

nuclear envelope in various species^{6,7}. Moreover, the convergence and attachment of chromatin fibers to the annuli of nuclear membrane have been reported for mammalian, avian and insect cells^{8,9}. On the light of these data, it seems reasonable to assume that rays of the wheel-like structure observed in interphase nuclei treated with trypsin represent the orderly attachment of g-bands to the nuclear envelope. Furthermore, the network of filaments and the perinucleolar masses which occupy the inner part of the nucleoplasm probably correspond to other g-bands not directly connected with the nuclear envelope.

Information regarding the mechanism of g-band production is still incomplete. However, several experiments strongly suggest that g-bands are the result of disruptions in the molecular structure of DNA-non-histone complexes^{1,10}. Such being the case, it is possible to assume that some of these complexes may be specifically involved in the attachment of chromatin to the nuclear membrane¹¹.

Resumen. En las preparaciones cromosómicas sometidas a digestión con tripsina se observa que la mayor parte de los nucleos celulares muestran una serie de rayos

oscuros que parten de la membrana celular y convergen formando un anillo. Esta imagen se hace presente en aquellos preparados que muestran bandas cromosómicas G y no se observa en aquellos casos con déficit o exceso de digestión enzimática. Estos hallazgos probablemente indican que las bandas G se hallan conectadas, ordenadamente, a la membrana nuclear durante la interfase.

N. O. BIANCHI

*Centro de Investigaciones Genéticas,
Instituto Fitotécnico de Santa Catalina, Llavallol,
Provincia de Buenos Aires, Calle 526 entre 10 y 11,
La Plata (Argentina), 16 April 1973.*

⁷ P. M. M. RAE and W. W. FRANKE, *Chromosoma* 39, 443 (1972).
⁸ D. E. COMINGS and T. A. OKADA, *Exptl. Cell Res.* 62, 293 (1970).
⁹ P. ENGELHARDT and K. PUSA, *Nature New Biol.* 240, 162 (1972).
¹⁰ D. E. COMINGS, E. AVELINO, T. A. OKADA and H. E. WYANDT, *Proc. Twelfth Ann. Meet. Am. Soc. Cell Biol. (St. Louis)*, *J. Cell Biol.* 55, 48a (1972).
¹¹ This work was supported by grants from CONICET and CIC.

Aflatoxin Production in some Varieties of Soybeans (*Glycine max* L.)

Aflatoxin contamination has been extensively studied on peanuts¹. Soybean (*Glycine max* (L) Merr.) is one of the next best sources of edible oil, and its meal is also useful as a source of proteins for consumption by humans and poultry. But information on the susceptibility of soybeans to aflatoxin contamination appears to be rather meagre, and also conflicting. Studies on aflatoxin production in several agricultural commodities such as rice, wheat, corn, sorghum, peanuts and soybeans showed that soybeans were a poor substrate for toxin production by toxigenic strains of *Aspergillus flavus*^{2,3}. It was therefore interesting to investigate the toxin production in soybeans and also to examine varietal differences, if any, in toxin production. Such a study seemed important in view of the crash programme currently in operation in India to boost the production of new and promising varieties of soybeans.

Methods. Five authentic varieties of soybeans (Lee, Bragg, Semmes, Punjab-1, and JS-2) were obtained from the production units of the Agricultural Universities at Pantnagar (Uttar Pradesh) and Jabbalpore (Madhya Pradesh) in this country. The toxin production in these varieties was assessed using 2 toxigenic isolates of *A. flavus* Link (NIN 25, NIN 169) and 2 toxigenic isolates of *A. parasiticus* Speare (NRRL 2999, RIB 4002). The toxin production of these fungal isolates were first graded by growing them on a synthetic medium described by ADYE and MATELES⁴. 20 g lots of each variety of soybeans were rehydrated with just enough water, sterilized by

autoclaving at 15 lbs pressure inch³ for 15 min. The flasks were then inoculated with a uniform spore suspension of the fungal isolates and incubated at 28°C for 7 days. At the end of this incubation period, the samples were sprayed with alcohol and dried overnight at 80°C. The dried samples were first defatted with n-hexane and then extracted with methanol. The aqueous methanolic extracts were extracted with chloroform and chloroform extracts were processed appropriately for thin layer chromatography using chloroform: methanol (95:5) as developing system. The aflatoxin B₁ content was quantified by the method described by PONS et al.⁵. Confirmation of the chemical nature of aflatoxin B₁ was made by the method of CRISAN⁶.

¹ L. A. GOLDBLATT, in *Aflatoxin, Scientific Background, Control and Implications* (Ed. L. A. GOLDBLATT; Academic Press, New York 1969), p. 1.
² C. W. HESSELTINE, O. L. SHOTWELL, J. J. ELLIS and R. D. STUBBLEFIELD, *Bact. Rev.* 30, 795 (1966).
³ R. W. DETROY, E. B. LILLEHOJ and A. CIEGLER, in *Microbial Toxins* (Eds. A. CIEGLER, S. KADIS and S. J. AJL; Academic Press, New York 1971), p. 3.
⁴ J. C. ADYE and R. I. MATELES; *Biochim. biophys. Acta* 86, 418 (1964).
⁵ W. A. PONS JR., A. F. CUCULU, L. S. LEE, J. A. ROBERTSON, A. O. FRANZ and L. A. GOLDBLATT, *J. Ass. off. agric. Chem.* 49, 554 (1969).
⁶ E. V. CRISAN, *Contr. Boyce Thompson. Inst. Pl. Res.* 24, 37 (1968).

Aflatoxin (B₁) production (in ppm) in synthetic medium and in Soybean varieties

Species/isolate	Synthetic medium	Soybean varieties				
		Lee	Semmes	Punjab-1	Bragg	JS-2
<i>A. flavus</i> , NIN 25	+ ^a	0.125	0.125	0.125	0.5	3.125
<i>A. flavus</i> , NIN 169	++	0.125	1.55	0.78	0.25	1.25
<i>A. parasiticus</i> ^b , RIB 4002	+++++	12.5	12.5	12.5	15.63	31.25
<i>A. parasiticus</i> , NRRL 2999	+++++	19.53	19.5	31.25	20.83	31.25

^a + is approximately equal to 250 µg of B₁ per 100 ml medium. ^b Designated as *A. toxicarius* by MURAKAMI¹⁵.

Results and discussion. The aflatoxin B₁ production by different fungal isolates on synthetic medium and the different varieties of soybeans is indicated in the Table. There were wide variations in aflatoxin production in different varieties of soybeans. The amount of the toxin produced was closely related to the toxin-producing potential of the fungal isolate used and the genotype of the soybean employed as natural substrate. The toxin production by the isolates of *A. flavus* was markedly lower than that compared to the production by *A. parasiticus*. The variety 'Lee' produced the lowest and the variety 'JS-2' generally resulted in the highest production of the toxin by *A. flavus* or *A. parasiticus*. The extent of difference in toxin production between less susceptible variety (Lee) and relatively more susceptible variety (JS-2) is of a higher degree in *A. flavus* series (0.125 to 1.55 or 3.125 ppm). Such a wide variation was, however, not demonstrable in the series using *A. parasiticus*.

It is generally believed that soybeans are a very poor substrate for aflatoxin production^{2,3,7}. In a field study involving a survey of 866 samples of soybeans, SHOTWELL et al.⁸ could observe only 0.8% incidence of aflatoxin positive, though 50% of samples showed evidence of contamination with *A. flavus*. The toxin level in the 2 positive samples was as low as 7 to 10 ppb. Again, CHONG et al.⁹ failed to demonstrate the presence of aflatoxin in moldy soybeans contaminated with toxigenic isolates of *A. flavus*. However, they could demonstrate measurable amounts of toxin production using another isolate of *A. flavus* (Weybridge V. 3734/-10) which is in fact *A. parasiticus* (NRRL 2999).

Under optimal laboratory conditions, HESSELTINE et al.² obtained very low toxin production (0.03 to 0.08 ppm) on pearly soybeans (Hawkeye) using 3 isolates of *A. flavus*. Two of these isolates were later designated as *A. parasiticus* (NRRL 2999 and NRRL 3000). On the other hand, DAVIS and DIENER¹⁰ obtained fairly good amounts of toxin (41 to 138 ppm) on Bragg variety of soybean after 21 days of incubation, using *A. parasiticus* (Ala-6). The results of the present series showed toxin yields ranging from 0.12 to 31.25 ppm using different varieties of soybeans infected with different isolates of *A. flavus* and *A. parasiticus*. It is interesting to note that HESSELTINE et al.² could get very low production (0.08 ppm) with *A. parasiticus* (NRRL 2999) using pearly soybeans (Hawkeye). It could be that this latter variety is highly resistant to toxin production, even when using one of the most virulently toxigenic isolates. The higher production of toxin in the series by DAVIS and DIENER¹⁰ might be due to the higher toxigenic potential of the isolate used and also probably due to longer period of incubation for 21 days.

From the present series, it is obvious that soybeans do support the production of aflatoxin under optimal conditions, but the extent of toxin production is dependent on the variety of the soybeans and the toxigenic potential of the fungal isolate used. From the limited studies reported here, it is apparent that Lee variety, which supports minimal toxin production, would be suitable for extensive cultivation. This variety, nevertheless, produces appreciable quantity of the toxin when infected with *A. parasiticus*. But all available evidence appears to suggest that prevalence of *A. parasiticus* contamination is rarely encountered in India^{11,12}. It is pertinent to note that the agro-economic factors, such as yield, oil and protein contents of the Lee variety compared quite favourably with the other varieties of soybeans^{13,14}.

Zusammenfassung. Es wurden 5 Varietäten von *Glycine max.* mit 2 toxinerzeugenden *Aspergillus*-stämmen beimpft und auf ihre Aflatoxinbildung untersucht. Alle Varietäten lieferten Substrate, die zur Biosynthese messbarer Aflatoxinmengen durch beide *Aspergillus*-stämme führten.

V. NAGARAJAN, R. V. BHAT and P. G. TULPULÉ¹⁵

National Institute of Nutrition, Indian Council of Medical Research, Hyderabad-7 (India), 21 August 1972.

⁷ R. W. HOWELL, in Proc. 1st U.S.-Japan Conference on Toxic Microorganisms (Ed. M. HERZBERG; U.S. Dept. of Interior 1970), p. 61.

⁸ O. L. SHOTWELL, C. W. HESSELTINE, H. R. BURMEISTER, W. F. KWOLEK, G. M. SHANNON and H. H. HALL, Cereal. Chem. 46, 454 (1969).

⁹ Y. H. CHONG, C. G. BENG, J. T. PONNAMPALAM and R. K. H. LIM, Far East med. J. 2, 298 (1966).

¹⁰ N. D. DAVIS and U. L. DIENER, in Proc. 1st U.S.-Japan Conference on Toxic Microorganisms (Ed. M. HERZBERG; U.S. Dept. of Interior 1970), p. 43.

¹¹ V. NAGARAJAN and V. R. BHAT, unpublished (1972).

¹² S. RAGHAVENDRA RAO, A. S. INDULKAR and H. S. VEDANAYAGAM, Final Rep. PL. 480 Project on Mycotoxins in Cottonseeds (Reg. Res. Lab., Hyderabad 1970).

¹³ H. SINGH, Indian Farming 19, 3 (1969).

¹⁴ M. C. SAXENA and R. K. PANDEY, Ind. J. agric. Sci. 41, 355 (1971).

¹⁵ H. MURAKAMI, J. gen. appl. Microbiol. 17, 281 (1971).

¹⁶ Acknowledgements. The authors are extremely grateful to Dr. C. GOPALAN, Director National Institute of Nutrition, Hyderabad, for his keen interest and valuable suggestions. The authors are also indebted to Dr. L. A. GOLDBLATT of New Orleans for the generous supply of pure aflatoxin standards; to Dr. S. RAGHAVENDRA RAO, Regional Research Laboratory, Hyderabad, for the culture of *A. parasiticus* (NRRL 2999) and to Dr. H. MURAKAMI of Tokyo for the culture of *A. toxicarius* (RIB 4002).

Improved Visualization of Wall Ultrastructure in *Saccharomyces cerevisiae*

Recently, *tris*-1 aziridinyphosphine oxide (TAPO) has been successfully used as a chemical fixative for biological electron microscopy¹⁻³. A prefixation with a mixture of TAPO and acrolein followed by aqueous osmium postfixation produced a significant amount of new information on the ultrastructure of *Candida albicans* wall^{2,4}. The results obtained in this organism cannot however be extrapolated to the generality of yeast and yeast-like forms owing to the differences in wall chemistry and organization existing between them⁵. In particular, it was of interest to see whether the fixation procedure described for *C. albicans* could be usefully applied to

Saccharomyces cerevisiae, a yeast 'paradigmatic' as far as wall structure is concerned.

¹ N. E. WILLIAMS and J. H. LUFT, J. Ultrastruct. Res. 25, 271 (1968).

² W. DJACZENKO and A. CASSONE, J. Cell Biol. 52, 186 (1972).

³ N. CALIO, R. CALIO, A. CASSONE, W. DJACZENKO and C. D. PESCE, Atti XVI Congresso Società Italiana Microbiologia, Pisa (1972).

⁴ A. CASSONE, N. SIMONETTI and V. STRIPPOLI, J. gen. Microbiol. 77, 417 (1973).

⁵ H. J. PHAFF in *The Yeasts* (Eds. A. H. ROSE and J. S. HARRISON); Academic Press, London and New York 1971), vol. 2, p. 135.