Carbendazim Resistance of *Fusarium graminearum* From Henan Wheat

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Abstract

Fusarium head blight, also called scab, is caused by *Fusarium graminearum* and is one of the most important destructive diseases of wheat. The frequency of carbendazim resistance in 1,132 isolates of *F. graminearum* recovered from fields in different regions of Henan Province in 2016, 2017, and 2018 was determined. A total of 31 *F. graminearum* isolates resistant to carbendazim were detected, including 30 moderately resistant isolates and one highly resistant isolate. The frequency of resistance of *F. graminearum* isolates to carbendazim was 2.7%. The range of effective concentration (EC₅₀) values of 1,101 sensitive isolates and 30 moderately resistant isolates was 0.08 to 0.98 $\mu\text{g ml}^{-1}$ and 2.73 to 13.28 $\mu\text{g ml}^{-1}$, respectively. The mean $\pm$ SD EC₅₀ value was $0.55 \pm 0.13 \mu\text{g ml}^{-1}$ and $5.61 \pm 2.58 \mu\text{g ml}^{-1}$, respectively. The EC₅₀ value of

the highly resistant isolate was 21.12 $\mu\text{g ml}^{-1}$. Point mutation types of the carbendazim-resistant isolates were characterized by cloning the β_2 -tubulin gene of 31 resistant isolates. Three point mutation types at amino acids F167Y, E198Q, and E198L in the β_2 -tubulin gene of resistant isolates were identified. Among 31 resistant isolates, the frequency of point mutation types in F167Y, E198Q, and E198L of the β_2 -tubulin gene was 71.0, 25.8, and 3.2%, respectively. The data indicate that *F. graminearum* has developed resistance to carbendazim in Henan Province, and single point mutations at amino acid F167Y were the predominant type of mutation detected.

Keywords: benzimidazole, fungicide

Fusarium head blight (FHB), also called scab, is caused by the *Fusarium graminearum* species complex and is one of the most important diseases of wheat and other cereals (Bai and Shaner 1994; McMullen et al. 1997; Parry et al. 1995; Starkey et al. 2007). In addition to loss of wheat yield, FHB also causes a decline in wheat quality as a result of contamination with trichothecene mycotoxins (e.g., 3-acetyl-deoxynivalenol, 15-acetyl-deoxynivalenol, nivalenol, and zearalenone) that threaten human and animal health (Chen et al. 2007, 2008; Duan et al. 2018a; Liu et al. 2010, 2013; Pirgozliev et al. 2003; Proctor et al. 1995; Qiu et al. 2012, 2014). In China, FHB occurs in warm and humid areas, such as the Yellow River basin, the Huaihe River basin, and the Yangtze River basin (Chen et al. 2015; Hao et al. 2017; Liu et al. 2010, 2013; Qu et al. 2008). The Yellow River basin and Huaihe River basin are located in Henan Province, which is the main wheat production area, where 82 million hectares are planted to wheat annually. In Henan Province, the primary causal organism of FHB is *F. graminearum*, and the pathogen is a serious threat to wheat production in the area (Hao et al. 2017).

Because of a lack of wheat varieties with high resistance or immunity to FHB, chemical application has become the main method of controlling FHB. The benzimidazole fungicide, carbendazim, became available in the late 1960s and has become one of the most effective fungicides for controlling FHB during the period of wheat heading and flowering (Chen et al. 2007; Liu et al. 2010; Zhou and Wang 2001). The mechanism of action of benzimidazole fungicides is to inhibit the assembly of microtubules by binding to β -tubulin in pathogenic fungi and then to prevent the mycelial growth of phytopathogenic fungi (Leroux et al. 2002; Ma and Michailides 2005; Zhou et al. 2016; Zhu et al. 2018). Use of carbendazim and other benzimidazole fungicides over several years has resulted in

resistance to carbendazim and other benzimidazole fungicides in several phytopathogenic fungi such as *Botrytis cinerea*, *Monilinia fructicola*, and *Sclerotinia sclerotiorum* (Bollen and Scholten 1971; Duan et al. 2018b; Liu et al. 2013, 2016, 2018; Ma and Michailides 2005; Ma et al. 2003; Schroeder and Provvidenti 1969; Zhu et al. 2016). In 1992, the first *F. graminearum* isolates resistant to carbendazim were detected in Zhejiang Province of China (Chen et al. 2007; Qiu et al. 2011; Yuan and Zhou 2005). Resistance of *F. graminearum* to carbendazim has been widely reported in other wheat growing areas in China (Chen et al. 2015; Yuan and Zhou 2005; Zhang et al. 2013). The mechanism of resistance to benzimidazole fungicides has been reported as a point mutation in the β -tubulin gene in *Tapesia yallundae*, *T. aciformis*, *B. cinerea*, *M. fructicola*, and *S. sclerotiorum* (Albertini et al. 1999; Liu et al. 2016, 2018; Ma and Michailides 2005; Ma et al. 2003; Zhu et al. 2016) or point mutation in the β_2 -tubulin gene of *F. graminearum* at different amino acid sites at codons 167, 198, and 200 (Chen et al. 2009, 2014, 2015; Duan et al. 2014; Qiu et al. 2011; Sevastos et al. 2016; Zhang et al. 2016).

However, to our knowledge, carbendazim resistance has not yet been reported in Henan Province. Control efficacy of carbendazim and other benzimidazole fungicides against *F. graminearum* has declined in Henan Province. We speculate that endemic *F. graminearum* may have developed resistance to carbendazim and other benzimidazole fungicides. Therefore, *F. graminearum* isolates were collected from Henan Province in 2016, 2017, and 2018, and their sensitivity to carbendazim was assessed by measuring mycelial growth on carbendazim-amended media. The β_2 -tubulin gene of isolates that were resistant to carbendazim was cloned and the point mutation types associated with resistance were characterized.

Materials and Methods

Fungal isolates. A total of 1,132 *F. graminearum* isolates were obtained from FHB diseased wheat in different regions of Henan Province, China, in 2016, 2017, and 2018 (Table 1). Two to five wheat ears with FHB symptoms were collected from each field ($n = 354$), and the distance between fields was >10 km. A single symptomatic wheat grain from each head was washed twice in sterile water, then soaked in 0.1% sodium hypochlorite disinfection solution for 30 s, washed twice in sterile water again, and finally blotted dry with sterile filter paper before it was transferred onto potato sucrose agar (PSA) medium (200 g of fresh potato tuber, 15 g of agar, and 20 g of sucrose per liter of distilled water) for 2 days at 25°C in the dark. After 2 days, 10 mycelial plugs (5-mm diameter) from each culture were transferred into 150 ml of mung bean broth (50 g of

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mung bean per liter of distilled water) and shaken (120 rpm, 25°C, 12-h photoperiod). Seven days later, double conventional microscope lens wiping paper (Taizhou Oak Filter Paper Factory, Taizhou, China) was used as a filter to line a glass funnel for spore collection. The lens wiping paper containing the mycelial plugs and hyphae was discarded, and the filtrate containing spores was collected and centrifuged at 10,000 rpm and diluted with sterile water to 10^3 spores ml^{-1} . Ten microliters of the spore suspension was spread across the surface of a 9-cm PSA plate. A single spore was picked out using a dissecting microscope (XSP-8C; Shanghai CSOIF Co. Ltd., Shanghai, China) and cultured on a new PSA plate for 2 days at 25°C in the dark. A single mycelial tip from the single spore colony was transferred to a PSA slope, allowed to grow for 2 days 25°C, and then stored at 4°C.

Fungicide. Technical-grade carbendazim (MBC, 98%) was provided by Jiangsu Rotam Chemistry Co. Ltd. (Suzhou, China). The fungicide was dissolved in 0.1 M of hydrochloric acid to obtain a 10 mg ml^{-1} stock solution and stored at 4°C.

Resistance to carbendazim. To determine the carbendazim resistance of each of 1,132 *F. graminearum* isolates, discriminatory doses of 1.4 and $100 \text{ } \mu\text{g ml}^{-1}$ were used (Chen et al. 2015; Duan et al. 2014). Mycelial plugs (5-mm diameter) were transferred to PSA plates containing 0, 1.4, and $100 \text{ } \mu\text{g ml}^{-1}$ of carbendazim. After 2 days at 25°C in the dark, growth of each of the *F. graminearum* isolates on PSA plates with and without carbendazim was observed. An isolate was considered sensitive if no growth occurred on PSA plates amended with $1.4 \text{ } \mu\text{g ml}^{-1}$ of carbendazim. An isolate was considered moderately resistant if growth occurred on PSA plates amended with $1.4 \text{ } \mu\text{g ml}^{-1}$ of carbendazim and not on PSA plates amended

with $100 \text{ } \mu\text{g ml}^{-1}$ of carbendazim. Growth of highly resistant isolates was observed on PSA plates amended with $100 \text{ } \mu\text{g ml}^{-1}$ of carbendazim. Each concentration was replicated four times, and the experiment was replicated three times.

Range of effective concentrations of *F. graminearum* isolates to carbendazim. Effective concentration (EC_{50}) values of *F. graminearum* isolates to carbendazim were calculated based on the level of inhibition of mycelial growth. For the sensitive isolates, the carbendazim concentrations were 0, 0.1, 0.3, 0.5, 0.7, 0.9, and $1.0 \text{ } \mu\text{g ml}^{-1}$. For the moderately resistant isolates, the carbendazim concentrations were 0, 1.25, 2.5, 5, 10, and $25 \text{ } \mu\text{g ml}^{-1}$. For the highly resistant isolates, the carbendazim concentrations were 0, 2.5, 5, 10, 25, 50, and $100 \text{ } \mu\text{g ml}^{-1}$. In this study, 5-mm mycelial plugs were taken from the edge of the 2-day-old colony and transferred to PSA plates amended with the carbendazim concentrations. After incubation at 25°C in the dark for 3 days, the average diameter of each colony was measured with a cross-section method. The inhibitory rate of mycelial growth against *F. graminearum* isolates was calculated for each concentration according to the following formula: (diameter of the control – diameter of each treatment)/(diameter of the control – 5 mm) $\times$ 100%. EC_{50} values were calculated based on the linear relationship between the $\log_{10}$ value of each concentration and the probability value of the inhibitory rate. Each treatment was repeated three times, and the experiment was repeated twice.

β_2 -tubulin gene clone. To identify the point mutation types of carbendazim-resistant isolates, the β_2 -tubulin gene of all resistant isolates and 10 randomly selected sensitive isolates was cloned. The genomic DNA of all target isolates was extracted using the cetyl

Table 1. Number of isolates of *Fusarium graminearum* recovered from wheat fields in Henan Province from 2016 to 2018 that were susceptible (S), moderately resistant (MR), or highly resistant (HR) to carbendazim

Wheat fields in cities ^y	Wheat fields sampled (n)	Isolates (n)	2016			2017			2018		
			S	MR	HR	S	MR	HR	S	MR	HR
Anyang	12	37	— ^z	—	—	13	0	0	24	0	0
Hebi	17	58	—	—	—	—	—	—	58	0	0
Jiaozuo	7	26	—	—	—	—	—	—	26	0	0
Jiyuan	8	29	—	—	—	—	—	—	29	0	0
Kaifeng	30	101	23	0	0	27	1	0	50	0	0
Luohe	16	48	11	0	0	—	—	—	35	1	1
Luoyang	20	58	—	—	—	17	0	0	41	0	0
Nanyang	32	112	30	1	0	22	0	0	59	0	0
Pingdingshan	14	38	25	0	0	—	—	—	13	0	0
Puyang	6	15	—	—	—	—	—	—	15	0	0
Shangqiu	29	92	—	—	—	46	0	0	43	3	0
Xinxiang	31	96	35	0	0	19	0	0	42	0	0
Xinyang	22	67	25	0	0	28	0	0	14	0	0
Xuchang	28	93	18	0	0	18	0	0	52	5	0
Zhengzhou	15	44	28	0	0	16	0	0	—	—	—
Zhoukou	37	121	8	0	0	62	0	0	44	7	0
Zhumadian	30	97	32	0	0	34	12	0	19	0	0
Total	354	1,132	235	1	0	302	13	0	564	16	1

^y Cities near the wheat fields that were sampled.

^z Dashes indicate that no samples were collected.

Table 2. Sensitivity indicated as EC_{50} values of *Fusarium graminearum* isolates to carbendazim in Henan Province

Year	S ^w			MR			HR	
	Isolates (n)	EC_{50} range ^x	Mean EC_{50} ^y	Isolates (n)	EC_{50} range	Mean EC_{50}	Isolates (n)	EC_{50}
2016	235	0.34–0.97	0.55 ± 0.10 A	1	8.84	8.84 A	0	— ^z
2017	302	0.08–0.90	0.47 ± 0.13 A	13	2.73–5.10	3.85 ± 0.71 C	0	—
2018	564	0.32–0.98	0.59 ± 0.15 A	16	2.73–13.28	7.63 ± 2.681 A	1	21.12
Total	1,101	0.08–0.98	0.55 ± 0.13 A	30	2.73–13.28	5.61 ± 2.581 B	1	21.12

^w S, MR, and HR indicate sensitive, moderately resistant, and highly resistant to carbendazim, respectively.

^x EC_{50} values are given in milligrams per liter.

^y Mean values with the same letters within the same column are not significantly different according to Fisher's least significant difference ($P = 0.01$).

^z Dashes indicate that no samples were collected.

trimethylammonium bromide method (Liu et al. 2016; Möller et al. 1992).

The primers beta2F (5'-GATGCGTGAGATTGTAAGA-3') and beta2R (5'-TGAGGGTAGTAGTGAGGATA-3') were designed based on the complete sequence of the β_2 -tubulin gene (NCBI Nucleotide Database reference sequence NC_026477.1; <https://www.ncbi.nlm.nih.gov/nucore>) using Primer Premier 6.0 software (Premier, Palo Alto, CA). Primers beta2F and beta2R were synthesized by Sangon Biotech (Shanghai, China). A 50- μ l PCR reaction mixture contained 25 ng of genomic DNA, 0.25 μ l of Takara LA Taq polymerase (5 U μ l⁻¹; Takara Bio Inc., Shiga, Japan), 2.5 μ l of 10 $\times$ LA PCR Buffer II (Mg²⁺ free; Takara Bio Inc.), 10 μ l of MgCl₂ (25 mM; Takara Bio Inc.), 8 μ l of dNTP mixture (2.5 mM each; Takara Bio Inc.), 1.0 μ M of each primer, and replenished sterile water to 50 μ l. PCR reactions were performed in a TP-600 DNA Engine System (Takara Bio Inc.). The PCR procedure was performed as follows for amplification of the β_2 -tubulin gene of *F. graminearum*: initial denaturation at 94°C for 5 min, followed by 35 cycles at 94°C for 1 min, 57°C for 1 min, and 72°C for 2 min, then a final extension at 72°C for 10 min, and holding at 4°C.

All target fragments of the β_2 -tubulin gene were separated using 2% agarose gel electrophoresis and purified with Takara MiniBEST Agarose Gel DNA Extraction Kit version 4.0. Thereafter, all fragments were cloned into the pMD18-T Simple Vector (Takara Bio Inc.) and gene sequencing was completed by Sangon Biotech. The β_2 -tubulin gene sequences of sensitive and resistant isolates were compared, and the mutant types of resistant isolates were analyzed with BioEdit version 7.0.5.3 software (Ibis Therapeutics, Carlsbad, CA).

Statistical analysis. The data were analyzed with SPSS 14.0 (Statistical Package for the Social Sciences; SPSS Inc., Chicago, IL). Standard errors and confidence intervals for the EC₅₀ values were determined and compared using Student's *t* tests. Fisher's protected

least significant difference ($P = 0.01$) was used to test the mean EC₅₀ values.

Results

Resistance to carbendazim. Of the 1,132 isolates of *F. graminearum* tested, 31 were characterized as resistant to carbendazim, which included 30 moderately resistant isolates and one highly resistant isolate (Table 1). Among the moderately resistant isolates, one (NY1630) was detected from Nanyang in 2016, 12 (ZMD1707, ZMD1708, ZMD1712, ZMD1713, ZMD1714, ZMD1717, ZMD1719, ZMD1740, ZMD1741, ZMD1742, ZMD1743, and ZMD1746) were detected from Zhumadian, one (KF1727) was detected from Kaifeng in 2017, and one (LH1845), three (SQ1814, SQ1837, and SQ1847), five (XC1802, XC1814, XC1819, XC1827, and XC1845), and seven (ZK1803, ZK1814, ZK1817, ZK1820, ZK1823, ZK1833, and ZK1864) were detected from Luohe, Shangqiu, Xuchang, and Zhoukou in 2018, respectively. In 2018, the only highly resistant isolate (LH1828) was detected from Luohe. The frequency of resistance to carbendazim in field isolates of *F. graminearum* from Henan Province was 2.7%.

Range of EC₅₀ values of *F. graminearum* isolates to carbendazim. The EC₅₀ values of 1,101 sensitive isolates ranged from 0.08 to 0.98 μ g ml⁻¹, with a mean EC₅₀ $\pm$ SD of 0.55 $\pm$ 0.13 μ g ml⁻¹. EC₅₀ values of 30 moderately resistant isolates ranged from 2.73 to 13.28 μ g ml⁻¹, whereas the mean EC₅₀ was 5.61 $\pm$ 2.58 μ g ml⁻¹ and the EC₅₀ of the highly resistant isolate was 21.12 μ g ml⁻¹ (Table 2). The range of the frequency distribution of EC₅₀ values for the 1,101 sensitive isolates was narrow (Fig. 1).

Sequence variation in the β_2 -tubulin gene. Comparison of the β_2 -tubulin gene from the 31 carbendazim-resistant isolates with the 10 sensitive isolates from which the gene was cloned and sequenced indicated a point mutation at amino acid position 167 or one of two point mutations at amino acid position 198 (Table 3). The point mutation at amino acid position 167 in 22 moderately resistant isolates resulted in a change from phenylalanine to tyrosine (TTT-TAT, Phe-Tyr, F167Y). The single point mutation at amino acid position 198 resulted in a change from glutamic acid to glutamine (GAG-CAG, Glu-Gln, E198Q) in the other eight moderately resistant isolates. The single point mutation at amino acid position 198 in the sequence of the β_2 -tubulin gene of the highly resistant isolate (LH1828) indicated a change from glutamic acid to leucine (GAG-CTG, Glu-Leu, E198L). Among 31 resistant isolates, the frequency of point mutation types of the β_2 -tubulin gene at F167Y, E198Q, and E198L was 71.0, 25.8, and 3.2%, respectively (Fig. 2).

Discussion

The frequency of carbendazim resistance of 1,132 *F. graminearum* isolates collected over 3 years from different regions of Henan Province along the Yellow River and Huaihe River was characterized in this study. Less than 3% of the isolates were resistant to the fungicide, which is lower than that reported in the Jiangsu and Anhui Provinces, where 15.3 and 8.2% of the isolates collected were resistant to carbendazim, respectively (Chen et al. 2015; Shao et al. 2011). The

Fig. 1. Distribution of EC₅₀ values for sensitivity to carbendazim of 1,101 *Fusarium graminearum* isolates recovered from symptomatic wheat heads in Henan Province.

Table 3. Point mutation types of the β_2 -tubulin gene detected in *Fusarium graminearum* isolates collected from Henan Province from 2016 to 2018

Phenotype ^x	Isolates (n)	Name of isolate	β_2 -tubulin gene mutation		
			167 ^y	198	200
S	10	NY1608, XY1624, KF1711, SQ1713, SQ1716, ZMD1710, LH1842, XC1858, ZMD1818, ZK1844	TTT F ^z	GAG E	TTC F
MR	22	NY1630, KF1727, ZMD1708, ZMD1713, ZMD1714, ZMD1717, ZMD1741, LH1845, SQ1814, SQ1837, SQ1847, XC1802, XC1814, XC1819, XC1827, ZK1803, ZK1814, ZK1817, ZK1820, ZK1823, ZK1833, ZK1864	TAT Y	GAG E	TTC F
MR	8	ZMD1707, ZMD1712, ZMD1719, ZMD1740, ZMD1742, ZMD1743, ZMD1746, XC1845	TTT F	CAG Q	TTC F
HR	1	LH1828	TTT F	CTG L	TTC F

^x S, MR, and HR indicate sensitive, moderately resistant, and highly resistant to carbendazim, respectively.

^y Amino acid position within the β_2 -tubulin gene.

^z Nucleotide sequence of the codon; underlining indicates the site of the point mutation.

reason for this phenomenon may be related to the different frequency of applications of carbendazim and other benzimidazole fungicides within a growing season in different regions of China. Since the late 1960s, growers in Jiangsu and Anhui Provinces have applied two to three applications of carbendazim and other benzimidazole fungicides per season during the period of wheat heading and flowering to manage FHB (Chen et al. 2015; Shao et al. 2011; Zhou and Wang 2001). The incidence of FHB in Henan Province is often slightly lower than that in Jiangsu and Anhui Provinces, and carbendazim and other benzimidazole fungicides are consequently applied less frequently in Henan Province. Fungicides exert selection pressure on pathogen populations; often, the more frequently a fungicide is used during a season, the greater the frequency of detecting fungicide-resistant isolates (Ma and Michailides 2005). Another reason for this phenomenon may be related to the different species of pathogens that cause FHB. FHB is mainly caused by *F. graminearum* in Henan Province and by *F. asiaticum* in Jiangsu and Anhui Provinces (Chen et al. 2015; Hao et al. 2017; Qu et al. 2008). Of the 31 carbendazim-resistant isolates of *F. graminearum* recovered, only one was highly resistant and able to grow on medium amended with 100 $\mu\text{g ml}^{-1}$ of carbendazim. The resistant phenotype of the *F. graminearum* population to carbendazim in Henan Province was predominantly moderately resistant (30 of 31 resistant isolates). These data are consistent with reports on Jiangsu and Anhui Provinces where resistance to carbendazim was also detected in the population of *F. asiaticum* and >90% of the resistant isolates were moderately resistant (Chen et al. 2015; Duan et al. 2014).

The mean EC_{50} value of carbendazim inhibiting the mycelial growth of 100 wild-type isolates of *F. graminearum*, which were collected from wheat fields in Jiangsu, Zhejiang, and Hubei Provinces in 1999, was $0.57 \pm 0.01 \mu\text{g ml}^{-1}$ (Zhou and Wang 2001). The mean EC_{50} values of our isolates were not different. All of our *F. graminearum* isolates were collected from fields of wheat to which carbendazim and other benzimidazole fungicides (e.g., benomyl and thiophanate-methyl) had been applied once or twice each season. To mitigate FHB, carbendazim and other benzimidazole fungicides are applied during the period of wheat heading and flowering. In Henan Province, this application typically occurs once per season and occasionally twice when conditions are very favorable for FHB development. The sensitive isolates in our study had a narrow range of frequency distribution of EC_{50} values, which indicated that carbendazim still had high activity to the majority of *F. graminearum* isolates (>97%) in Henan Province, and thus these data could be used for monitoring susceptibility to carbendazim and other benzimidazole fungicides in the *F. graminearum* population of this area.

Previous studies identified point mutations that occur within the β_2 -tubulin gene in *F. graminearum* and *F. asiaticum* at codons 167 (TTT→TAT, F167Y), 198 (GAG→CAG, E198Q; GAG→CTG,

E198L; GAG→AAG, E198K), and 200 (TTC→TAC, F200Y) as the mechanism of resistance to benzimidazole fungicides (Chen et al. 2009, 2014, 2015; Duan et al. 2014; Qiu et al. 2011; Sevastos et al. 2016; Zhang et al. 2016). The point mutation of amino acids at different sites or different types of mutations within an amino acid at the same site can lead to different levels of resistance of *F. graminearum* to carbendazim (Chen et al. 2009; Duan et al. 2014). The point mutation types at codon F167Y, E198Q, or F200Y of the β_2 -tubulin gene can result in moderate resistance to carbendazim, whereas E198L or E198K of the β_2 -tubulin gene can result in high resistance to carbendazim in *F. graminearum* and *F. asiaticum* (Chen et al. 2009, 2015). Among carbendazim-resistant isolates in the field, the point mutation type in F167Y is the main type and has been confirmed in Jiangsu and Anhui Provinces by previous studies (Chen et al. 2009, 2015; Duan et al. 2014). In this study, we identified three of the reported point mutations at codons F167Y, E198Q, and E198L, of which F167Y was the most frequent. The single point mutation type at amino acid F167Y was the main type of *F. graminearum* in Henan Province, and this result is consistent with reports from Jiangsu and Anhui Provinces where the F167Y mutation was detected in >90% of the resistant isolates of *F. asiaticum* (Chen et al. 2009, 2015; Duan et al. 2014). These data suggest the predominant single point mutation type is similar between *F. graminearum* and *F. asiaticum*.

In conclusion, FHB caused by *F. graminearum* is one of the most important destructive diseases of wheat in Henan Province of China. Fungicide spray application remains the most important way of controlling FHB during the period of heading and flowering. Detection of resistance within the *F. graminearum* population to benzimidazole fungicides is a concern for wheat production in Henan Province. Our data are important for monitoring carbendazim resistance in *F. graminearum* in Henan Province and providing disease management recommendations for local wheat growers. To delay the more frequent occurrence of fungicide resistance, benzimidazole fungicides should be mixed or rotated with fungicides having different mechanisms or belonging to different FRAC Fungicide Resistance Action Committee groups. In Henan Province, products containing single fungicide tebuconazole, epoxiconazole and phenamacril, and fungicide mixes such as carbendazim plus tebuconazole, carbendazim plus epoxiconazole, thiophanate-methyl plus tebuconazole, tebuconazole plus prochloraz, trifloxystrobin plus tebuconazole, and phenamacril plus tebuconazole have been registered to control FHB (Chen et al. 2008, 2011, 2015; <http://www.chinapesticide.org.cn/>). The fungicides pydiflumetofen, metconazole, and metconazole plus phenamacril have all been more effective at reducing FHB than benzimidazole fungicides (Duan et al. 2019; Hou et al. 2017).

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Fig. 2. Frequency of point mutation types in the β_2 -tubulin gene of 31 *Fusarium graminearum* isolates that were recovered from symptomatic wheat heads in Henan Province and tested resistant to carbendazim.

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