Morphogenetic and Cytogenetic Effects of 7,12-Dimethylbenz(a)anthracene on *Haworthia* Callus in vitro[1]

Recent investigations have shown that Benz(a)anthracene[2] and other related tobacco smoke components[3,4] induce vegetative buds on the callus derived from haploid plants of 'Burley 21' tobacco. The effects of the chemical carcinogen[5], 7,12-dimethylbenz(a)anthracene (DMBA), on plant tissue cultures have not been studied. In the present investigation undifferentiated *Haworthia* callus cells were used to determine the effects of DMBA on their growth, morphogenesis and the chromosomes.

The callus cells used in this study were derived from the flower axes of *Haworthia variegata* by culturing inflorescences on White's modified medium[6-8]. The callus was induced to defferentiate into many shoots and a few roots by subculturing on the White's basal medium containing indole-3-acetic acid (IAA, 1 mg/l), kinetin (0.5 mg/l), coconut milk (20%), vitamins (thiamine-HCl, 0.1 mg/l; pyridoxine-HCl. 0.1 mg/l; niacin, 0.5 mg/l), sucrose (2%) and Difco Bacto-agar (0.8%). This served as the control medium. The second medium has all the nutrient supplements found in the control but no IAA, kinetin or coconut milk was added. The experimental nutrient media were supplemented with 1, 5, 10 and 20 mg/l of DMBA but lacking in IAA, kinetin and coconut milk. The carcinogen[9] was dissolved in benzene before adding to the agar nutrient media and the pH of the media was adjusted to 5.8. The media were autoclaved at 15 lb pressure for 15 min and the cultures were kept at 26° ± 1 °C under continuous light (40 watt, Gro-Lux fluorescent Sylvania lamp). 25 culture tubes were set up for each treatment and morphological changes were studied for 10 weeks.

Chromosome slides were prepared from callus and root tips using the sqash technique after fixing the material in 1:3 acetic acid-alcohol and staining with 1.5% orcein or carmine. Some 35–40 metaphases and anaphases were examined for each treatment.

The callus growth and organ formation on the control medium were vigorous over the 10-week period. By 4 weeks, the cultures had formed many nodules, and developmental stages of many plantlets were visible in each tube. Very few nodules were visible after 8 weeks when nodule differentiation and organ formation were found to be extensive. On the average, 10 week-old cultures contained somewhere between 15–30 leafy shoots but generally no more than 2 roots per tube. The progression of culture differentiation in the control medium is given in the Table.

Very little growth of the callus was observed on medium lacking in IAA, kinetin and coconut milk. In addition no organogenesis was observed in the medium; therefore, it was felt unnecessary to include the results in the Table.

Calluses on the DMBA media produced many nodules and some vegetative buds in a few tubes (Table) 2 weeks after inoculation. By the fourth week, most of the concentrations of the DMBA group produced shoots and roots in over half of the 25 tubes inoculated for each treatment. As can be seen from the Table, the increase in differentiation percentage rose with the increase in the concentration of DMBA in the medium. After 6 weeks, the growth and differentiation of the calluses continued in the higher concentrations but leveled off in the lower concentrations (1 and 5 mg/l). In these 2 lower concentrations some bud differentiation was seen during the 8 and 10 weeks period and on the average each culture tube contained 3–10 shoots and 2–4 roots at the end of 10 weeks. On both the media containing DMBA at 10 mg and 20 mg/l, the percentage of tubes with vegetative buds increased to 88% at the end of 10 weeks of growth compared to 93% in the control. About 6–20 shoots and 4–8 roots were visible from each tube at this stage, but comparatively more roots tended to form in 20 mg/l DMBA media. However, the roots in the DMBA media were frequently feeble in appearance.

Chromosome studies of calluses and root tips grown in the control and the DMBA media showed 14 normal chromosomes, 8 long and 6 short. The karyotype was perfectly normal and the separation of the chromosomes in anaphase was equal. The cells in the dividing areas appeared healthy, and no chromosomal aberrations such as chromatid breaks, fragments or any interchanges were seen. The cytogenetic effects of DMBA on plants have received no attention. The chemical carcinogen, 7,12-dimethylbenz(a)anthracene, is well known for its leukegenic and chromosome-breaking effects in animals[5,10]. Our study presented here shows for the first time that this carcinogen stimulates callus growth and induces callus differentiation to produce many shoots and roots in the absence of IAA, kinetin and coconut milk. This chemical definitely stimulated root formation as evidenced by the remarkable extent of root production on all of the DMBA media. Interestingly, the chemical did not alter the chromosome structure or the number in *Haworthia* culture cells. We cannot explain with certainty why this animal

Progression of *Haworthia* callus differentiation on the control and on different concentrations of DMBA media

Treatment	% culture[a] differentiation in weeks				
	2	4	6	8	10
Control[b]	36	67	82	93	93
DMBA[c] (1 mg/l)	4	42	78	78	78
DMBA (5 mg/l)	8	57	82	82	82
DMBA (10 mg/l)	16	67	82	86	88
DMBA (20 mg/l)	16	71	86	88	88

[a] Each treatment consisted of 25 replicates. [b] Supplemented with IAA, kinetin and coconut milk. [c] No IAA, kinetin or coconut milk was added to the media.

[1] This study was supported in part by a summer research grant from Lafayette College Faculty Research Funds.

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chromosome breaking agent was unable to induce chromosomal aberrations in this plant species. We know, however, that many chemicals which damage animal chromosomes, may not induce chromosomal abnormalities in plants as was found in connection with the cytogenetic studies of cyclamates[11–14], and different plant species may react differently to the same chemical as was revealed by a study of RILEY and NEUROTH[15] on the effect of LSD on plant chromosomes.

Zusammenfassung. 7,12-Dimethylbenz(a)anthracen (DMBA) in verschiedenen Konzentrationen anstelle von Auxin, Kinetin und Kokosmilch im Nährmedium induziert die Bildung von Wurzeln und beblätterten Sprossen auf den Kalli von *Haworthia variegata*. Dieses Ergebnis gleicht demjenigen im vollständigen Kontrollmedium ohne DMBA, mit dem Unterschied, dass unter dem Einfluss von DMBA weniger Sprosse und eine grössere Anzahl von Wurzeln gebildet werden. Cytologische Untersuchungen an Kallus- und Wurzelzellen aus dem DMBA-Medium zeigten eine normale Konstitution des Chromosomensatzes ohne chromosomale Aberrationen.

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Induced Morphogenetic Variations in *Aspergillus oryzae* by 8-Azaguanine

One of the most potent modifiers of genetic transcription in metazoan systems is 8-azaguanine, which is known to replace guanine in messenger RNA (m-RNA)[1]. The knowledge that such replacement can be environmentally programmed, led the authors to investigate the impact of 8-azaguanine on diverse strains of *A. oryzae*, cultured in defined nutrient milieu. This communication reports the new[2] morphological variations induced in *A. oryzae* by this aza-base.

Materials and methods. The diverse cultures of *A. oryzae* figuring in this investigation are from KAKKAR's personal stock, and these strains were originally isolated from the soil and rhizosphere microflora of wheat fields of Allahabad and suburbs in 1960–1961. During their entire period of retention under cultural conditions, these strains have retained their rigid structural pattern and general morphology, as described by RAPER and FENNELL[2] without any apparent discernible abnormality.

The basal medium of the present investigation was the modified Czapek's medium containing: Sucrose, 10 g; KH_2PO_4, 1 g; $NaNO_3$ 3.0 g; KCl, 0.5 g; $MgSO_4$, $7H_2O$, 0.5 g; $FeSO_4$, 0.01 g; and pyrex thrice distilled water to make 1 l. 8-azaguanine was added to the basal medium in the concentration of 85 mg/l. The reinforced medium was apportioned in pyrex (white label) 150 ml Erlenmeyer culture flasks, each flask containing 25 ml of the culture solution. The flasks containing the culture solution were subjected to fractional sterilization in Arnold's steamer, by steaming them for 30 min each day for 3 consecutive days. The pH of the culture fluid was 6.5.

The inoculum was prepared from the conidia of diverse strains of *A. oryzae*, which were washed by centrifugation and finally suspended in double distilled water. Inoculation was performed by pipetting 1 ml. of the standardized spore suspensions (approx. 25,000 spores) into the flasks containing the culture solution. After inoculation, the

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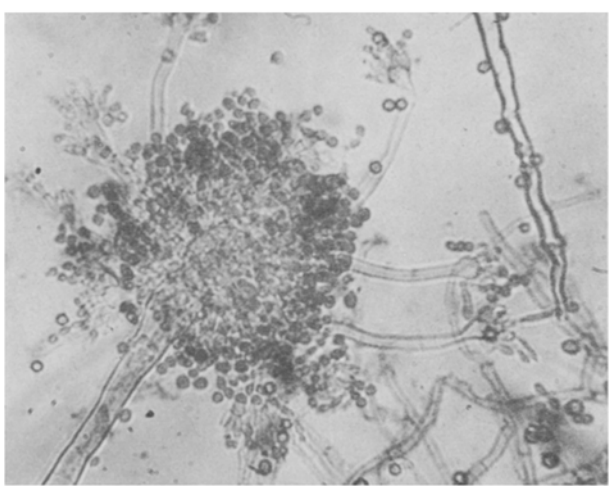

Fig. 1. An abnormal head of *A. oryzae* with 6 secondary septate stalks, terminating into fertile miniature vesicles bearing chains of globose or sub-globose conidia. ×800.

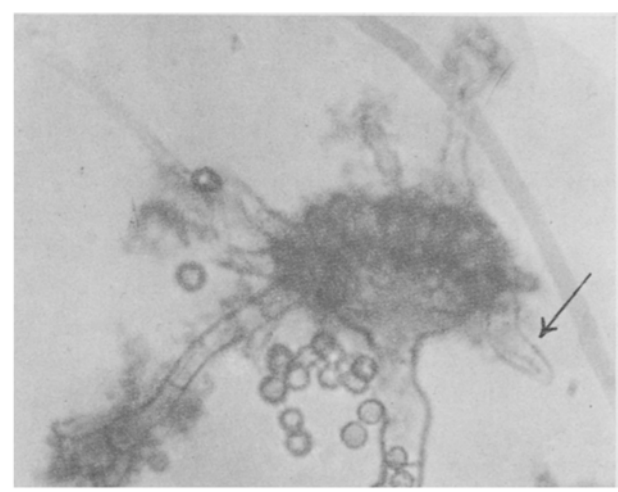

Fig. 2. An abnormal head of *A. oryzae* with secondary stalks in various stages of development. ×800.