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EFFECTS OF ANTIBIOTICS STRAIN ON ROOT GROWTH AND SEEDLING GROWTH IN MUSTER (SINAPIS ALBA L.)

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ABSTRACT: The veterinary antibiotics contamination in agroseosystems is a substantial problem globally. However, little is known about their to toxicity to crops, especially in Muster. This education assessed the phytotoxic properties of the two greatest illustrative antibiotics, namely doxycycline and ciprofloxacin, on seed germination, seedling growth, root elongation and antioxidant status in Muster, The results showed that doxycycline and ciprofloxacin under the experimental concentrations (5, 10, 20, 40 and 80 mg/L-1) had no influence on seed germination of muster. The reduced root length, fresh weight and surface area were observed when the concentrations of doxycycline and ciprofloxacin were higher than 10 mg·L^{-1} and 5 mg/L^{-1}, respectively. High concentrations (>20 mg/L^{-1}) of antibiotics dramatically decreased the root length, fresh weight, root numbers and surface area as well as the number of stele cells and stele area. The activity of catalase, superoxide dismutase (SOD) and peroxidase (POD) and malondial-dehyde (MAD) content in shoots and roots were increased with the increasing doxycycline and ciprofloxacin concentrations.

Keywords: Sinapis alba L. (Muster), root-shoot ratio, seed germination.

I. INTRODUCTION

Antibiotics are widely used for the therapy of infectious diseases in veterinary medicine. The vast majority (about 75%) of antibiotics ingested by animals are directly released into the environment through urine or feces, and indirectly to the farmland as fertilizer through bio- solids containing excreted antibiotics. Antibiotics can also be introduced to agricultural land through irrigation of crops with wastewater. Hence, a variety of veterinary an- tibiotics especially tetracyclines, fluorchinolones and sulfonamides have been detected in manure contaminated soil, water and sediment samples. Most excreted antibiotics and their metabolites are still bioactive and are potential hazards to the environment, animals and human beings. Because of their broad usage and persistence in the environment, negative environ- mental impacts caused by antibiotic pollutants have become a mounting concern in recent years, especially in developing countries. Overuse of antibiotics can lead to the accumulation of various antibiotic-resistant bacteria and the occurrence of antibiotic resistance genes in the environment, animals, crops, etc.. Antibiotic contamination in arable land can also impact soil microbial activity, plant growth and development. The detrimental effects of anti- biotics on seed germination, seedling growth and plant development have been reported in several vegetable species, including lettuce, carrot, cabbage and tomato. However, research work on the influence of various antibiotics on seed germination and root elongation of crops is rather limited, especially in muster. Abscisic acid is one of the important phytohormones involved in seed germination, plant development and abiotic stress responses in plants.

The critical roles of Abscisic acid signaling in seed germination and seedling growth of plants have been identified. It has also been reported that environmental stress can influence Abscisic acid biosynthesis and catabo- lism in plants by regulating Abscisic acid -related genes to enhance their stress tolerance. However, the response of Abscisic acid to antibiotic stress and its underlying mechanism remain unclear. The majority of studies on anti- biotic contamination focused on the accumulation characteristics of antibiotics in plants or the effects of antibiotic uptake on the presence of antibiotic-resistant bacteria) and antibiotic-resistance genes. Little is known about the effects of antibiotic stress on root development and Abscisic acid biosynthesis of crops, especially in Muster. Among the two most commonly used tetracyclines and quinolones antibiotics in veterinary medicine, oxytetracycline and enrofloxacin are frequently detected at relatively high concentrations in livestock products and environment.

II. MEASUREMENT OF MUSTER HEAT PHYSIOLOGICAL INDEXES

The following equations were used to calculate wheat physiological indexes including germination percentage, germination potential, germination index and vigor index.

$$\text{Germinationpotential}(\%) = \frac{\text{the number of germinated seeds in7th}}{\text{daytotal number of seeds for testing}} \times 100$$

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$$\text{Germination index}(\%) = \sum \frac{Gt}{Dt}$$

$$\text{Vigor index}(\%) = \sum \frac{Gt}{Dt} \times S$$

where Gt is the germination percentage at day t, Dt is the number of days needed for germination and S is the length of the seedlings

Root morphology analysis

Root morphology was quantified using a root analysis instrument. A× Safranin O/Fast Green staining method was used for root morphology observation. In brief, the embedded root samples were sectioned at 5.5 mm using a Leica microtome. The sections were deparaffinized with two fresh changes of Tulene and rehydrated by gradient ethanol. all sections were examined under an optical microscope (BX53, Olympus, Japan) at 400 magnification using the Image-pro plus software (version 7.0, Media Cybemetics, Inc., USA).

Determination of antioxidant status

Fresh shoot and root tissues were used a 0.5 g sample was ho- mogenized in 5 mL very cold solution containing 0.1 M HCl buffer (pH 7.8), 1 mM EDTA, 1 mM dithiothreitol and 4% (w/v) poly- vinylpyrrolidone. The homogenate was centrifuged at 13,000 g for 20 min at 4 ◦C and the supernatant was used for the following analysis.

III. TABLE 1:

Effects of Doxycycline and ciprofloxacin germination of muster

Treatment ($mg.L^{-1}$)		Germination Percentage	Germination potential	Germination index	Vigor index
Doxy	Control	95.50	93.25	27.98	1.67
	5	97.52	94.98	27.89	1.84
	10	95..98	89.56	26.26	1.49
	20	94.87	76.68	24.52	1.25
	40	96.26	72.59	22.02	0.89
	80	94.44	70.52	19.89	0.45
Cipro	Control	95.56	94.12	20.21	1.87
	5	90.89	94.89	24.79	1.83
	10	97.78	95.23	25.58	1.65
	20	82.59	96.01	26.87	1.42
	40	91.23	96.88	26.99	1.32
	80	96.65	99.32	27.25	1.13

TABLE 2:
Effects of Doxycycline and ciprofloxacin root development of muster

Treatment (mg.L^{-1})		Total root length (cm)	Root fresh weight (mg.explant^{-1})	Root number	Average diameter of root (mm)
Doxy	Control	27.13	16.72	5.89	0.52
	5	26.56	15.26	5.52	0.46
	10	25.49	13.54	5.23	0.49
	20	19.25	10.83	5.12	0.50
	40	14.59	7.28	4.82	0.53
	80	7.84	4.46	4.56	0.59
Cipro	Control	27.13	16.72	5.89	0.52
	5	21.23	12.26	5.79	0.50
	10	20.56	10.89	5.99	0.48
	20	18.25	8.56	4.54	0.44
	40	16.51	7.20	4.72	0.39
	80	12.28	4.28	3.21	0.22

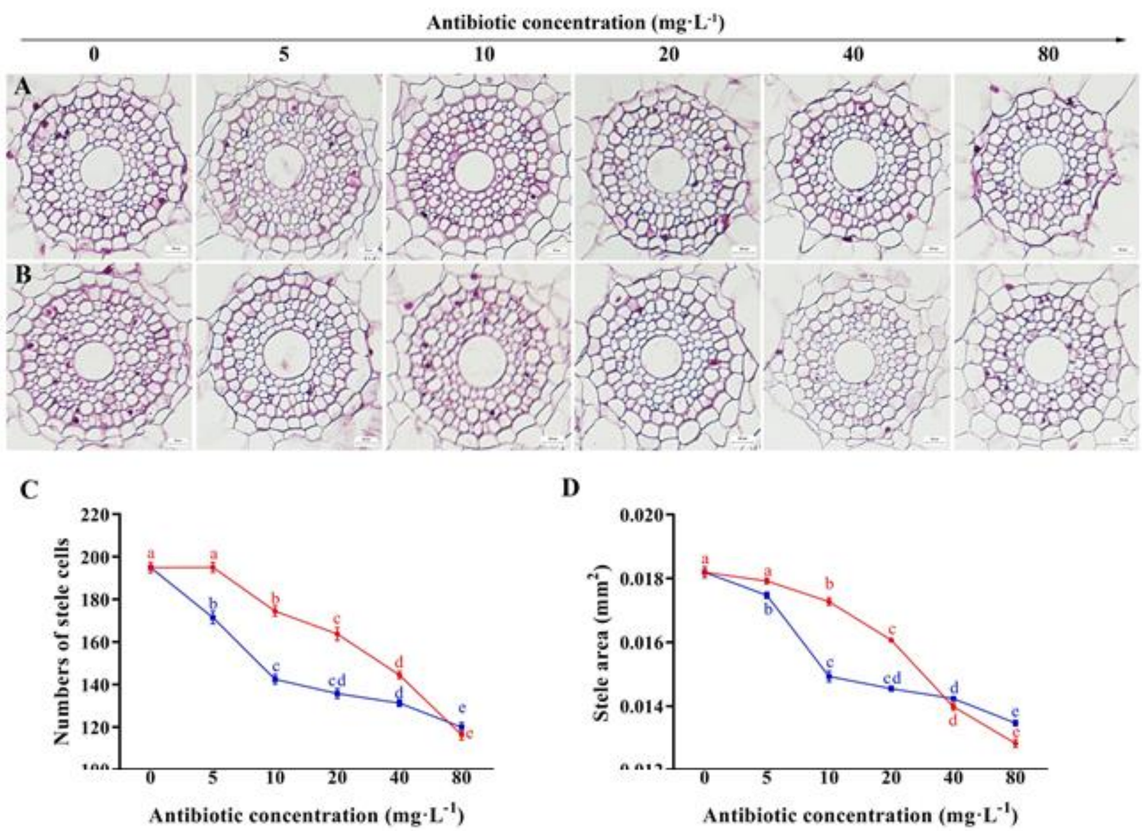

Fig. 1. Effects of Doxycycline and ciprofloxacin on root histomorphology, stele cells numbers (C) and stele area (D) of muster.

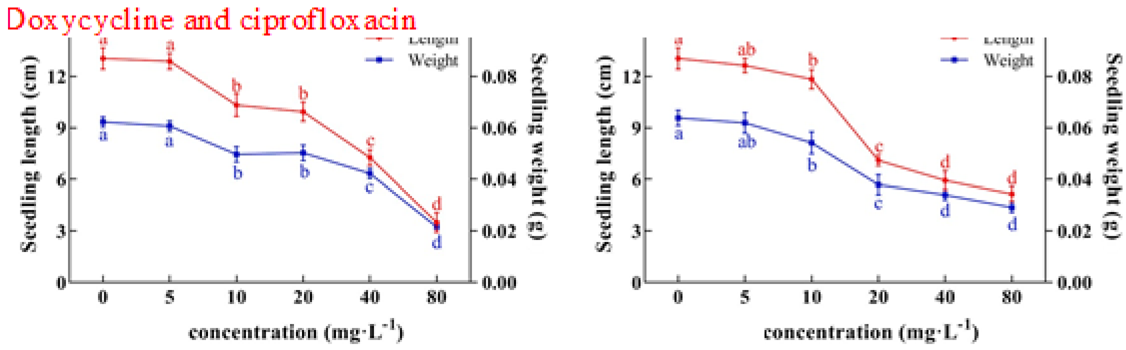

Fig. 2. Seedling length and fresh weight of muster exposed to various concentrations of Doxycycline and ciprofloxacin. Different superscript letters show statistical differences ($P < 0.05$).

Seed germination rate was not affected as muster seeds were exposed to various concentrations of (Table 1). The insignificant difference in germination rate indicates that doxycycline and ciprofloxacin under the experimental concentrations (5–80 mg/L^{-1}) have limited toxic effects on seed germination of muster. The maximum concentration of doxycycline and ciprofloxacin (80 mg/L) used in the present study was applicated according to the inhibition rate (80%) of root elongation. absorption cracked seed coat, the plumules and radicles can still grow and reach to the evaluation criteria (over 2 mm) for seed germination. In the present study, seed germination likely was radically inhibited when the concentration of doxycycline was higher than 10 mg/L^{-1}. Vigor index was also decreased with the increasing oxytetracycline concentration. The lowest vigor index was observed in the muster seeds treated with 80 mg/L^{-1} of doxycycline and ciprofloxacin As compared with the control group, seed vigor index in the doxytetracycline and ciprofloxacin groups decreased by 75% and 44%, respectively. No significant differences in the germinal potential and germinal index were observed among the ciprofloxacin treatment groups. These results suggest that high concentrations of antibiotics can negatively affect the germination of muster seeds with cracked coat and muster seeds are more sensitive to doxycycline.

The results of root development are depicted in Table 2 and Fig.2. As compared to the control, the decreased total root length and fresh weight in doxycycline and ciprofloxacin indicates that root elongation of muster seedlings was sensitive to low concentrations of antibiotics. This point can also be verified from the decreased vigor index of the seeds exposed to low concentrations of antibiotics. The lowest root length, fresh weight and root numbers was observed in the seeds exposed to 80 mg/L^{-1} doxycycline and ciprofloxacin. Total root length in the doxycycline and ciprofloxacin was significantly decreased by an average of 80% and 69%, respectively. The relatively greater reduction (76% of doxycycline vs. 69% of ciprofloxacin) in root weight was obtained in the muster seedlings exposed to 80 mg/L^{-1} antibiotics. Negative effects induced by the higher concentrations of doxycycline and ciprofloxacin were also observed in the number of stele cells and stele area (Fig. 1). These results indicate that high concentrations of antibiotics stress can inhibit the growth and development of roots in Muster. It is worth noting that treatment with 80 mg/L^{-1} doxycycline resulted in a more significant decrease in root length and fresh weight when compared to the ciprofloxacin Similar results were also observed in the number of stele cells, stele area, seedling length and weight. It is suggested that the toxic effects caused by high concentrations of doxycycline on muster were much greater than the same concentrations of ciprofloxacin.

In the present study, the effects of doxycycline and ciprofloxacin on antioxidant status of muster were concentration-dependent. However, the sensitivity of muster seedling to various antibiotics was different. It is indicated that muster seedlings were more sensitive to low concentration of ciprofloxacin. As compared to the Doxycycline, The cellular and molecular response of plants to Abscisic acid under abiotic stress conditions such as drought and high salinity has been extensively studied However, Abscisic acid action and its molecular mechanism in antibiotics stress response are still not well defined. In consideration of the critical roles of Abscisic acid in seedling growth, Abscisic acid content in shoot and root tissues of Muster exposed to different concentrations of doxycycline and ciprofloxacin was examined in the present study (Fig. 2). Abscisic acid content in roots was generally elevated with the increasing antibiotics concentration.

IV. CONCLUSION

In conclusion, high concentrations of doxycycline and ciprofloxacin did not affect seed germination rate of muster, but dramatically inhibited seedling growth and root elongation. muster seedlings have the relatively strong sensitivity to low concentration of ciprofloxacin. ROS overproduction induced by antibiotic stress could elevate MDA content of muster seedlings and activate the antioxidant defense systems by enhancing the activity of SOD and POD. Antibiotic stress can also influence ABA levels in shoots and roots by regulating the key genes related to ABA biosynthesis and metabolism. Further studies will be required to investigate the underlying mechanism of ABA signaling involved in seedling growth and root elongation of crops under antibiotic stress.

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