September 2010 ISSN : 0974 - 7435 Volume 4 Issue 3

Trade Science Inc.

BioTechnology

An Indian Journal

FULL PAPER

BTAIJ, 4(3), 2010 [136-139]

Changes in level of biochemical constituents and fumonisin production by *F.moniliforme* in relation to seed variety

K.Narasimha Rao*, B.Vijaypal Reddy, S.Girisham, S.M.Reddy

Microbial Biotechnology Research Laboratory, Dept. of Microbiology, Kakatiya University, Warangal, A.P., (INDIA)

E-mail : kollu2006@yahoo.com

Received: 26th March, 2010 ; Accepted: 5th April, 2010

ABSTRACT

The response of *F.moniliforme* towards, different varieties of sorghum and maize for infestation and fumonisins production was investigated. In local variety of sorghum phenol content increased to 0.62mg/g in the infested seeds followed by 0.38mg/g to 0.81mg/g in CSH6. Increased reducing sugars in infested seeds was observed in local yellow variety (272mg/g). Free amino acids content decreased however the degree of decrease varied with variety. High amounts of fumonisins was recorded in local yellow sorghum variety (0.73µg/ml). Increase in total phenols was more in white maize variety (0.38 to 0.90mg/g), free fatty acids was maximum in white variety (0.31 to 0.83). Kargil variety supported with low amounts on fumonisins (0.38µg/ml). A careful consideration has to be made in selecting a seed variety for cultivation.

KEYWORDS

Fusarium moniliforme;
Fumonisins;
Maize;
Sorghum;
Biochemical constituents of seeds.

INTRODUCTION

The natural contamination of agricultural commodities by fusarial toxins in India, may be fairly higher because of socio economic conditions, unhygienic and outdated storage practices, prevalence of unfavourable environmental conditions through major part of the year. In addition to these conditions, periodic cyclones aggravate the problem[1]. The environment is reported to play a vital role in keeping conditions of different agricultural commodities during storage. Fungal infestation is reported to be directly proportional to moisture content of the seed. Water activity in food commodity is reported to have significant influence on the activity of microorganisms. Similarly temperature and relative humidity of storage place are also reported to influence the stored grains.

Maize and sorghum are most frequently infected with *Fusarium moniliforme* a cosmopolitan fungus that grows well saprophytically and survives effectively in live asymmetric kernels. *F.moniliforme* has been shown to produce a group of mycotoxins called fuminosins[2]. Fuminosins are found to occur naturally throughout the world in maize and have been associated with different human and animal diseases[3]. Fumonisin B1 has been found to be stable at low temperatures but degrades at higher temperatures and chemical treatment. Fumonisin B, the major mycotoxin produced by *F.moniliforme* has been found to occur in Indian maize and sorghum. Rain damazed maize and sorghum containing fuminosin B_1 has been shown to cause food borne disease outbreaks in humans[4].

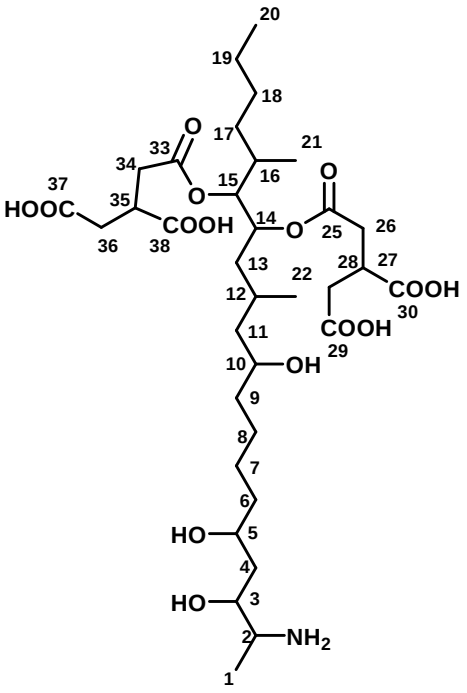

Figure 1 : Structure of fumonisin B_1

Exploitation of genetic resource is being advocated by several workers to control the fungal infestation and mycotoxin production in different crop plants. Nagarajan and bhat[5], bilgrami et al.[6] have attempted to select a maize variety, which is resistant to aflatoxin. However there are no such studies with the incidence and production of fumonisins by *F.moniliforme*. Hence it was considered worth while to screen different crop varities grown in Andhra Pradesh against the proliferation of mycotoxigenic *fusaria*. The present investigation was undertaken to examine the response of *F.moniliforme* towards, different varieties of sorghum and maize for infestation and fumonisins production.

MATERIALS AND METHODS

Chemicals and microorganisms

All chemicals and media used in the present investigation are procured at the highest purity available from Himedia, Mumbai, India.

Fusarium moliliforme MRC 826 was was procured from *Fusarium* research centre collection (Pennsylvania State University, University Park, South Africa). Stock cultures were maintained on potato dextrose agar slants at 4^0C and subcultured for every three months.

Seed material

Seeds of Maize varieties like Local red, Kargil, Pioneer, Bio seed, White and sorghum varieties like Local, CSH1, CSH6, CSV1, Swarna, CSV4 and Local yellow were collected from different fields of Andhra Pradesh. 100g of sterilized Seeds were treated with spore suspension of *F.moliforme* in 250ml conical flasks and incubated for 21 days under laboratory conditions.

Quantitative estimation of biochemicals

At the end of incubation period, biochemical changes in seeds with respect to proteins[7], free amino acids[8], free fatty acids[9], reducing sugars[10] were estimated.

Qualitative and quantitative estimation of fumonisins

At the end of incubation period the seeds were light heated and extracted with water: acetonitrile (100:50). The filtrate was extracted twice with 100ml of chloroform. The combined extracts were then passed through anhydrous Na_2SO_4 bed to remove moisture and evaporated to dryness and redissolved in 1ml of chloroform and spotted on the plates and developed in water: Methanol (1:3) mixture. The plates thus developed weresprayed with p-anisaldehyde (0.2% in ethanol) and heated at 120°C until color developed. Fumonisins appeared as brown coloured spots. Fumonisins were estimated quantitatively as suggested by sydenham[11].

RESULTS AND DISCUSSION

The response of *F.moniliforme* towards, different varieties of sorghum and maize collected from Andhra Pradesh for infestation, biochemical composition and fumonisin production was investigated and the results are tabulated in TABLE 1 and 2.

Significant increase in the phenol content was observed in the infested seeds compared to healthy seeds. How ever the degree of accumilation of phenols varied with the variety (TABLE 1). In local variety phenol content increased to 0.62mg/g in the infested seeds with 0.40mg/g in the healthy seeds. Like wise phenol concentration increased from 0.38mg/g to 0.81mg/g in CSH6 variety followed by Local variety where phenol content increased from 0.54 to 0.68mg/g. Reducing

FULL PAPER

TABLE 1 : Biochemical changes and fumonisins production by *F. moniliforme* in different varieties of sorghum

Seed variety		Proteins (mg/g)	R.S (mg/g)	Phenols (mg/g)	F.A.A (mg/g)	F.F.A (mg/100g)	Fumonisins (µg/ml)
Local	H	46	158	0.40	0.61	0.62	--
	I	49	191	0.62	0.24	0.69	0.72
CSH1	H	58	210l	0.61	0.82	0.41	--
	I	57	258	0.64	0.48	0.54	0.59
CSH6	H	60	220	0.38	0.92	0.38	--
	I	58	240	0.81	0.68	0.72	0.60
CSV1	H	53	216	0.71	1.52	0.34	--
	I	51	224	0.92	0.38	0.81	0.72
Swarna	H	64	281	0.96	0.54	0.39	--
	I	60	298	0.10	0.22	0.42	0.69
CSV4	H	58	240	0.58	0.58	0.43	--
	I	53	260	0.79	0.28	0.68	0.59
Local yellow	H	61	230	0.54	0.49	0.38	--
	I	59	272	0.68	0.38	0.54	0.73

H = Healthy seeds, I = Infected seeds, R.S. = Reducing sugars; F.A.A = Free amino acids; F.F.A. = Free fatty acids

sugar content increased in all the seed varieties infested in the present study. Maximum reducing sugars in infested seeds was observed in local yellow variety (272mg/g) and marginal in local variety (191mg/g). Total proteins increased only in local variety from 46mg/g to 49mg/g in infested seeds. Free amino acids content decreased due to the infestation of *F.moniliforme*, however the degree of decrease varied with the variety. Maximum decrease was recorded in CSV1 and minimum in CSH6. Increase of free fatty acids was recorded in all seed varieties infested by *F.moniliforme*.

High amounts of fuminosins was recorded in local yellow variety (0.73µg/ml). CSV1 and local red variety supported almost same amount of fumonisins (0.72µg/ml), while CSH1 (0.59µg/ml) and CSV4 (0.59µg/ml) were responsible for inhibition of fuminosins elaboration to a significant level. Rest of the varieties supported the fuminosins production to an intermediate degree. *F.moniliforme* has been shown to produce a group of mycotoxins called fuminosins[2]. Considerable changes have been noticed in the level of biochemical constituents in all the infested sorghum varieties under present study which coincide with the earlier findings of King and Scott[12] who reported that *F.moniliforme* has been found to be strongly under genetic control.

Protein content of maize local variety and pioneer

TABLE 2 : Biochemical changes and fumonisins production by *F. moniliforme* in different varieties of maize

Seed variety		Proteins (mg/g)	R.S (mg/g)	Phenols (mg/g)	F.A.A (mg/g)	F.F.A (mg/100g)	Fumonisins (µg/ml)
Local Red	H	48.0	170	0.80	0.84	0.59	--
	I	51.0	230	1.12	0.23	0.62	0.81
Kargil	H	61.0	220	0.58	1.51	0.39	--
	I	59.0	320	0.81	0.42	0.61	0.38
Pioneer	H	68.0	301	0.61	1.50	0.38	--
	I	83.0	295	1.05	1.15	0.41	0.80
Bio-seed	H	63.0	280	0.51	1.31	0.37	--
	I	59.2	138	0.84	1.05	0.53	0.71
White	H	58.0	142	0.38	1.20	0.31	--
	I	53.0	231	0.90	0.92	0.83	0.84

H = Healthy seeds, I = Infected seeds, R.S. = Reducing sugars; F.A.A = Free amino acids; F.F.A. = Free fatty acids

variety increased due to *F.moniliforme* infestation, while in other varieties only marginal decrease in total protein content was recorded (TABLE 2). Reducing sugars increased due to the infestation of *F.moniliforme*. However, degree of increase varied with the variety. Total phenols increased under the influence of *F.moniliforme*. Increase in total phenols was more in white variety (0.38 to 0.90mg/g) followed by pioneer variety (0.61 to 1.05mg/g). Considerable increase of phenols was also observed in Local red variety with 0.80mg/g to 1.12mg/g). The increase of phenols may be attributed to the defence reaction of host against the infecting fungus. Increase in free fatty acids was only marginal in pioneer variety from 0.38 to 0.41mg/g while it was maximum in white variety (0.31 to 0.83). Rest of the varieties showed changes in total phenols to an intermediate degree. Free amino acids decreased considerably due to the infestation of *F.moniliforme*. The decrease was minimum in bioseed variety (1.31 to 1.05mg/g), while it was more significant in Kargil variety (1.51 to 0.42mg/g). Rest of the varieties showed intermediate level of change in FAA. Parallel to this study Krishna Reddy and Reddy have recorded response of different cultivars of maize towards infestation of *Penicillium griseofulvum* and cyclopiazonic acid production[13].

White variety followed by local red variety supported comparatively high amount of fumonisins production with 0.84 and 0.81µg/ml respectively. Kargil variety supported with low amounts on fumonisins 0.38µg/ml. A considerable variation exists in fuminosin production in maize varieties grown in different geo-

graphic locations. In the present investigation too all the maize seed varieties supported fuminosins production, however amount of fuminosin production varied.

Critical perusal of TABLE 1 and 2 reveals that the resistance towards *F.moniliforme* infestation varied in different varieties. For instance, CSH1 and CSV4 varieties of sorghum were resistant to *F.moniliforme* infestation and fumonisins production, while local yellow variety supported maximum amount of fumonisins production. Priyadarshini and Tulpule[14] have attempted to select a maize variety, which is resistant to aflatoxin. In the present investigation Kargil variety which supports low amounts of fumonisins (0.38μg/ml) can be considered as resistant variety. White variety was susceptible to infestation of *F.moniliforme* and supported maximum amount of fuminosins production. Thus a careful consideration has to be made to control diseases in the field and infestation and mycotoxin elaboration in storage and selecting a variety for cultivation.

ACKNOWLEDGEMENTS

I am greatful to Council of Scientific and Industrial Research (CSIR), New Delhi for financial assistance.

REFERENCES

[1] K.S.Bilgrami, S.S.Sahay, A.K.Shrivasthava, M.F.Rahman; Proc.Indian Natl.Sci.Acad., **56**, 223 **(1990)**.
[2] W.C.A.Gelderblom, K.Jackiewicz, W.F.O.Marasas, P.G.Thiel, R.M.Horak, R.Vieggar, N.P.J.Kriek; Appl.Env.Microbiol., **54**, 1806 **(1988)**.
[3] W.F.O.Marasas; Adv.Exp.Med.Biol., **392**, 1 **(1996)**.
[4] R.V.Bhatt, P.H.I.Shetty, R.P.Amruth, R.V.Sudharshan; Clinical Toxicol., **35**, 249 **(1997)**.
[5] V.Nagarajan, R.V.Bhatt; J.Agric.Food Chem., **20**, 911 **(1972)**.
[6] K.S.Bilgrami, K.K.Sinha, P.L.Sinha; Curr.Sci., **51**, 138 **(1982)**.
[7] O.H.Lowry, N.J.Rosebrough, L.Ferr, R.J.Randall; J.Biochem.Chem., **193**, 265 **(1951)**.
[8] S.Moore, W.H.Stein; J.Biol.Chem., 176 **(1948)**.
[9] AOAC; Official Methods of the Analysis of the Association of Agricultural Chemists, Benjamin, Franken Station, Washington, 832 **(1960)**.
[10] M.Dubois, K.Gilles, J.K.Hamilton, P.A.Rebers, F.Smith; Nature, **168**, 167 **(1951)**.
[11] E.W.Sydenham; 'The Chromatographic Determination of Fusarium Toxons in Maize Associated with Human/Oesophageal Cancer', M.Sc. Thesis, University of Cape Town, South Africa, **(1989)**.
[12] S.B.King, G.E.Scott; Phytopathology, **7**, 1245 **(1981)**.
[13] V.Krishna Reddy, S.M.Reddy; Acta Bot.Indica., **16**, 122 **(1988)**.
[14] E.Priyadarshini, P.G.Tulpule; Food Cosmet.Toxicol., **14**, 293 **(1978)**.