FACTORS AFFECTiNG FORMATION OF AFLATOXIN B_1 BY *ASPERGILLUS FLAVUS* ON GANGA-5 MAIZE HYBRID

MOULDY maize poisoning in early 1950's was perhaps because of Aflatoxin (Forgacs[5]. During 1964–1968 in Alabama (U.S.A.), mouldy maize was associated with the death of swine. Examination of contaminated samples of the maize revealed the presence of aflatoxin B_1 (Diener and Davis[4]. Krishnamachari *et al.*[6] attributed the death of the people in Banswara and Dungarpur Districts of Rajasthan due to aflatoxicosis. Since maize is a staple food of the people in many states and the storage conditions are very poor, it is possible that the hazard of aflatoxin in maize may exceed the same in other food-stuffs.

Banswara District of Rajasthan has maximum rainfall in the State. It also has very mild winters and summers, very congenial for fungal growth on food-stuffs. This study was undertaken with an objective to know if aflatoxin B_1 formation on maize has similar requirements.

Fifty gram seeds of hybrid Ganga–5 were sterilized and were inoculated with 1·5 ml spore suspension of *Aspergillus flavus* Link ex Fries having 4×10^4 spores/ml. The flasks were incubated at 30° ± 1° C or as specified. The seeds so incubated were ground. A sample of 25 g of the ground material was used to extract aflatoxin B_1 as described by de Iongh *et al.*[3] Quantity of aflatoxin B_1 in each gram of the material was estimated as described by Detroy *et al.*[2]

Inoculated seeds were incubated for 4, 8 and 12 days to study effect of time on aflatoxin B_1 formation. Effect of temperatures was studied at 10°, 15°, 20°, 30° and 35° C for 20 days. Inoculated seeds were also kept at 25° C for 20 days at relative humidity of 0, 12·5, 25, 50, 75, and 100 per cent using sulphuric acid (Buxton and Mellanby[1]), to control the relative humidity. Effect of light and darkness was also studied on aflatoxin B_1 production by subjecting an inoculated sample to day light and another to darkness for 20 days at room temperature.

Maximum aflatoxin B_1 was produced in the sample incubated at 30° ± 1° C for 12 days. The inoculated sample incubated for 4 days gave very poor yield of aflatoxin B_1 in comparison to 8 and 12 days of incubation period. Optimal temperature for aflatoxin B_1 production was 25° C. Lowest yield was recorded at 10° C.

Maximum aflatoxin B_1 formation was observed at 100 per cent followed by 75 per cent relative humidity. No aflatoxin B_1 formation could be detected at 50, 25, 12·5 and 0 per cent relative humidity when the grains were incubated for 20 days.

It was observed that there was more aflatoxin B_1 formation when the inoculated sample was exposed to normal day light than in the dark (Table I).

TABLE I

Effect of incubation period, temperature, humidity and light on aflatoxin B_1 (Aspergillus flavus) formation on Ganga-5 maize hybrid

Treatments	Aflatoxin B_1 µg/g of grains
Incubation period (incubated at 30 ± 1° C)	
4 days	0·66
8	2·40
12	3·77
Temperature ° C (incubated for 20 days)	
10	0·068
15	0·343
20	20·260
25	27·110
30	23·350
35	23·700
Relative humidity % (incubated at room temperature for 20 days)	
100	14·42
75	1·71
50	0·00
25	0·00
12·5	0·00
0·0	0·00
Light : (incubated at room temperature for 20 days)	36·40
Dark : (incubated at room temperature for 20 days)	24·38

Optimal conditions for aflatoxin B_1 formation are thus a temperature of about 25° C, incubation period of about 12 days, relative humidity of about 100 per cent and normal day light. It is interesting to note that under optimum conditions of temperature and humidity aflatoxin could be formed even within 4 days. In general, after rains during September-October in Banswara District these conditions do prevail. During 1974 October there was about 10" of rainfall during October. In Banswara District grains are left on cobs and are removed only when these are to be consumed. This method of storage is certainly harmful as once the cob gets wet, it takes more time to get dry and thus is helpful for fungal growth and ultimately for aflatoxin production. Thus removal of grains from

the cobs after harvest, and storage in a dry place would cut down aflatoxin B_1 formation on maize.

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MEIOSIS IN THE INTERSPECIFIC HYBRID OF TWO SPINOUS SOLANUMS AND ITS BEARING ON THEIR AFFINITIES

IT *is recognised from the cytogenetic point of view* that data obtained on chromosome pairing and chiasma formation in interspecific hybrids elucidate the evolutionary affinities between the concerned taxa. As the mutual relationships among many of the spinous Solanums, which are of economic importance[1,2], are poorly understood[3–5], studies were initiated in this *direction and the chromosome behaviour in the* hybrid *S. integrifolium* × *S. surattense* is reported now.

Following a newly devised hand pollination technique[7] and the method of screening for *functional pistillate* flowers, 85 pollinations were made and a solitary fruit with a single seed was obtained which germinated to yield the interspecific hybrid. Obviously the cross-ability barriers between the two species have developed to the extreme such that less than 1% of ovules were capable of post-fertilisation development.

The solitary hybrid resembled one or the other of the parents in some exomorphic characters and was intermediate in others. This hybrid, however, could not be maintained for more than a few weeks after flowering. The development to the extreme of cross-ability barriers suggested earlier and the post-zygotic isolating mechanisms appear to have been strongly developed such that the F–1 heterozygote cannot be sustained for long, even in its vegetative phase[6].

In the hybrid, the chromosome associations at meiosis I varied in the different PMCs analysed. They ranged *from a maximum of* 12 II to I–IV + 10 II per PMC. Other kinds of associations were also met with. A total of 22 PMCs were analysed at diakinesis and the relative frequencies of different kinds of associations are summarised in Table I. The average chiasma frequency of 1·37 per bivalent in the F–1 was significantly lower than that in either of the parents (1·61 for *S. integrifolium* and 1·57 for *S. surattense*).

TABLE I

The different chromosome associations and their frequencies at diakinesis in the F–1 hybrid of S. integrifolium × S. surattense

Frequency	Chromosome associations			
	I	II	III	IV
8	..	10	..	1
3	2	9	..	1
1	1	10	1	..
1	3	9	1	..
5	..	12	..	..
4	2	11	..	..
Total 22				

The occurrence of 22·8% of PMCs with twelve bivalents suggests that the chromosomes of the two species have retained *sufficient ancestral homologies* to permit their intergenomic pairing in the F–I heterozygote. The occurrence of higher chromosome associations of at least one per PMC (59·1%) also indicates that a given chromosome of one species has homeologies with more than one of them in the other species. Obviously chromosomal repatterning has *contributed to the cytological divergence of the two* species.

Results on selfing the F–1 and back-crossing it with both the parents revealed that the hybrid is about 98% sterile both ways. With regular chromosome pairing, followed by normal anaphase I segregation and meiosis II as observed now, the sterility is apparently attributable to genetic imbalances brought about by segregational events following intergenomic recombination between one or more of chromosomes of the parental genomes, *when included in a common* nucleus.