UNIVERSITY OF KWAZULU-NATAL

INYUVESI YAKWAZULU-NATALI

Study on *Fusarium* toxins contamination of cereal grains in Jiangsu Province, China

Submitted in fulfilment of the academic requirements for the degree of Doctor of Philosophy (PhD) in the Discipline of Microbiology; School of Life Sciences; College of Agriculture, Engineering and Science at the University of KwaZulu-Natal (Westville Campus), Durban.

Fang Ji

JULY 2018

As the candidate's supervisors, we have approved this thesis for submission

Supervisor: Dr. M.P. Mokoena 

Co-Supervisors: Prof. A.O. Olaniran _____

Prof. J. Shi _____

PREFACE

The experimental work described in this thesis was carried out in Jiangsu Academy of Agricultural Sciences, Nanjing, China and School of Life Sciences, University of KwaZulu-Natal (Westville Campus), Durban, South Africa from July 2015 to May 2018, under the supervision of Dr. M.P. Mokoena, Professor A.O. Olaniran and Professor J. Shi.

The studies represent original work by the author and have not otherwise been submitted in any form for any degree to any tertiary institution. Where use has been made of the work of others, it is duly acknowledged in the text.

Form EX1-5

COLLEGE OF AGRICULTURE, ENGINEERING AND SCIENCE

DECLARATION 1 – PLAGIARISM

I, **Fang Ji** declare that:

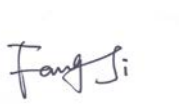
1. The research reported in this dissertation, except where otherwise indicated, is my original research.
2. This thesis has not been submitted for any degree or examination to any other university.
3. This thesis does not contain other persons' data, pictures, graphs or other information, unless specifically acknowledged as being sourced from other persons.
4. This thesis does not contain other persons' writing, unless specifically acknowledged as being sourced from other researchers. Where other written sources have been quoted, then:

a. Their words have been re-written but the general information attributed to them has been referenced

b. Where their exact words have been used, their writing has been placed inside quotation marks, and referenced.

5. This thesis does not contain text, graphics or tables copied and pasted from the Internet, unless specifically acknowledged, and the source being detailed in the dissertation and in the References sections.

Signed:



MC Feb-2012

Form EX1-6

COLLEGE OF AGRICULTURE, ENGINEERING AND SCIENCE

DECLARATION 2 – PUBLICATIONS

Details of contributions to publications that form part and/or include research presented in this thesis (include publications in preparation, submitted, in press and published and give details of the contributions of each author to the experimental work and writing of each publication).

Publication 1

Title: Development of an immunochromatographic strip test for the rapid detection of zearalenone in wheat from Jiangsu province, China

Journal: Plos One

Authors:

Fang Ji - Conceptualisation, Experimental design, Data analysis, Drafting and editing of manuscript

Mduduzi P. Mokoena - Conceptualisation, Experimental design, and editing of manuscript

Hongyan Zhao - Data collection and editing of manuscript

Ademola O. Olaniran - Conceptualisation, Experimental design, and editing of manuscript

Jianrong Shi - Conceptualisation, Experimental design, and editing of manuscript

Signed: 

CMC Feb-2012

ABSTRACT

Jiangsu Province is located in the transition zone from subtropical to warm temperate zone, with the characteristics of monsoon climate. The province's natural ecological conditions are suitable for the production of winter wheat and rice. In this region, the warm and humid climate is very suitable for the plant epidemics such as *Fusarium* head blight (FHB). *Fusarium* mycotoxins are secondary metabolites produced by *Fusarium* species; and can cause acute and chronic toxic effects on the body and are a threat to humans and animal health. Therefore, knowledge about the prevalence of FHB-producing *Fusarium* species, incidence of deoxynivalenol, zearalenone and fumonisins as well as possible influencing factors is imperative for preventing influx of contaminated grains into food supply chain. The present study focuses on the occurrence of *Fusarium* species coupled with contamination levels of *Fusarium* mycotoxins from different Jiangsu Province, China for three years, and the influences of rainfall and temperature on accumulation of DON. In addition, *Fusarium* strains were isolated from rice and assessed for the potential to produce fumonisins and beauvericin. The findings of this study increase the knowledge on important rice fungal pathogens and provide relevant information on the high variability of these pathogens, as well as their implications for the development of further diseases. The ICS test developed in our study has advantages, such as rapid and efficient screening of samples. The data obtained from the ICS test shows good agreement with LC-MS/MS data. These results showed that the ICS test is suitable for on-site monitoring of ZEN.

DEDICATION

This thesis is dedicated to:

My husband, Ahua Gu and my son, Chenpeng Gu

for their love, encouragement and support during the course of my academic career

ACKNOWLEDGEMENTS

The author records appreciation to the following:

- Prof. J. Shi for his supervision, advice and support.
- Dr. M.P. Mokoena and Prof. A.O. Olaniran, for their supervision, constructive suggestions and assistance throughout the course of this study.
- Staff and students of the *Fusarium* mycotoxins Lab, for your kindly help in my research.
- Jiangsu Academy of Agricultural Sciences and University of KwaZulu-Natal, for their support that I have this opportunity to continue my PhD study.
- The National Natural Science Foundation (31271988) and Jiangsu province science and technology support program-Social development (BE2014738) for their support.
- My family for their love, encouragement and supply.

TABLE OF CONTENTS

LIST OF TABLES

CHAPTER TWO

CHAPTER THREE

CHAPTER FOUR

CHAPTER FIVE

LIST OF FIGURES

CHAPTER TWO

CHAPTER THREE

CHAPTER FOUR

CHAPTER FIVE

CHAPTER ONE

Introduction

1.1 Background

The *Fusarium* genus comprises the most common pathogenic fungi of cereals and other crops in the world. They do not only bring obvious economic losses, but also produce mycotoxins that endanger the health of humans and animals (Backhhouse, 2014). *Fusarium fujikuroi* species complex (FFSC) and *Fusarium graminearum* species complex (FGSC) are the main genera of toxin production. *Fusarium verticillioides* and *Fusarium proliferatum* belongs to the genus of FFSC and are capable of producing fumonisins. *Fusarium verticillioides* is a frequent contaminant of maize while *Fusarium proliferatum* occurs in many different crops. FGSC consists of at least 16 species, which can produce a group of mycotoxins known as trichothecenes (Xu and Nicholson, 2009). Trichothecenes, zearalenone (ZEN) and fumonisins (FBs) are the most frequent *Fusarium* mycotoxins. However, other mycotoxins, such as moniliformin, enniatins, fusaproliferin and beauvericin can be identified in combination with the toxins described above. Trichothecene mycotoxins that include type A and type B are the most important group in terms of prevalence and contamination levels. Type A trichothecenes include T-2 and HT-2, and Type B trichothecenes include deoxynivalenol (DON) and nivalenol (NIV) which are found to be potent in cereals. The type of *Fusarium* mycotoxins produced by microorganisms can be predicted based on genetic markers coming from gene cluster (Lee *et al*., 2012).

The worldwide rise in demand of different varieties of foods/food products due to rapid urbanization and industrial growth leads to increased risks in food chain by contamination of foods and feeds with *Fusarium* mycotoxins (Leblanc *et al*., 2005). This leads to a serious threat to the health of humans

and animals, because they are responsible for different toxicological effects as well as huge economic losses(Smyth *et al*., 2014). As Escriva reported, many factors will affect the toxicological effects. Toxicities of different types of mycotoxins are different. Exposure level and duration, exposure to animal species and animal age all affect the toxicological effects (Escriva *et al*., 2015). Pigs exposed to high concentrations of DON can experience fatigue, vomiting, diarrhea, abdominal pain, and even shock or death. Exposure to fumonisin can cause pulmonary edema. The toxic effects of fumonisin on horses can be attributed to equine leukoencephalomalacia (ELM). *Fusarium* mycotoxins can affect the immune system by passing through the intestinal epithelium and reach the systemic compartment (Escriva *et al*., 2015). Although mycotoxins pose a risk to human health, due to the natural occurrence of these metabolites, it is impossible to completely ban these pollutants. Therefore, the protection of consumers is to maintain low levels of mycotoxins through good agricultural practices, good storage and processing measures (Gilbert, 2000). Many countries have adopted regulations that limit the exposure of mycotoxins, particularly to DON and ZEN, and the maximum allowable levels are significantly different in different countries (EFSA, 2011; GB/T, 2011). Therefore, the establishment of appropriate analytical methods to assess compliance with regulations and to monitor the emergence of mycotoxins in food and feed has become a worldwide priority.

In recent years, several methods have been established to detect *Fusarium* mycotoxins in foods and feeds (Anfossi *et al*., 2016). Conventional techniques such as thin layer chromatography (TLC); gas chromatography (GC) combined with electron capture, flame ionization or mass spectrometry detectors; high performance liquid chromatography (HPLC) combined with ultraviolet, diodearray, fluorescence or mass spectrometry detectors and enzyme-linked immunoassay (ELISA) are employed for detection of mycotoxins (Visconti *et al*., 2005; Lippolis *et al*., 2008; Li *et al*., 2013). These methods are accurate and precise for detection of mycotoxins in food or feed samples. However, these methods require skilled operators, large sample pretreatment, inaccurate equipment at low

concentration of analyte (Chauhan *et al*., 2016). Therefore, the major goals of this study influence of environmental factors on the prevalence of *Fusarium* mycotoxins in the Jiangsu Province of China, and to develop a rapid, sensitive and specific assay for routine analysis of mycotoxins in foods and feeds (Chen *et al*., 2016).

1.2 Scope of this study

The prevalence of wheat scab coupled with global climate change and farming system in China results in contamination of the wheat by *Fusarium* toxins, which have become an important issue that restricts the development of wheat industry (Xu and Berrie, 2005). Qiu and Shi, (2014) reported that *Fusarium asiaticum* is the primary pathogen causing scab in Asian countries and Brazil, and *Fusarium graminearums* is distributed throughout the world. In order to better predict *Fusarium* mycotoxins, Zeller *et al*. studied the genetic map of the pathogen population and the relationship between *Fusarium* species complexes and mycotoxin profiles (Zeller *et al*., 2003). Several studies have been reported on the influence of environmental conditions such as pH, humidity and drought on the occurrence of diseases produced by *Fusarium* species. Since these environmental factors affect the performance of both plant growth and infection, the study of climatic conditions and their changes have important implications for the control of *Fusarium* species and their mycotoxins (Fallah *et al*., 2016). Due to the widespread distribution of *Fusarium* mycotoxins and the analytical complexity of food matrices, there is an urgent need fo ra rapid, on-site, high-throughput mycotoxins detection technique.

This research provides a rapid immuno-chromatographic strip approach for screening wheat field for DON and ZEN contamination in Jiangsu Province, China. It further elucidates influences of

temperature, humidity, wheat variety on DON accumulation. All these will be helpful for the prediction and control of *Fusarium* mycotoxins.

1.3 Hypotheses

It is hypothesized that *Fusarium* species, wheat varieties and climate change will affect the accumulation of *Fusarium* mycotoxins. It is further hypothesized that immuno-chromatographic strip approach for detecting mycotoxins will be helpful in monitoring *Fusarium* mycotoxins contamination.

1.4 Aim

To elucidate the kinds of *Fusarium* species and mycotoxins that are found in Jiangsu Province, and to further assess the influence of environmental conditions (temperature, humidity, wheat varieties) on mycotoxin accumulation.

1.5 Specific objectives

The objectives of this study were:

1.5.1 To evaluate the occurrence of DON in wheat in different counties over a period of time (2014-2016) and correlate with the prevailing dominant *Fusarium* species;

1.5.2 To determine potential climatic factors that influence DON contamination in wheat;

1.5.3 To identify *Fusarium* strains and carry out phylogenetic differentiation of *Fusarium proliferatum* and *Fusarium fujikuroi;*

1.5.4 To evaluate the ability of *Fusarium proliferatum* and *Fusarium fujikuroi* strains to produce fumonisins and cause elongation of rice seeds;

1.5.5 To develop immuno-chromatographic strip technique that can rapidly detect ZEN in wheat samples.

1.6 Key research questions

1.6.1 What kinds of *Fusarium* mycotoxins are found in Jiangsu Province, China? What is the occurrence of *Fusarium* mycotoxins in different years and counties?

1.6.2 What kinds of *Fusarium* strains are isolated from rice?

1.6.3 What is the difference in fumonisins production from *Fusarium proliferatum* and *Fusarium fujikuroi* strains isolated from rice?

1.6.4 What is the detection limit of immuno-chromatographic strip test (ICST) in detection of ZEN in wheat?

1.6.5 Are the results obtained from ICST for ZEN consistent with those from HPLC-MS/MS?

1.7 References

Anfossi, L., Giovannoli, C., and Baggiani, C. (2016). Mycotoxin detection. Current Opinion in Biotechnology **37,** 120-126.

Backhhouse, D. (2014). Global distribution of Fusarium graminearum, F. asiaticum and F. boothii from wheat in relation to climate. Eur J Plant Pathol **139,** 13-16.

Chauhan, R., Singh, J., Sachdev, T., Basu, T., and Malhotra, B.D. (2016). Recent advances in mycotoxins detection. Biosensors & Bioelectronics **81,** 532-545.

Chen, Y., Chen, Q., Han, M., Zhou, J., Gong, L., Niu, Y., Zhang, Y., He, L., and Zhang, L. (2016). Development and optimization of a multiplex lateral flow immunoassay for the simultaneous determination of three mycotoxins in corn, rice and peanut. Food Chemistry **213,** 478-484.

EFSA. (2011). Scientific opinion on the risks for animal and public health related to the presence of T-2 and HT-2 toxin in food and feed. EFSA J **9,** 1.

Escriva, L., Font, G., and Manyes, L. (2015). In vivo toxicity studies of fusarium mycotoxins in the last decade: a review. Food and chemical toxicology : an international journal published for the British Industrial Biological Research Association **78,** 185-206.

Fallah, B., Zaini, F., Daei Ghazvini, R., Kachuei, R., Kordbacheh, P., Safara, M., and Mahmoudi, S. (2016). The antagonistic effects of Candida parapsilosis on the growth of Fusarium species and fumonisin production. Current Medical Mycology **2,** 1-6.

GB/T. (2011). China N.C.o. Maximum levels of mycotoxins in foods.

Gilbert, J.T., A. (2000). Review: Recent developments in research on fusarium head blight of wheat in Canada. Can. J. Plant Pathol **22,** 8-15.

Leblanc, J.C., Guerin, T., Noel, L., Calamassi-Tran, G., Volatier, J.L., and Verger, P. (2005). Dietary exposure estimates of 18 elements from the 1st French Total Diet Study. Food Additives and Contaminants **22,** 624-641.

Lee, J., Kim, H., Jeon, J.J., Kim, H.S., Zeller, K.A., Carter, L.L., Leslie, J.F., and Lee, Y.W. (2012). Population structure of and mycotoxin production by Fusarium graminearum from maize in South Korea. Applied and Environmental Microbiology **78,** 2161-2167.

Qiu, J., and Shi, J. (2014). Genetic relationships, carbendazim sensitivity and mycotoxin production of the Fusarium graminearum populations from maize, wheat and rice in eastern China. Toxins **6,** 2291-2309.

Smyth, S.J., McDonald, J., and Falck-Zepeda, J. (2014). Investment, regulation, and uncertainty: managing new plant breeding techniques. GM Crops & Food **5,** 44-57.

Xu, X., and Nicholson, P. (2009). Community ecology of fungal pathogens causing wheat head blight. Annual Review of Phytopathology **47,** 83-103.

Xu, X.M., and Berrie, A.M. (2005). Epidemiology of mycotoxigenic fungi associated with Fusarium ear blight and apple blue mould: a review. Food Additives and Contaminants **22,** 290-301.

Zeller, K.A., Summerell, B.A., Bullock, S., and Leslie, J.F. (2003). Gibberella konza (Fusarium konzum) sp. nov. from prairie grasses, a new species in the Gibberella fujikuroi species complex. Mycologia **95,** 943-954.

CHAPTER TWO

OCCURRENCE, TOXICITY, PRODUCTION, AND DETECTION OF *FUSARIUM* MYCOTOXINS: A REVIEW

Occurrence, toxicity, production, and detection of *Fusarium* mycotoxins: A Review

Fang Ji[1,2], A. O. Olaniran[2], M.P. Mokoena[2] and J. Shi[1*]

1. Key Lab of Food Quality and Safety of Jiangsu Province-State Key Laboratory Breeding/Key Laboratory of Control Technology and Standard for Agro-Product Safety and Quality/Key Laboratory of Agro-Product Safety Risk Evaluation (Nanjing), Ministry of Agriculture, China/ Institute of Food Safety and Nutrition, Jiangsu Academy of Agricultural Sciences, 210014, China
2. Discipline of Microbiology, School of Life Science, College of Agriculture, Engineering and Science, University of KwaZulu-Natal, Private Bag X54001, Durban, 4000, South Africa.

∗Corresponding author. Tel: +25 84392001; Fax: Tel: +25 84392001

E-mail address: jianrong63@126.com (Jianrong Shi)

Abstract

Fusarium mycotoxin contamination of both foods and feeds is an inevitable phenomenon worldwide. Deoxynivalenol, nivalenol, zearalenone, T-2 toxin and fumonisin B1 are the most studied *Fusarium* mycotoxins. Co-contamination of mycotoxins has also been studied frequently. *Fusarium* mycotoxins occur frequently in foods at very low concentrations, so there is a need to provide sensitive and reliable methods for their early detection. The present review provides insight on the types, toxicology and occurrence of *Fusarium* mycotoxins. It further elucidates the various detection methods for mycotoxin production from *Fusarium* strains, with a special focus on chromatographic and immunochemical techniques.

Keywords: *Fusarium* mycotoxins, toxicology, occurrence, detection

1. Introduction

Annually, 25-50% of crops harvested worldwide are contaminated with mycotoxins (Ricciardi *et al*., 2013). *Fusarium* head blight (FHB), also known as ear disease or scab, is a worldwide disease of wheat, corn, barley, rice and other small grains. Over the past decades, FHB has become one of the most serious fungal diseases, attributable to climate change and modern agricultural practices, causing tremendous economic losses worldwide (Osborne and Stein, 2007). *Fusarium* mycotoxins are secondary metabolite produced by *Fusarium* species during growth and storage. They also have chemical and thermal stability. Furthermore, mycotoxins are passed from the contaminated feed to animals and eventually to humans. Mycotoxins exhibit both acute and chronic toxic effects in humans and animals. The outbreak of the *Fusarium* toxicity has been reported in many countries, such as Europe, Asia, Africa, New Zealand and South America (Marin *et al*., 2013). Therefore, to protect human health, some countries have continuously monitored the maximum levels of mycotoxins in foods and other commodities to (Table 1) (Selvaraj *et al*., 2015; Ferrigo *et al*., 2016; Moretti *et al*., 2017).

Table 1: Allowable limits of *Fusarium* mycotoxins in food and feeds in certain countries and regions

Range	Toxin	Country	Applicable Products	Limit (μg/kg)
Food	DON	China	Cereals and their products	1,000
			Raw durum and oats, wet-milled corn	1,750
			Unprocessed cereals other than hard wheat, oats, and corn	1,250
		EU	Cereal that can be consumed directly and cornflakes less than or equal to 500 microns in size	750
			Bread, snacks, desserts, and breakfast cereals	500
			Cereal-based foods for infants and young children	200
		America	Wheat for food milling	2,000
			Final products made using edible wheat	1,000
			Unpurified soft wheat in China	2,000
		Canada	Soft wheat flour (adult food)	1,200
			Soft wheat flour (baby food)	600
		Armenia	Wheat	700
			Barley	1,000
		Belarus	Wheat	700
			Baby food	Prohibited
		Bulgaria	Grain and products made from grain for direct consumption or as processed food ingredients	1,000
			Cereals which will be stored or subjected to further physical processing prior to consumption	2,000
			Corn and corn products	1,000
		Cuba	Imported cereals	300
		Cyprus	Grain	1,200
		Serbia	Raw corn	1,750
	ZEN	China	Wheat and flour	60

		Corn and corn flour (slag and slice)	60
		Processed cereals for infants and young children	20
		Bread and breakfast cereals	50
		Grain products that can be eaten directly	75
	EU	Corn, corn snacks, and corn breakfast cereals that can be eaten directly	100
		Corn flakes larger than 500 microns in size	200
		Corn flakes less than or equal to 500 microns in size	300
		Corn treated via wet grinding	350
		Refined corn oil	400
	Armenia	All foods	1,000
	Austria	Wheat, rye, and hard wheat	60
	Belarus	Barley, wheat, and corn	1,000
		Baby foods	Prohibited
	Bulgaria	Grain and processed grain products for direct consumption or for use as processed food ingredients	200
		Corn and corn products	200
	Chile	All foods	200
	Columbia	Sorghum	1,000
	France	Grain and grain products	50
		Corn-based baby foods	200
		Corn snacks and corn breakfast cereals	800
FUMS	EU	Corn, corn snacks, and corn breakfast cereals that can be eaten directly	1,000
		Corn flakes larger than 500 microns in size	1,400
		Corn flakes less than or equal to 500 microns in size	2,000
		Corn treated via wet grinding	4,000
	America	Edible corn	2,000
FB1 & FB2	Bulgaria	Corn and corn products	1,000

Feed	FB1	Cuba	Corn and rice	1,000
	FB1	France	Grain and grain products	1,000
	T-2	China	Distiller's dried grain with corn solubles for feed	100
			Formulated feeds for pigs and poultry	1,000
		Armenia	All foods	100
		Belarus	Cereal, flour, and shelled oats	100
			Infant food	Prohibited
		Bulgaria	Grain and grain products for direct consumption and for use as processed food ingredients	100
		China	Formulated feeds for pigs, calves, and lactating animals	1,000
			Formulated feeds for cattle and poultry	3,000
		Austria	Pannage	500
			Feed for fattening poultry	1,500
			Feeds for breeding poultry and laying fowl	1,000
	DON	Canada	Feeds for beef cattle	1,000
			Feeds for livestock and poultry	5,000
			Feeds for pigs, calves, and cows	1,000
		Cuba	All feedstuffs	300
		Cyprus	All feedstuffs except coarse grain	7,000
			Complete feeds for pigs	1,000
			Complete feeds for poultry and fattening calves	5,000
			Complete feeds for other animals	3,000
		Serbia	Feeds	8,000
	ZEN	China	Feeds and distiller's dried grain with corn solubles	500
		Austria	Feeds for breeding swine	50
		Canada	Feeds for gilts and sows	3,000
		Cyprus	Feedstuffs	2,000

		Complete feeds for piglets	1,000
		Complete feeds for all pigs except piglets	1,500
T-2	Canada	Feeds for pigs and poultry	1,000
HT-2	Canada	Feeds for livestock and poultry	100

1.1 Types and toxicities of *Fusarium* mycotoxins

Fusarium species produce three most important classes of mycotoxins namely: trichothecenes, zearalenone (ZEN), and fumonisins (FBs).

1.1.1 Trichothecenes

Trichothecenes are the most important class of *Fusarium* mycotoxins, and they are also of most diverse chemical composition. They are a large family that contains many chemically related mycotoxins. *Fusarium*, *Myrothecium*, and *Stachybotrys* can produce trichothecenes, although they come from taxonomically different genera. Trichothecenes are one of the potential threats to the health of humans and animals worldwide (Li *et al*., 2011).

Trichothecenes are extremely prevalent with molecular weights ranging from 200 to 500 Da. They include more than 200 toxins, which have a substantial sesquiterpenoid structure, with or without macrocyclic esters or ester ether bridges between C-4 and C-15. In addition, trichothecenes consist of 12,13-epoxyalkylene groups that are responsible for cytotoxicity, as well as 9,10 double bonds with different side-chain substitutions (McCormick *et al*., 2011).

Trichothecenes have been subdivided into four groups (A-D) based on the substitution mode of the core structure of 9- ene (EPT) by tricyclic 12,13- epoxidation. Type A toxins include T-2, HT-2,

neosolaniol (ENNS), and diacetoxyscirpenol (DAS). Type B toxins include deoxynivalenol (DON) and its 3-acetyl and 15-acetyl derivatives, nivalenol (NIV), together with acetylated precursor of NIV [4-acetylnivalenol, also termed Fusarenon-X (FUX)]. Type C trichothecenes contain a C-7/C-8 epoxide, such as crotocin. Type D trichothecenes include roridin A, verrucarin A, and satratoxin H which have an extra loop that can link C-4 and C-15 (McCormick *et al*., 2011; Pinton and Oswald, 2014). The structures of the trichothecenes are shown in Figure 1and Table 2.

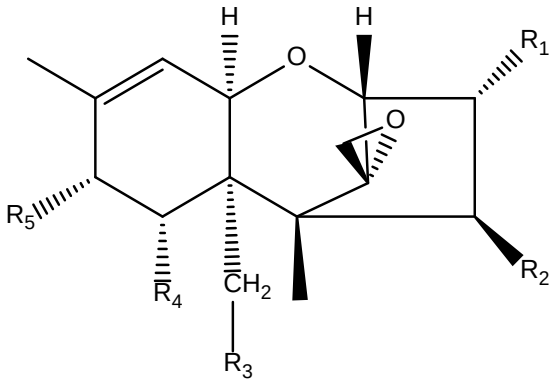


Fig. 1. Structures of trichothecenes (Marin *et al*., 2013).

Table 2: Representation of different groups contained in trichothecenes structures

Type	Trichothecene	R_1	R_2	R_3	R_4	R_5	Chemical Formula	Molecular mass (amu)
A	Diacetoxyscirpenol	OH	$OCOCH_3$	$OCOCH_3$	H	H	$C_{19}H_{26}O_7$	366
A	Neosolaniol	OH	$OCOCH_3$	$OCOCH_3$	H	OH	$C_{19}H_{26}O_8$	382
A	T-2 Toxin	OH	$OCOCH_3$	$OCOCH_3$	H	$OCOCH_2CH(CH_3)_2$	$C_{24}H_{34}O_9$	466
A	HT-2 Toxin	OH	OH	$OCOCH_3$	H	$OCOCH_2CH(CH_3)_2$	$C_{22}H_{32}O_8$	424
B	Deoxynivalenol	OH	H	OH	OH	=O	$C_{15}H_{20}O_6$	296
B	3-Acetyldeoxynivalenol	$OCOCH_3$	H	OH	OH	=O	$C_{17}H_{22}O_7$	338
B	15-Acetyldeoxynivalenol	OH	H	$OCOCH_3$	OH	=O	$C_{17}H_{22}O_7$	338
B	Fusarenon X	OH	$OCOCH_3$	OH	OH	=O	$C_{17}H_{22}O_8$	354

B	Nivalenol	OH	OH	OH	OH	=O	$C_{15}H_{20}O_7$	312

1.1.1.1 Deoxynivalenol

In recent years, FHB has once again become a major disease threatening food security, and this has led to renewed interest in trichothecenes, such as deoxynivalenol (DON) (Goswami and Kistler, 2004; van Egmond *et al*., 2007).

DON is mainly produced by *Fusarium graminearum* and *Fusarium culmorum*. DON composed of 12,13-epoxy-3α,7α,15-trihydroxytrichothec-9-en-8-one ($C_{15}H_{20}O_6$), crystallizes as colorless needles, stable at extreme temperatures (120-180 °C) and soluble in polar organic solvents such as aqueous acetonitrile, chloroform, methanol, ethanol and ethyl acetate (EFSA, 2004c). DON causes vomiting (that is why it is also known as vomitoxin), digestive disorders, oxidative damage, and reproductive toxicities in animals and humans, however, this mycotoxin is not a human carcinogen (Berthiller *et al*., 2011). The International Agency for Research on Cancer (IARC) classified DON in group 3 (non-carcinogenic substances) (Ostry *et al*., 2017). DON causes biological barriers and affects cell and organ functions and viability (Maresca, 2013). At cellular level, DON binds ribosomal peptide transferase active sites and activates cell kinases to inhibit protein and nucleic acid synthesis(Ueno *et al*., 1973; Shifrin and Anderson, 1999). Many kinases have been affected, including extracellular signal-regulated kinases, mitogen-activated protein kinases (MAPKs) p38 and c-jun N-terminal kinases (Shifrin and Anderson, 1999). DON triggers MAPK-mediated up-regulation of pro-inflammatory cytokine and chemokine expression, and apoptosis (Shifrin and Anderson, 1999; Zhou et al., 2003; Islam *et al*., 2006). The effects of DON on the immune system are manifold. Due to the different mycotoxin concentrations, timing and duration of exposure, effects can be achieved from immunosuppression to immunostimulation. According to Peraica report, DON is a potent protein

synthesis inhibitor that depresses the immune system, and causes dysphagia (Peraica *et al*., 1999). DON is regarded as a teratogen, neurotoxin, and immunosuppressant agent by The World Health Organization (WHO). In general, DON has been associated with chronic and fatal human and animal intoxication by eating contaminated food and feed (Rotter *et al*., 1996).

1.1.1.2 Nivalenol

Nivalenol (NIV) was detected from a virulent *Fusarium nivale* (Fn-2B), isolated from a farmland by Kokoda in 1963 in the Kumamoto region of Japan. Subsequently, Tani and Shigata (1979) found that the organism was lethal to rice, as it produced both NIV and FUX (Tatsuno *et al*., 1979). NIV (3,4,7,15-tetrahydroxy-12,13-epoxytrichothec-9-en-8-one) is produced mainly by *Fusarium graminearum, Fusarium crookwellense,* and *Fusarium nivale*. It co-occurs with FUX and DON in crops such as wheat, barley, and maize. NIV has been recently found in cereal-based products of European countries, and those of Brazil, Japan, Southeast Asia, and China (Turner, 2010).

NIV and DON are similar in terms of chemical structure, and also share many toxicological properties such as causing nausea, vomiting, diarrhea, and eventually death. Both toxins inhibit protein synthesis, and increase the levels of stress-activated MAPKs and serum alkaline phosphatase. Gerez *et al*. (2015) found that the overall liver and kidney weights of female mice were reduced when NIV was added to feeds at up to 0.7 mg/kg body weight (bw)/day for 2 years. After NIV administration to mice at 12 ppm for up to 8 weeks, the serum IgA concentration increased and IgA became deposited on the glomerular mesangium, mirroring human IgA nephropathy (Gerez *et al*., 2015).

Of the various *Fusarium* mycotoxins tested, NIV exerted one of the highest *in vitro* immunosuppressive effects on human peripheral blood mononuclear cells. NIV can inhibit the proliferation of human male and female mitogen-stimulated lymphocytes (Nagashima, 2014). At the mRNA level, NIV and DON modulate Th1-type cytokine expression differently at various doses, interacting with lymphocytes to inhibit cell proliferation by stimulating apoptosis (Severino *et al*., 2006). NIV is more toxic to human promyelocytic leukemia cell line HL60, human lymphoblastic leukemia cell line MLT-4 and rat aortic myoblast cell line A10 than DON (Nagashima *et al*., 2014). The chronic effects of low oral NIV doses in animal models have been seldom explored, but several countries tolerate only low levels of trichothecenes in cereals (Gouze *et al*., 2007). China imposes no NIV limit on foods or feeds.

1.1.1.3 T-2 and HT-2

The T-2 toxin [3-hydroxy-4-15-diacetoxy-8ct-(3-methyl butyryloxy) 12,13 epoxytrichothec-9-ene] contains an epoxy trichothecene loop. HT-2, a deacetylated form of T-2, is the principal metabolite of T-2. The toxicities of T-2 and HT-2 are similar, since both contain the epoxy sesquiterpenoid moiety. Consequently, the toxicity of T-2 may be partly attributable to HT-2 for T-2 is rapidly metabolized to HT-2 (Ndossi *et al*., 2012). Of all *Fusarium species*, *Fusarium langsethiae* seems to be the major producer of T-2 and HT-2 followed by *Fusarium poae* and *Fusarium sporotrichioides* (Thrane *et al*., 2004; Glenn and Quillin, 2007). T-2 and HT-2 contaminate many grains, such as maize, oat, barley, wheat, rice, and soybeans.

T-2 is considered one of the most acutely toxic trichothecenes, causing a wide range of toxic effects in animals. Acute T-2 toxicity has been studied in rats, mice, guinea pigs, and pigeons; with the toxin administered intravenously, orally, subcutaneously, intraperitoneally, or intratracheally (Bouaziz *et*

al., 2013). Symptoms of acute poisoning include nausea, vomiting, abdominal pain, diarrhea, bloody stools, cartilage tissue damage, weight loss, decreased immunity, decreased plasma glucose levels, and pathological changes in the liver and stomach. (Li *et al*., 2011). T-2 at 2 $mg \cdot kg^{-1}$ reduced lymphocyte numbers and caused hepatopancreatic necrosis in the black tiger shrimp. In addition, T-2 at 2.5 $mg \cdot kg^{-1}$ reduced body weight, feed ingestion, feed conversion, and hemoglobin concentration in rainbow trout. T-2 at 1 $mg \cdot kg^{-1}$ dose in catfish reduced intestinal immunity and increased mortality by up to 84% (Sehata *et al*., 2004). The main action of T-2 is to inhibit protein synthesis and secondary destruction of DNA and RNA synthesis (Doi *et al*., 2008).

T-2 can affect cell cycle, and induce chondrocytes, human astrocytes, mouse embryonic stem cells, pig primary hepatocytes, hematopoietic cells in bone marrow and spleen red pulp and epidermal basal cell apoptosis, indicating that T-2 can induce cell death with high proliferation activity (Shinozuka *et al*., 1998; Fang *et al*., 2012; Weidner *et al*., 2013).

In addition, T-2 targets the immune system, alters leukocyte counts, triggers delayed-type hypersensitivity, leads to depletion of certain hematopoietic progenitor cells, reduces antibody formation, and enhances allograft rejection and lectin promotion (Creppy, 2002). Pigs and horses are among the animals that are most sensitive to T-2, the major effects of which are immunological and hematological in nature. In quail, T-2 reduced the activity of blood alkaline phosphatase, an enzyme that plays an important role in the innate immune response, increased the levels of glutamic-pyruvic transaminase and glutamic-oxaloacetic transaminase (Nemcsok and Boross, 1982; Madheswaran *et al*., 2004).

1.1.2 Zearalenone

Zearalenone (ZEN), also known as F-2 toxin, is a resorcyclic acid lactone [6-(10-hydroxy-6-oxo-*trans*-1-undecenyl)-β-resorcyclic acid lactone ($C_{18}H_{22}O_5$, MW: 318.36, CAS 17924-92-4)]. In mammals, the ketones in C-8 are reduced to two stereoisomeric metabolites (the a- and b-isomers). The structures of ZEN and its derivatives are shown in Figure 2. Various ZEN metabolites are produced by fungi, but at lower concentrations. The relative concentrations of the individual toxins vary among host plants and geographical regions. These include several *Fusarium* species (*Fusarium graminearum, Fusarium culmorum, Fusarium crookwellense,* and *Fusarium equiseti*) that are known to also produce other toxins including DON, NIV, and FUX (Frizzell *et al.*, 2011). ZEN is a whitish, crystalline toxin with a melting point of 164°C -165 °C. ZEN is fat-soluble, insoluble in water, but soluble in alkalis and various organic solvents. ZEN is thermostable during storage, milling, processing, and cooking (EFSA., 2004d). ZEN contaminates corn, barley, oats, wheat, sorghum, millet, rice, flour, malt, soybeans, and beer. ZEN derivatives [α-zearalenol (α-ZEA), β-zearalenol (β-ZEA), *α*-zearalanol (α-ZAL), β-zearalanol (β-ZAL), and zearalanone] have been detected in corn stems, rice cultures, corn silage, corn products, and soya meal (Marin *et al*., 2011). The ZEN limits in corn and other cereals are currently in the range of 50 to 1,000 μg/kg (Table 1).

Several *in vivo* studies found that ZEN principally targeted the reproductive system. In laboratory animals, the toxic effects included changes in reproductive tract, uterine enlargement, reduced fertility, increased embryo-lethal resorption, and changes in serum levels of progesterone and estradiol (Koraichi *et al*., 2012). ZEN and its metabolites α-ZOL and β-ZOL exert estrogenic effects, since they are structurally similar to estrogen; the toxins bind competitively to estrogen receptors, as

found in pigs and sheep. In addition, ZEN exhibits relatively low acute toxicity (oral LD_{50} values >2,000-20,000 mg/kg bw) after oral administration in mice, rats, and guinea pigs (Schoevers *et al*., 2012). Furthermore, ZEN is immunotoxic, hepatotoxic, hematotoxic, nephrotoxic and enhances lipid peroxidation (Choi *et al*., 2012). ZEN induces liver lesions and subsequent hepatocarcinoma, and alters hepatic function in rabbits, rats, and gilts (Pistol *et al*., 2014). Recent studies indicate that ZEN may stimulate the growth of human breast cancer cells that express the estrogen receptors (Ahamed *et al*., 2001).

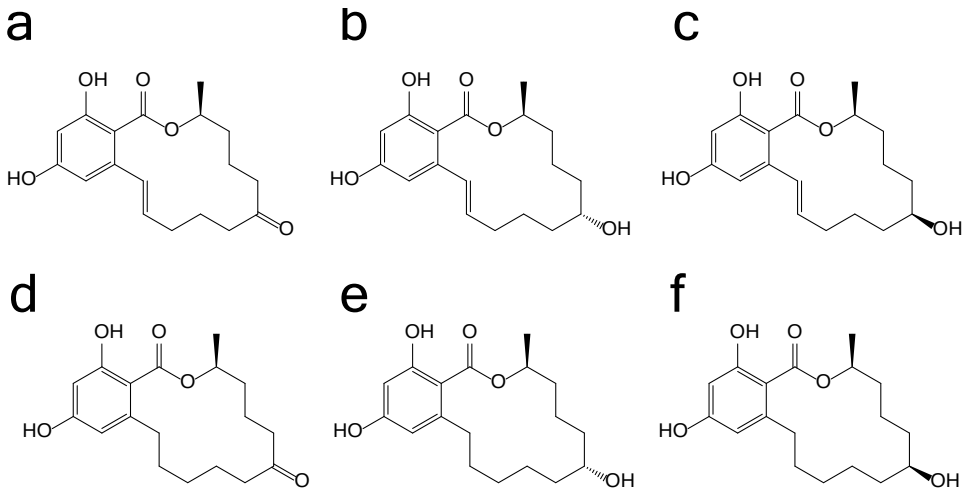


Fig. 2. Chemical structures of ZEN and its derivatives: (a) zearalenone, (b) α-zearalenol, (c) β-zearalenol, (d) zearalanone, (e) α-zearalanol, and (f) β-zearalanol (Marin *et al*., 2013).

1.1.3 Fumonisins

Fumonisins (FBs) were initially isolated from corn cultures of *Fusarium moniliforme* in South Africa (Gelderblom *et al*., 1988). The structures of these mycotoxins as shown in Fig. 3 and Table 3 were first reported by Marasas *et al*. in 1988 (Scott, 2012). Subsequently, fumonisins have been isolated

from other *Fusarium* species, such as *Fusarium verticillioides, Fusarium proliferatum* and *Alternaria alternata* f. sp. *lycopersici* (Bezuidenhout SC, 1988). It is divided into three: FB1, FB2, and FB, and are present as natural contaminant in foods. The molecular structures of fumonisins are shown in Fig. 1 (Soriano JM, 2004). FB1 often contaminates corn and its products, and is the most abundant and most toxic FB. FB1 is a diester of propane-1,2,3-tricarboxylic acid and 2S-amino-12S,16R-dimethyl-3S,5R,10R,14S,15R-pentahydroxyeicosane, where the C-14 and C-15 hydroxy groups are esterified with the terminal carboxy group of propane-1,2,3-tricarboxylic acid (TCA). FB2 is a 10-deoxy FB1 while FB3 is a 5-deoxy FB1 (Soriano *et al*., 2005). The structures of the principal fumonisins are shown in Fig. 3. The symptoms induced by FBs are very broad, including neural tube defects in newborns, brain lesions in horses, pulmonary edema in pigs and cancer in experimental animals. Although FBs have no mutagenicity, they promote cancer development(Summerell, 2011). FBs are associated with human apoptosis, esophageal cancer and neural tube defects (Scott, 2012; Ahangarkani, 2014). FBs can affect the progress of liver cancer in rats, cause bleeding in rabbit brains and have nephrotoxicity to other animals. In addition, FBs are also toxic to pigs, chickens and other farm animals (Ahangarkani, 2014). FB1 interferes with myelin synthesis, causes leukoencephalomalacia and liver necrosis in horses, leading to death. Pig intake of FB1 contaminated feed will cause pulmonary edema (Scott, 2012). In rodent studies, liver and kidney are the main FB1 targets.

The mechanism by which fumonisin exerts toxic effects is complex. Structurally, fumonisins are similar to sphingoid base (a sphingolipid). They can inhibit the synthesis of ceramide synthase and block the biosynthesis of complex sphingolipids, thereby promoting the accumulation of sphingosine and sphinganine 1-phosphate (Wan *et al*., 2013). As sphingolipids play key roles in cellular regulation, dysfunctional sphingolipid metabolism may account for the observed toxicity. These lipids play an important role at the cellular level. They can maintain cell morphology, promote cell

differentiation, regulate growth factor levels, and affect cell carcinogenicity and apoptosis. In addition, they also play a role in maintaining cell membrane structure, enhancing cell interaction and extracellular interaction.

It also plays a role in maintaining cell membrane structure, enhancing cell interaction and extracellular interaction. Moreover, sphingolipids also act as secondary messengers in various signal transduction pathways (Ahangarkani, 2014).

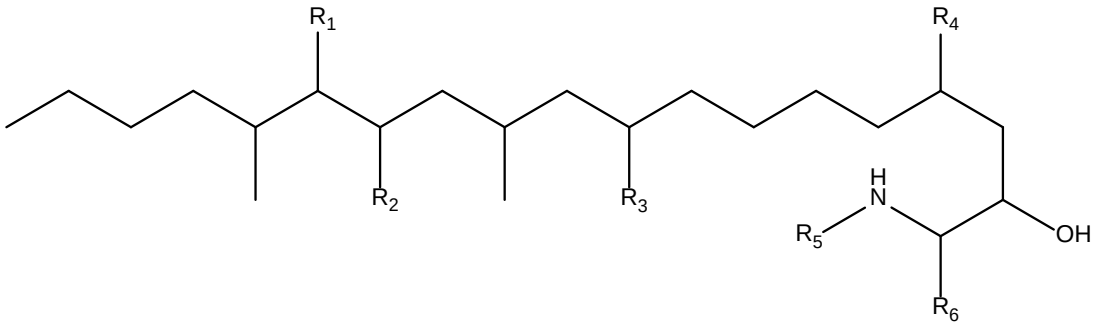


Fig. 3. Structures of the principal fumonisins in foods (FB: fumonisins of group B; AP: aminopentol) (Marin *et al*., 2013).

Table 3: Representation of different groups contained in fumonisins structures

	R_1	**R_2**	**R_3**	**R_4**	**R_5**	**R_6**
FB1	TCA	TCA	OH	OH	H	CH_3
FB2	TCA	TCA	H	OH	H	CH_3
FB3	TCA	TCA	OH	H	H	CH_3
FB4	TCA	TCA	H	H	H	CH_3
AP1	OH	OH	OH	OH	H	CH_3

1.2 *Fusarium* mycotoxins in China

China has been recognized as a major country suffering from *Fusarium* mycotoxin contamination. This is typical of FHB, causing serious problems in cereal grains, especially in 2003, 2010, and 2015 (Zheng Zhang, 2010). Cereals are the staple foods of Asians, and play important roles in healthy diets. Temperature and rainfall are the key climatic factors that affect plants and their associated pathogens as well as mycotoxin concentrations in infected plants. In the middle-to-low valleys of the Huaihe and Yangtze Rivers, the most developed agro-production regions of China, the (typical) humid warm climate encourages FHB epidemics. In 2010, rainfall promotes wheat flowering, leading to the development of FHB, found as the common disease of wheat in Southern China. The total amount of wheat produced in 2010 in Jiangsu and An-hui was 100.81 and 120.65 million kg, respectively.

Li *et al*. (2014) sampled 76 cereals and oil products of the Yangtze Delta of China, and found that ZEN is the most prevalent toxin, with an incidence of 27.6% (9.2% higher than the legal limit). DON was detected in 7.9% of the samples (Rui Li, 2014). Han *et al*. reported the levels of DON, 3-ADON, and 15-ADON in wheat and maize samples from Shanghai, China. From 2009 to 2012, 58% of all maize samples and 80% of all wheat samples were contaminated by DON. In 2011 to 2012, all 50 wheat and maize samples evaluated were contaminated with low levels of 3-ADON and 15-ADON (Han *et al*., 2014). The authors collected 180 samples in Jiangsu Province from 2010 to 2012. The percentage of DON-positive samples was 74.4%, and that of ZEA-positive samples was 12.8%. The highest DON concentration was 41,157 μg/kg, far above the allowable limits (Ji *et al*., 2014). Li *et al*. (2015) reported that 39.7% of maize samples were contaminated by FB1 and FB2 in Southwest China (Renjie Li., 2015). Recent studies have found that 30-80% corn grains have FB1 and FB2 in the corn grains planted in some provinces in China, and the mean mycotoxin concentration range is range from 11 to 13,110 μg/kg(Feng, 2011; Wei, 2013). Several authors have investigated mycotoxin

levels in various cereals and feeds. Table 4 summarizes data obtained over the past 28 years on *Fusarium* mycotoxin contamination of foods and feeds in China.

Table 4: Contamination of *Fusarium* mycotoxins in foods and feeds in China

Product	Number	toxin	% of positive samples	Province	Reference
Corn	120	DON	74.2	Shanxi	Wei *et al*., 2017
		3-A-DON	16.7		
		15-A-DON	74.2		
		NIV	27.5		
		ZEN	49.2		
		FB1	74.2		
		FB2	82.5		
		FB3	70.0		
		T-2	5.0		
		HT-2	17.5		
	215	DON	84.65	Twelve provinces	Ma *et al*., 2011
		ZEN	69.30		
		T-2	46.05		
		HT-2	16.28		
	42	DON	47.6	Anhui and Henan	Xiong *et al*., 2009
		ZEN	78.6		
	111	DON	16.2	Anhui	Lu *et al*., 1994
	105	DON	61.9	Hebei	Liu *et al*., 1993
	284	DON	66.6	Six provinces: Henan, Hubei, Sichuan, Jilin,	Wang *et al*., 2007

				Guangxi, and Guangdong	
		DON	50.5	Seven provinces: Henan, Hebei, Guangxi, Anhui, Sichuan, Ghongqing, and Jiangsu	Li *et al*., 2011
	204	ZEA	41.7		
	50	FBs	26	Shangdong	Yan *et al*., 1999
	70	FBs	44.3	Jilin	Sun *et al*., 2003
	50	FBs	38.00	Hubei	Lv *et al*., 2005
	100	NIV	35	Shanghai	Li *et al*., 1997
	100	DON	53.0		
	41	DON	97.6	Anhui and Henan	Xiong *et al*., 2009
		ZEN	68.3		
	439	ZEN	31.9	National	Luo *et al*., 1989
	815	DON	49.2		
	329	DON	69.3	Anhui	Lu *et al*., 1994
		ZEN	61.0		
	200	DON	89.0	Twenty-six provinces	Cheng *et al*., 2014
		T-2	42.0		
Wheat	190	DON	66.3	Six provinces: Henan, Hubei, Sichuan, Jilin, Guangxi, and Guangdong	Wang *et al*., 2007
		DON	88.8	Seven provinces: Henan, Hebei, Guangxi, Anhui, Sichuan, Ghongqing, and Jiangsu	Li *et al*., 2011
	162	ZEN	22.9		
	183	DON	37.99	National	Wu *et al*., 2009
		FBs	87.34		

		T-2	97.38		
		ZEN	16.02		
	56	DON	89.3	Anhui and Jiangsu	Cui *et al*., 2013
	50	DON	30	Ten regions of China, including Shandong, Hebei, Jilin, et al.	Wang. *et al*., 2014
	50	FBs	94	Shangdong	Yan *et al*., 1999
	40	FBs	72.5	Jilin	Sun *et al*., 2003
	52	FBs	55.77	Hubei	Lv *et al*., 2005
	330	T-2	80	Nine provinces: Shandong, Henan,Hebei, Hubei, Liaoning, Shanxi, Anhui,Jiangsu, and Shanghai	Yang *et al*., 1992
	37	T-2	76.9	Guizhou	Chen *et al*., 1995
	174	T-2	58.05	Beijing	He *et al*., 1998
		DON	84		
		DON-3-G	24		
	158	3-A-DON	84	Anhui, Beijing, Henan, Jilin, Shandong	Han *et al*., 2017
		15-A-DON	61		
Flour		NIV	22		
		ZEN	77		
		DON	96.80		
	125	ZEN	72.80	Twelve provinces	Ma *et al*., 2011
		T-2	74.40		
		HT-2	24.80		
	50	DON	54	Hebei	Liu *et al*., 1993

Rice	132	DON	92.4	Anhui	Lu *et al*., 1994
	18	DON	44.44	Twelve provinces	Ma *et al*., 2011
		ZEN	38.89		
		T2	61.11		
		HT2	11.11		
	51	ZEN	3.9	Guangxi	Li *et al*., 2011
	40	FBs	95	Shangdong	Yan *et al*., 1999
	60	FBs	38.3	Jilin	Sun *et al*., 2003
	49	FBs	32.65	Hubei	Lv *et al*., 2005
Feeds	205	ZEN	62.5	National	Zhou *et al*., 2014
		DON	85.83		
	341	DON	45.4	Twenty-eight provinces	Chen *et al*., 1997
		ZEN	35.8		
		T2	24.2		
Combined feeds	47	DON	100	Guangxi	Jiang *et al*., 2011
		ZEN	100		
		T2	100		
		FBs	100		

1.3 Production of *Fusarium* mycotoxins

The *Fusarium fujikuroi* species complexes (FFSC) and *Fusarium graminearum* species complexes (FGSC) are the major mycotoxin producers, respectively (O'Donnell *et al*., 2000). The FFSC produces fumonisins. *Fusarium verticillioides* is the main contaminant of corn, while *Fusarium proliferatum* is a polyphagous species that found in many different crops.

Qiu *et al*. (2014) isolated *Fusarium* species from maize kernels from Jiangsu and Anhui Provinces, China. They also found that *Fusarium verticillioides* was the most prevalent species, followed by *Fusarium proliferatum*, and finally *Fusarium graminearum*. FUM1 is a gene that plays a key role in fumonisin biosynthesis. Qiu *et al*. (2014) reported that most *Fusarium verticillioides* strains have been detected the presence of FUM1 (Qiu and Shi, 2014).

The FGSC contains 16 phylogenetically distinct species at least, which can cause FHB of a variety of crops and produce trichothecenes (O'Donnell *et al*., 2004). In North America and Europe, *Fusarium graminearum* is predominated in a survey of *Fusarium* species composition and population structure (Starkey *et al*., 2007). The distribution of *Fusarium asiaticum* and *Fusarium graminearum* is different in location, they are the main etiological agents of FHB in Japan and Korea (Gale *et al*., 2002; Suga *et al*., 2008; Lee *et al*., 2012). In China, both *Fusarium graminearum* and *Fusarium asiaticum* are widespread. In the colder northern regions of China, *Fusarium graminearum* isolates are the predominated. In the warm wheat growing areas, *Fusarium asiaticum* is found principally (Wang *et al*., 2008). *Fusarium* species differ in their responses to temperature and moisture, which perhaps influence their distributions in causing infections (Parikka *et al*., 2012). FGSC strains are usually classified into three trichothecene profiles according to the difference in the production of mycotoxins: (i) DON and 3-acetyldeoxynivalenol (3-ADON chemotype); (ii) DON and 15-acetyldeoxynivalenol (15-ADON chemotype), or (iii) NIV, its acetylated derivatives (NIV chemotype) (Ward *et al*., 2002). The analysis of the distribution of FGSC and trichothecene chemotypes in cereal crops will help to correctly understand the relationship between disease and mycotoxin pollution, so as to develop effective management strategies for controlling disease and mycotoxin pollution.

1.4 Detection of Fusarium mycotoxins

Mycotoxins can be detected by various techniques, which are broadly divided into instrumental and bioanalytical methods. However, each approach has merits and drawbacks; the method of choice is dictated by the detection requirements.

1.4.1 Chromatographic methods

There are many kinds of instrument detection methods for mycotoxins. Thin layer chromatography (TLC) is a qualitative or semi quantitative method with the longest history in the detection of mycotoxins. High-performance liquid chromatography (HPLC) can couple with different detectors. These detectors include ultraviolet (UV) detection, diode array detection, fluorescence detection or mass spectrometric detection. Gas chromatography can couple with electron capture detection, flame ionization detection (FID), or mass spectrometry (MS) detection (Visconti and De Girolamo, 2005; Lippolis *et al*., 2008). These methods afford high accuracy and precision, and are used for both quantitative and qualitative analyses. However, they are expensive, require skilled personnel and longer periods for sophisticated sample preparation (Elliott, 2011). Thus, instrumental methods are not suitable for normal laboratories or field environments. Chromatographic techniques involving UV and FID are principally employed in confirmatory contexts, thus facilitating compliance with regulations. Occasionally, such techniques serve as reference methods for validation of immunochemical tests.

MS has indisputable advantages of high sensitivity, high selectivity, high throughput and accuracy, making multi-residue analysis possible. Quick, easy, cheap, effective, rugged, and safe (QuEChERS)

approaches for sample preparation allow analysis of a wide range of matrices and analytes, and further allowing the simultaneous extraction of the amount of mycotoxins. However, QuEChERS approaches reduce analytical sensitivity, and require pre-concentration steps. Alternatively, isotope dilution quantification can improve sensitivity in the absence of pre-concentration (Anfossi *et al*., 2016).

High resolution MS (HRMS) and tandem MS/MS allow (possibly) identification of unknown compounds by analyzing structural information of the compounds. The use of non-selective extraction protocols followed by mass screening employing HRMS or MS/MS has allowed identification of new masked mycotoxins and new members of known groups. The rapid multi-residue LC-MS/MS methods have been used to evaluate mycotoxins level in food and feed.

1.4.2 Immunochemical methods

Immunoassays based on antibody-antigen reactions are very useful for routine analyses, as these techniques are simple and have been used for rapid mycotoxin detection (V.Zherdev, 2014). Recently, several immunological techniques have been developed, including enzyme-linked immunosorbent assays, time-resolved immunochromatographic assays, enzyme-linked aptamer assays, chemiluminescence immunoassays, fluorescence immunoassays, fluorescence resonance energy transfer immunoassays, and metal-enhanced fluorescence assays (Chauhan *et al*., 2016). Aptamers are an important parameter in these detection techniques. They can bind a variety of peptides, proteins, amino acids, and organic or inorganic molecules, all of which have high affinity and specificity (Torres-Chavolla and Alocilja, 2009). Jodra *et al*. (2015) developed an electrochemical magneto-immunosensor to detect FB1 and FB2. The sensor was made of magnetic beads and disposable carbon screen-printed electrodes. Liu *et al*. (2014) constructed an ultrasensitive

immunosensor based on mesoporous carbon and trimetallic nanorattles with special Au cores. The lower detection limit of ZEN was 1.7 pg mL^{-1}, and the assay was found to exhibit good stability and reproducibility.

Because of the strong selectivity of molecular recognition mechanisms, it is difficult to simultaneously assay different compounds or discover new toxins. Osward *et al*. (2013) designed an analytical array that can detect several targets separately in spatially distinct regions. Song *et al*. (2014) developed an immuno-chromatographic strip test device that simultaneously detect at least 10 different toxins (AFs, DON and analogs thereof, and ZON and analogs thereof). Wang *et al*. (2013) reported that they developed a unique spectral addresses which can simultaneous detection of many mycotoxins in peanuts. Those mycotoxins include AFB1, DON, ZON, and T-2.

In comparison to chromatographic methods, immunochemical methods afford greater selectivity in terms of monitoring mycotoxin levels which is very important to ensure food safety in developing countries. In addition, due to global changes in climate and the environment, the level of contamination by fungi and their mycotoxins will increase in the future. Risk management requires routine application of efficient control programs such as optimally employing immunoassays.

1.5 Conclusion

In conclusion, the study of *Fusarium* mycotoxins has attracted increasing attention. Many studies have addressed the toxicokinetic profile, mycotoxin persistence and accumulation. The progress of mycotoxin analysis highlights the limitations currently being understood due to their effective impact on animal and human health in food. Co-contamination by several toxic compounds and identification of new compounds in the mycotoxin family both require new toxicological studies to assess. In

addition, food from crops is susceptible to fungal contamination, and it has been clearly demonstrated that animals fed the contaminated feed can transmit mycotoxins. Some regulations, especially those established by the European Union, have gradually recognized the risk of contamination by mycotoxins in the food chain. Mycotoxin levels should be monitored routinely and continuously, as the annual levels may vary depending on environmental moisture, climate, temperature changes, plant disease status, and insect pest numbers. Effective management of food safety risks is required, especially including the use of rapid and sensitive immunological techniques.

1.6 References

Ahamed, S., Foster, J.S., Bukovsky, A., and Wimalasena, J. (2001). Signal transduction through the Ras/Erk pathway is essential for the mycoestrogen zearalenone-induced cell-cycle progression in MCF-7 cells. Molecular Carcinogenesis **30,** 88-98.

Ahangarkani, F., Rouhi, S., Azizi, I.G. (2014). A review on incidence and toxicity of fumonisins. Toxin Rev. **33,** 6-11.

Anfossi, L., Giovannoli, C., and Baggiani, C. (2016). Mycotoxin detection. Current Opinion in Biotechnology **37,** 120-126.

Backhhouse, D. (2014). Global distribution of Fusarium graminearum, F. asiaticum and F. boothii from wheat in relation to climate. Eur J Plant Pathol **139,** 13.

Berthiller, F., Krska, R., Domig, K.J., Kneifel, W., Juge, N., Schuhmacher, R., and Adam, G. (2011). Hydrolytic fate of deoxynivalenol-3-glucoside during digestion. Toxicology Letters **206,** 264-267.

Bezuidenhout SC, G.W., Gorst-Allman CP, Horak RM, Marasas WFO, Spiteller G, et al. (1988). Structure elucidation of the fumonisins, mycotoxins from Fusarium moniliforme. Chem Commun **19,** 3-9.

Bouaziz, C., Bouslimi, A., Kadri, R., Zaied, C., Bacha, H., and Abid-Essefi, S. (2013). The in vitro effects of zearalenone and T-2 toxins on Vero cells. Experimental and toxicologic pathology : official journal of the Gesellschaft fur Toxikologische Pathologie **65,** 497-501.

Chauhan, R., Singh, J., Sachdev, T., Basu, T., and Malhotra, B.D. (2016). Recent advances in mycotoxins detection. Biosensors & Bioelectronics **81,** 532-545.

Chen, Y., Chen, Q., Han, M., Zhou, J., Gong, L., Niu, Y., Zhang, Y., He, L., and Zhang, L. (2016). Development and optimization of a multiplex lateral flow immunoassay for the simultaneous determination of three mycotoxins in corn, rice and peanut. Food Chemistry **213,** 478-484.

Choi, B.K., Cho, J.H., Jeong, S.H., Shin, H.S., Son, S.W., Yeo, Y.K., and Kang, H.G. (2012). Zearalenone affects immune-related parameters in lymphoid organs and serum of rats vaccinated with porcine parvovirus vaccine. Toxicological Research **28,** 279-288.

Creppy, E.E. (2002). Update of survey, regulation and toxic effects of mycotoxins in Europe. Toxicology Letters **127,** 19-28.

Doi, K., Ishigami, N., and Sehata, S. (2008). T-2 toxin-induced toxicity in pregnant mice and rats. International Journal of Molecular Sciences **9,** 2146-2158.

EFSA. (2004c). Opinion of the scientific panel on contaminants in the food chain on a request from the commission related to deoxynivalenol(DON) as undesirable substance in animal feed. EFSA **73,** 41.

EFSA. (2011). Scientific opinion on the risks for animal and public health related to the presence of T-2 and HT-2 toxin in food and feed. EFSA J **9,** 1.

EFSA. (2004d). Opinion of the scientific panel on contaminants in the food chain on a request from the commission related to zearalenone as undesirable substance in animal feed. EFSA **89,** 35.

Escriva, L., Font, G., and Manyes, L. (2015). In vivo toxicity studies of fusarium mycotoxins in the last decade: a review. Food and chemical toxicology : an international journal published for the British Industrial Biological Research Association **78,** 185-206.

Fallah, B., Zaini, F., Daei Ghazvini, R., Kachuei, R., Kordbacheh, P., Safara, M., and Mahmoudi, S. (2016). The antagonistic effects of Candida parapsilosis on the growth of Fusarium species and fumonisin production. Current Medical Mycology **2,** 1-6.

Fang, H., Wu, Y., Guo, J., Rong, J., Ma, L., Zhao, Z., Zuo, D., and Peng, S. (2012). T-2 toxin induces apoptosis in differentiated murine embryonic stem cells through reactive oxygen species-mediated mitochondrial pathway. Apoptosis : an International Journal on Programmed Cell Death **17,** 895-907.

Feng, Y.Z., Lu, X. H., Tao, B., Pang, M. H., Liu, Y. C., & Dong, J. G. (2011). Natural occurrence of fumonisins b1 and b2 in corn from three main production provinces in China. Journal of Food Protection, **74,** 5-11.

Ferrigo, D., Raiola, A., and Causin, R. (2016). Fusarium Toxins in Cereals: Occurrence, Legislation, Factors Promoting the Appearance and Their Management. Molecules **21**, 24-29.

Frizzell, C., Ndossi, D., Verhaegen, S., Dahl, E., Eriksen, G., Sorlie, M., Ropstad, E., Muller, M., Elliott, C.T., and Connolly, L. (2011). Endocrine disrupting effects of zearalenone, alpha- and beta-zearalenol at the level of nuclear receptor binding and steroidogenesis. Toxicology Letters **206,** 210-217.

Gale, L.R., Chen, L.F., Hernick, C.A., Takamura, K., and Kistler, H.C. (2002). Population Analysis of Fusarium graminearum from Wheat Fields in Eastern China. Phytopathology **92,** 1315-1322.

GB/T. (2011). China N.C.o. Maximum levels of mycotoxins in foods.

Gelderblom, W.C., Thiel, P.G., and van der Merwe, K.J. (1988). The chemical and enzymatic interaction of glutathione with the fungal metabolite, fusarin C. Mutation Research **199,** 207-214.

Gerez, J.R., Pinton, P., Callu, P., Grosjean, F., Oswald, I.P., and Bracarense, A.P. (2015). Deoxynivalenol alone or in combination with nivalenol and zearalenone induce systemic histological changes in pigs. Experimental and toxicologic pathology : official journal of the Gesellschaft fur Toxikologische Pathologie **67,** 89-98.

Gilbert, J.T., A. (2000). Review: Recent developments in research on fusarium head blight of wheat in Canada. Can. J. Plant Pathol **22,** 8-15.

Glenn, L.L., and Quillin, S.I. (2007). Opposing effects of maternal and paternal socioeconomic status on neonatal feeding method, place of sleep, and maternal sleep time. The Journal of Perinatal & Neonatal Nursing **21,** 165-172.

Goswami, R.S., and Kistler, H.C. (2004). Heading for disaster: Fusarium graminearum on cereal crops. Molecular Plant Pathology **5,** 515-525.

Gouze, M.E., Laffitte, J., Pinton, P., Dedieux, G., Galinier, A., Thouvenot, J.P., Loiseau, N., Oswald, I.P., and Galtier, P. (2007). Effect of subacute oral doses of nivalenol on immune and metabolic defence systems in mice. Veterinary Research **38,** 635-646.

Han, Z., Nie, D., Ediage, E.N., Yang, X., Wang, J., Chen, B., Li, S., On, S.L., De Saeger, S., and Wu, A. (2014). Cumulative health risk assessment of co-occurring mycotoxins of deoxynivalenol and its acetyl derivatives in wheat and maize: case study, Shanghai, China. Food and chemical toxicology : an international journal published for the British Industrial Biological Research Association **74,** 334-342.

Nagashima H., Nakagawa, H. (2014). Differences in the Toxicities of Trichothecene Mycotoxins, Deoxynivalenol and Nivalenol, in Cultured Cells. Japan Agricultural Research Quarterly: JARQ **48,** 5-12.

Islam, Z., Gray, J.S., and Pestka, J.J. (2006). p38 Mitogen-activated protein kinase mediates IL-8 induction by the ribotoxin deoxynivalenol in human monocytes. Toxicology and Applied Pharmacology **213,** 235-244.

Ji, F., Xu, J., Liu, X., Yin, X., and Shi, J. (2014). Natural occurrence of deoxynivalenol and zearalenone in wheat from Jiangsu province, China. Food Chemistry **157,** 393-397.

Koraichi, F., Videmann, B., Mazallon, M., Benahmed, M., Prouillac, C., and Lecoeur, S. (2012). Zearalenone exposure modulates the expression of ABC transporters and nuclear receptors in pregnant rats and fetal liver. Toxicology Letters **211,** 246-256.

Leblanc, J.C., Guerin, T., Noel, L., Calamassi-Tran, G., Volatier, J.L., and Verger, P. (2005). Dietary exposure estimates of 18 elements from the 1st French Total Diet Study. Food Additives and Contaminants **22,** 624-641.

Lee, J., Kim, H., Jeon, J.J., Kim, H.S., Zeller, K.A., Carter, L.L., Leslie, J.F., and Lee, Y.W. (2012). Population structure of and mycotoxin production by Fusarium graminearum from maize in South Korea. Applied and Environmental Microbiology **78,** 2161-2167.

Li, Y., Wang, Z., Beier, R.C., Shen, J., De Smet, D., De Saeger, S., and Zhang, S. (2011). T-2 toxin, a trichothecene mycotoxin: review of toxicity, metabolism, and analytical methods. Journal of Agricultural and Food Chemistry **59,** 3441-3453.

Lippolis, V., Pascale, M., Maragos, C.M., and Visconti, A. (2008). Improvement of detection sensitivity of T-2 and HT-2 toxins using different fluorescent labeling reagents by high-performance liquid chromatography. Talanta **74,** 1476-1483.

Madheswaran, R., Balachandran, C., and Murali Manohar, B. (2004). Influence of dietary culture material containing aflatoxin and T(2) toxin on certain serum biochemical constituents in Japanese quail. Mycopathologia **158,** 337-341.

Maresca, M. (2013). From the gut to the brain: journey and pathophysiological effects of the food-associated trichothecene mycotoxin deoxynivalenol. Toxins **5,** 784-820.

Marin, D.E., Taranu, I., Burlacu, R., Manda, G., Motiu, M., Neagoe, I., Dragomir, C., Stancu, M., and Calin, L. (2011). Effects of zearalenone and its derivatives on porcine immune response. Toxicology in vitro : an international journal published in association with BIBRA **25,** 1981-1988.

Marin, S., Ramos, A.J., Cano-Sancho, G., and Sanchis, V. (2013). Mycotoxins: occurrence, toxicology, and exposure assessment. Food and chemical toxicology : an international journal published for the British Industrial Biological Research Association **60,** 218-237.

McCormick, S.P., Stanley, A.M., Stover, N.A., and Alexander, N.J. (2011). Trichothecenes: from simple to complex mycotoxins. Toxins **3,** 802-814.

Moretti, A., Logrieco, A.F., and Susca, A. (2017). Mycotoxins: An Underhand Food Problem. Methods in Molecular Biology **1542,** 3-12.

Ndossi, D.G., Frizzell, C., Tremoen, N.H., Faeste, C.K., Verhaegen, S., Dahl, E., Eriksen, G.S., Sorlie, M., Connolly, L., and Ropstad, E. (2012). An in vitro investigation of endocrine disrupting effects of trichothecenes deoxynivalenol (DON), T-2 and HT-2 toxins. Toxicology Letters **214,** 268-278.

Nemcsok, J., and Boross, L. (1982). Comparative studies on the sensitivity of different fish species to metal pollution. Acta biologica Academiae Scientiarum Hungaricae **33,** 23-27.

O'Donnell, K., Kistler, H.C., Tacke, B.K., and Casper, H.H. (2000). Gene genealogies reveal global phylogeographic structure and reproductive isolation among lineages of Fusarium graminearum, the fungus causing wheat scab. Proceedings of the National Academy of Sciences of the United States of America **97,** 7905-7910.

O'Donnell, K., Ward, T.J., Geiser, D.M., Corby Kistler, H., and Aoki, T. (2004). Genealogical concordance between the mating type locus and seven other nuclear genes supports formal recognition of nine phylogenetically distinct species within the Fusarium graminearum clade. Fungal genetics and biology **41,** 600-623.

Osborne, L.E., and Stein, J.M. (2007). Epidemiology of Fusarium head blight on small-grain cereals. International Journal of Food Microbiology **119,** 103-108.

Ostry, V., Malir, F., Toman, J., and Grosse, Y. (2017). Mycotoxins as human carcinogens-the IARC Monographs classification. Mycotoxin Research **33,** 65-73.

Parikka, P., Hakala, K., and Tiilikkala, K. (2012). Expected shifts in Fusarium species' composition on cereal grain in Northern Europe due to climatic change. Food Additives & Contaminants. Part A, Chemistry, Analysis, Control, Exposure & Risk Assessment **29,** 1543-1555.

Peraica, M., Radic, B., Lucic, A., and Pavlovic, M. (1999). Toxic effects of mycotoxins in humans. Bulletin of the World Health Organization **77,** 754-766.

Pinton, P., and Oswald, I.P. (2014). Effect of deoxynivalenol and other Type B trichothecenes on the intestine: a review. Toxins **6,** 1615-1643.

Pistol, G.C., Gras, M.A., Marin, D.E., Israel-Roming, F., Stancu, M., and Taranu, I. (2014). Natural feed contaminant zearalenone decreases the expressions of important pro- and anti-inflammatory mediators and mitogen-activated protein kinase/NF-kappaB signalling molecules in pigs. The British Journal of Nutrition **111,** 452-464.

Qiu, J., and Shi, J. (2014). Genetic relationships, carbendazim sensitivity and mycotoxin production of the Fusarium graminearum populations from maize, wheat and rice in eastern China. Toxins **6,** 2291-2309.

Renjie Li., B.T., Minhao Pang., Yingchao Liu., Jingao Dong. (2015). Natural occurrence of fumonisins B1 and B2 in maize from three main maize-producing provinces in China. Food Control **5,** 11-16.

Ricciardi, C., Castagna, R., Ferrante, I., Frascella, F., Marasso, S.L., Ricci, A., Canavese, G., Lore, A., Prelle, A., Gullino, M.L., and Spadaro, D. (2013). Development of a microcantilever-based immunosensing method for mycotoxin detection. Biosensors & Bioelectronics **40,** 233-239.

Rotter, B.A., Prelusky, D.B., and Pestka, J.J. (1996). Toxicology of deoxynivalenol (vomitoxin). Journal of Toxicology and Environmental Health **48,** 1-34.

Rui Li, X.W., Ting Zhou, Dongxu Yang, Qi Wang, Yu Zhou. (2014). Occurrence of four mycotoxins in cereal and oil products in Yangtze Delta region of China and their food safety risks. Food Control **35,** 7-13.

Schoevers, E.J., Santos, R.R., Colenbrander, B., Fink-Gremmels, J., and Roelen, B.A. (2012). Transgenerational toxicity of Zearalenone in pigs. Reproductive Toxicology **34,** 110-119.

Scott, P.M. (2012). Recent research on fumonisins: a review. Food Additives & Contaminants. Part A, Chemistry, Analysis, Control, Exposure & Risk Assessment **29,** 242-248.

Sehata, S., Kiyosawa, N., Makino, T., Atsumi, F., Ito, K., Yamoto, T., Teranishi, M., Baba, Y., Uetsuka, K., Nakayama, H., and Doi, K. (2004). Morphological and microarray analysis of T-2 toxin-induced rat fetal brain lesion. Food and chemical toxicology : an international journal published for the British Industrial Biological Research Association **42,** 1727-1736.

Selvaraj, J.N., Wang, Y., Zhou, L., Zhao, Y., Xing, F., Dai, X., and Liu, Y. (2015). Recent mycotoxin survey data and advanced mycotoxin detection techniques reported from China: a review. Food Additives & Contaminants. Part A, Chemistry, Analysis, Control, Exposure & Risk Assessment **32,** 440-452.

Severino, L., Luongo, D., Bergamo, P., Lucisano, A., and Rossi, M. (2006). Mycotoxins nivalenol and deoxynivalenol differentially modulate cytokine mRNA expression in Jurkat T cells. Cytokine **36,** 75-82.

Shifrin, V.I., and Anderson, P. (1999). Trichothecene mycotoxins trigger a ribotoxic stress response that activates c-Jun N-terminal kinase and p38 mitogen-activated protein kinase and induces apoptosis. The Journal of Biological Chemistry **274,** 13985-13992.

Shinozuka, J., Suzuki, M., Noguchi, N., Sugimoto, T., Uetsuka, K., Nakayama, H., and Doi, K. (1998). T-2 toxin-induced apoptosis in hematopoietic tissues of mice. Toxicologic Pathology **26,** 674-681.

Smyth, S.J., McDonald, J., and Falck-Zepeda, J. (2014). Investment, regulation, and uncertainty: managing new plant breeding techniques. GM Crops & Food **5,** 44-57.

Soriano, J.M., Gonzalez, L., and Catala, A.I. (2005). Mechanism of action of sphingolipids and their metabolites in the toxicity of fumonisin B1. Progress in Lipid Research **44,** 345-356.

Soriano JM, D.S. (2004). Occurrence of fumonisins in foods [Review]. Food Research International **37,** 6-11.

Starkey, D.E., Ward, T.J., Aoki, T., Gale, L.R., Kistler, H.C., Geiser, D.M., Suga, H., Toth, B., Varga, J., and O'Donnell, K. (2007). Global molecular surveillance reveals novel Fusarium head blight species and trichothecene toxin diversity. Fungal genetics and biology **44,** 1191-1204.

Suga, H., Karugia, G.W., Ward, T., Gale, L.R., Tomimura, K., Nakajima, T., Miyasaka, A., Koizumi, S., Kageyama, K., and Hyakumachi, M. (2008). Molecular characterization of the Fusarium graminearum species complex in Japan. Phytopathology **98,** 159-166.

Summerell, B.A., Leslie, J.F. (2011). Fifty years of Fusarium: how could nine species have ever been enough? Fungal Divers **50,** 10-15.

T.Elliott, J.P.M.F.H.P.v.E.C. (2011). Current methods of analysis for the determination of trichothecene mycotoxins in food. TrAC Trends in Analytical Chemistry **30,** 12-19.

Tatsuno, T., Inoue, T., and Tanimura, A. (1979). [Hygienic chemical studies on plastics (II). Residual acrylonitrile in ABS resin or AS resin and its migration into water (author's transl)]. Eisei Shikenjo hokoku. Bulletin of National Institute of Hygienic Sciences**,** 93-97.

Thrane, U., Adler, A., Clasen, P.E., Galvano, F., Langseth, W., Lew, H., Logrieco, A., Nielsen, K.F., and Ritieni, A. (2004). Diversity in metabolite production by Fusarium langsethiae, Fusarium poae, and Fusarium sporotrichioides. International Journal of Food Microbiology **95,** 257-266.

Torres-Chavolla, E., and Alocilja, E.C. (2009). Aptasensors for detection of microbial and viral pathogens. Biosensors & Bioelectronics **24,** 3175-3182.

Turner, P. (2010). Deoxynivalenol and nivalenol occurrence and exposure assessment. World Mycotoxin Journal **3,** 7-16.

Ueno, Y., Nakajima, M., Sakai, K., Ishii, K., and Sato, N. (1973). Comparative toxicology of trichothec mycotoxins: inhibition of protein synthesis in animal cells. Journal of Biochemistry **74,** 285-296.

V.Zherdev, B.B.D.N.A.B.A.E.U.A. (2014). Immunochromatographic methods in food analysis. TrAC Trends in Analytical Chemistry **55,** 13-19.

van Egmond, H.P., Schothorst, R.C., and Jonker, M.A. (2007). Regulations relating to mycotoxins in food: perspectives in a global and European context. Analytical and Bioanalytical Chemistry **389,** 147-157.

Visconti, A., and De Girolamo, A. (2005). Fitness for purpose--ochratoxin A analytical developments. Food Additives and Contaminants **22,** 37-44.

Wan, L.Y., Turner, P.C., and El-Nezami, H. (2013). Individual and combined cytotoxic effects of Fusarium toxins (deoxynivalenol, nivalenol, zearalenone and fumonisins B1) on swine jejunal epithelial cells. Food and chemical toxicology : an international journal published for the British Industrial Biological Research Association **57,** 276-283.

Wang, J.H., Li, H.P., Qu, B., Zhang, J.B., Huang, T., Chen, F.F., and Liao, Y.C. (2008). Development of a generic PCR detection of 3-acetyldeoxy-nivalenol-, 15-acetyldeoxynivalenol- and nivalenol-chemotypes of Fusarium graminearum Clade. International Journal of Molecular Sciences **9,** 2495-2504.

Ward, T.J., Bielawski, J.P., Kistler, H.C., Sullivan, E., and O'Donnell, K. (2002). Ancestral polymorphism and adaptive evolution in the trichothecene mycotoxin gene cluster of phytopathogenic Fusarium. Proceedings of the National Academy of Sciences of the United States of America **99,** 9278-9283.

Wei, T., Zhu,W., Pang, M., Liu, Y., & Dong, J. . (2013). Natural occurrence of fumonisins B1 and B2 in corn in four provinces of China. Food Additives & Contaminants: Part B **6,** 5-10.

Weidner, M., Lenczyk, M., Schwerdt, G., Gekle, M., and Humpf, H.U. (2013). Neurotoxic potential and cellular uptake of T-2 toxin in human astrocytes in primary culture. Chemical Research in Toxicology **26,** 347-355.

Xu, X., and Nicholson, P. (2009). Community ecology of fungal pathogens causing wheat head blight. Annual Review of Phytopathology **47,** 83-103.

Xu, X.M., and Berrie, A.M. (2005). Epidemiology of mycotoxigenic fungi associated with Fusarium ear blight and apple blue mould: a review. Food Additives and Contaminants **22,** 290-301.

Zeller, K.A., Summerell, B.A., Bullock, S., and Leslie, J.F. (2003). Gibberella konza (Fusarium konzum) sp. nov. from prairie grasses, a new species in the Gibberella fujikuroi species complex. Mycologia **95,** 943-954.

Zheng Zhang, H.Z., Theo van der Lee, Wan-Quan Chen, Paul Arens, Jin Xu, Jing-Sheng Xu, Li-Jun Yang, Da-Zhao Yu, Cees Waalwijk, Jie Feng. (2010). Geographic substructure of Fusarium asiaticum isolates collected from barley in China. European Journal of Plant Pathology **127,** 10-18.

Zhou, H.R., Lau, A.S., and Pestka, J.J. (2003). Role of double-stranded RNA-activated protein kinase R (PKR) in deoxynivalenol-induced ribotoxic stress response. Toxicological sciences : an official journal of the Society of Toxicology **74,** 335-344.

CHAPTER THREE

INFLUENCE OF CLIMATIC FACTORS AND WHEAT VARIETIES ON DEOXYNIVALENOL CONTAMINATION OF WHEAT IN JIANGSU PROVINCE, CHINA

Influence of climatic factors and wheat varieties on Deoxynivalenol contamination of wheat in Jiangsu Province, China

Fang Ji[1,2], Fei Dong[1], A. O. Olaniran[2], M.P. Mokoena[2] and J. Shi[1]∗

[1]Key Lab of Food Quality and Safety of Jiangsu Province-State Key Laboratory Breeding/Key Laboratory of Control Technology and Standard for Agro-Product Safety and Quality/Key Laboratory of Agro-Product Safety Risk Evaluation (Nanjing), Ministry of Agriculture, China/ Institute of Food Safety and Nutrition, Jiangsu Academy of Agricultural Sciences, 210014, China[2]Discipline of Microbiology, School of Life Science, College of Agriculture, Engineering and Science, University of KwaZulu-Natal, Private Bag X54001, Durban, 4000, South Africa.

∗Corresponding author. Tel: +25 84392001; Fax: Tel: +25 84392001

E-mail address: jianrong63@126.com (Jianrong Shi)

Abstract

Deoxynivalenol (DON) is produced by some *Fusarium graminearum* species complex (FGSC), such as *F. graminearum* and *F. culmorum,* and are found as common mycotoxins worldwide in wheat. The DON contamination of wheat has caused serious economic losses, not only causing food shortage, but also a threat to human health. The present study focuses on the occurrence of *Fusarium* species coupled with contamination levels of DON in wheat from different areas of Jiangsu Province in China for three years, and the influences of rainfall and temperature on accumulation of DON. A total of 428 *Fusarium* spp isolated from 2014 to 2016 were identified morphologically. A total of 178 samples of wheat were collected in 2014, 2015, 2016 after harvest period from eight counties and analyzed for DON using HPLC-MS/MS. The results obtained showed that most of the isolates (88%-94%) belong to *F. asiatium.* The highest prevalence and mean levels of DON were found in 2016 in Sihong county. The correlation between the climatic factors (rainfall and temperature) and actual presence of DON in wheat was different due to the different wheat variety and areas.

Keywords: *Fusarium* species, Deoxynivalenol, Wheat, Climatic, Correlation

1. Introduction

Wheat is one of the most important cereal grains in the world, providing over 20% of the calorific energy consumed by humans (Tester and Langridge, 2010). *Fusarium* head blight (FHB) also known as scab, is an important fungal disease of wheat, barley and many other small cereal grains. This disease is mostly caused by *Fusarium graminearum* species complex (FGSC) (Marcia McMullen, 2012). Among the infected grains, Type B trichothecenes are the most common toxic metabolites. They can be divided into three chemotypes, such as (i) deoxynivalenol (DON) and 3-acetyldeoxynivalenol (3ADON chemotype); (ii) deoxynivalenol and 15-acetyldeoxynivalenol (15ADON chemotype); or (iii) nivalenol (NIV), its acetylated derivatives and low levels of DON (NIV chemotype) (Ward *et al*., 2002). The chemical types of these mycotoxins are mainly related to the toxigenic species and are distributed in different geographical regions (Starkey *et al*., 2007). DON chemical type is very common in the world, while NIV is relatively rare (Desjardins *et al*., 2004). Gale et al. (2011) have reported that NIV chemotype has been found in several countries in Africa, Asia, Europe, and America (Gale *et al*., 2011).

Deoxynivalenol, one of the most important type B trichothecene, can cause vomiting in pigs, so it is also called vomit toxin. It is mainly produced by *F. graminearum* and *F. culmorum* (Rotter *et al*., 1996). This mycotoxin affects humans and animals by binding to the 60S ribosomal subunit and inhibiting protein synthesis (Ma and Guo, 2008). In addition, DON activates mitogen-activated protein kinases (MAPKs) and cause apoptosis through a process known as ribotoxic stress response. Exposure to DON usually causes animals to resist feed intake, and it also damages the immune function of many animals (Pestka, 2010).

For the widespread contamination of DON in crops, together with its potential toxicological implications in animal models as well as in humans, it has aroused great concern in public health in the past few years. Some national and international food safety organizations and expert groups have emphasized the need for DON risk assessment in food. In China, the highest permitted content of DON in grain and their products is 1000 μg/kg (China, 2017).

Resistance to FHB in wheat is controlled by polygenes, however, these genes usually have lesser effects and are vulnerable to environmental influences (Brunner *et al*., 2009). This further leads to complications including, type I resist the initial penetration of FHB, type II prevent the spread of fungal in spikes, type III promote the decomposition of mycotoxins, and reduce kernel infection (Mesterhazy, 1995). Therefore, it is necessary to develop FHB resistant cultivars to reduce the risk of mycotoxin contamination in wheat.

Jiangsu province is the main wheat growing area in the North South transition area of China, spanning two main ecological zones suitable for wheat planting. In this region, the typical humid and warm climate facilitates epidemics of FHB. Therefore, knowledge about the prevalence of FHB-producing *F*. species, incidence of DON, as well as possible influencing factors is imperative for preventing influx of contaminated grains into food supply chain. Therefore, the present study aimed at evaluating the occurrence of DON in wheat samples in different counties and years in Jiangsu Province, China and correlate with the prevailing dominant *F*. species. Influence of climatic factors on DON contamination of wheat samples is further assessed.

2. Material and methods

2.1 Chemicals and reagents

Deoxynivalenol (100µg/mL in acetonitrile) was purchased from Sigma-Aldrich (China). Acetonitrile and methanol are all HPLC-grade. (Merck, Germany). Deionised water requiring the resistivity less than 8 MX cm^{-1}, and was generated by the Milli-Q water purification system (Millipore, Bedford, MA). Filtration of all other reagents was carried out using a 0.22-µm cellulose filter (Jinteng, Tianjin, China).

2.2 Molecular identification of *F*. species and detection of trichothecene genotypes

To identify the *F.* species and the trichothecene genotype, we performed DNA extraction according to the method of Pan *et al*. (2013). Fg16 F/R primers were used to amplify DNA from all the isolates by PCR. The primers previously produced polymorphic products (400-500bp) from DNA extracted from FGSC members (Shi, 2014). Single and multiplex PCR were used to detect trichothecene genotypes. The chemical types of FGSC isolates were determined by specific primers described by Li *et al*. (2005). Another primer group targeting *Tri3* gene Tri303F/Tri303R and Tri315F/Tri315R were used to further characterize chemotype of the *F. graminearum* sensu stricto complex as 3ADON chemotype or 15ADON chemotype (Jennings *et al*., 2004).

The multiple PCR assay developed by Wang *et al*. (2012) uses primer pairs based on the sequence of *Tri11* gene. The *Tri11* gene encodes the key enzymes involved in the biosynthesis of DON, 3ADON, 15ADON , NIV, and T-2 in *Fusarium* species (Alexander *et al.*, 1998; Wang *et al*., 2012). These

primers produced a 279 bp fragment for the identification of 15ADON chemotype, a 334 bp fragment for the identification of 3ADON chemotype, and a 497 bp for the identification of NIV chemotype.

Primers used in this study (5′ → 3′):

1. Fg16F: CTCCGGATATGTTGCGTCAA
   Fg16R: GGTAGGTATCCGACATGGCAA
   (reaction procedure: pre-degeneration at 95 ℃ for 5min, 94 ℃ for 40s, 57 ℃ for 40s, 72 ℃ for 50s, 30 cycles at 72 ℃ for 10min and 10℃ for 5min)
2. Tri11CON: GACTGCTCATGGAGACGCTG
   Tri11-3AcDON：TCCTCATGCTCG GTGGACTCG
   Tri11-15AcDON：TGGTCCAGT TGTCCGTATT
   Tri11-NIV：GTAGGTTCCATTGC TTGTTC
   (reaction procedure: pre-degeneration at 95 ℃ for 5min, 94 ℃ for 40s, 58 ℃ for 30s, 72 ℃ for 50s, 25 cycles at 72 ℃ for 5 min and 10℃ for 5min)
3. Tri13F:TACGTGAAACATTGTTGGC
   Tri13R:TGGTGTCCCAGGATCTGCG
   (reaction procedure: pre-degeneration at 94 ℃ for 5min, 94 ℃ for 30s, 57 ℃ for 45s, 72 ℃ for 1min, 35 cycles at 72 ℃ for 10 min and 4 ℃ for 5min)
4. Tri303F: GATGGCCGCAAGTGGA
   Tri303R：GCCGGACTGCCCTATTG
   (reaction procedure: pre-degeneration at 94 ℃ for 5min, 94 ℃ for 30s, 52 ℃ for 45s, 72 ℃ for 1min, 35 cycles at 72 ℃ for 10 min and 10℃ for 5min)
5. Tri315F: CTCGCTGAAGTTGGACGTAA
   Tri315R: GTCTATGCTCTCAACGGACAAC
   (reaction procedure: pre-degeneration at 94 ℃ for 5min, 94 ℃ for 30s, 58 ℃ for 45s, 72 ℃ for 1min, 35 cycles at 72 ℃ for 10 min and 10℃ for 5min)

2.3 Acquisition and presentation of meteorological data

The rainfall and temperature profiles during the month of April that grain flowering takes place in Jiangsu province of China were provided by the Jiangsu Center of Meteorological Information.

2.4 Sample collection and storage

In 2014-2016 years, 178 wheat grain samples were collected from different regions of 8 counties in Jiangsu Province of China (Table 1). All the samples were collected from farms after wheat grain harvested. These samples include 4 wheat varieties, and the presence of DON was analyzed (Fig. 1). Sampling was carried out according to the European Union guidelines (EC No. 401/2006), in which ten samples each of 100 g were collected. After homogenization, the samples were packed in paper bags and taken to the laboratory. About 1 kg of the sample was milled and passed through a 20 meshes sieve. Thereafter, 200 g from each sample was packed in a sealed polyethylene bag and kept in the refrigerator at -20 ℃ for a maximum of 60 d until further analysis.

Fig. 1 The map of Jiangsu province shows the sample collection points in different research areas.

Table 1: Number of samples collected in different years and areas

Areas	Number of samples		
	2014	2015	2016
Donghai	13	10	10
Guannan	5	7	7
Jingjiang	5	8	5
Jurong	5	8	5
Pizhou	10	5	5
Rugao	8	7	7
Sihong	10	6	9
Taicang	5	10	8

2.5 Sample preparation and mycotoxin (DON) analyses

A 10 g sample of wheat grain was dissolved in 40 mL of extract, and the extract was composed of acetonitrile, water and acetic acid, respectively, at 79:20:1 (v / v / v). It was then shaken for 30 minutes in a 180 rpm shaker (Sulyok *et al*., 2006). After 10 minutes of 3000rpm centrifugation, the final extracts of each 0.5mL were diluted with a combined solvent acetonitrile, water and acetic acid (20:79:1v/v/v, respectively) and filtered through a nylon filter with 13mm diameter, 0.22 μm pore size (Sulyok et al., 2006; Spanjer *et al*., 2008). The content of DON was quantified by high pressure liquid chromatography/electrospray ionization tandem mass spectrometry (LC-MS / MS) system. The LC-MS/MS system is composed of Agilent 1200 HPLC, Agilent 6410B triple-quadrupole mass spectrometer and Agilent Mass Hunter workstation running qualitative analysis B.01.03 software, which is used for data acquisition and analysis. The analytical column used in this study was XDB-C18, with a size of 2.1*150 mm, 3.5μm bead diameter (Agilent), and the column temperature was kept at 30 ℃. Nitrogen was used as the drying gas at a flow rate of 10 L/min. The mass spectrometric

parameters of mycotoxin and the composition and proportion of mobile phase are shown in references (Soleimany *et al*., 2012). The detection ability of DON was revealed by the detection limit (LOD) and the quantitative limit (LOQ). Based on the signal-to-noise ratios of 3/1 and 10/1, LOD (10 µg/kg) and LOQ (20 µg/kg) were estimated respectively.

2.6 Statistical analysis

The DON concentrations were analyzed using MS Excel and expressed as percentages and means ± SD. Use the "correlate" procedure in SPSS 23 for correlation. The results were presented as means of three independent samples. Differences in mycotoxins content between wheat samples were confirmed using one-way analysis of variance and Duncan's test of ANOVA.

3. Results

3.1 Production of DON from wheat by *F. asiaticum* strains

A total of 428 *Fusarium* spp isolated from 2014 to 2016 were morphologically identified as members of FGSC. As illustrated in Table 2, most of the isolates belong to *F. asiatium,* accounting for between 88% and 94% of the isolates during the three years of sampling. In *F. asiatium*, two trichothecene types were identified, with about 90% being of the 3ADON type and 10% being of the NIV over the three years. In *F. graminearum* isolates, all identified DON were found to belong to the 15ADON type. The frequency patterns of species and chemotype compositions were stable over the 3 years.

Table 2: Frequency of different chemotypes among the *Fusarium* species isolated from wheat grains of different years of Jiangsu Province, China

Year	Total	Species and Trichothecene chemotype		
		F.asiaticum		*F.graminearum*
		3ADON	NIV	15ADON
2014	102	82	8	12
2015	84	68	6	10
2016	242	204	24	14

3.2 Occurrence of DON in wheat samples from 2014 to 2016

The prevalence of DON contamination of wheat grains collected from 2014 to 2016 is depicted in Table 3. The occurrence of DON was found in the wheat samples throughout the sampling years, with 73.7 to 100% of the samples testing positive for DON. In 2014, only 73.7% of the samples tested positive for DON, while the highest prevalence was observed in 2015 with all the wheat samples tested positive. Of the samples tested, highest median and average values of DON were obtained in 2016 whereas, lowest incidence and contamination occurred in 2014. In 2015, the highest maximum level of DON was found to be far more than that obtained for the other years. Except for 2014, the average values of DON in other years were higher than the maximum permissible level of DON in China.

Table 3：Incidence of Deoxynivalenol (DON) contamination of wheat grains collected in Jiangsu Province, China from 2014 to 2016

Growing seasons	Positive samples/ DON Conc (μg/kg)					
	%	Mean	Median	Max	Rainfall (mm)	Temp (℃)
2014	73.7	434.8	218.9	2859.3	86.4	15.5

2015	100	1411.2	656.7	12204.2	78.6	14.6
2016	97.1	1992.9	1252.36	9425.3	96.6	16.6

3.3 Occurrence of DON in different counties of wheat grains from 2014 to 2016

In the present study, the incidence and contamination of DON in different counties of wheat grains from 2014 to 2016 is presented in Table 4. Eight counties were sampled to measure the occurrence of DON over three consecutive years. The eight counties were divided into three regions, Southern (2), Northern (4) and Central (2) regions of Jiangsu Province. As shown in Table 3, 100% positive samples were found in five counties. It was also found that the highest average, median and maximum of DON occurred in Sihong while lowest average, median and Maximum of DON were found in Pizhou. Except the Donghai and Pizhou, the average values of DON in other counties were higher than the limited level of DON in China, while compare the median values of DON, only Taicang and Sihong that are higher than the limited level of DON in China. In general, the incidence and contamination level of DON in Southern region is serious, followed by Central and Northern regions.

Table 4： Deoxynivalenol (DON) content of naturally contaminated wheat grains collected in different regions of Jiangsu Province from 2014-2016, China

			Positive samples (μg/kg)					
	County	Number	%	Mean	Median	Max	Rainfall (mm)	Temp (℃)
Southern	Jurong	17	100	1793.2	875.5	9425.3	138.1	16.0
	Taicang	23	100	1530.0	1275.1	3224.5	139.7	15.9

Northern	Donghai	33	75.8	410.2	161.28	6424.9	33.1	14.9
	Guannan	19	100	1373.9	988.9	5737.1	47.9	15.1
	Pizhou	20	70	299.9	116.5	1041.3	42.6	15.3
	Sihong	25	100	2643.37	1930.3	12204.2	72.7	15.9
Central	Jingjiang	18	89.9	1061.36	766.6	2859.3	111.9	16.6
	Rugao	22	100	1239.7	530.3	5510.6	111.5	14.9

3.4 Effect of climatic factors on accumulation of DON in wheat

Overall, the whole set of data from climatic factors and natural occurrence of DON in wheat was analyzed in order to investigate if there was any correlation between the climatic factors (rainfall and temperature) and actual presence of DON in wheat. The rainfall and temperature profiles for April (wheat flowering month) each year and county were also shown in Table 2 and Table 3. As indicated by a significant interaction between DON contamination and rainfall, the dynamics of DON accumulation differed in each year. As show in Fig 2, the contamination levels of DON in the eight counties was significant and positively correlated with rainfall in 2014 ($r = 0.689$, $p<0.05$) and in 2016 ($r=0.74$, $p<0.05$).

In 2015, rainfall in Northern region was the least while high rainfall was observed in Southern. However, this was found to be inconsistent with contamination of DON. The highest rainfall occurred in Jurong, while the contamination level of DON is relatively low. The highest contamination of DON was observed in Sihong with less rainfall. Remarkably, a stronger correlation between DON concentration and rainfall level was observed in central Jiangsu, with a correlation coefficient of 0.991($p<0.05$).

Temperature, another climatic factor, was measured in order to investigate the relationship between the environmental factor and DON contamination in wheat. Results obtained showed no correlation between DON contamination of wheat and temperature within the years and regions studied.

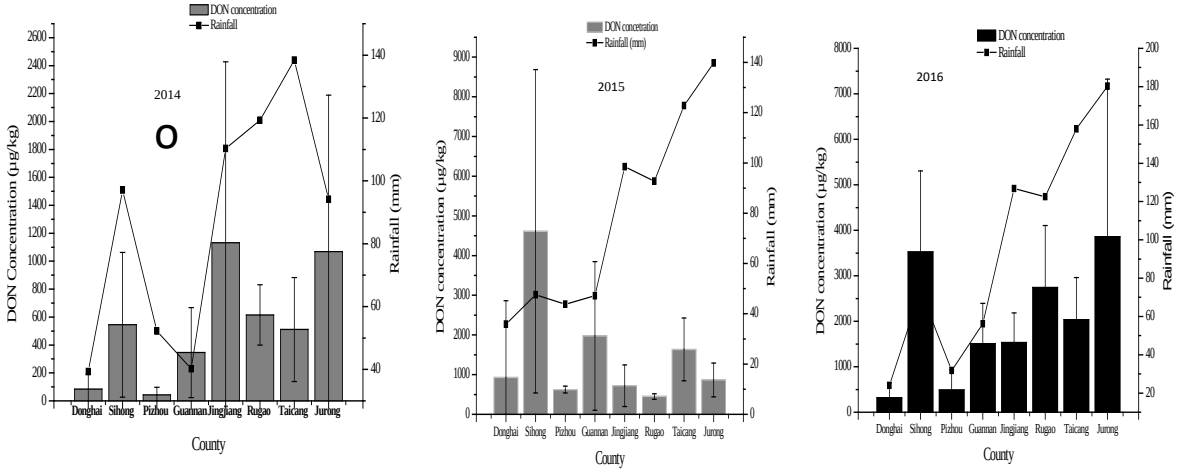


Fig. 2 Effect of rainfall on DON contamination of wheat in different years (2014: r=0.689, p< 0.05; 2016: r=0.74, p<0.05; 2015: no correlation).

3.5 Effect of rainfall on DON contamination of different wheat varieties

Four wheat varieties were selected for assessment of DON contamination, followed by investigating the influence of rainfall on contamination of these wheat varieties for three consecutive years. Two planting sites selected for each variety include Jimai 22 and Yannong 19 (susceptible cultivars) and Yangmai 13 and Yangmai 16 (resistant cultivars). The average value of DON recorded in Yangmai 16 wheat samples for three consecutive years was 1641.9μg/kg, found to be higher than those of other varieties. However, in Jimai 22 wheat samples, lowest DON contamination was recorded.

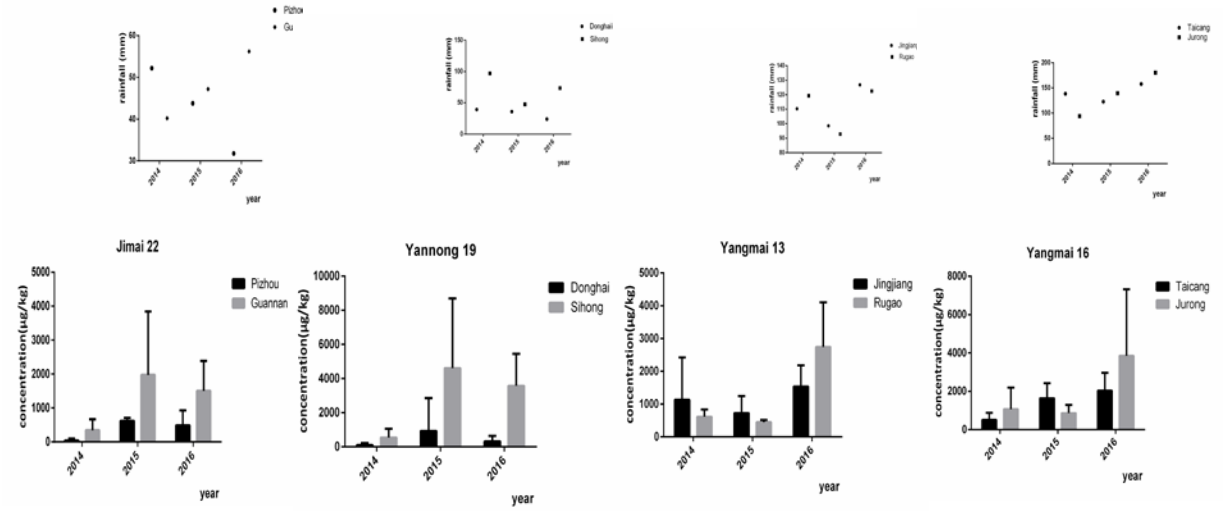


Fig 3. Effect of rainfall on DON contamination of different wheat varieties. Jimai 22 and Yannong 19 are susceptible cultivars, Yangmai 13 and Yangmai 16 are resistant cultivars.

The contamination of cereals by DON was found to be different, even though the wheat cultivars were the same. As illustrated in Fig. 3, the contamination of Jimai 22 wheat by DON was significant and positively correlate with rainfall ($r=0.85$, $P<0.05$), Yangmai 13 and Yangmai 16 were also both significant and positively correlate with rainfall ($r=0.83$, $P<0.05$ and $r=0.74$, $P<0.05$, respectively). However, in Yannong 19 wheat samples, the occurrence of DON was observed to be insignificant relative to rainfall.

4. Discussion

The current study investigated the infection of wheat by *Fusarium* species, deoxynevalenol (DON) occurrence in wheat from Jiangsu Province, China and the correlation between DON contamination of wheat and climatic factors over three consecutive years. Results obtained from this study indicated that *F. asiaticum* was by far the dominant species isolated, while the 3ADON chemotype is the mainly

type of *F. asiaticum* from wheat in Jiangsu Province from 2011-2016. These results were consistent with previous studies.

Zhang *et al*. (2012) found that among the wheat samples tested in Jiangsu, 92.1% of the 82 isolates were *F. asiaticum* and 5.6% were *F. graminearums.* They also reported that all strains of *F. graminearum* collected from Jiangsu province belonged to the 15ADON chemotype. In a similar study, 83 of 891 *Fusarium* species isolated from Jiangsu and Anhui were *F. graminearum*, and 808 were *F. asiaticum* (Qiu *et al*., 2014). In China, *F. graminearum* was frequently isolated from the colder northern region and *F. asiaticum* was found mainly in warm wheat planting areas, of which the FHB epidemic is prevalent (Qu *et al*., 2008). This is consistent with our results, indicating that *F. asiaticum* was the main pathogenic strain in Jiangsu Province, and Jiangsu is also found to be an endemic area of FHB.

The relationship between the outbreak of FHB and the moisture condition during the flowering season has been reported (Pan *et al*., 2007). Precipitation during grain flowering will increase *Fusarium* infection in mature grains. Anthers with rain and/or high humidity are associated with the incidence of FHB (Moschini *et al*., 2001; De Wolf *et al*., 2003). The mycelia can infect small flowers under moist conditions and grow along the surface of the spikelets, especially when the anthers are extruded from the wheat ears during flowering. Notably, the risks of major FHB epidemics significantly increase when the relative humidity increases above the threshold of 70%. Del Ponte *et al*. (2012) reported that the level of DON in the growing season of 2007 and 2008 was higher than that in 2006, and indicated that the higher prevalence of DON may be partly associated with the increase in the risk of the FHB epidemic in some years.

Fusarium mycotoxins seem to be generally stimulated by a narrower window of climatic factors than the *Fusarium* infestation (Hope *et al.*, 2005; Medina and Magan, 2011). It is reported that the impact of DON depends largely on climate conditions, crop systems, cultural practices, and the management

of grain, feed and food after harvest, and climate conditions are the most influential (Reyneri, 2006). Stankovic *et al*. (2012) found that DON level in 2005 was higher than that in 2007 and concluded that rainfall played an important role in the DON pollution of wheat. Changing water activity and temperature has been reported to affect the ratio of type B trichothecenes, such as DON, 3-acetyl and 15-acetyl DON both *in vitro* and in wheat grain (Leite, 2014). Paterson and Lima (2011) maintain that the relationship between humidity and rainfall and contamination is ambiguous, but high rainfall is usually more conducive to pollution, while temperatures ≤10 °C are relatively safe (Paterson and Lima, 2011). Medina and Magan (2011) suggest that high moisture can promote the toxin production of *F. langsethiae*, which is far beyond the effect of temperature on toxin production. This is consistent with the findings in the current study, since increased DON concentration was found in those years with high rainfall level.

Though, there was no significant correlation between flowering temperature and DON contamination in this study. Xu *et al*. (2007) suggested that trichothecene production was enhanced by warm temperatures during initial infection of wheat heads. Li *et al*. (2009) also found that storage temperature had a significant influence on mycotoxin production in both resistant and susceptible cultivars of potato tubers. High temperature accelerates the accumulation of trichothecenes to the adjacent asymptomatic tissue, compared to low temperature (Xue *et al*., 2014). Similarly, Hui and Kushalappa (2002) reported that warmer temperatures tend to cause dry rot during initial infection, and there was a strong positive correlation between disease incidence and storage temperature in potato tubers. However, low temperatures are often associated with wet weather, and high water activity has a greater impact on toxicity than temperature (Hope *et al*., 2005).

5. Conclusion

Deoxynivalenol (DON) contamination is prevalent in wheat and wheat products worldwide, causing serious economic and health problems. Human exposure to DON can cause serious health risks. Current research shows that DON is common in various parts of China. Further research is needed to prevent and control the potential risks of DON exposure to humans. These studies will provide more information on the contamination of DON in wheat and other cereal products in different parts of China. It is also necessary to study the accumulative mechanism of DON pollution and better identify the climatic conditions and other factors that affect the DON production. In addition, good agricultural practices, such as the use of disease resistant wheat seeds, early sowing, crop rotation and the removal of the remaining residue of the previous crop, should be used to minimize the risk to consumers.

6. References

Ahamed, S., Foster, J.S., Bukovsky, A., and Wimalasena, J. (2001). Signal transduction through the Ras/Erk pathway is essential for the mycoestrogen zearalenone-induced cell-cycle progression in MCF-7 cells. Molecular Carcinogenesis **30,** 88-98.

Ahangarkani, F., Rouhi, S., Azizi, I.G. (2014). A review on incidence and toxicity of fumonisins. Toxin Rev. **33,** 6.

Alexander, N.J., Hohn, T.M., and McCormick, S.P. (1998). The TRI11 gene of Fusarium sporotrichioides encodes a cytochrome P-450 monooxygenase required for C-15 hydroxylation in trichothecene biosynthesis. Applied and Environmental Microbiology **64,** 221-225.

Anfossi, L., Giovannoli, C., and Baggiani, C. (2016). Mycotoxin detection. Current Opinion In Biotechnology **37,** 120-126.

Backhhouse, D. (2014). Global distribution of Fusarium graminearum, F. asiaticum and F. boothii from wheat in relation to climate. Eur J Plant Pathol **139,** 13-20.

Berthiller, F., Krska, R., Domig, K.J., Kneifel, W., Juge, N., Schuhmacher, R., and Adam, G. (2011). Hydrolytic fate of deoxynivalenol-3-glucoside during digestion. Toxicology Letters **206,** 264-267.

Bezuidenhout SC, G.W., Gorst-Allman CP, Horak RM, Marasas WFO, Spiteller G, et al. (1988). Structure elucidation of the fumonisins, mycotoxins from Fusarium moniliforme. Chem Commun **19,** 3-11.

Bouaziz, C., Bouslimi, A., Kadri, R., Zaied, C., Bacha, H., and Abid-Essefi, S. (2013). The in vitro effects of zearalenone and T-2 toxins on Vero cells. Experimental and toxicologic pathology : official journal of the Gesellschaft fur Toxikologische Pathologie **65,** 497-501.

Brunner, K., Paris, M.P.K., Paolino, G., Burstmayr, H., Lemmens, M., Berthiller, F., Schuhmacher, R., Krska, R., and Mach, R.L. (2009). A reference-gene-based quantitative PCR method as a tool to determine Fusarium resistance in wheat. Analytical and Bioanalytical Chemistry **395,** 1385-1394.

Castanares, E., Albuquerque, D.R., Dinolfo, M.I., Pinto, V.F., Patriarca, A., and Stenglein, S.A. (2014). Trichothecene genotypes and production profiles of Fusarium graminearum isolates obtained from barley cultivated in Argentina. International Journal of Food Microbiology **179,** 57-63.

Chauhan, R., Singh, J., Sachdev, T., Basu, T., and Malhotra, B.D. (2016). Recent advances in mycotoxins detection. Biosensors & Bioelectronics **81,** 532-545.

Chen, Y., Chen, Q., Han, M., Zhou, J., Gong, L., Niu, Y., Zhang, Y., He, L., and Zhang, L. (2016). Development and optimization of a multiplex lateral flow immunoassay for the simultaneous determination of three mycotoxins in corn, rice and peanut. Food Chemistry **213,** 478-484.

China, N.C.o. (2017). Maximum levels of mycotoxins in foods. GB/T 2761-2011.

Choi, B.K., Cho, J.H., Jeong, S.H., Shin, H.S., Son, S.W., Yeo, Y.K., and Kang, H.G. (2012). Zearalenone affects immune-related parameters in lymphoid organs and serum of rats vaccinated with porcine parvovirus vaccine. Toxicological Research **28,** 279-288.

Creppy, E.E. (2002). Update of survey, regulation and toxic effects of mycotoxins in Europe. Toxicology Letters **127,** 19-28.

Cruz, A., Marin, P., Magan, N., and Gonzalez-Jaen, M.T. (2014). Combined effects of benomyl and environmental factors on growth and expression of the fumonisin biosynthetic genes FUM1 and FUM19 by Fusarium verticillioides. International Journal of Food Microbiology **191,** 17-23.

De Wolf, E.D., Madden, L.V., and Lipps, P.E. (2003). Risk assessment models for wheat Fusarium head blight epidemics based on within-season weather data. Phytopathology **93,** 428-435.

Desjardins, A.E., Jarosz, A.M., Plattner, R.D., Alexander, N.J., Brown, D.W., and Jurgenson, J.E. (2004). Patterns of trichothecene production, genetic variability, and virulence to wheat of Fusarium graminearum from smallholder farms in Nepal. Journal of Agricultural and Food Chemistry **52,** 6341-6346.

Doi, K., Ishigami, N., and Sehata, S. (2008). T-2 toxin-induced toxicity in pregnant mice and rats. International Journal of Molecular Sciences **9,** 2146-2158.

EFSA. (2004c). Opinion of the scientific panel on contaminants in the food chain on a request from the commission related to deoxynivalenol(DON) as undesirable substance in animal feed. EFSA **73,** 41.

EFSA. (2011). Scientific opinion on the risks for animal and public health related to the presence of T-2 and HT-2 toxin in food and feed. EFSA J **9,** 1.

EFSA. (2004d). Opinion of the scientific panel on contaminants in the food chain on a request from the commission related to zearalenone as undesirable substance in animal feed. EFSA **89,** 35.

Escriva, L., Font, G., and Manyes, L. (2015). In vivo toxicity studies of fusarium mycotoxins in the last decade: a review. Food and chemical toxicology : an international journal published for the British Industrial Biological Research Association **78,** 185-206.

Fallah, B., Zaini, F., Daei Ghazvini, R., Kachuei, R., Kordbacheh, P., Safara, M., and Mahmoudi, S. (2016). The antagonistic effects of Candida parapsilosis on the growth of Fusarium species and fumonisin production. Current Medical Mycology **2,** 1-6.

Fang, H., Wu, Y., Guo, J., Rong, J., Ma, L., Zhao, Z., Zuo, D., and Peng, S. (2012). T-2 toxin induces apoptosis in differentiated murine embryonic stem cells through reactive oxygen species-mediated mitochondrial pathway. Apoptosis : An International Journal on Programmed Cell Death **17,** 895-907.

Feng, Y.Z., Lu, X. H., Tao, B., Pang, M. H., Liu, Y. C., & Dong, J. G. (2011). Natural occurrence of fumonisins b1 and b2 in corn from three main production provinces in China. Journal of Food Protection, **74,** 5-11.

Ferrigo, D., Raiola, A., and Causin, R. (2016). Fusarium Toxins in Cereals: Occurrence, Legislation, Factors Promoting the Appearance and Their Management. Molecules **21,** 24-29.

Frizzell, C., Ndossi, D., Verhaegen, S., Dahl, E., Eriksen, G., Sorlie, M., Ropstad, E., Muller, M., Elliott, C.T., and Connolly, L. (2011). Endocrine disrupting effects of zearalenone, alpha- and beta-zearalenol at the level of nuclear receptor binding and steroidogenesis. Toxicology Letters **206,** 210-217.

Gale, L.R., Chen, L.F., Hernick, C.A., Takamura, K., and Kistler, H.C. (2002). Population Analysis of Fusarium graminearum from Wheat Fields in Eastern China. Phytopathology **92,** 1315-1322.

Gale, L.R., Harrison, S.A., Ward, T.J., O'Donnell, K., Milus, E.A., Gale, S.W., and Kistler, H.C. (2011). Nivalenol-type populations of Fusarium graminearum and F. asiaticum are prevalent on wheat in southern Louisiana. Phytopathology **101,** 124-134.

GB/T. (2011). China N.C.o. Maximum levels of mycotoxins in foods.

Gelderblom, W.C., Thiel, P.G., and van der Merwe, K.J. (1988). The chemical and enzymatic interaction of glutathione with the fungal metabolite, fusarin C. Mutation Research **199,** 207-214.

Gerez, J.R., Pinton, P., Callu, P., Grosjean, F., Oswald, I.P., and Bracarense, A.P. (2015). Deoxynivalenol alone or in combination with nivalenol and zearalenone induce systemic histological changes in pigs. Experimental and toxicologic pathology : official journal of the Gesellschaft fur Toxikologische Pathologie **67,** 89-98.

Gilbert, J.T., A. (2000). Review: Recent developments in research on fusarium head blight of wheat in Canada. Can. J. Plant Pathol **22,** 8.

Glenn, L.L., and Quillin, S.I. (2007). Opposing effects of maternal and paternal socioeconomic status on neonatal feeding method, place of sleep, and maternal sleep time. The Journal of Perinatal & Neonatal Nursing **21,** 165-172.

Goswami, R.S., and Kistler, H.C. (2004). Heading for disaster: Fusarium graminearum on cereal crops. Molecular Plant Pathology **5,** 515-525.

Gouze, M.E., Laffitte, J., Pinton, P., Dedieux, G., Galinier, A., Thouvenot, J.P., Loiseau, N., Oswald, I.P., and Galtier, P. (2007). Effect of subacute oral doses of nivalenol on immune and metabolic defence systems in mice. Veterinary Research **38,** 635-646.

Han, Z., Nie, D., Ediage, E.N., Yang, X., Wang, J., Chen, B., Li, S., On, S.L., De Saeger, S., and Wu, A. (2014). Cumulative health risk assessment of co-occurring mycotoxins of deoxynivalenol and its acetyl derivatives in wheat and maize: case study, Shanghai, China. Food and chemical toxicology : an international journal published for the British Industrial Biological Research Association **74,** 334-342.

Hitoshi NAGASHIMA, H.N. (2014). Differences in the Toxicities of Trichothecene Mycotoxins, Deoxynivalenol and Nivalenol, in Cultured Cells. Japan Agricultural Research Quarterly: JARQ **48,** 5-11.

Hope, R., Aldred, D., and Magan, N. (2005). Comparison of environmental profiles for growth and deoxynivalenol production by Fusarium culmorum and F-graminearum on wheat grain. Lett Appl Microbiol **40,** 295-300.

Islam, Z., Gray, J.S., and Pestka, J.J. (2006). p38 Mitogen-activated protein kinase mediates IL-8 induction by the ribotoxin deoxynivalenol in human monocytes. Toxicology and Applied Pharmacology **213,** 235-244.

Jennings, P., Coates, M.E., Walsh, K., Turner, J.A., and Nicholson, P. (2004). Determination of deoxynivalenol- and nivalenol-producing chemotypes of Fusarium graminearum isolated from wheat crops in England and Wales. Plant Pathology **53,** 643-652.

Ji, F., Xu, J., Liu, X., Yin, X., and Shi, J. (2014). Natural occurrence of deoxynivalenol and zearalenone in wheat from Jiangsu province, China. Food Chemistry **157,** 393-397.

Koraichi, F., Videmann, B., Mazallon, M., Benahmed, M., Prouillac, C., and Lecoeur, S. (2012). Zearalenone exposure modulates the expression of ABC transporters and nuclear receptors in pregnant rats and fetal liver. Toxicology Letters **211,** 246-256.

Leblanc, J.C., Guerin, T., Noel, L., Calamassi-Tran, G., Volatier, J.L., and Verger, P. (2005). Dietary exposure estimates of 18 elements from the 1st French Total Diet Study. Food Additives and Contaminants **22,** 624-641.

Lee, J., Kim, H., Jeon, J.J., Kim, H.S., Zeller, K.A., Carter, L.L., Leslie, J.F., and Lee, Y.W. (2012). Population structure of and mycotoxin production by Fusarium graminearum from maize in South Korea. Applied and Environmental Microbiology **78,** 2161-2167.

Li, Y., Wang, Z., Beier, R.C., Shen, J., De Smet, D., De Saeger, S., and Zhang, S. (2011). T-2 toxin, a trichothecene mycotoxin: review of toxicity, metabolism, and analytical methods. Journal of Agricultural and Food Chemistry **59,** 3441-3453.

Lippolis, V., Pascale, M., Maragos, C.M., and Visconti, A. (2008). Improvement of detection sensitivity of T-2 and HT-2 toxins using different fluorescent labeling reagents by high-performance liquid chromatography. Talanta **74,** 1476-1483.

Ma, Y.Y., and Guo, H.W. (2008). Mini-review of studies on the carcinogenicity of deoxynivalenol. Environmental Toxicology and Pharmacology **25,** 1-9.

Madheswaran, R., Balachandran, C., and Murali Manohar, B. (2004). Influence of dietary culture material containing aflatoxin and T(2) toxin on certain serum biochemical constituents in Japanese quail. Mycopathologia **158,** 337-341.

Marcia McMullen, G.B., Erick De Wolf, Ruth Dill-Macky, Don Hershman, Greg Shaner, Dave Van Sanford. (2012). A Unified Effort to Fight an Enemy of Wheat and Barley: Fusarium Head Blight. Plant Disease **96,** 27-35.

Maresca, M. (2013). From the gut to the brain: journey and pathophysiological effects of the food-associated trichothecene mycotoxin deoxynivalenol. Toxins **5,** 784-820.

Marin, D.E., Taranu, I., Burlacu, R., Manda, G., Motiu, M., Neagoe, I., Dragomir, C., Stancu, M., and Calin, L. (2011). Effects of zearalenone and its derivatives on porcine immune response. Toxicology in vitro : an international journal published in association with BIBRA **25,** 1981-1988.

Marin, S., Ramos, A.J., Cano-Sancho, G., and Sanchis, V. (2013). Mycotoxins: occurrence, toxicology, and exposure assessment. Food and Chemical Toxicology : an International Journal Published for the British Industrial Biological Research Association **60,** 218-237.

McCormick, S.P., Stanley, A.M., Stover, N.A., and Alexander, N.J. (2011). Trichothecenes: from simple to complex mycotoxins. Toxins **3,** 802-814.

Medina, A., and Magan, N. (2011). Temperature and water activity effects on production of T-2 and HT-2 by Fusarium langsethiae strains from north European countries. Food Microbiol **28,** 392-398.

Mesterhazy, A. (1995). Types and components of resistance to Fusarium head blight of wheat. Plant Breeding **114,** 377-386.

Moretti, A., Logrieco, A.F., and Susca, A. (2017). Mycotoxins: An Underhand Food Problem. Methods in Molecular Biology **1542,** 3-12.

Moschini, R.C., Pioli, R., Carmona, M., and Sacchi, O. (2001). Empirical predictions of wheat head blight in the northern Argentinean Pampas region. Crop Sci **41,** 1541-1545.

Ndossi, D.G., Frizzell, C., Tremoen, N.H., Faeste, C.K., Verhaegen, S., Dahl, E., Eriksen, G.S., Sorlie, M., Connolly, L., and Ropstad, E. (2012). An in vitro investigation of endocrine disrupting effects of trichothecenes deoxynivalenol (DON), T-2 and HT-2 toxins. Toxicology Letters **214,** 268-278.

Nemcsok, J., and Boross, L. (1982). Comparative studies on the sensitivity of different fish species to metal pollution. Acta biologica Academiae Scientiarum Hungaricae **33,** 23-27.

O'Donnell, K., Kistler, H.C., Tacke, B.K., and Casper, H.H. (2000). Gene genealogies reveal global phylogeographic structure and reproductive isolation among lineages of Fusarium graminearum, the fungus causing wheat scab. Proceedings of the National Academy of Sciences of the United States of America **97,** 7905-7910.

O'Donnell, K., Ward, T.J., Geiser, D.M., Corby Kistler, H., and Aoki, T. (2004). Genealogical concordance between the mating type locus and seven other nuclear genes supports formal recognition of nine phylogenetically distinct species within the Fusarium graminearum clade. Fungal genetics and biology **41,** 600-623.

Osborne, L.E., and Stein, J.M. (2007). Epidemiology of Fusarium head blight on small-grain cereals. International Journal of Food Microbiology **119,** 103-108.

Ostry, V., Malir, F., Toman, J., and Grosse, Y. (2017). Mycotoxins as human carcinogens-the IARC Monographs classification. Mycotoxin Research **33,** 65-73.

Pan, D., Bonsignore, F., Rivas, F., Perera, G., and Bettucci, L. (2007). Deoxynivalenol in barley samples from Uruguay. International Journal of Food Microbiology **114,** 149-152.

Parikka, P., Hakala, K., and Tiilikkala, K. (2012). Expected shifts in Fusarium species' composition on cereal grain in Northern Europe due to climatic change. Food additives &

contaminants. Part A, Chemistry, analysis, control, exposure & risk assessment **29,** 1543-1555.

Paterson, R.R.M., and Lima, N. (2011). Further mycotoxin effects from climate change. Food Research International **44,** 2555-2566.

Peraica, M., Radic, B., Lucic, A., and Pavlovic, M. (1999). Toxic effects of mycotoxins in humans. Bulletin of the World Health Organization **77,** 754-766.

Pestka, J.J. (2010). Deoxynivalenol: mechanisms of action, human exposure, and toxicological relevance. Archives of Toxicology **84,** 663-679.

Pinton, P., and Oswald, I.P. (2014). Effect of deoxynivalenol and other Type B trichothecenes on the intestine: a review. Toxins **6,** 1615-1643.

Pistol, G.C., Gras, M.A., Marin, D.E., Israel-Roming, F., Stancu, M., and Taranu, I. (2014). Natural feed contaminant zearalenone decreases the expressions of important pro- and anti-inflammatory mediators and mitogen-activated protein kinase/NF-kappaB signalling molecules in pigs. The British Journal of Nutrition **111,** 452-464.

Qiu, J., and Shi, J. (2014). Genetic relationships, carbendazim sensitivity and mycotoxin production of the Fusarium graminearum populations from maize, wheat and rice in eastern China. Toxins **6,** 2291-2309.

Qiu, J.B., Xu, J.H., and Shi, J.R. (2014). Molecular characterization of the Fusarium graminearum species complex in Eastern China. Eur J Plant Pathol **139,** 811-823.

Qu, B., Li, H.P., Zhang, J.B., Xu, Y.B., Huang, T., Wu, A.B., Zhao, C.S., Carter, J., Nicholson, P., and Liao, Y.C. (2008). Geographic distribution and genetic diversity of Fusarium graminearum and F-asiaticum on wheat spikes throughout China. Plant Pathology **57,** 15-24.

Renjie Li., B.T., Minhao Pang., Yingchao Liu., Jingao Dong. (2015). Natural occurrence of fumonisins B1 and B2 in maize from three main maize-producing provinces in China. Food Control **5**, 11-19.

Reyneri, A. (2006). The role of climatic condition on micotoxin production in cereal. Vet Res Commun **30,** 87-92.

Ricciardi, C., Castagna, R., Ferrante, I., Frascella, F., Marasso, S.L., Ricci, A., Canavese, G., Lore, A., Prelle, A., Gullino, M.L., and Spadaro, D. (2013). Development of a microcantilever-based immunosensing method for mycotoxin detection. Biosensors & Bioelectronics **40,** 233-239.

Rotter, B.A., Prelusky, D.B., and Pestka, J.J. (1996). Toxicology of deoxynivalenol (vomitoxin). Journal of Toxicology and Environmental Health **48,** 1-34.

Rui Li, X.W., Ting Zhou, Dongxu Yang, Qi Wang, Yu Zhou. (2014). Occurrence of four mycotoxins in cereal and oil products in Yangtze Delta region of China and their food safety risks. Food Control **35,** 7-15.

Schoevers, E.J., Santos, R.R., Colenbrander, B., Fink-Gremmels, J., and Roelen, B.A. (2012). Transgenerational toxicity of Zearalenone in pigs. Reproductive Toxicology **34,** 110-119.

Scott, P.M. (2012). Recent research on fumonisins: a review. Food Additives & Contaminants. Part A, Chemistry, Analysis, Control, Exposure & Risk Assessment **29,** 242-248.

Sehata, S., Kiyosawa, N., Makino, T., Atsumi, F., Ito, K., Yamoto, T., Teranishi, M., Baba, Y., Uetsuka, K., Nakayama, H., and Doi, K. (2004). Morphological and microarray analysis of T-2 toxin-induced rat fetal brain lesion. Food and chemical toxicology : an international journal published for the British Industrial Biological Research Association **42,** 1727-1736.

Selvaraj, J.N., Wang, Y., Zhou, L., Zhao, Y., Xing, F., Dai, X., and Liu, Y. (2015). Recent mycotoxin survey data and advanced mycotoxin detection techniques reported from China: a review. Food Additives & Contaminants. Part A, Chemistry, Analysis, Control, Exposure & Risk Assessment **32,** 440-452.

Severino, L., Luongo, D., Bergamo, P., Lucisano, A., and Rossi, M. (2006). Mycotoxins nivalenol and deoxynivalenol differentially modulate cytokine mRNA expression in Jurkat T cells. Cytokine **36,** 75-82.

Shi, J.Q.X. (2014). Molecular characterization of the Fusarium graminearum species complex in Eastern China. European Journal of Plant Pathology **139,** 13-20.

Shifrin, V.I., and Anderson, P. (1999). Trichothecene mycotoxins trigger a ribotoxic stress response that activates c-Jun N-terminal kinase and p38 mitogen-activated protein kinase and induces apoptosis. The Journal of Biological Chemistry **274,** 13985-13992.

Shinozuka, J., Suzuki, M., Noguchi, N., Sugimoto, T., Uetsuka, K., Nakayama, H., and Doi, K. (1998). T-2 toxin-induced apoptosis in hematopoietic tissues of mice. Toxicologic Pathology **26,** 674-681.

Smyth, S.J., McDonald, J., and Falck-Zepeda, J. (2014). Investment, regulation, and uncertainty: managing new plant breeding techniques. GM Crops & Food **5,** 44-57.

Soleimany, F., Jinap, S., and Abas, F. (2012). Determination of mycotoxins in cereals by liquid chromatography tandem mass spectrometry. Food Chemistry **130,** 1055-1060.

Soriano, J.M., Gonzalez, L., and Catala, A.I. (2005). Mechanism of action of sphingolipids and their metabolites in the toxicity of fumonisin B1. Progress in Lipid Research **44,** 345-356.

Soriano JM, D.S. (2004). Occurrence of fumonisins in foods [Review]. Food Research International **37,** 6-11.

Spanjer, M.C., Rensen, P.M., and Scholten, J.M. (2008). LC-MS/MS multi-method for mycotoxins after single extraction, with validation data for peanut, pistachio, wheat, maize, cornflakes, raisins and figs. Food Additives & Contaminants. Part A, Chemistry, Analysis, Control, Exposure & Risk Assessment **25,** 472-489.

Starkey, D.E., Ward, T.J., Aoki, T., Gale, L.R., Kistler, H.C., Geiser, D.M., Suga, H., Toth, B., Varga, J., and O'Donnell, K. (2007). Global molecular surveillance reveals novel Fusarium head blight species and trichothecene toxin diversity. Fungal Genetics and Biology **44,** 1191-1204.

Suga, H., Karugia, G.W., Ward, T., Gale, L.R., Tomimura, K., Nakajima, T., Miyasaka, A., Koizumi, S., Kageyama, K., and Hyakumachi, M. (2008). Molecular characterization of the Fusarium graminearum species complex in Japan. Phytopathology **98,** 159-166.

Sulyok, M., Berthiller, F., Krska, R., and Schuhmacher, R. (2006). Development and validation of a liquid chromatography/tandem mass spectrometric method for the determination of 39 mycotoxins in wheat and maize. Rapid Commun Mass Sp **20,** 2649-2659.

Summerell, B.A., Leslie, J.F. (2011). Fifty years of Fusarium: how could nine species have ever been enough? Fungal Divers **50,** 10-18.

T.Elliott, J.P.M.F.H.P.v.E.C. (2011). Current methods of analysis for the determination of trichothecene mycotoxins in food. TrAC Trends in Analytical Chemistry **30,** 12-19.

Tatsuno, T., Inoue, T., and Tanimura, A. (1979). [Hygienic chemical studies on plastics (II). Residual acrylonitrile in ABS resin or AS resin and its migration into water (author's transl)]. Eisei Shikenjo hokoku. Bulletin of National Institute of Hygienic Sciences**,** 93-97.

Tester, M., and Langridge, P. (2010). Breeding technologies to increase crop production in a changing world. Science **327,** 818-822.

Thrane, U., Adler, A., Clasen, P.E., Galvano, F., Langseth, W., Lew, H., Logrieco, A., Nielsen, K.F., and Ritieni, A. (2004). Diversity in metabolite production by Fusarium langsethiae, Fusarium poae, and Fusarium sporotrichioides. International Journal of Food Microbiology **95,** 257-266.

Torres-Chavolla, E., and Alocilja, E.C. (2009). Aptasensors for detection of microbial and viral pathogens. Biosensors & Bioelectronics **24,** 3175-3182.

Turner, P. (2010). Deoxynivalenol and nivalenol occurrence and exposure assessment. World Mycotoxin Journal **3,** 7-10.

Ueno, Y., Nakajima, M., Sakai, K., Ishii, K., and Sato, N. (1973). Comparative toxicology of trichothec mycotoxins: inhibition of protein synthesis in animal cells. Journal of Biochemistry **74,** 285-296.

V.Zherdev, B.B.D.N.A.B.A.E.U.A. (2014). Immunochromatographic methods in food analysis. TrAC Trends in Analytical Chemistry **55,** 13-19.

van der Lee, T., Zhang, H., van Diepeningen, A., and Waalwijk, C. (2015). Biogeography of Fusarium graminearum species complex and chemotypes: a review. Food Additives & Contaminants. Part A, Chemistry, Analysis, Control, Exposure & Risk Assessment **32,** 453-460.

van Egmond, H.P., Schothorst, R.C., and Jonker, M.A. (2007). Regulations relating to mycotoxins in food: perspectives in a global and European context. Analytical and Bioanalytical Chemistry **389,** 147-157.

Visconti, A., and De Girolamo, A. (2005). Fitness for purpose--ochratoxin A analytical developments. Food Additives and Contaminants **22 Suppl 1,** 37-44.

Wan, L.Y., Turner, P.C., and El-Nezami, H. (2013). Individual and combined cytotoxic effects of Fusarium toxins (deoxynivalenol, nivalenol, zearalenone and fumonisins B1) on swine jejunal epithelial cells. Food and chemical toxicology : an international journal published for the British Industrial Biological Research Association **57,** 276-283.

Wang, J.H., Zhang, J.B., Chen, F.F., Li, H.P., Ndoye, M., and Liao, Y.C. (2012). A multiplex PCR assay for genetic chemotyping of toxigenic Fusarium graminearum and wheat grains for 3-acetyldeoxynivalenol, 15-acetyldeoxynivalenol and nivalenol mycotoxin. J Food Agric Environ **10,** 505-511.

Wang, J.H., Li, H.P., Qu, B., Zhang, J.B., Huang, T., Chen, F.F., and Liao, Y.C. (2008). Development of a generic PCR detection of 3-acetyldeoxy-nivalenol-, 15-acetyldeoxynivalenol- and nivalenol-chemotypes of Fusarium graminearum Clade. International Journal of Molecular Sciences **9,** 2495-2504.

Ward, T.J., Bielawski, J.P., Kistler, H.C., Sullivan, E., and O'Donnell, K. (2002). Ancestral polymorphism and adaptive evolution in the trichothecene mycotoxin gene cluster of phytopathogenic Fusarium. Proceedings of the National Academy of Sciences of the United States of America **99,** 9278-9283.

Wegulo, S.N. (2012). Factors influencing deoxynivalenol accumulation in small grain cereals. Toxins **4,** 1157-1180.

Wei, T., Zhu,W., Pang, M., Liu, Y., & Dong, J. . (2013). Natural occurrence of fumonisins B1 and B2 in corn in four provinces of China. Food Additives & Contaminants: Part B **6,** 5-11.

Weidner, M., Lenczyk, M., Schwerdt, G., Gekle, M., and Humpf, H.U. (2013). Neurotoxic potential and cellular uptake of T-2 toxin in human astrocytes in primary culture. Chemical Research in Toxicology **26,** 347-355.

Xu, X., and Nicholson, P. (2009). Community ecology of fungal pathogens causing wheat head blight. Annual Review of Phytopathology **47,** 83-103.

Xu, X.M., and Berrie, A.M. (2005). Epidemiology of mycotoxigenic fungi associated with Fusarium ear blight and apple blue mould: a review. Food Additives and Contaminants **22,** 290-301.

Xue, H.L., Yang, B., Tang, Y.M., Ying, Z., and Yi, W. (2014). Effect of cultivars, Fusarium strains and storage temperature on trichothecenes production in inoculated potato tubers. Food Chemistry **151,** 236-242.

Zeller, K.A., Summerell, B.A., Bullock, S., and Leslie, J.F. (2003). Gibberella konza (Fusarium konzum) sp. nov. from prairie grasses, a new species in the Gibberella fujikuroi species complex. Mycologia **95,** 943-954.

Zheng Zhang, H.Z., Theo van der Lee, Wan-Quan Chen, Paul Arens, Jin Xu, Jing-Sheng Xu, Li-Jun Yang, Da-Zhao Yu, Cees Waalwijk, Jie Feng. (2010). Geographic substructure of Fusarium asiaticum isolates collected from barley in China. European Journal of Plant Pathology **127,** 10-16.

Zhou, H.R., Lau, A.S., and Pestka, J.J. (2003). Role of double-stranded RNA-activated protein kinase R (PKR) in deoxynivalenol-induced ribotoxic stress response. Toxicological sciences : an official journal of the Society of Toxicology **74,** 335-344.

CHAPTER FOUR

GENETIC DIVERSITY, TOXIN PRODUCTION AND PATHOGENICITY OF *GIBBERELLA FUJIKUROI* SPECIES COMPLEX ISOLATED FROM RICE

Genetic diversity, toxin production and pathogenicity of *Gibberella fujikuroi* species complex isolated from rice

Fang Ji[1,2], Jianbo Qiu[1], A. O. Olaniran[2], M.P. Mokoena[2] and J. Shi[1*]

[1]Key Lab of Food Quality and Safety of Jiangsu Province-State Key Laboratory Breeding/Key Laboratory of Control Technology and Standard for Agro-Product Safety and Quality/Key Laboratory of Agro-Product Safety Risk Evaluation (Nanjing), Ministry of Agriculture, China/ Institute of Food Safety and Nutrition, Jiangsu Academy of Agricultural Sciences, 210014, China

[2]Discipline of Microbiology, School of Life Science, College of Agriculture, Engineering and Science, University of KwaZulu-Natal, Private Bag X54001, Durban, 4000, South Africa.

∗Corresponding author. Tel: +25 84392001; Fax: Tel: +25 84392001

E-mail address: jianrong63@126.com (Jianrong Shi)

Abstract

Gibberella fujikuroi species complex (GFSC) are the most frequent fungal pathogens affecting cereals all over the world, producing various mycotoxins. In this study, a total of 61 strains were isolated from rice (*Oryza sativa* L.) seed. The *FUM1* gene sequences were used to identify the species characteristics of the isolates. The fungal pathogens in rice samples were mainly *F. fujikuroi, F. proliferatum and F. verticillioides*. Phylogenetic trees based on the *FUM1* gene from all isolates revealed higher intraspecific variability in *F. proliferatum*. The production of fumonisin and beauverin from all strains in rice medium were detected. The results indicated that *F. proliferatum* and *F. verticillioides* belong to the fumonisin producing species, while *F. fujikuroi* strains produced extremely low amounts of fumonisin. Beauverin was detected in *F. fujikuroi* and *F. proliferatum* strains with low levels. Pathogenicity results suggest that the three species affected seed germination with similar degrees and that *F. fujikuroi* could cause elongated seedlings.

Keywords: Beauverin, Fumonisin, *Gibberella fujikuroi*, Rice.

1. Introduction

Gibberella fujikuroi species complex (GFSC) consists of at least 50 distinct species or phylogenetic lineages (O'Donnell *et al*., 2015), and is the causative agent of various diseases in plants and animals. Among GFSC, *Fusarium verticillioides* (*G. fujikuroi* mating population A), *F. fujikuroi* (*G. fujikuroi* mating population C) and *F. proliferatum* (*G. fujikuroi* mating population D) are well known for their ability to cause devastating diseases of many cereal crops and considerable reduction in crop yields and quality.

Members in the GFSC can produce a variety of mycotoxins including fumonisins, beauvericin, gibberellic acid, and fusaric acid, which contaminate food. And they are harmful to the health of humans and animals. However, there are significant differences in the toxic metabolites production by individual species. Fumonisins (FBs) are the most frequently detected mycotoxins in grains and grains-based products. Researche has shown that fumonisins inhibit sphingosine -N- acyltransferase that participates in sphingolipid biosynthesis (Ho and Durst, 2000). To date, although there is no direct evidence that fumonisins are associated with serious human and animal health problems, it has been found that the high incidence of human esophageal cancer is strongly related to the consumption of fumonisins contaminated corn products (Gong *et al*., 2009). Beauvericin (BEA) has been shown to be toxic to some human cell lines and can induce apoptosis (Lu *et al*., 2016). It has also been found in grain-producing areas all over the world (Luz *et al*., 2016).

The enzymes-encoding *FUM* gene cluster has been proved to be source of fumonisins biosynthesis. The pathway starts with the key iterative polyketide synthase (encoded by *FUM1* gene) and synthesizes the toxin skeleton, modified subsequently by other enzymes (Proctor *et al*., 1999). *F.*

verticillioides, the model species of *FUM* gene cluster, has been widely studied and found to be composed of 21 co-regulated genes that show a common expression pattern during the biosynthesis of fumonisins (Brown *et al*. 2005). Recent studies suggested that the fumonisin gene clusters of *F. fujikuroi*, *F. proliferatum* and *F. Verticillioides* are highly conserved (Wiemann *et al*., 2013; Rösler *et al*., 2016).

The translation elongation factor (*TEF-1α*) sequence is one of many molecular markers for phylogenetic reconstruction. It is most useful in taxonomic studies of fungi, especially in the *G. fujikuroi* species complex classification, and other *Fusarium* species (Geiser *et al*., 2004; Kristensen *et al*., 2005). Recent studies have shown that genes and other sequences involved in the mycotoxin biosynthetic pathway have the advantage of a combinatorial approach that can be used to diagnose mycotoxin production capacity and are a good target in phylogenetic studies (Stępień, 2013; Susca *et al*., 2017). Thus, genes from the *FUM* cluster have been used as good markers for phylogenetic studies of *Fusarium* species producing fumonisins (Baird *et al*., 2008; González-Jaén *et al*., 2004; Stępień *et al*., 2011).

In the previous study, we have isolated and characterized *F. verticillioides* and *F. proliferatum* strains from maize, but little information is available about GFSC members from the rice. Therefore, the main purpose of this study was to identify *Fusarium* strains and phylogenetically differentiate three populations by analyzing the sequence divergences of *Fum1*. In addition, the ability of all strains to produce fumonisins and cause elongation of rice seeds was evaluated.

2. Materials and methods

2.1 Isolation of *Fusarium* species

98rice seeds were collected in several counties of Jiangsu provinces in 2013. They were randomly chosen, and then sterilized with 1% sodium hypochlorite for 1 minutes, and then washed with sterile distilled water for twice, and finally placed in a Petri dish containing PDA (potato glucose agar) for 5 days at 25°C. Recovered *Fusarium* isolates were purified on fresh PDA plates and incubated for 5 days at 25°C. Conidia produced by mung bean broth (MBB) were spread on PDA and isolated the single conidia according to Jurado *et al*. (2007). All strains were grown on potato dextrose agar (PDA) as the regular culture medium and stored in 20% glycerol solution at -80 °C.

2.2 Sequence analysis and phylogeny reconstruction

Colonies of *Fusarium* species were grown on PDA at 25°C for 5 days and the mycelium produced was scraped from the surface. Extraction of DNA using cetyltrimethylammonium bromide (2% CTAB) method (Leslie and Summerell, 2006). Primers Fum1F1 (CACATCTGTGGGCGATCC) and Fum1R2 (ATATGGCCCCAGCTGCATA) were used for the amplification of *Fum1* gene fragments (Stępień *et al*., 2011). PCR-amplified DNA fragments were sequenced by Shanghai Shenggong Biotechnological Ltd. Sequences of *FUM1* of several *F. verticillioides*, *F. fujikuroi* and *F. proliferatum* isolates were also included in the analysis. Phylogenetic relationships were reconstructed with MEGA 4 software package using Maximum Parsimony approach (Tamura *et al*., 2007). No gap-containing positions were considered in phylogeny analysis. The Bootstrap method used 1000 repeated heuristic searches.

2.3 PCR assays with species-specific primers

F. verticillioides and *F. proliferatum* were confirmed by PCR, which was previously described using species-specific primers VERT-1 (5′-GTCAGAATCCATGCCAGAACG-3′) and VERT-2 (5′-CACCCGCAGCAATCCATCAG -3′) for *F. verticillioides* (Patiño *et al*., 2004) and PRO1 (5′-CTTTCCGCCAAGTTTCTTC-3′) and PRO2 (5′-TGTCAGTAACTCGACGTTGTTG-3′) for *F. Proliferatum* (Mulé *et al.*, 2004).

2.4 Design of species-specific PCR primers

28S ribosomal RNA gene and intergenic spacer region of nine strains, including three *F. fujikuroi* strains (AJ879945.1, HQ165889.1, AY249382.1), three *F. verticillioides* strains (AJ880004.1, HQ165881.1, AY249379.1) and three *F. proliferatum* strains (GU737458.1,AY249383.1, DQ831905.1), were compared by multiple sequence alignments. SNPs between strains of different species were translated into PCR amplicons of different lengths (Fig. 1). This allowed for the design of a set of primers-GF1 (5′-ACGAGCGGGGTCAAATCCT-3′), GF2 (5′-GCACGGAAGCCAACATCAG-3′), GF3 (5′- ACAGCCGCACACACTCGC-3′), and GF4 (5′-CCAGATAATTCTCTTCCCCG-3′)-that generated 952 bp fragment from *F. proliferatum* strains, a 397bp fragment from *F. verticillioides* strains, and a 260 bp fragment from *F. fujikuroi* strains, respectively. Validation of the species-specific PCR assay was conducted using 9 strains from rice seeds after phylogenetic analysis with *Fum1* gene.

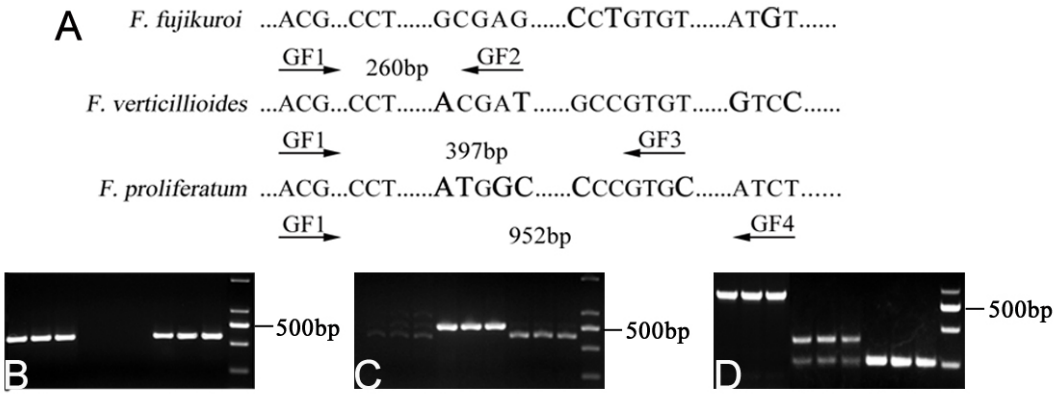


Figure 1. A schematic map of the polymerase chain reaction of 28S ribosomal RNA gene was established to identify species in GFSC. B, Amplifications using primers PRO1/PRO2.C, Amplifications using primers VERT1/VERT2. D, Amplifications using primers for complex. The first three lanes were *F. fujikuroi* strains, the middle three lanes were *F. Verticillioides* strains, and the last three strains were *F. proliferatum* strains.

2.5 Toxins production

The ability of all strains to produce fumonisin B1 and beauverin was evaluated on autoclaved rice grains. After 20 day's culture at 25°C, rice grains were dried and ground to a fine powder. Five grams of each sample were shaken with 25 mL methanol/water (1:1, v/v) (ROE Scientific Inc. Newmark, DE, USA; Ultrapure water was produced by a Millipore Milli-Q system Millipore, Bedford, MA, USA) at 180 rpm for 30min.). After centrifugation at 6000 rpm for 5 min, 5 ml supernatant was purified on a solid phase extraction column (CNWBOND SAX SPE Cartridge, 500 mg, 6 mL). The column was then washed with methanol (5 mL) and water (5 mL), and toxins were eluted with 1% (v/v) acetic acid in methanol (10 mL). The extracts concentrated by nitrogen were diluted with 1 mL methanol/water (1:1, v/v) and filtered through a nylon filter with a diameter of 13mm and 0.22μm pore size. Toxins were analyzed with LC-20ADXR liquid chromatograph (Shimadzu, Kyoto, Japan)

which was coupled to an AB SCIEX Triple Quad mass spectrometer (Applied Biosystems, Foster City, CA, USA). The analytical column used was a Kinetex 100A C18 column (100 × 2.3 mm, 2.6 μm), from Phenomenex (USA), and the column temperature was held at 40°C. The flow rate was 0.5 mL/min, and the injection volume was 2 μL. The mass spectrometric analyses were performed with the following operation parameters: gas temperature, 500 °C; gas flow rate, 10 L/min; nebulizer gas pressure, 50 psi; and capillary voltage, 5500 V. Nitrogen was used in the ion source and the collision cell. Mycotoxins were analyzed via multiple reactions monitoring (MRM).

The mobile phase for FB1 consisted of water/acetic acid (99.9:0.1, v/v) (A) and methanol (B). Separate conditions of high performance liquid Chromatography were as follows: 0-1.6 min, solvent A was linearly decreased from 90% to 60%; 1.6-10 min, solvent A was linearly decreased from 60% to 40%; 10-11 min, solvent A was linearly increased from 40% to 90%; 11-12 min, solvent A was linearly decreased from 90% to 40%; 12-15 min, solvent A was kept constant at 90%.

About 5 nM ammonium acetate (A) and methanol (B) form the mobile phase of BEA. Separate conditions of high performance liquid Chromatography were as follows: 0-1 min, solvent A was linearly increased to 75%; solvent A was down to 0% at 1min and kept constant for 3 min; solvent A was increased to 90% for 7min and kept constant for 5 min; solvent A was decreased to 90% at 12 min and kept constant for 3 min.

2.6 Pathogenicity tests

All the strains were grown at 25°C for 5 days on PDA and then cultured in 5% sterile MBB under shaking condition (175 rpm) for 5 days at 25°C. The conidia were collected and the spore concentration was adjusted to approximately 10^6/ml in sterile water. The seeds of susceptible rice varieties Xinliangyou6308 were used to test the pathogenicity of the fungal isolates. The seeds were sterilized by 1% sodium hypochlorite for 10 minutes and rinsed for three times with sterile distilled water for 48 hours. The seeds were transferred to sterile filter papers in Petri plates moistened with sterile distilled water and finally incubated at 25°C. After 2 days, 20 sprouting seeds were soaked in 10 ml of inoculum suspension at 175 rpm at 25°C for 24 h. Control seeds were soaked in sterile water. Thereafter, inoculated and control seeds were placed in Petri plate and incubated at room temperature (two plates per isolate/ten seeds per plate). The excessive growth of seedlings is relative to the invasive measure of the control (Ahmed *et al*., 1988). Measurements of elongation and growth retardation in millimeter units were performed and compared with controls to detect differences in the 5% significant levels. The experiment was repeated twice.

2.7 Statistical analysis

Statistical comparisons of pathogenicity parameters and toxin production were made with *t* test. All statistical analyses were performed with the Sigma Stat statistical software package (SPSS, version 11).

3. Results

3.1 Phylogenetic analysis

Partial sequences of 1004 bp long of *FUM1* of all isolates were amplified and analyzed. Of the 1004 nt analyzed for the *FUM1* sequence, 85 nt were polymorphic sites and 0 parsimonic-informative sites. Phylogenetic analysis was performed by comparing the sequences with several sequences obtained from the GenBank database. Dendrogram constructed with the *FUM1* sequences differentiated the isolates into two main clusters (Fig. 2). All the *F. fujikuroi* isolates fell firmly into one cluster, unlike those closely related to *F. proliferatum.* The cluster containing strains of *F. proliferatum* showed higher intraspecific variability than *F. fujikuroi*. After verification, there were 16, 16, and 29 strains of *F. proliferatum*, *F. fujikuroi*, and *F. verticillioides* used for the future research, respectively.

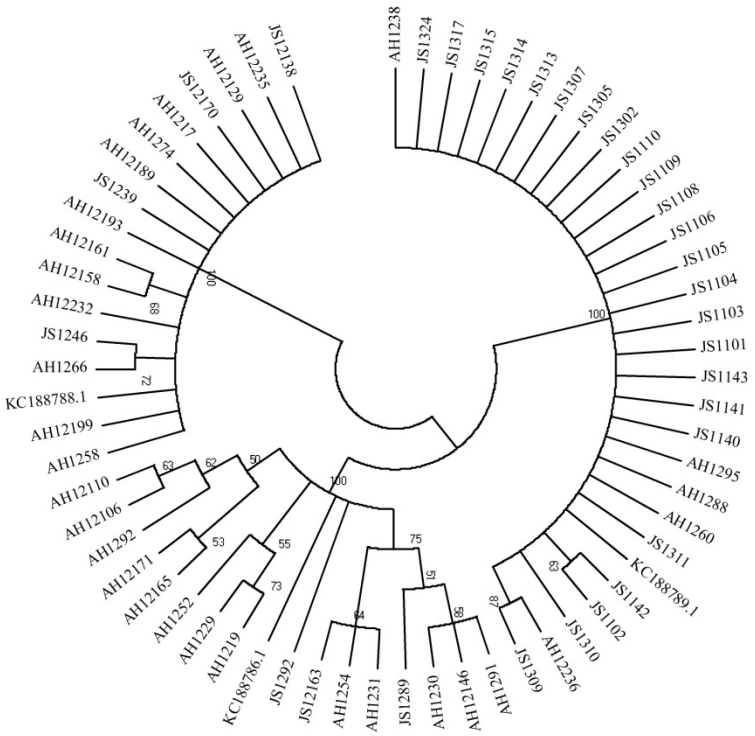


Figure 2. Consensus phylogenetic tree of sickle isolates was generated based on *Fum1* sequences. The dendrogram was constructed by the neighbor-joining approach and tested by bootstrapping (10,000 replicates) with a cut-off value of 50%. *Fum1* of *F. proliferatum* (KC188786.1), *F. verticillioides* (KC188788.1), and *F. fujikuroi* (KC188789.1) were used as the reference.

3.2 Pathogenicity

Most of the strains in this study are pathogenic to rice. All the pathogenic strains inhibited seed germination compared with the 100% set of germination control treatments. The number of germinated seeds treated by different strains is between 25% to 85%. There are no significant difference in the ability to inhibit seed germination between different populations. The average number of germinated seed is between 50% and 60%. The severity of the disease was expressed as

the elongation of seedlings relative to the control. The ability to cause elongation of seedlings varied widely among the strains tested, with only one strain observed to have no effect on rice seedlings. The consistence of the effect of all isolates in two tests was observed in some isolates (Table. 1). The production of fumonisins was not related to pathogenicity.

Table 1: Pathogenicity and toxin production of *F. verticillioides*, *F. fujikuroi*, and *F. proliferatum* populations tested in this study

Species	Seedling length (cm)	Seed germination (%)	Fumonisin B1(*mg/kg*)	Beauverin *(mg/kg)*
F. verticillioides	3.45±0.71b*	52.22±18.96a	257.17±249.98b	0b
F. proliferatum	3.66±0.75b	58.82±17.09a	453.81±400.67a	0.21±0.16a
F. fujikuroi	4.06±0.65a	56.51±14.88a	2.43±5.97c	0.24±0.34a
Control	3.55±0.59	100%		

*The same letter in each column is not significantly different ($P< 0.05$).

3.3 Fumonisin production

Fumonisin B1 was produced by toxigenic *Fusarium* isolates in the concentration range 2.34-977.58 mg/kg for all *F. verticillioides* strains, 0.65-1375.99 mg/kg for all *F. proliferatumstrains*, and 0.023-34.48 mg/kg for all *F. fujikuroi* strains. FB1 productions of *F. proliferatum* were significantly higher than that of *F. verticillioides*, while *F. fujikuroi* produced much smaller amount of fumonisin than the other two populations. Beauverin (BEA) was produced by toxigenic *Fusarium* isolates in the concentration range 0.044-0.54 mg/kg for 14/16 *F. proliferatum* strains, and 0.04-1.58 mg/kg for 18/29 *F. proliferatum* strains. There were no significant difference of Beauverin production between *F. proliferatum*. However, BEA was not detected in all *F. verticillioides* strains tested.

4. Discussion

F. fujikuroi, *F. verticillioides*, and *F. proliferatum* are common pathogens infecting several hosts and are the main producers of fumonisins. The translation elongation factor (*TEF-1α*) gene has been successfully applied to various studies of *Fusarium* species molecular taxonomy. Recently, studies on the genetic relationship between *Fusarium* species using secondary metabolite biosynthesis genes are increasing (Stępień, 2014). Stępień *et al*. (2011) suggested that the intra-species divergence of the partial *Fum1* sequences was enough to distinguish more clear clades of individual *F. proliferatum* isolate in connection with different original hosts than the use of *TEF-1α* sequences. Similar findings were reported for *F. proliferatum* strains isolated from date palm and banana by Jurado *et al*. (2010), although a longer fragment of *TEF-1α* gene was used. Stępień *et al*. (2011) also reported that *FUM1* or *FUM8* showed major capability to distinguish between *Fusarium* species, though the phylogenetic tree based on *FUM* cluster genes also reflected the scenarios presented by *TEF-1α* sequences. *TEF-1α* and *FUM1* gene sequences divergence was simultaneously analyzed in pea-associated *Fusarium* isolates and similar result was obtained, with all strains forming two separate clades of *F. proliferatum* and *F. verticillioides* (Waśkiewicz *et al*., 2013). *TEF-1α* is a classical target gene widely and successfully applied in phylogenetic differentiation of fungi since it displays a high level of polymorphism. In some special cases about populations of some less polymorphic species, different genomic regions with high genetic diversity could be used to resolve the problem. In this study, the sequence of the *FUM1* gene was used for phylogenetic analysis besides the *TEF-1α* gene. The intraspecific variability of all *F. fujikuroi* isolates appeared to be rather low, while a certain level of sub-specific polymorphism was observed in the clade of *F. proliferatum* strains. Phylogenetic trees clearly grouped *F. fujikuroi*, *F. verticillioides*, and *F. proliferatum* with high bootstrap values based on *TEF-1α* sequences. Similar finding from analysis of the gene divergence were reported by Amatulli *et al*. (2010), Wulff *et al*. (2010) and Cruz *et al*. (2013). Our result confirmed that the *FUM1*

gene is a good marker for species identification and phylogenetic reconstruction of *Fusarium* species producing fumonisins.

In this study, the correlation between the diversity of the fumonisin biosynthesis and the structure of the *FUM*1 gene was not found in the FUM1 sequence of many strains from different hosts. However, in some cases isolates of high and low fumonisin production are grouped together in one clade (Fig. 2). This is consistent with our previous studies, in which some non-producing mutants of *Fusarium* strains were identified, although at least a part of FUM clusters existed (Stępień *et al*. 2011).

Pathogenicity of *F. fujikuroi, F. verticillioides,* and *F. proliferatum* isolates on rice was observed in form of reduction of seed germination and bakanae symptoms in the seedlings. However, different strains caused symptoms with different severity. Typical elongation of bakanae symptoms was caused by all *F. fujikuroi* isolates, except one. In contrast with the finding of Zainudin *et al*. (2008), no infected seedlings were stunned. Amoah *et al*. (1995) found that although growth retardation and elongation were observed in rice seedlings, they were separated according to their ability to elongate. Both stunning and elongation were also observed by the *F. fujikuroi* isolates in the Philippines and all the isolates varied in the degree of disease indices (Cruz *et al*., 2013). Amatulli *et al*. (2010) found that only *F. fujikuroi* isolates could cause classical bakanae symptoms and these isolates showed different disease severity. In this study, there was no significant difference in the pathogenicity based on inhibition of seed germination between *F. fujikuroi, F. verticillioides, and F. proliferatum* isolates. Wulff *et al*. (2010) observed that the majority of *G. fujikuroi* species complex showed pathogenicity on rice with reduction of seed germination. *F. fujikuroi* strains showed stronger aggressiveness in causing symptoms of elongation seedlings. Even in the case of high seed colonization, the *Fusarium verticillioides* strains did not affect seed germination (Venturini*et al*., 2013). As the variation in pathogenicity of these isolates could be attributed to their physiological features or environment

conditions, further research should be conducted, especially on the effect of temperature and relative humidity.

Amoah *et al* (1995) studies show that the ability of *G. fujikuroi* species complex to cause crop symptoms may depend on the balance of secondary metabolites and growth regulators affected by fungal strains, environmental factors and nutritional conditions. The abnormal elongation of the rice seedlings could be as a result of gibberellic acid produced by *F. fujikuroi* isolates (Johnson and Coolbaugh, 1990). Wulff *et al*. (2010) reported the similar idea that more symptoms of chlorotic or slender leaves were examined in gibberellin-producing *F. fujikuroi* isolates. Variations in gibberellic acid production led to the diversity of the height of the rice seedlings. On the other hand, fumonisins production was determined to study the relationship with the symptoms. Our results suggested that there is no direct correlation between fumonisins production by the *Fusarium* strains and disease occurrence in the rice seedlings. Similar findings were previously reported about *F. proliferatum* (Busman *et al*., 2012) and *F. fujikuroi* (Cruz *et al*., 2013). Wulff *et al*. (2010) found that *F. fujikuroi* strains with the smallest amount of fumonisins production were still able to cause disease and the authors suggested that unknown factors could play a stronger role in the aggressiveness of those low-producing strains. All these researches indicated that fumonisins production was not closely related to pathogenicity, at least for *F. proliferatum* and *F. fujikuroi*. The generation of fumonisins and gibberellic acid can help pathogens colonize plant tissue and compete with other fungi during infection, but their involvement in disease symptoms may be minimal (Jurado *et al*., 2008; Marín *et al*., 2010). The production of mycotoxins, including fumonisins and Beauverin, may play an important role in the competition with other fungal species during plant tissue colonization and infection (Marin et al, 2013), but their effects on the symptoms of the bakanae disease may be reduced. However, in the case of *F. verticillioides*, the conflicting results suggested that our

understanding about the role of fumonisins is very little and might depend on the host considered (Cumagun *et al*., 2008; Glenn *et al*., 2008).

The ability of *F. verticillioides*, *F. fujikuroi* and *F. proliferatum* strains to produce fumonisin *in vitro* is consistent with observations results of Lee *et al*. (2012), Wulff et al. (2010), Desjardins et al. (2000) and Stępień*et al*. (2011). Among these strains, it has been reported that *F. fujikuroi* produces little or no fumonisin compared to *F. verticillioides* and *F. proliferatum.* However, there were several recent reports mentioned that some strains of *F. fujikuroi* from rice and wine grape have the ability to produce the equivalent fumonisin level with *F. Verticillioides* and *F. proliferatum (*Matić *et al*., 2013*)*. The differential regulation of fumonisin biosynthetic gene cluster in this population can be the basis for enhancing mycotoxin production. Host or related pest management practices can create an environment conducive to the activation of biosynthetic genes, which may be related to the availability of nitrogen, which shows the influence of the expression of the gene cluster (Shim *et al*., 1999).

5. Conclusion

In this study, *F. fujikuroi*, *F. proliferatum*, and *F. verticillioides* are shown to be main species of GFSC contaminating rice samples. The phylogenetic analysis, the mycotoxigenic profile (fumonisin and beauvericin) and the pathogenicity showed high variability on GFSC species, but no correlations could be observed between the latter two parameters. Overall, these findings increase the knowledge on character of the most important rice fungal pathogens worldwide. Moreover, information about the high variability of pathogens is important for further development of disease management strategies. Our future studies will be aimed to identify GFSC species associated with different crops, such as barley, rice and maize, and soybeans and comparing genetic and phenotypic variation of various host populations.

6. References

1. **Ahmed HU, M. Mia AT and Miah SA.** (1986). Standard test-tube inoculation for bakanae disease. IRRI Newsl **11**, 21-22.
2. **Amatulli, M. T., Spadaro, D., Gullino, M. L., & Garibaldi, A.** (2010). Molecular identification of *Fusarium* spp. associated with bakanae disease of rice in Italy and assessment of their pathogenicity. Plant Pathology **59**, 839-844.
3. **Amoah, B. K., Rezanoor, H. N., Nicholson, P., & MacDonald, M. V.** (1995). Variation in the Fusarium section Liseola: pathogenicity and genetic studies of isolates of *Fusarium moniliforme* Sheldon from different hosts in Ghana. Plant Pathology **44**, 563-572.
4. **Baird R, Abbas HK, Windham G, Williams P, Baird S, Ma P, Kelley R, Hawkins L, Scruggs M.** (2008). Identification of select fumonisin forming *Fusarium* species using PCR applications of the polyketide synthase gene and its relationship to fumonisin production in vitro. Int J Mol Sci **9**, 554-570.
5. **Bolton, S. L., Brannen, P. M., & Glenn, A. E.** (2016). A novel population of *Fusarium fujikuroi* isolated from southeastern US winegrapes reveals the need to re-evaluate the species' fumonisin production. Toxins **8**, 254-260.
6. **Brown DW, Cheung F, Proctor RH, Butchko RAE, Zheng L, Lee Y, Utterback T, Smith S, Feldblyum T, Glenn AE, Plattner RD, Kendra DF, Town CD, Whitelaw CA.** (2005). Comparative analysis of 87,000 expressed sequence tags from the fumonisin-producing fungus *Fusarium verticillioides.* Fungal Genet Biol **42**, 848–861.
7. **Busman M, Desjardins AE and Proctor RH.** (2012). Analysis of fumonisin contamination and the presence of *Fusarium* in wheat with kernel black point disease in the United States.Food Addit Contam A **29**, 1092-1100.
8. **Cruz, A., Marín, P., González-Jaén, M. T., Aguilar, K. G. I., & Cumagun, C. J. R.** (2013).

Phylogenetic analysis, fumonisin production and pathogenicity of *Fusarium fujikuroi* strains isolated from rice in the Philippines. Journal of the Science of Food and Agriculture **93**, 3032-3039.

9. **Cumagun, C. J. R., Ramos, J. S., Dimaano, A. O., Munaut, F. and Van Hove, F.** (2009). Genetic characteristics of *Fusarium verticillioides* from corn in the Philippines. Journal of General Plant Pathology **75**, 405-413.
10. **Desjardins, A. E., Manandhar, H. K., Plattner, R. D., Manandhar, G. G., Poling, S. M., & Maragos, C. M.** (2000). *Fusarium* species from Nepalese rice and production of mycotoxins and gibberellic acid by selected species. Applied and Environmental Microbiology **66**, 1020-1025.
11. **Geiser DM, Jiménez-Gasco MdM, Kang S, Makalowska I, Veeraraghavan N, Ward TJ, Zhang N, Kuldau GA, O'Donnell K.** (2004). FUSARIUM-ID v. 1.0: a DNA sequence database for identifying *Fusarium*. Eur J Plant Pathol **110**, 473-479.
12. **Glenn, A. E., Zitomer, N. C., Zimeri, A. M., Williams, L. D., Riley, R. T. and Proctor, R. H**. (2008). Transformation-mediated complementation of a FUM gene cluster deletion in *Fusarium verticillioides* restores both fumonisin production and pathogenicity on maizeseedlings. Molecular Plant-Microbe Interactions **21**, 87-97.
13. **Gong HZ, Ji R, Li YX, Zhang HY, Li B, Zhao Y.** (2009). Occurrence of fumonisin B1 in corn from the main corn-producing areas of China. Mycopathologia **167,** 31-36.
14. **González-Jaén, M. T., Mirete, S., Patiño, B., López-Errasquín, E., & Vazquez, C.** (2004). Genetic markers for the analysis of variability and for production of specific diagnostic sequences in fumonisin-producing strains of *Fusarium verticillioides*. Eur. J. Plant Pathol **110**, 525-532.
15. **Ho JA, Durst RA.** (2000). Preparation of reagents for the determination of fumonisin B1 by flow-injection immunoanalysis. Anal Chim Acta **5**, 51-60.

16. **Johnson SW and Coolbaugh RC.** (1990). Light stimulated giberellin biosynthesis in *Gibberella fujikuroi*. Plant Physiol **94**, 1696-1701.
17. **Jurado M, Marín P, Callejas C, Moretti A, Vázquez C, González-Jaén MT**. (2010). Genetic variability and fumonisin production by *Fusarium proliferatum*. Food Microbiol **27**, 50-57.
18. **Jurado, M., Marín, P., Magan, N. and González-Jaén, M. T.**(2008).Relationship between solute and matric potential stress, temperature, growth and *FUM1* gene expression in two *Fusarium verticillioides* strains from Spain. Appl Environ Microbiol **74**, 2032-2036.
19. **Kristensen R, Torp M, Kosiak B, Holst-Jensen A.** (2005). Phylogeny and toxigenic potential is correlated in *Fusarium* species as revealed by partial translation elongation factor 1 alpha gene sequences. Mycol Res **109**, 173-186.
20. **Lee, S. H., Kim, J. H., Son, S. W., Lee, T. and Yun, S. H.** (2012). Fumonisin Production by Field Isolates of the *Gibberella fujikuroi* Species Complex and *Fusarium* commune Obtained from Rice and Corn in Korea. Research in Plant Disease **18**, 310-316.
21. **Leslie JF and Summerell BA.** (2006).The *Fusarium* Laboratory Manual. Blackwell, Ames, IA.
22. **Lu, C. L., Lin, H. I., Chen, B. F. and Jow, G. M.** (2016). Beauvericin-induced cell apoptosis through the mitogen-activated protein kinase pathway in human nonsmall cell lung cancer A549 cells. The Journal of Toxicological Sciences **41**, 429-437.
23. **Luz, C., Saladino, F., Luciano, F. B., Mañes, J. and Meca, G.** (2017). Occurrence, toxicity, bioaccessibility and mitigation strategies of beauvericin, a minor *Fusarium* mycotoxin. Food and Chemical Toxicology **107**, 430-439.
24. **Marín, P., Magan, N., Vázquez, C. and González-Jaén, M. T.** (2010). Differential effect of environmental conditions on the growth and regulation of the fumonisin biosynthetic gene FUM1 in the maize pathogens and fumonisin producers *Fusarium verticillioides* and *Fusarium*

proliferatum. FEMS Microbiology Ecology **73**, 303-311.

25. **Matić, S., Spadaro, D., Prelle, A., Gullino, M. L. and Garibaldi, A.** (2013). Light affects fumonisin production in strains of *Fusarium fujikuroi*, *Fusarium proliferatum*, and *Fusarium verticillioides* isolated from rice. International Journal of Food Microbiology **166**, 515-523.
26. **Mulè, G., Susca, A., Stea, G. and Moretti, A.** (2004). Specific detection of the toxigenic species *Fusarium proliferatum* and *F. oxysporum* from asparagus plants using primers based on calmodulin gene sequences. FEMS Microbiology Letters **110**, 495–502.
27. **O'Donnell, K., Ward, T. J., Robert, V. A., Crous, P. W., Geiser, D. M. and Kang, S.** (2015). DNA sequence-based identification of *Fusarium*: current status and future directions. Phytoparasitica, 43(5), 583-595.
28. **Patiño, B., Mirete, S., Gonzàlez-Jaèn, M.T., Mulè, G., Rodrìguez, T., Vàzquez, C.** (2004). PCR detection assay of the fumonisin producing *Fusarium verticillioides* strains. Journal of Food Protection **67**, 1278-1283.
29. **Proctor, R. H., Desjardins, A. E., Plattner, R. D. and Hohn, T. M.** (1999). A polyketide synthase gene required for biosynthesis of fumonisin mycotoxins in *Gibberella fujikuroi* mating population A.Fungal Genetics and Biology **27,** 100-112.
30. **Rösler, S. M., Sieber, C. M., Humpf, H. U. and Tudzynski, B.** (2016). Interplay between pathway-specific and global regulation of the fumonisin gene cluster in the rice pathogen *Fusarium fujikuroi*. Applied Microbiology and Biotechnology **100**, 5869-5882.
31. **Shim, W. B. and Woloshuk, C. P.** (1999). Nitrogen repression of fumonisin B1 biosynthesis in *Gibberella fujikuroi*. FEMS Microbiology Letters **177**, 109-116.
32. **Stępień Ł.** (2013). The use of *Fusarium* secondary metabolite biosynthetic genes in chemotypic and phylogenetic studies. Critical Reviews in Microbiology **40,** 176-185.
33. **Stępień, Ł.** (2014). The use of *Fusarium* secondary metabolite biosynthetic genes in chemotypic and phylogenetic studies. Critical Reviews in Microbiology **40**, 176-185.

34. **Stępień, Ł., Koczyk, G., & Waśkiewicz, A.** (2011).*FUM* cluster divergence in fumonisins-producing *Fusarium* species. Fungal Biol **115**, 112-123.

35. **Tamura K, Dudley J, Nei M, Kumar S.** (2007). MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. Mol Biol Evol **24**, 1596-1599.

36. **Thompson JD, Higgins DG, Gibson TJ.** (1994). Clustal W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice.Nucleic Acids Research **22**, 4673-4680.

37. **Venturini, G., Assante, G., Toffolatti, S. L. and Vercesi, A.** (2013). Pathogenicity variation in *Fusarium verticillioides* populations isolated from maize in northern Italy. Mycoscience **54**, 285-290.

38. **Waśkiewicz, A., Stępień, Ł., Wilman, K. and Kachlicki, P.** (2013). Diversity of pea-associated F. proliferatum and F. verticillioides populations revealed by FUM1 sequence analysis and fumonisin biosynthesis. Toxins **5**, 488-503.

39. **Wiemann, P., Sieber, C. M., Von Bargen, K. W., Studt, L., Niehaus, E. M., Espino, J. J. and Bergner, S. V.** (2013). Deciphering the cryptic genome: genome-wide analyses of the rice pathogen *Fusarium fujikuroi* reveal complex regulation of secondary metabolism and novel metabolites. PLoS Pathogens **9**, e1003475.

40. **Wulff, E. G., Sørensen, J. L., Lübeck, M., Nielsen, K. F., Thrane, U. and Torp, J.** (2010). *Fusarium* spp. associated with rice Bakanae: ecology, genetic diversity, pathogenicity and toxigenicity. Environmental Microbiology **12**, 649-657.

41. **Zainudin, N. A. I. M., Sidique, S. N. M. and Salleh, B.** (2008). Genetic diversity of Fusarium fujikuroi isolated from bakanae disease of rice on the basis of vegetative compatibility. Pertanika J. Trop. Agric. Sci **31**, 271-278.

CHAPTER FIVE

DEVELOPMENT OF AN IMMUNOCHROMATOGRAPHIC STRIP TEST FOR THE RAPID DETECTION OF ZEARALENONE IN WHEAT FROM JIANGSU PROVINCE, CHINA

This chapter has been published in PLOS ONE with the following citations:

(Fang Ji, Mduduzi P. Mokoena, Hongyan Zhao, Ademola O. Olaniran and Jianrong Shi (2017). Development of an immunochromatographic strip test for the rapid detection of zearalenone in wheat from Jiangsu province, China. PLoS ONE, 12(5): e0175282, doi.org/10.1371/journal.pone.0175282)

CHAPTER SIX

GENERAL DISCUSSION AND CONCLUSION

6.1 The Research in Perspective

Fusarium mycotoxins are secondary metabolites produced by members of *Fusarium graminearum* species complex (FGSC), consisting of at least 16 different species of phylogenetically during growth and crop storage, and are chemically and thermally stable (van der Lee *et al.*, 2015). The strains of FGSC usually produce one of three types of trichothecene profiles, that is, the 3ADON chemotype which produce DON and 3ADON, the 15ADON chemotype that produce DON and 15ADON, and the NIV chemotype that produce NIV and little DON (Castanares *et al*., 2014). Most species seem to grow only in specific geographical areas. In China, most of the *Fusarium graminearum* isolates were found in the colder northern regions, and *Fusarium asiaticum* is mainly from warmer regions where the most prevalent regions of the FHB epidemic (Wang *et al*., 2008).

The occurrence of *Fusarium* mycotoxin contamination in both foods and feeds is inevitable worldwide. National and international organizations, such as the World Health Organization (WHO), the Food and Agriculture Organization (FAO), the US Food and Drug Administration (FDA) and the European Commission (EC), have recognized the potential risks of *Fusarium* mycotoxins on human and animal health. Humans can be poisoned by mycotoxins transmitted through food and feed. Furthermore, *Fusarium* mycotoxin contaminations could have a significant economic impact, too. Among the known mycotoxins, DON, ZEA, NIV, T-2 toxin and FB1 are of greatest concern due to their frequent occurrence in foods and feeds. Hence, the need for monitoring of mycotoxin levels routinely and continuously using rapid, sensitive, and reliable techniques for their detection. The aim of the present study was to elucidate the kinds of *Fusarium* species and mycotoxins that are found in Jiangsu Province of China and to further assess the influence of environmental conditions (temperature, humidity, wheat varieties) on mycotoxin accumulation.

It was established that:

Approximately 90% of the 428 *Fusarium* strains isolated from 2014-2016 belong to *F. asiatium;* while approximately 9.8% belong to *F. graminearum.* In *F. asiatium*. Two trichothecene types were identified, with about 90% being of the 3ADON type and 10% being of the NIV. In *F. graminearum* isolates, all identified DON were found to belong to the 15ADON type.

A total of 178 wheat samples were collected in 2014, 2015 and 2016 after harvest period from eight counties and analyzed for DON using HPLC-MS/MS as described in Chapter 3. The highest prevalence and mean levels of DON were found in 2016, and the incidence and contamination level of DON was more prevalent in Southern region, followed by Central and Northern regions. The correlation between the climatic factors (rainfall and temperature) and actual presence of DON in wheat differ due to variation in wheat samples and counties (Chapter 3). The contamination levels of DON in the eight counties was significant and positively correlated with rainfall in 2014 ($r = 0.689$, $p<0.05$) and in 2016 ($r=0.74$, $p<0.05$), while in 2015, there was no correlation between them. No correlation was found between temperature and DON contamination of wheat. Yannong 19, Jimai 22, Yangmai 13 and Yangmai 16 significantly and positively correlated with rainfall ($r=0.85$, $P<0.05$, $r=0.83$, $P<0.05$ and $r=0.74$, $P<0.05$, respectively).

In this study, *F. fujikuroi*, *F. proliferatum*, and *F. verticillioides* are shown to be main species of GFSC contaminating rice samples as detailed in chapter 4. *F. proliferatum* and *F. verticillioides* were among the fumonisin producing species, while *F. fujikuroi* strains produced extremely low amounts of fumonisin. Beauverin was detected in *F. fujikuroi* and *F. proliferatum* strains with low levels.

Pathogenicity results suggested three species affected seed germination with similar degrees and *F. fujikuroi* could cause elongated seedlings.

Chapter 5 published in PLOS ONE. The colloidal gold (ICS) test has been demonstrated, providing an important high-throughput method for ZEN monitoring. 30nm colloidal gold nanoparticles were optimized to obtain the best performance. Millipore 135 was selected as NC membrane for its good sensitivity. The optimal amounts of coated antigen ZEN-OVA and anti-ZEN mAb were 0.5 mg/mL and 8 μg/mL, respectively. In this study, the ICS test can detect ZEN in 5 minutes with a detection limit of 15 ng/mL. The mAb has high affinity for ZEN and its metabolites, and no cross-reactivity with other mycotoxins such as aflatoxins B1, T-2, DON and HT-2. Analysis of ZEN in 202 wheat samples over three consecutive years revealed that data obtained from the ICS test were in a good agreement with LC-MS/MS data. This result demonstrated that the ICS test could be used as a qualitative tool to screen ZEN in the field.

6.2 Potential for future development of the study

The current study demonstrated that *Fusarium* mycotoxins are prevalent in various areas in China. In order to obtain more data on the incidence and pollution levels of Fusarium toxins in wheat and other grain products in different regions of China, further scientific research needs to be conducted, which will help to prevent and control the potential risk of the exposure of *Fusarium* mycotoxin to humans. Studies on the mechanisms of other *Fusarium* mycotoxins contamination and better understanding of climatic conditions and other factors influencing their production are also essential (Cruz *et al*., 2014). In addition, in order to reduce mycotoxins pollution and reduce the harm of pollution to consumers, some good agricultural practices will be adopted. For example, the use of resistant wheat seeds, early

sowing time, crop rotation and removal of residues from the previous crop should minimize the risk to consumers (Wegulo, 2012).

The phylogenetic analysis, the mycotoxigenic profile (fumonisin and beauvericin) and the pathogenicity showed high variability on GFSC species, but no correlations could be observed between the latter two parameters. Overall, these findings increase the knowledge on characteristics of the most important rice fungal pathogens worldwide. Moreover, information about the high variability of pathogens may be important for further development of disease management strategies. Future studies may also focus on identification of GFSC species that are associated with different crops, such as barley, rice and maize, and soybeans coupled with genetic and phenotypic variation of various host populations.

6.3 References

Castanares, E., Albuquerque, D.R., Dinolfo, M.I., Pinto, V.F., Patriarca, A., and Stenglein, S.A. (2014). Trichothecene genotypes and production profiles of Fusarium graminearum isolates obtained from barley cultivated in Argentina. International Journal of Food Microbiology **179,** 57-63.

Cruz, A., Marin, P., Magan, N., and Gonzalez-Jaen, M.T. (2014). Combined effects of benomyl and environmental factors on growth and expression of the fumonisin biosynthetic genes FUM1 and FUM19 by *Fusarium verticillioides*. International Journal of Food Microbiology **191,** 17-23.

van der Lee, T., Zhang, H., van Diepeningen, A., and Waalwijk, C. (2015). Biogeography of *Fusarium graminearum* species complex and chemotypes: a review. Food Additives & Contaminants. Part A, Chemistry, Analysis, Control, Exposure & Risk Assessment **32,** 453-460.

Wang, J.H., Li, H.P., Qu, B., Zhang, J.B., Huang, T., Chen, F.F., and Liao, Y.C. (2008). Development of a generic PCR detection of 3-acetyldeoxy-nivalenol-, 15-acetyldeoxynivalenol- and nivalenol-chemotypes of *Fusarium graminearum* Clade. International Journal of Molecular Sciences **9,** 2495-2504.

Wegulo, S.N. (2012). Factors influencing deoxynivalenol accumulation in small grain cereals. Toxins **4,** 1157-1180.