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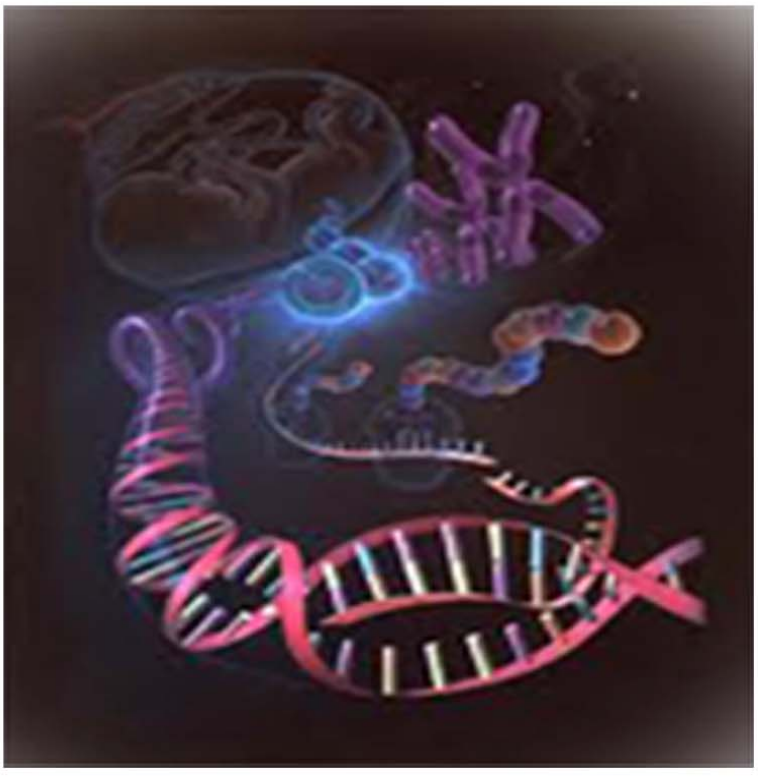

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Research Paper

LEVELS OF AFLATOXIN B1 PRODUCTION IN SEEDS OF SOME SELECTED VARIETIES OF PADDY AND THEIR RELATION WITH TOTAL STARCH, AMYLOSE AND AMYLOPECTIN CONTENTS

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Eleven varieties of paddy seeds were infested with a highly toxigenic strain of *Aspergillus flavus* under laboratory conditions. None of the varieties was totally immune to aflatoxin production but they facilitated aflatoxin production at varying levels. Minimum amount (52.37 µg/kg) was elaborated on Swarna variety whereas maximum amount of aflatoxin B_1 (573.63 µg/kg) was elaborated on Bauna Mansuri. On the basis of the amount of aflatoxin B_1 produced, these varieties were conveniently grouped into three categories; highly resistant (below 100 µg/kg), moderately resistant (from 100-200 µg/kg) and susceptible (above 200 µg/kg). Only Swarna was considered highly resistant while seven varieties viz., Rajendera Sweta, Bhagalpuri Katarni, Rajendera Kasturi, Sita, JKRH-401, Birsa Dhan-201, Birsa Dhan-202, were found to be moderately resistant. Parvati, Tulsi Manjeri and Bauna Mansuri were grouped as susceptible varieties. It was further observed that varieties containing higher levels of starch supported higher levels of aflatoxin B_1. A positive correlation was found between aflatoxin B_1 production and total starch as well as amylopectin content of the seeds whereas there was negative correlation between aflatoxin B_1 elaboration and amylose level of the seeds.

Keywords: Aflatoxin B1, Toxigenic strain, Amylopectin content

INTRODUCTION

Paddy (*Oryza sativa* L.) ranks first among the cereals in crop cultivation and yield in India. In Bihar and most other states it is directly consumed simply after hulling, polishing and boiling.

Paddy, botanically called *caryopsis*, consists of a loose husk enclosing a kernel. The kernel is made up of three parts: the outer layer includes pericarp (seed coat) with underlying aleurone layer, starchy endosperm, and the germ or embryo. 90% of kernel is occupied by endosperm, which is full of starch, and the rest is formed by aleuronic layer and germ, collectively called bran is rich in other nutrients like fat, protein, vitamins and minerals. Starch includes amylose and amylopectin the amounts of which vary according

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to grain types. The problem of aflatoxin contamination in agricultural product is worldwide but in India it is of greater magnitude due to congenial climate, socioeconomic backwardness, traditional and unscientific agronomic practices including storage of commodities (Bhat, 1998).

From Bihar also high incidence of aflatoxin has been reported in cereals (Bilgrami and Sinha, 1984). Amongst the cereals rice is a moderately susceptible commodity to aflatoxin (Bars and Bars, 1992). *A. flavus* can infect rice grains only when its moisture content is more than 12% (Reddy and Raghavender, 2006). Generally the infection resides on the surface of rice grains. Milling of rice has been found to be beneficial so far as minimization of aflatoxin is concerned. Jayaraman and Kalyansundaram (1990) observed that milling of husked rice contaminated with aflatoxin yielded grain having 60-80% less aflatoxin present in original husked rice. This could be one of the physical methods to remove aflatoxin though in the process some vital nutrients may be lost. Bran obtained during polishing needs to be suitably diluted before they are used as cattle feed (Sales and Yoshizawa, 2005a).

Since rice is consumed by all the sections of our society, an attempt has been made in this investigation to estimate the levels of Aflatoxin B_1 production in seeds of some selected varieties of paddy and find out their relation, if any, with total starch, amylose and amylopectin contents.

MATERIALS AND METHODS

Paddy seeds of 11 varieties were collected from different sources *viz*. Bihar Agriculture College Sabour, Bihar (Sita, Rajendera Kasturi), Directorate of Rice Research, IARI, New Delhi (JKRH-401), Rajendra Agriculture University, Pusa, Samastipur (Rajendera Sweta), Birsa Agriculture University, Kanke, Ranchi (Birsa Dhan-201, Birsa Dhan-202), Local market viz. JK Seeds Pvt. Ltd., Bhagalpur (Swarna, Bhagalpuri Katarni, Tulsi Manjeri, Parvati, Bauna Mansuri).

Kernels of each of these varieties were taken in 250 mL conical flask. At first, these seeds were soaked in sterilized distilled water for 2 h. Extra water was discarded and these were infested with 1 mL spore (6×10^6 spores/ mL) suspension of a highly toxigenic strain of *A. flavus* (AF39) obtained after extensive screening of eighty isolates of *A. flavus* (Personal Communication). After incubation period, these samples were then transferred to an oven fixed at 55±2 ^oC for three days. Properly dried seeds were ground for the chemical extraction of aflatoxin as well as for the estimation of various biochemical parameters.

The method of Thomas *et al.* (1975) was followed for the extraction of aflatoxin form the rice seeds infested with the toxigenic strain of *A. flavus*.

Qualitative estimation of aflatoxin was done by Thin Layer Chromatographic (TLC) technique. 50 µL of chloroform extract was spotted on TLC plate along with the standard of aflatoxins. The spotted chromatoplate was developed in TLC tank containing toluene: iso-amyl alcohol: methanol (90:32:3, v/v/v). Developed plates were dried and observed under long wave ultraviolet light. Initial identification of aflatoxin was made on visual basis by color and intensity of fluorescence of the sample and standard spots. The quantity of aflatoxin was estimated by spectrophotometer (Table 1).

The method of Snell *et al.* (1961) was followed with slight modifications for starch estimation. 100 mg of powdered sample was homogenized with

Table 1: Varietal Screening of Paddy Seeds Against Aflatoxin Production by *A. Flavus* Under Laboratory Conditions

S. No.	Paddy Variety	B_1 (μg/kg)	Group
1.	Swarna	52.37	Highly Resistant (<100 μg/Kg)
2.	Rajendera Sweta	117.19	Moderately Resistant (100-200 μg/kg)
3.	Bhagalpuri Katarni	137.63	,,
4.	Rajendera Kasturi	142.16	,,
5.	Sita	162.22	,,
6.	JKRH-401	178.25	,,
7.	Birsa Dhan-201	180.23	,,
8.	Birsa Dhan-202	188.75	,,
9.	Parvati	219.20	Suseptible (above 200 μg/kg)
10.	Tulsi Manjeri	292.62	,,
11.	Bauna Mansuri	573.63	,,

2 mL of distilled water, 1 mL of 10% $ZnSO_4$ and 1 mL 0.5 N NaOH. After 1 h the sediment was then mixed with 2 mL 52% perchloric acid for acid hydrolysis of storage polysaccharides into sugar molecules. The volume was ultimately dilute 100 times. To 2 mL of this stock solution, 8 mL of 0.1% anthrone reagent was added and placed in water bath. Extract was heated on water bath for 10 min at 100 °C and then cooled rapidly in running water to room temperature. The optical density was recorded at 625 nm in Vis-UV 117 Systronics Spectrophotometer against the blank prepared in distilled water.

For amylose estimation method of Juliano (1971) was followed. 100 mg of fine powdered sample was poured in 1 mL of 70% ethanol and left for 30 min. To this solution, 10 mL of 1 N NaOH was added and left overnight. The volume was subsequently raised to 100 mL with distilled water. 2.5 mL of the extract was then mixed with 20 mL distilled water and three drops of 0.1% phenolphthalein after which the solution became pink. In order to neutralize the solution few drops of 0.1 N HCl were added until the pink color got disappeared. 1 mL iodine reagent (1 g iodine and 10 g KI in 50 mL distilled water) was then poured in experimental solution and the volume was raised up to 50 mL with distilled water. Optical density of this solution was recorded at 590 nm in Vis-UV 117 Systronics Spectrophotometer against the blank prepared in distilled water.

Amylopectin content of different varieties of rice was calculated by subtracting the value of amylose content from total starch content.

RESULTS AND DISCUSSION

Table 1 shows that none of the varieties was found totally immune to aflatoxin production but they facilitated aflatoxin production at varying levels. Swarna variety elaborated minimum aflatoxin B_1 production (52.37 µg/kg) while the Bauna Mansuri produced maximum aflatoxin B_1 (573.63 µg/kg). On the basis of aflatoxin production screened variety were grouped into three categories, i.g.,

Table 2: Total Starch, Amylose and Amylopectin Contents of the Paddy Varieties

Paddy Variety	Total Starch (mg/G) ± SE_m	Amylose Content (mg/G) ± SE_m	Amylopectin Content (mg/G) ± SE_m
Bauna Mansuri	600.4±0.45	146.7±0.22	453.7±0.56
Tulsi Manjeri	633.2±0.36	165.4±0.76	467.8±0.13
Parvati	631.2±0.76	186.3±0.16	444.9±0.29
Birsa Dhan 201	657.5±0.45	116.8±0.12	539.7±0.23
Birsa Dhan 202	662.2±0.23	119.5±0.19	542.7±0.23
JKRH-401	665.1±0.24	110.6±0.15	554.5±0.23
Sita	698.6±0.21	119.5±0.14	579.1±0.27
Rajendera Kasturi	704.6±0.31	145.4±0.16	559.28±0.23
Bhagalpuri Katarni	707.3±0.31	131.4±0.11	575.9±0.21
Rajendera Sweta	710.5±0.51	121.4±0.13	589.1±0.23
Swarna	713.4±0.24	111.2±0.12	602.2±0.24

highly resistant (< 100 µg/kg), moderately resistant (100-200 µg/kg) and susceptible (> 200 µg/kg). The Swarna variety was considered resistant one while six varieties comprising Rajendera Sweta, Bhagalpuri Katarni, Rajendr Kasturi, Sita, JKTH-401, Birsa Dhan-1 and Birsa Dhan-2 were treated as moderately resistant. The remaining three varieties, viz., Parvati, Tulsi Manjeri and Bauna Mansuri were placed in susceptible category.

Total starch level of different paddy varieties varied to a great extant (Table 2). Maximum amount (713.4 mg/g) of starch was estimated in highly resistant Swarna variety whereas minimum amount (600.4 mg/g) was found in Bauna Mansuri which is the most susceptible variety. As far as sharing of amylose content as starch constituent of paddy varieties is concerned, minimum concentrations, i.e., 110.6 and 111.2 mg/g were estimated in the moderately and highly resistant varieties, viz., JKRH-401 and Swarna, respectively. All three susceptible varieties, viz., Parvati, Tulsi Manjeri and Bauna Mansuri had amylose concentration above 140 mg/g which is fairly high. Obviously amylopectin content of total starch of different seed varieties showed negative correlation with amylose content. In contrast to amylose content, the amylopectine level was found to be the highest (602.2 mg/g) in Swarna variety and the lowest (111.2 mg/g) in the Bauna Mansuri variety. However, there is slight variation in amylopectin level of highly and moderately resistant varieties. Though the total starch, amylopectin and amylose level of different paddy varieties showed substantial variation, there is positive correlation between aflatoxin B_1 elaboration and total starch and amylopectin content of the paddy varieties.

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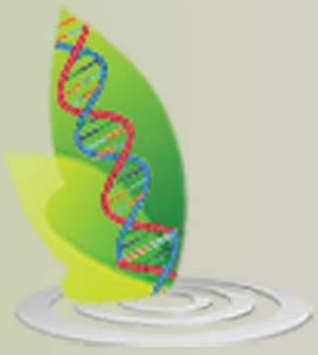

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