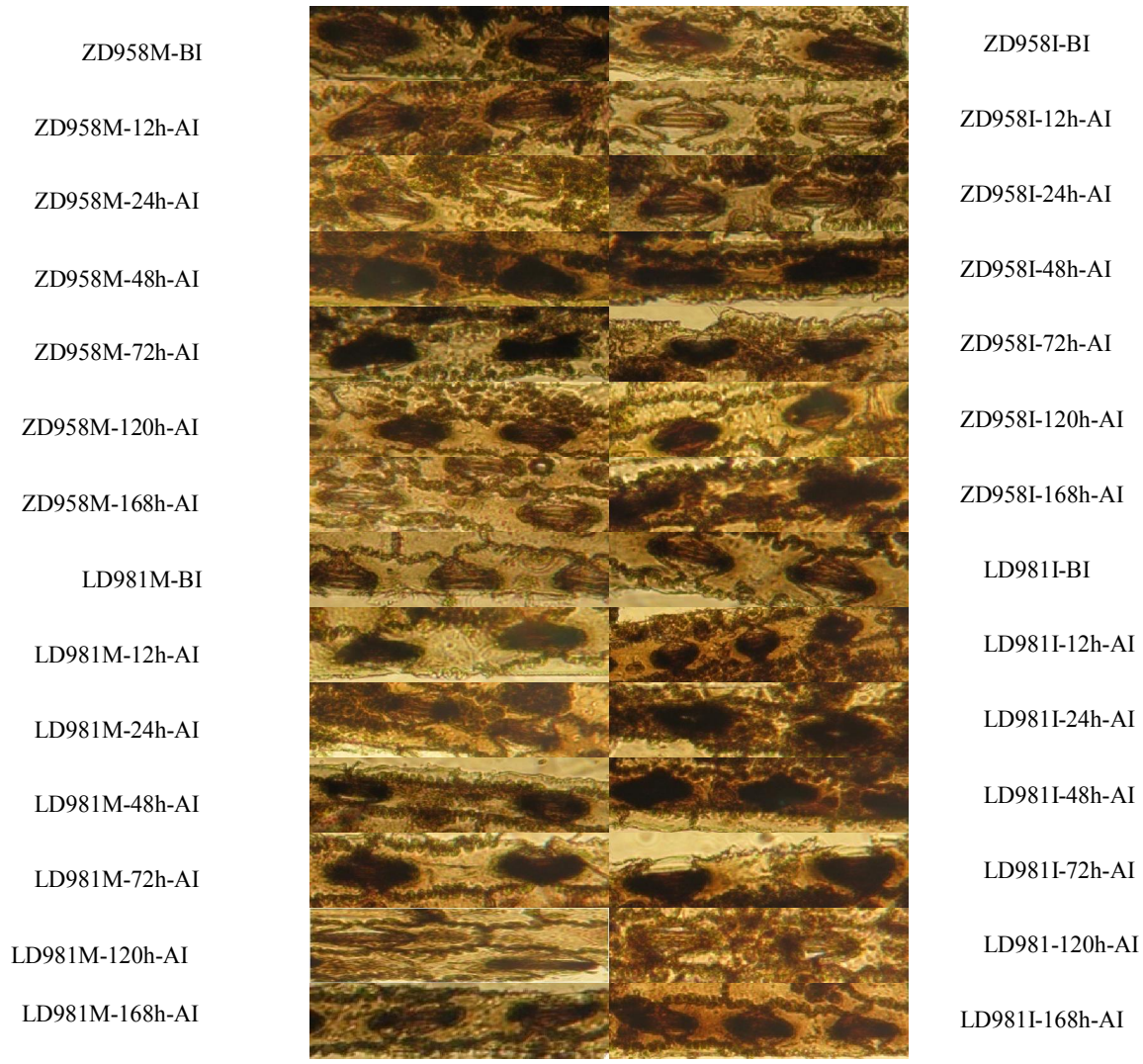

Figure 5 The activity changes of PPO in stoma guard cell of maize leaf after Puccinia polysora Underw inoculationNote: BI and AI indicate before/ after inoculation of *Puccinia polysora* Underw, respectively.

4 Discussion

Inter-cropping maize hybrids with complementary SCR resistance can control SCR infection effectively, and the control effect is more effective in more severe conditions. Other studies have already demonstrated that inter-cropping can effectively control either bacteria or fungi caused plant disease (Fernández-Aparicio et al., 2010; Sapoukhina et al., 2010; Sikirou and Wydra, 2008). This result supports the view that intra-specific diversity also can be used as an effective cultivation practise to control disease in sustainable crop production (Zhu et al., 2000). By our enzymological analysis, the effective control of SCR by complementary SCR resistant maize genotype was attributed partly to the variation of LOX, PAL and PPO activity.

LOX primarily participated in unsaturated fatty acid metabolism. Some of the low molecular volatile products of this metabolism, such as C3-, C6-, C9- and C12-aldehyde, ketone, alcohol, ester, and JA (or MeJA), constitute the signal molecule of plant resistance system which can induce resistance protein synthesis and a serious defence response(Kishimoto et al., 2006; Noordermeer et al., 2001). And some of these metabolisms can inhibit fungi growth. LOX gene expression can be significantly induced both by biotic and abiotic stresses, such as wounds, insect herbivores or pathogen infection (Elizabeth, 1998), while repress LOX activity increased plant sensitivity

to pests and fungi infection (Feussner and Wasternack, 2002). Our result showed that after inoculation with SCR, LOX activity increased more in SCR sensitive maize hybrid ZD958 than SCR resistant maize hybrid LD981, which indicate that SCR sensitive maize hybrid responds more forcefully to SCR infection. The activity of LOX in the inter-cropped maize leaves of ZD958 was a little lower than the mono-cropped plants but increased more rapidly after SCR inoculation. The rapid increase of LOX activity in the inter-cropped SCR sensitive hybrid could be one of the reasons for lower SCR infection rate when they were inter-cropped with the resistant hybrid.

The activity of PAL can be induced by pathogen infection and is closely correlated to plant disease resistance (Chandra et al., 2007; Geetha et al., 2005; Nagarathna et al., 1993; Xu et al., 2011) and the expression of PAL were higher in the infected leaves (Xu et al., 2011; Yu et al., 2010). However, the response of PAL activity to pathogen infection is different. For example, PAL activity exhibits a first maximum when the rust fungus *Puccinia graminis* f. sp. *tritici* race 32 just growing on the surface of the inoculated wheat leaves with a second increase at the time of hypersensitive resistant reaction of the resistant isoline and a continued decline of the susceptible plants (Moerschbacher et al., 1988). While Cahill and McComb found no increase in PAL activity in the susceptible *Eeucalyptus.* after *Phytophthora cinnamomi* zoospores inoculation (Cahill and McComb, 1992). Another study found that the activity of PAL was time-, tissue and pathotype-specific (Nagarathna et al., 1993). Our result showed a notable increased of PAL activity in the susceptible hybrid leaves, which kept steady in the resistant hybrid leaves. The activity of PAL increased more rapidly in the inter-cropped plants than the mono-cropped plants of the susceptible hybrid. The rapidly increased PAL activity in the inter-cropped susceptible plants may contribute to the higher defence response to SCR inoculation and lead to a lower degree of infection.

PPO is assumed to be participated in plant disease resistance by catalyzing the oxygen-dependent oxidation of phenols to quinones by a quantity of studies (Li and Steffens, 2002; Mohammadi and Kazemi, 2002; Ngadze et al., 2011). In the present studies here, a great variation of the PPO activity was observed (Figure 5). Considering only one susceptible and one resistance genotypes to SCR were used here, we cannot conclude that there is a relationship between SCR resistance and PPO activity. However, neither for the susceptible nor the resistant genotype, we did find that most of the sampling time PPO activity in the inter-cropped plants was higher than in the mono-cropped plants under SCR inoculation. That is, inter-cropping with different SCR resistant genotypes can increase PPO activity under SCR inoculation. Based on the role of PPO in disease resistance, the increase of PPO activity of the inter-cropped susceptible genotype could contribute to the significant decrease of SCR severity of ZD958.

5 Conclusion

Effective control of SCR infection by inter-cropping with different resistance genotype was observed in the field conditions. And under SCR inoculation, the activities of LOX, PAL and PPO varied not only between the two different resistance genotypes, but also between mono- and inter-cropped plants of the same genotype. This result indicates that the disease resistance of plants is not only related to its hereditary but also could be affected by the genetic diversity of the cropping community. By intercropping the SCR susceptible genotype with the SCR resistant genotype can significantly enhance the defense related metabolism of the susceptible genotype under SCR inoculation and improve the resistance to SCR.

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