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# PHYSIOLOGICAL AND BIOCHEMICAL BASES OF RESISTANCE OF FLAX (*LINUM USITATISSIMUM* L.) TO PASMO

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**Abstract**

This study preliminarily aimed to discuss the relationship between the dynamic changes in physiological and biochemical indexes of flax (*Linum usitatissimum* L.) resistant and susceptible varieties (lines) and their resistance to pasmo pathogen (*Septoria linicola*), and provide the basis for exploring the mechanism of disease resistance in flax. The activities of defensive enzymes and the content of malondialdehyde (MDA) in the leaves of two flax cultivars, one resistant and another susceptible to pasmo, were detected at different stages after inoculation with the pathogen. The results showed that in the early stages of pathogen infection, the activity of superoxide dismutase of resistant varieties (lines) was significantly higher than that of susceptible varieties (lines). In addition, the changes in peroxidase, catalase, and polyphenol oxidase activities in the inoculated leaves of different flax varieties positively correlated with their varied resistance to pasmo, but with no significant difference between resistant and susceptible varieties. However, the changes in phenylalanine ammonia-lyase activity in the two cultivars were assumed to be negatively correlated with their resistance to pasmo. For most of the stages after inoculation with *S. linicola*, the changes in the MDA content were negatively correlated with host resistance, but with no significant difference between resistant and susceptible varieties.

**Key words:** Defensive enzyme; Flax; Malondialdehyde; Resistance; *Septoria linicola*.

## Introduction

Flax (*Linum usitatissimum* L.) is an annual herb belonging to the Linaceae family. It has good tensile strength, fineness, and moisture absorption, and is harmless to the human body. It plays an important role in building materials, textile, paper making, energy, and other fields. It is the first natural plant fiber used by humans. Flax pasmo, also known as spot blight or spot disease, is caused by *Septoria linicola* Gar. The disease is mainly transmitted by seeds. In fields with mild disease, the yield is seriously reduced, while with severe disease, it could often result in loss of yield. *S. linicola* can infect all parts of a flax plant, including cotyledon, true leaf, petiole, stem, flower bud, ovary, and capsule. Hence, it causes flower and bud falling; especially branches and fruit stalks are seriously injured. Recent studies showed that the incidence rate in Heilongjiang, Yunnan, and other places was 10%~30%, and only less than 20% of healthy plants were found in the seriously damaged plots during harvest, posing a great threat to the flax production (Yang, 2004).

Plant disease resistance is closely related to material metabolism, including hormone and enzyme metabolism. When plants are stressed by pathogens, changes are seen not only in tissue structure but also in physiology and biochemistry. In this series of changes, the activity of defense enzymes is closely related to plant disease resistance (Baker *et al*., 1997). Defense enzymes are produced during the development of plant diseases. They mainly include superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), polyphenol oxidase (PPO), and phenylalanine ammonia-lyase (PAL) (Ma *et al*., 2012). When host plants are infected by the pathogen, they produce a large number of reactive oxygen species, including hydrogen peroxide ($H_2O_2$), hydroxyl radicals (OH), and superoxide anion radicals ($O^{2-}$), which affect the stability of the plant cell membrane. The defense enzymes SOD, CAT, and POD can scavenge the oxide, thus maintaining the stability of the plant cell membrane and reducing the severity of plant diseases. PPO can oxidize monophenols and diphenols to produce quinones. It has inhibitory and toxic effects on pathogens. It plays an important role in the process of plant disease resistance and also an important indicator in the study of disease physiology (Dai *et al*., 2007). PAL is an enzyme closely related to disease resistance in the host. It participates in the metabolism of phenylpropanes and can form disease-resistant secondary biomass in plants, such as phenols, lignin, phytoalexins, and chlorogenic acids (Ma *et al*., 2007). Defense enzymes are commonly reported in the study of disease resistance and defense response of plants under stress. Wang *et al*., (2013) determined the role of CAT, SOD, POD, and PPO in the study of the relationship between the physiological indexes of apple cultivars and the resistance to *Eriosoma lanigerum* in summer. They considered that SOD, CAT, and POD could remove harmful substances and change the activity to repair the damage to plants. Liu & Cao (2015) discussed the changes in SOD, CAT, POD, and other physiological indexes of Palmae in the study of physiological and biochemical traits of cold and drought resistance. Wang *et al*., (2016) studied the physiological and biochemical indexes of cold resistance of *Averrhoa carambola* cultivars under low-temperature stress and determined the cold resistance of four different varieties of *A. carambola* by measuring SOD, POD, and other indicators. Li (2017) measured the activities of POD, PPO, and PAL in cassava leaves infected with bacterial wilt. The results showed that the activities of these three enzymes increased. Therefore, the activities of these three enzymes were believed to be related to the disease resistance of cassava.

Malondialdehyde (MDA) is one of the most important products of membrane lipid peroxidation. Its accumulation can aggravate membrane damage. MDA is a common index of plant senescence and resistance. Therefore, the degree of membrane lipid peroxidation can be measured using MDA, which indirectly indicates the degree of membrane damage and the resistance of host plants. The MDA content is related to disease resistance (Liu & Li, 2015; Jiang *et al*., 2006). Recently, Kong *et al*., (2016) used hyperspectral imaging technology to rapidly and nondestructively detect the MDA content in plant leaves.

At present, no study explored the physiology and biochemistry of flax pasmo at home and abroad. In this study, the activities of five defense enzymes and the MDA content in the leaves of flax varieties with different resistances were determined after inoculation with *S. linicola*. Also, the relationship between the changes in physiological and biochemical indexes and the resistance was explored. This study aimed to reveal the mechanism of resistance of flax to pasmo based on the changes in physiological indexes of the host, and also to find out the physical indicators that could reflect the resistance of flax. The findings might provide the basis for developing flax disease resistance.

## Materials and Methods

**Test materials:** Tested flax varieties (lines) [Resistant variety (line) y62-9 and susceptible variety (line) y64-5] were provided by the Industrial Crops Institute of Heilongjiang Academy of Agricultural Sciences.

**Test pathogen:** In July 2018, flax plants with pasmo under natural conditions were collected from the Minzhu Experimental Station of Heilongjiang Academy of Agricultural Sciences. The stalks of flax were smashed, put into the flowerpot, and then seeded on a layer of disease-free soil so as to establish a disease transmission environment (Chen *et al*., 2019).

**Planting and sampling of flax:** The experiment was carried out in a potted field of Heilongjiang Academy of Agricultural Sciences. y62-9 and y64-5 varieties sown in the diseased soil were used as the treatment group, while those sown in the healthy soil were used as the control group. The test variety was planted in 5 pots, with a sowing amount of 100 seeds/pot, and 3 repetitions were set. The leaves of plants were taken in the emergence stage, fir like stage, fast growing stage, bud stage, flowering stage, and early yellow stage. They were put into liquid nitrogen for quick freezing and stored in an ultra-low temperature refrigerator at –80°C for further use.

**Determination of defense enzyme activity:** The flax leaves in each stage were washed with sterile distilled water, dried with a sterilized absorbent paper, and kept aside. The extraction of crude enzyme solution of CAT, SOD, POD, and PPO and the determination of their activity were in accordance with the method proposed by Guo (2018). For the measurement of PAL activity, the method introduced by Kamdee *et al*., (2009) was used.

**Extraction and determination of MDA:** The determination of the MDA content was based on the thiobarbituric acid colorimetric method proposed by Li (2000).

**Data processing and analysis:** Experiments were repeated three times with similar results. Data shown are from one of the experiments with three biological replicates. The experimental data were plotted and statistically analyzed using Microsoft Excel 2010 and SPSS 22.0 (one-way analysis of variance).

## Results

**Activity analysis of SOD in flax leaves inoculated with *S. linicola*:** Table 1 shows that the SOD activity in the leaves of resistant variety (line) y62-9 was significantly higher than that in the leaves of the uninoculated control group from the flowering stage to the early yellow stage after inoculation with *S. linicola*, but lower in other stages. The SOD activity in the leaves of susceptible variety (line) y64-5 inoculated with *S. linicola* was significantly higher than that in the leaves of the uninoculated control group in the fast growing stage and early yellow stage, but lower in other stages. The SOD activity in the leaves of resistant variety (line) y62-9 was significantly lower than that in the leaves of susceptible variety (line) y64-5 in the emergence stage and fast growing stage after inoculation with *S. linicola*, but significantly higher in the fir like stage and bud stage. The SOD activity in resistant and susceptible varieties (lines) showed a double-peak curve change of rising, falling, rising, and falling again before and after inoculation with *S. linicola*. The first peaks in the resistant and susceptible varieties appeared in the fir like stage (985.49 U $g^{-1}$ and 837.66 U $g^{-1}$, respectively). The SOD activity in the resistant variety (line) was 1.18 times higher than that in the susceptible variety (line), and the difference was significant. The second peak of the SOD activity in the leaves of resistant and susceptible varieties (lines) appeared in the flowering stage (711.52 U $g^{-1}$ and 571.9 U $g^{-1}$, respectively); the activity was significantly higher than that in the uninoculated control group (Table 1 and Fig. 1).

The results showed that the SOD activity in the resistant variety (line) was significantly higher than that in the susceptible variety (line) in the early stages of infection, indicating that the increase in the SOD activity was a kind of stress resistance reaction of flax after inoculation with *S. linicola*. Therefore, the SOD activity could be used as a physiological index to reflect the difference in resistance among resistant and susceptible varieties (lines) of flax.

**POD activity analysis of flax leaves inoculated with *S. linicola*:** After inoculation with *S. linicola*, the POD activity in the leaves of resistant and susceptible varieties (lines) showed a double-peak curve change of increase, decrease, increase, and decrease (Table 2); the first peak in the resistant and susceptible varieties appeared in the fir like stage (21704.93 U·$g^{-1}$ and 19782.4 U·$g^{-1}$, respectively). The POD activity in the resistant variety (line) was 1.1 times higher than that in the susceptible variety (line), with no significant difference between them (Fig. 2). The second peak of the POD activity in the leaves of the resistant and susceptible varieties (lines) appeared in the flowering stage (15372.15 U·$g^{-1}$ and 13813.17 U·$g^{-1}$, respectively), with a significant difference. The activity in the resistant variety (line) was 2.02 times higher than that in the uninoculated control group, while the activity in the susceptible variety (line) was 1.16 times higher than that in the uninoculated

control group. The POD activity in the leaves of the susceptible variety (line) y64-5 was significantly higher than that in the leaves of the uninoculated control group in the fast growing stage and early yellow stage after inoculation with *S. linicola*, but lower in other stages. The POD activity in the leaves of the resistant variety y62-9 was significantly lower than that in the leaves of the uninoculated control group before the bud stage and significantly higher after the bud stage.

The results showed that the POD activity in the resistant and susceptible varieties of flax changed greatly before and after inoculation with *S. linicola*, but with no significant difference between them. Therefore, the POD activity could not reflect the difference between resistant and susceptible varieties (lines) of flax.

**CAT activity analysis of flax leaves after inoculation with *S. linicola*:** The changing trend of the CAT activity of flax inoculated with *S. linicola* is shown in Fig 3. Throughout the growth period after inoculation with *S. linicola*, y62-9 showed a double-peak trend of increase, decrease, increase, and decrease in the activity; the CAT activity reached the first peak in the fir like stage (477.17 U $g^{-1}$). At this time, the SOD activity in the resistant variety (line) was 1.18 times higher than that in the susceptible variety (line), with no significant difference between them (Table 3). The second peak appeared in the bud stage (439.23 U $g^{-1}$). The SOD activity in the resistant variety (line) was 1.13 times higher than that in the susceptible variety (line). The CAT activity in the leaves of y62-9 was higher than that in the control group in the emergence stage, bud stage, flowering stage, and early yellow stage, but lower in the fir like stage and fast growing stage. The CAT activity in the leaves of y64-5 increased and then decreased after inoculation with *S. linicola*, and reached the peak in the fir like stage (476.79 U $g^{-1}$). The CAT activity in the leaves of the susceptible variety (line) after inoculation with *S. linicola* was significantly higher than that in the leaves of the uninoculated control group in the fast growing stage, bud stage, and early yellow stage, and lower than that in the leaves of the control group in other stages.

Fig. 3 and Table 3 show that the CAT activity in the resistant and susceptible varieties (lines) of flax changed greatly throughout the growth period except in the emergence stage and fir like stage, with a significant difference between them. However, no significant difference was found in the CAT activity in the leaves of resistant and susceptible varieties (lines) in the early stages of infection (emergence stage and fir like stage). Therefore, the CAT activity in the early stages of infection could not reflect the differences among flax resistant and susceptible varieties (lines).

**Table 1. SOD activity in the leaves of two varieties of flax infected with *S. linicola* in different stages after different treatments.**

| Treatment | Emergence stage | Fir like stage | Fast growing stage | Bud stage | Flowering stage | Early yellow stage |
|---|---|---|---|---|---|---|
| y62-9CK | 748.97 ± 32.22bB | 1343.58 ± 85.07aA | 810.61 ± 66.91aA | 647.9 ± 12.46aA | 320.05 ± 20.26cC | 86.18 ± 4.20cC |
| y62-9+P | 759.43 ± 24.60bB | 985.49 ± 58.58bB | 486.18 ± 21.44cC | 664.07 ± 27.06abA | 711.52 ± 14.63aA | 371.63 ± 3.82aA |
| y64-5CK | 859.67 ± 32.31aA | 1279.99 ± 17.50aA | 614.12 ± 39.89bB | 605.3 ± 11.94bA | 695.42 ± 9.27aA | 152.61 ± 4.33bB |
| y64-5+P | 814.82 ± 46.23abAB | 837.66 ± 29.03cB | 795.15 ± 30.20aA | 505.9 ± 37.98cB | 571.9 ± 8.47bB | 373.71 ± 12.83aA |

Data are expressed as mean ± SD. Different lowercase letters in the same column indicate a significant difference in the 0.05 level as revealed by the Duncan test. Different uppercase letters in the same column indicate a significant difference in the 0.01 level as revealed by the Duncan test. y62-9CK, y62-9+P, y64-5CK, and y64-5+P indicate control group of y62-9 of flax, y62-9 of flax infected with *S. linicola*, control group of y64-5 of flax, and y64-5 of flax infected with *S. linicola*

**Table 2. POD activity in the leaves of two varieties of flax infected with *S. linicola* in different stages after different treatments.**

| Treatment | Emergence stage | Fir like stage | Fast growing stage | Bud stage | Flowering stage | Early yellow stage |
|---|---|---|---|---|---|---|
| y62-9CK | 17139.7 ± 572.68bBC | 31937.68 ± 2282.26aA | 15797 ± 1269.61bA | 14211.48 ± 606.50aA | 7597.53 ± 392.79cB | 2035.56 ± 49.03dD |
| y62-9+P | 16795.76 ± 313.33bC | 21704.93 ± 2062.06bB | 11803.75 ± 478.05cB | 14442.65 ± 471.71aA | 15372.15 ± 535.47aA | 8020.47 ± 678.17bB |
| y64-5CK | 20135.26 ± 1330.16aAB | 32256.31 ± 2353.94aA | 11807.73 ± 1157.54cB | 13927.47 ± 855.63aA | 15964.1 ± 1176.00aA | 3499.72 ± 155.51cC |
| y64-5+P | 19101.59 ± 625.94aA | 19782.4 ± 1137.16bB | 18376.03 ± 918.79aA | 11032.15 ± 945.64bB | 13813.17 ± 672.79bA | 10199.59 ± 518.26aA |

Data are expressed as mean ± SD. Different lowercase letters in the same column indicate a significant difference in the 0.05 level, as revealed by the Duncan test. Different uppercase letters in the same column indicate a significant difference in the 0.01 level, as revealed by the Duncan test. y62-9CK, y62-9+P, y64-5CK, and y64-5+P indicate control group of y62-9 of flax, y62-9 of flax infected with *S. linicola*, control group of y64-5 of flax, and y64-5 of flax infected with *S. linicola*

**Table 3. CAT activity in the leaves of two varieties of flax infected with *S. linicola* in different stages after different treatments.**

| Treatment | Emergence stage | Fir like stage | Fast growing stage | Bud stage | Flowering stage | Early yellow stage |
|---|---|---|---|---|---|---|
| y62-9CK | 445.35 ± 12.45aA | 513.38 ± 8.02aA | 439.45 ± 7.71abA | 435.08 ± 11.24aA | 318.1 ± 10.54cC | 100.43 ± 10.42dC |
| y62-9+P | 455.08 ± 16.15aA | 477.17 ± 13.31bB | 385.26 ± 7.376cB | 439.23 ± 19.13aA | 418.85 ± 10.39aA | 334.5 ± 21.93bA |
| y64-5CK | 464.83 ± 7.09aA | 498.82 ± 7.66aAB | 419.01 ± 12.21bAB | 383.64 ± 11.03bB | 425.31 ± 5.27aA | 176.9 ± 13.99cB |
| y64-5+P | 453.77 ± 1.50aA | 476.79 ± 3.85bB | 458.87 ± 23.07aA | 388.77 ± 6.16bB | 385.45 ± 0.55bB | 367.64 ± 5.94aA |

Data are expressed as mean ± SD. Different lowercase letters in the same column indicate a significant difference in the 0.05 level, as revealed by the Duncan test. Different uppercase letters in the same column indicate a significant difference in the 0.01 level, as revealed by the Duncan test. y62-9CK, y62-9+P, y64-5CK, and y64-5+P indicate control group of y62-9 of flax, y62-9 of flax infected with *S. linicola*, control group of y64-5 of flax, and y64-5 of flax infected with *S. linicola*

**Analysis of the PPO activity in flax leaves inoculated with *S. linicola*:** Fig 4 shows that in the four treatments, the changing trends of the PPO activity in the leaves of flax resistant and susceptible varieties (lines) y62-9 and y64-5 were basically the same: they showed the trend of increasing, decreasing, and then increasing. After inoculation with *S. linicola*, the PPO activity in the leaves of resistant variety (line) was higher than that in the leaves of the susceptible variety (line) in the fir like stage, flowering stage, and early yellow stage, but the difference was significant only in the flowering stage (Table 4). After inoculation with *S. linicola*, the peak values of the PPO activity in the leaves of resistant and susceptible varieties (lines) appeared in the fir like stage (930.89 U $g^{-1}$ and 886.70 U $g^{-1}$, respectively). The rate of increase in the PPO activity in the resistant and susceptible varieties was 72.90% and 60.26%, respectively, compared with the control group. The PPO activity in the leaves of the resistant variety (line) was 1.73 times higher than that in the leaves of the control group. The PPO activity in the leaves of the susceptible variety (line) was 1.03 times higher than that in the leaves of the control group. After inoculation with *S. linicola*, the PPO activity in the resistant variety (line) was 2.77 times higher than that in the susceptible variety (line). After inoculation with *S. linicola*, the PPO activity in the leaves of the resistant variety (line) was higher than that in the leaves of the uninoculated control group in the fir like stage, bud stage, and flowering stage, but lower in other stages. The PPO activity in the leaves of the susceptible variety (line) was higher than that in the leaves of the uninoculated control group only in the fir like stage, but lower than that in the leaves of the uninoculated control group in other stages.

The results showed that the PPO activity in the resistant and susceptible varieties (lines) of flax inoculated with *S. linicola* was significantly different only in the fast growing stage and flowering stage, but not in other stages. Therefore, the PPO activity could not reflect the difference between resistant and susceptible varieties (lines) of flax.

**Analysis of the PAL activity in flax leaves after inoculation with *S. linicola*:** The PAL activity in the leaves of resistant and susceptible varieties (lines) showed a trend of increasing, decreasing, and increasing again after four treatments (Fig. 5). In the control treatment without inoculation with *S. linicola*, the PAL activity in the resistant cultivar (line) y62-9 was lower than that in the susceptible cultivar (line) y64-5 all the stays, but higher than that in the susceptible cultivar (line) y64-5 in other stages. After inoculation with *S. linicola*, the PAL activity in the leaves of the resistant variety (lines) was significantly higher than that in the leaves of the control group in the fir like stage, bud stage, and flowering stage, and lower than that in the leaves of the control group in other stages. The PAL activity in the leaves of the susceptible variety (lines) was higher than that in the leaves of the control group only in the emergence stage and flowering stage, and lower than that in the leaves of the control group in most other stages. After inoculation with *S. linicola*, the PAL activity in the resistant cultivar (line) y62-9 was significantly higher than that in the susceptible cultivar (line) in the emergence stage, bud stage, flowering stage, and early yellow stage. However, the PAL activity in the resistant cultivar (line) was significantly lower than that in the susceptible cultivar (line) only in the fir like stage and fast growing stage, both of which reached the peak value in the fir like stage (157.88 U $g^{-1}$ and 195.89 U $g^{-1}$, respectively). The rate of increase in the PAL activity in the resistant variety (line) was 22.59%; the activity was 1.23 times higher than that in the uninoculated control group. The PAL activity reduction rate in the susceptible variety (line) was 13.6%. The PAL activity in the leaves of the susceptible variety (line) was 1.24 times higher than that in the resistant variety (line) and the difference was significant (Table 5).

The results showed that PAL in the susceptible variety (line) responded more quickly to the pathogen than to the resistant variety (line) in most stages. Hence, it was speculated that PAL was negatively correlated with disease resistance. The increase in the PAL activity after inoculation with *S. linicola* could be used as a physiological and biochemical indicator for the infectivity of flax to pasmo.

**Analysis of the MDA content in the flax leaves after inoculation with *S. linicola*:** Fig. 6 shows the changing trend of the MDA content before and after flax inoculation with *S. linicola*. After inoculation with *S. linicola*, the changes in the MDA content in the leaves of resistant and susceptible varieties (lines) showed an overall upward trend. After inoculation with *S. linicola*, the MDA content in the leaves of the resistant variety (line) was higher than that in the leaves of the control group in the fir like stage and fast growing stage, and lower than that in the leaves of the control group in other stages, and the difference between them was not significant in most stages. The MDA content in the leaves of the susceptible variety (line) was higher than that in the leaves of the control group in the emergence stage, fir like stage, and flowering stage, and lower in the fast growing stage, bud stage, and early yellow stage. After inoculation with *S. linicola*, the MDA content in the leaves of the resistant varieties (lines) was lower than that in the leaves of the susceptible variety (line) in the emergence stage, bud stage, flowering stage, and early yellow stage, but higher than that in the leaves of the susceptible variety (line) in the fir like stage and fast growing stage, with no significant difference between them in most stages. The MDA content in the leaves of resistant and susceptible varieties (lines) was 32.28 and 33.29 nmol·$g^{-1}$, respectively, in the early yellow stage. Compared with the control group, the increase in the MDA content after inoculation with *S. linicola* was slightly less throughout the growth stages. For the resistant varieties (lines) not inoculated with *S. linicola*, the MDA content in the leaves increased significantly after the flowering stage and reached 75.91 and 54.63 nmol·$g^{-1}$ in the early yellow stage (Table 6).

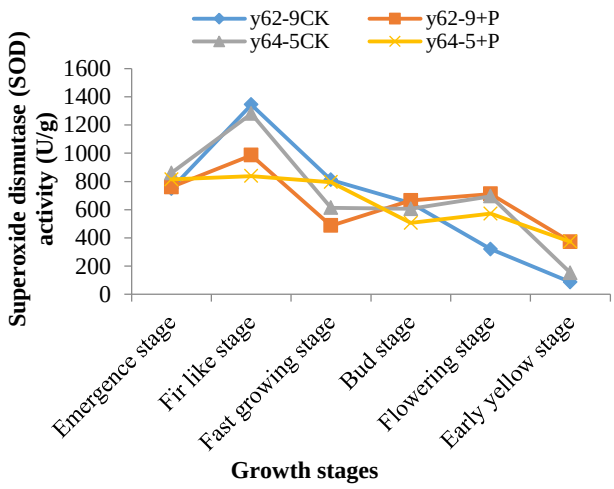


Fig. 1. The changing trend of the SOD activity in the leaves of flax in different growth stages. y62-9CK, y62-9+P, y64-5CK, and y64-5+P indicate control group of y62-9 of flax, y62-9 of flax infected with *S. linicola*, control group of y64-5 of flax, and y64-5 of flax infected with *S. linicola*.

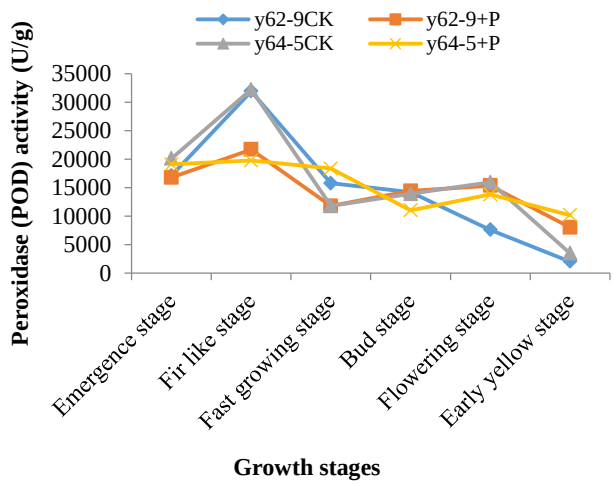


Fig. 2. The changing trend of the POD activity in the leaves of flax in different growth stages. y62-9CK, y62-9+P, y64-5CK, and y64-5+P indicate control group of y62-9 of flax, y62-9 of flax infected with *S. linicola*, control group of y64-5 of flax, and y64-5 of flax infected with *S. linicola*.

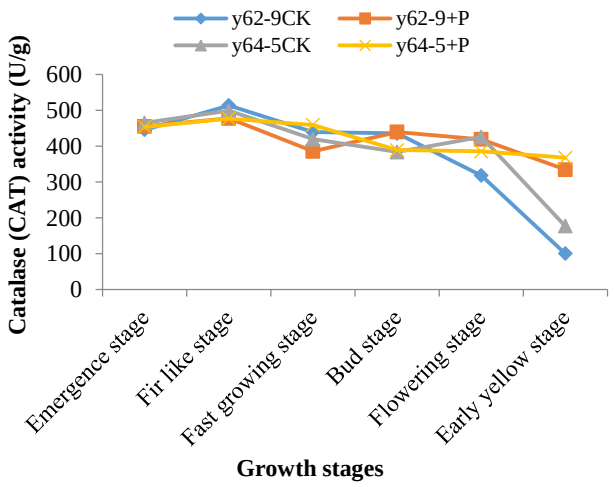


Fig. 3. The changing trend of the CAT activity in the leaves of flax in different growth stages. y62-9CK, y62-9+P, y64-5CK, and y64-5+P indicate control group of y62-9 of flax, y62-9 of flax infected with *S. linicola*, control group of y64-5 of flax, and y64-5 of flax infected with *S. linicola*.

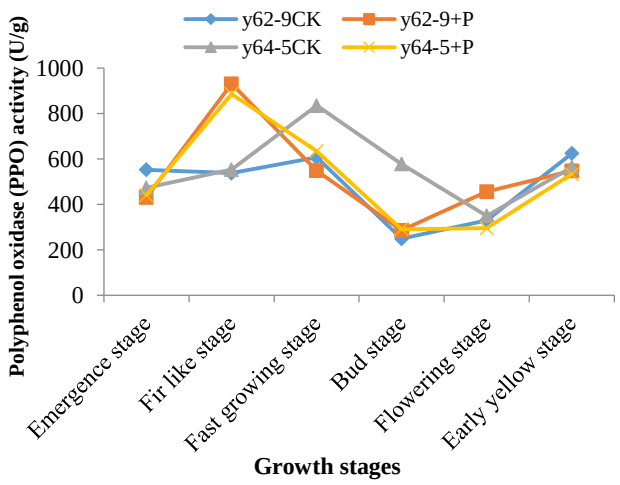


Fig. 4. The changing trend of the PPO activity in the leaves of flax in different growth stages. y62-9CK, y62-9+P, y64-5CK, and y64-5+P indicate control group of y62-9 of flax, y62-9 of flax infected with *S. linicola*, control group of y64-5 of flax, and y64-5 of flax infected with *S. linicola*.

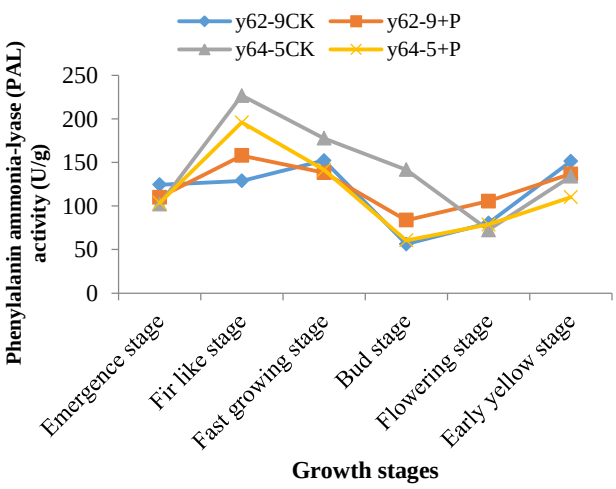


Fig. 5. The changing trend of the PAL activity in the leaves of flax in different growth stages. y62-9CK, y62-9+P, y64-5CK, and y64-5+P indicate control group of y62-9 of flax, y62-9 of flax infected with *S. linicola*, control group of y64-5 of flax, and y64-5 of flax infected with *S. linicola*.

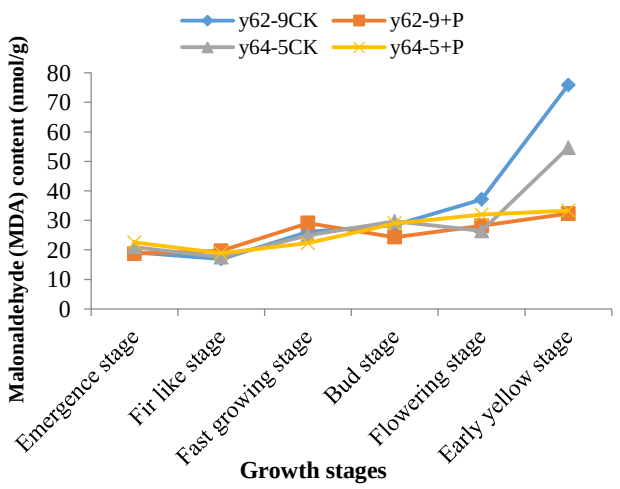


Fig. 6. The changing trend of the MDA content in the leaves of flax in different growth stages. y62-9CK, y62-9+P, y64-5CK, and y64-5+P indicate control group of y62-9 of flax, y62-9 of flax infected with *S. linicola*, control group of y64-5 of flax, and y64-5 of flax infected with *S. linicola*.

**Table 4. PPO activity in the leaves of two varieties of flax infected with *S. linicola* in different stages after different treatments.**

| Treatment | Emergence stage | Fir like stage | Fast growing stage | Bud stage | Flowering stage | Early yellow stage |
|---|---|---|---|---|---|---|
| y62-9CK | 522.51 ± 5.56aA | 538.41 ± 26.01bB | 607.06 ± 26.51bcB | 249.1 ± 16.73bB | 329.78 ± 6.57bcB | 624.75 ± 11.82aA |
| y62-9+P | 430.89 ± 42.28bB | 930.89 ± 48.18aA | 548.69 ± 48.87cB | 285.73 ± 25.63bB | 456.63 ± 16.83aA | 548 ± 14.61bB |
| y64-5CK | 473.04 ± 13.91bAB | 553.28 ± 31.62bB | 835.58 ± 29.63aA | 577.96 ± 38.54aA | 350.17 ± 31.80bB | 564.09 ± 39.15bAB |
| y64-5+P | 440.95 ± 21.81bB | 886.70 ± 18.55aA | 634.63 ± 13.18bB | 290.79 ± 10.15bB | 295.98 ± 23.15cB | 534.16 ± 22.56bB |

Data are expressed as mean ± SD. Different lowercase letters in the same column indicate a significant difference in the 0.05 level, as revealed by the Duncan test. Different uppercase letters in the same column indicate a significant difference in the 0.01 level, as revealed by the Duncan test. y62-9CK, y62-9+P, y64-5CK, and y64-5+P indicate control group of y62-9 of flax, y62-9 of flax infected with *S. linicola*, control group of y64-5 of flax, and y64-5 of flax infected with *S. linicola*

**Table 5. PAL activity in the leaves of two varieties of flax infected with *S. linicola* in different stages after different treatments.**

| Treatment | Emergence stage | Fir like stage | Fast growing stage | Bud stage | Flowering stage | Early yellow stage |
|---|---|---|---|---|---|---|
| y62-9CK | 124.42 ± 9.78aA | 128.79 ± 3.85dB | 152.16 ± 1.00bB | 56.35 ± 1.83cC | 80.34 ± 6.28bB | 151.3 ± 4.17aA |
| y62-9+P | 109.84 ± 7.67abA | 157.88 ± 5.01cB | 138.13 ± 1.54cB | 83.71 ± 10.98bB | 105.49 ± 7.62aA | 136.67 ± 2.03bB |
| y64-5CK | 102.02 ± 8.70bA | 226.73 ± 19.09aA | 177.74 ± 11.64aA | 141.71 ± 4.19aA | 72.41 ± 7.25bB | 134.04 ± 5.62bB |
| y64-5+P | 103.54 ± 8.96bA | 195.89 ± 10.41bA | 141.56 ± 7.70bcB | 60.54 ± 6.40cC | 78.61 ± 2.37bB | 110.05 ± 5.72cC |

Data are expressed as mean ± SD. Different lowercase letters in the same column indicate a significant difference in the 0.05 level, as revealed by the Duncan test. Different uppercase letters in the same column indicate a significant difference in the 0.01 level, as revealed by the Duncan test. y62-9CK, y62-9+P, y64-5CK, and y64-5+P indicate control group of y62-9 of flax, y62-9 of flax infected with *S. linicola*, control group of y64-5 of flax, and y64-5 of flax infected with *S. linicola*

**Table 6. MDA content in the leaves of resistant and susceptible varieties of flax infected with *S. linicola* in different stages after different treatments.**

| Treatment | Emergence stage | Fir like stage | Fast growing stage | Bud stage | Flowering stage | Early yellow stage |
|---|---|---|---|---|---|---|
| y62-9CK | 19.19 ± 3.52aA | 16.94 ± 3.83aA | 26.14 ± 2.68abA | 28.39 ± 3.61aA | 37.12 ± 5.54aA | 75.91 ± 12.69aA |
| y62-9+P | 18.68 ± 2.96aA | 19.67 ± 3.23aA | 29.06 ± 3.49aA | 24.3 ± 2.74aA | 28.13 ± 2.21bA | 32.28 ± 6.68bB |
| y64-5CK | 20.93 ± 6.73aA | 17.50 ± 1.80aA | 24.94 ± 3.78abA | 29.68 ± 3.04aA | 26.42 ± 4.23bA | 54.63 ± 20.58abAB |
| y64-5+P | 22.5 ± 11.15aA | 18.86 ± 1.27aA | 22.28 ± 2.91cA | 28.97 ± 0.94aA | 31.93 ± 3.32abA | 33.29 ± 3.11bB |

Data are expressed as mean ± SD. Different lowercase letters in the same column indicate a significant difference in the 0.05 level as revealed by the Duncan test. Different uppercase letters in the same column indicate a significant difference in the 0.01 level as revealed by the Duncan test. y62-9CK, y62-9+P, y64-5CK, and y64-5+P indicate control group of y62-9 of flax, y62-9 of flax infected with *S. linicola*, control group of y64-5 of flax, and y64-5 of flax infected with *S. linicola*

The results showed that the MDA content in the leaves negatively correlated with the disease resistance of the host in most of the stages after inoculation with *S. linicola*, but with no significant difference between the resistant and susceptible varieties, especially in the early stages of infection. Therefore, the MDA content could not be used as a physiological index to reflect the difference between the resistant and susceptible varieties (lines) of flax.

## Discussion

The research on plant defense enzymes has a long history. The defense enzymes include mainly POD, CAT, PAL, SOD, PPO, β-1,3-glucanase, and chitinase. These enzymes can participate in the synthesis of disease-resistant secondary substances or reactive oxygen species metabolism in plants or directly inhibit and kill pathogens during an interaction between plants and pathogens so that plants become resistant (Li, 2015). CAT is an enzyme that widely exists in organisms and scavenges mainly the hydrogen peroxide produced during plant metabolism, thus playing a protective role in plants (Zhang & Tian, 2007). POD is one of the key enzymes in the plant enzymatic defense system. It participates in the synthesis of lignin and in quality assurance. It can improve the mechanical strength, reduce the sensitivity to the degradation of extracellular enzymes, prevent the invasion of pathogens, induce phenol oxidation, and promote plant browning. SOD is one of the main antioxidant enzymes for scavenging superoxide anion free radicals in plants; it can prevent cell membrane peroxidation (Mittler, 2002). PPO is widely found in animals and plants, and can catalyze the oxidation of polyphenols to quinones, which can inhibit and poison the pathogen (Zhao, 2005). PAL is considered to be an important physiological index of plant resistance. The phenylpropane metabolic pathway controlled by PAL can synthesize a variety of phenols, flavonoids, terpenes, and other substances. It is of great significance for plant growth and development, resistance to diseases and insect pests, and other aspects (Huang & Zhao, 2017).

MDA is a strong oxidant that can reduce the electric resistance and membrane fluidity of the cell membrane system, and ultimately destroy the structure and physiological integrity of the membrane. It is directly related to the degree of damage of the cell membrane because of the toxicity of MDA to cells that it can cause the disorder of cell membrane function and damage many functional molecules. Therefore, the increase in the MDA content is the direct cause of plant cell damage. As the final product of membrane lipid peroxidation, MDA reflects the degree of membrane peroxidation in plants to some extent (Ding *et al.*, 2013; Zhao *et al.*, 2012). Therefore, MDA is closely related to plant disease resistance.

In this study, the changes in many biochemical indexes in flax leaves before and after the infection and the relationship between biochemical indexes and disease resistance were analyzed. The results showed that the SOD activity in the resistant varieties (lines) was significantly higher than that in the susceptible varieties (lines) in the early stages of infection. Hence, the SOD activity could be used as a physiological index to reflect the differences between resistant varieties (lines) and susceptible varieties (lines) of flax. After inoculation with *S. linicola*, the POD, CAT, and PPO activities in the leaves of the resistant and susceptible varieties could not reflect the differences among varieties (lines) of flax. Hence, they were not used as the screening indexes of early resistance. During infection, PAL in the susceptible varieties (lines) responded more quickly to pathogens compared with resistant varieties (lines). It was speculated that the PAL activity was negatively correlated with the resistance to pasmo. Therefore, the improvement in the PAL activity after inoculation with *S. linicola* could be used as a physiological and biochemical index for the infectivity of flax to pasmo. After inoculation with *S. linicola*, the MDA content in the leaves was negatively correlated with the resistance of the host, with no significant difference between the resistant and susceptible varieties (lines), especially in the early stages of the infection. Therefore, the MDA content could not be used as a physiological index reflecting the difference between the resistant and susceptible varieties (lines).

In this study, the SOD activity in the resistant varieties of flax inoculated with *S. linicola* was significantly lower than that in the untreated control group in the early stages of the infection, indicating that the nonaffinity interaction between plants and pathogens might activate the $O^{2-}$ production system. The plant produced a large amount of $O^{2-}$, leading to the accumulation of $O^{2-}$. However, the low level of SOD activity in plants was not enough to eliminate the excessive $O^{2-}$. The imbalance in $O^{2-}$ metabolism resulted in oxidative stress to plants, leading to the allergic reaction and thus playing a role in disease resistance.

The results showed that in the early stages of the infection, the increase in CAT and PPO activities in the resistant and susceptible varieties was very close. The increase in the resistant varieties (lines) was higher than that in the susceptible varieties (lines), but with no significant difference between them. Therefore, the increase in the enzyme activity could indicate that the resistance of the host to pathogens was enhanced. However, the CAT and PPO activities could not reflect the differences between the resistant varieties (lines) of flax very well, and hence they were not used as the physiological indicators to reflect the differences between the resistant varieties (lines) of flax.

In the early stages of infection, the POD activity in the leaves of the resistant varieties (lines) increased more rapidly than that in the susceptible varieties (lines). It was speculated that the increase in the POD activity positively correlated with the resistance of flax to pasmo, but with no significant difference between them. The results showed that POD was less affected by environmental stress, and less PODS was used to play a defensive role. The POD activity could not reflect the differences between flax resistant and susceptible varieties (lines), and therefore it could not be used as an indicator in early disease resistance screening.

In this study, the PAL activity in the flax leaves infected with pathogens was negatively correlated with resistance to pasmo, indicating that an increase in the PAL activity in the flax leaves inoculated with *S. linicola* was not sufficient to inhibit the continued spread of hyphae. This reflected the susceptibility to pathogenic toxins. The more susceptible the genotype, the stronger the sensitivity, and the greater the increase in PAL activity after infection. Therefore, it was presumed that the increase in the PAL activity was a concomitant effect of the infection of flax with *S. linicola*. The degree of increase in the PAL activity after inoculation with *S. linicola* could be used as a physiological and biochemical index of the susceptibility of flax to pasmo, and hence as a prescreening index.

The results showed that the MDA content in the leaves of the resistant and susceptible varieties in the early stages of the infection after inoculation with *S. linicola* was higher than that in the leaves of the control group, which was consistent with previous reports (Li, 2015; Xu, 2009). However, the MDA content in the leaves of susceptible varieties (lines) was significantly lower than that in the resistant varieties (lines) in the early stage of infection (fir like stage), which was different from previous studies (Yao, 2007; Liang *et al*., 2008; Gao *et al*., 2012) but consistent with the results of Jiang *et al*., (2006). The possible reason was that the SOD, POD, and PPO activities in the host were high when host–pathogen interaction occurred. A lot of active oxygen was removed, and the membrane lipid peroxidation was not strong; hence, the MDA content was low.

## Conclusions

This study showed that SOD and PAL in the defense enzyme system were related to resistance. Therefore, the determination of the SOD and PAL activity in different varieties at an appropriate time could be used as the biochemical index for identifying the resistance of flax to pasmo. However, this conclusion was obtained based on only two flax varieties. To further explore the relationship between the defense enzyme system and host resistance accurately, different flax varieties with different resistances should be selected for identifying a more accurate correlation between disease resistance of flax and defense enzyme activity. The next step is to measure and analyze the content of chlorophyll, soluble protein, soluble sugar, and ascorbic acid in different periods before and after inoculation with *S. linicola*; explore the relationship between the dynamic changes in physiological and biochemical indexes of flax and the resistance to pasmo; and investigate the early identification and mechanism of resistance to pasmo.

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