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Susceptibility to Infection by *Aspergillus parasiticus* in Barley

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보리의 *Aspergillus parasiticus* 感受性

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Abstract: The seeds of 20 barley cultivars were tested for aflatoxin contamination and susceptibility to infection by an aflatoxin-producing mold. When the samples were tested as they arrived, no aflatoxin was detected on any of them. When their moisture was raised to 25% and they were kept as 25°C for 2 weeks, all expect 2 cultivars showed aflatoxin contamination. Aflatoxins B_2 and G_2 were not detected in this incubation period. After wetting (25% moisture) the samples, inoculating them with *Aspergillus parasiticus* conidia and storing them at 25°C for 2 weeks, all cultivars were found heavily contaminated with aflatoxin, those with seedcoats more so than those without seedcoats.

Introduction

Aflatoxins are potent hepatotoxic and carcinogenic substances produced primarily by some strains of *Aspergillus flavus* and almost all strains of *Aspergillus parasiticus* (Chang and Markakis, 1981). Aflatoxin production depends not only on the strain of the mold, but also on the conditions for mold growth (temperature, humidity etc.) and the susceptibility of the host tissue, when the mold invades plants or animals (Anderson *et al.*, 1975; Diener and Davis, 1969; Ellis, 1969; Hesseltine *et al.*, 1976; Jarvis, 1971; Lillehoj *et al.*, 1976b; Shotwell *et al.*, 1978; Trenk and Hartman, 1970). The greatest hazard to human and animal health from aflatoxins appear to be related to the ingestion of contaminated grains, oilseeds and nuts. Among the grains, barley has not been studied extensively (Chang and Markakis, 1981), although it is an important feedstuff, the raw material for beer brewing and a staple food in certain countries.

The objective of this work was twofold: a) to obtain a rough estimate of the extent of natural contamination with aflatoxin-producing molds of barley grown in Korea and b) to compare the susceptibilities to 20 Korean cultivars to aflatoxin production by *A. parasiticus*.

Materials and Methods

Ten covered (with seedcoats) and ten naked (without seedcoats) cultivars of barley, grown in 1979, were used. The moisture of the samples did not exceed 10% when they arrived at the laboratory.

In the natural contamination study first the barley samples were tested for aflatoxin contamination as they arrived, and subsequently retested after providing quasi-optimum conditions for the growth of aflatoxin-producing organisms that may be lodged on the grain. To reach these conditions, 50g of barley from each cultivar were transferred into a 4 oz bottle and sufficient distilled, sterile water was added to raise the moisture of the sample to 25%. The bottles were then shaken mechanically for 3 min and placed in a refrigerator (0~3°C) for 3 days to allow uniform distribution of moisture in the samples. They were subsequently stored for 2 weeks at 25°C in desiccators which were converted to 100 % RH chambers by filling the space under the perforated plates with water. The caps of the bottles were not tightened. This arrangement resulted in stabilizing the moisture content of the barley to 25±0.3%, as showen by actual moisture analysis (AACC methods, 1969).

In the susceptibility study, again 50g barley samples were placed in 4 oz. bottles but the moisture content was raised to 25% by adding 1 ml spore suspension of *A. parasiticus* along with the water. In order to prepare the spore suspension, *A. parasiticus* NRRL 2999 was grown on potato-dextrose agar (Difco, Inc.) at 25°C for 9 days, and the spores (conidia) were harvested with sterile water, washed and resuspended in a 0.01% sterilized solution of Tween 80 in a way to reach the concentration of 10^6 spores per ml. The inoculated samples were equilibrated in regard to moisture and subsequently incubated exactly as described for the uninoculated wet samples.

The aflatoxins were assayed by the AOAC method (1980); the extraction was performed according to procedure 26.029 and the quantitative estimation by the densitometric procedure 26.059, using precoated silica gel plates (marketed by Brinkman Instr., Inc., as G-HR 25) and a double beam, scanning-recording-integrating spectrodensitometer (Model SD 3000-4, Schoeffel Instr., Inc.). The combined extracts of 3 bottles were used for the thin-layer chromatography and the plates were scanned twice.

Results and Discussion

When the barley samples were examined as they arrived at the laboratory, no aflatoxin was detected on any of them. When, however their moisture was raise to 25% and they were subsequently incubated for two weeks at 25°C, aflatoxin was found in all cultivars except two. Only aflatoxins B_1 and G_1 were present in detectable quantities. The concentrations shown in Table I are rather small, compared to

Table I. Aflatoxin content of barley cultivars stored at 25°C and 25% moisture (100 % RH) for 2 weeks. Average from duplicate densitometry scores.

Variety	Aflatoxin(μg/kg)		
	B_1	G_1	B_1+G_2
	Covered cultivars		
Dongbori #1	1.0	0.9	1.9
Dongbori #2	1.3	ND*	1.3
Bunong	0.9	0.6	1.5
Neulbori	1.1	2.1	3.2
Milyang #6	1.1	0.6	1.7
Kangbori	1.3	0.9	2.2
Olbori	2.0	1.0	3.0
Bokdae	1.2	1.0	2.2
Suweon #18	1.0	1.9	2.9
Suweon #182	1.9	1.4	3.3
Group mean	1.3	1.0	2.3
	Naked cultivars		
Bakdong	ND	ND	—
Sedohadaka	0.5	0.5	1.0
Mokpo #51	0.4	0.7	1.1
Mokpo #53	0.6	0.4	1.0
Mokpo #54	0.4	0.7	1.1
Mokpo #55	0.4	0.5	0.9
Mokpo #56	0.2	0.4	0.6
Suweon #185	0.5	1.2	1.7
Suweon #187	ND	ND	—
Kwangsung	0.6	0.8	1.4
Group mean	0.4	0.5	0.9

* ND: Not detected.

Table II. Yields of aflatoxin in barley cultivars inoculated by *A. parasiticus* NRRL 2999 and stored at 25°C and 25% moisture (100% RH) for 2 weeks.

Variety	Aflatoxin(μg/kg)				
	B_1	B_2	G_1	G_2	Total
	Covered cultivars				
Dongbori #1	164	8	358	18	548
Dongbori #2	197	8	371	20	596
Bunong	269	13	578	31	891
Neulbori	42	3	105	6	156
Milyang #6	289	16	540	32	877
Kangbori	300	17	594	30	941
Olbori	522	28	1864	58	1094
Bokdae	407	21	935	52	1451
Suweon #18	128	6	248	11	393
Suweon #182	55	5	142	9	211
Group mean	238	13	496	27	772
	Naked cultivars				
Bakdong	28	2	58	6	94
Sedohadaka	99	5	154	18	276
Mokpo #51	25	2	46	6	79
Mokpo #53	16	1	35	2	54
Mokpo #54	20	1	30	3	54
Mokpo #55	140	4	222	21	387
Mokpo #56	16	1	32	3	52
Suweon #185	115	3	192	19	329
Suweon #187	15	1	27	3	46
Kwangsung	56	2	77	8	143
Group mean	53	2	87	9	151

those of Table Ⅱ, and indicate that the covered cultivars are likely to contain more aflatoxin than the naked ones. This observation is consistent with the susceptibility results discussed below.

Large quantities of aflatoxins B_1, B_2, G_1 and G_2 were found in all of the samples which were inoculated with *A. parasiticus* condia and incubated under conditions favorable for the growth of this organism (Table II). Again the covered cultivars were more heavily contminated than the naked ones, in general. It may be concluded that although various barley cultivars differ in susceptibility to the growth of a typical aflatoxin-producing mold, they all are subject to aflatoxin contamination when favorable conditions for mold growth prevail. In another paper (Chang and Markakis, 1981), we showed that the moisture of the grain is a critical such conditon.

摘 要

보리品種別 aflatoxin汚染과 aflatoxin生成菌의 感受性을 調査하였다.

供試된 모든 試料에서는 aflatoxin이 檢出되지 않았으나 25%로 加水處理된 보리를 2週間 25°C에서 100% RH 狀態下에서 貯藏하였을때 2個品種을 除外하고는 aflatoxin B_1과 G_1이 微量檢出되었으며 B_2와 G_2는 檢出되지 않았다.

*Aspergillus parasiticus*에 依한 aflatoxin 生成에 對한 보리의 感染 感受性은 대단히 컸으며, 겉보리가 쌀보리보다 aflatoxin의 生成 및 蓄積이 더욱 높았다.

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