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EFFICACY OF DIFFERENT MUTAGENIC AGENTS IN INDUCING VARIATION IN *ASPERGILLUS NIGER*

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Out of 12 mutagenic agents/chemicals used, only 10 were effective in inducing variation in 38 isolates (31.4%) of *Aspergillus niger.* Forty five variants (59.2%) were stable while 31 reverted (40.8%) to their parent culture. Maximum variants were induced by mannitol, followed by malachite green, colchicine, methylene blue, sodium nitrite, ninhydrin, potassium bichromate, potassium iodide, mannitol + sodium nitrite and aniline blue. Twenty five variants (out of 76) produced more oxalic acid than their parent cultures. In 56 variants conidial production was depressed by mutagens while in 3 variants there was no conidial production at all.

INTRODUCTION

Mutants had been produced by subjecting a strain of *Aspergillus niger* to various concentrations of potassium bichromate (Chohan, 1970b). Biological stains and dyes have also been used by various workers in agar slants and petri dish cultures to study the variation in *A. niger* (Chohan, 1970b; Das, 1973; Raper and Fennell, 1965; Schiemann, 1912; Steinberg and Thom, 1940 a, b; Thom and Steinberg, 1939). In the present studies 12 mutagenic agents/ chemicals were used to study variability in *A. niger*.

MATERIALS AND METHODS

Aspergillus spp. were isolated on Czapek's solution agar from: (A) cotyledons and collar regions of the collar rot affected groundnut seedlings from different groundnut growing localities of the Punjab; (B) from groundnut seeds; and (C) from soil samples plated on modified Czapek's Dox agar (Warcup, 1950). The isolates were purified by single spore isolation technique (Gibson, 1953). Five mm bits from different isolates (7-day old) were plated in the centre of petri plates with different mutagenic agents and incubated for 7 days at $27 \pm 1^{0}C$. The variants observed on the basis of the differentiating characters from the parent culture in various treatments picked up, purified, described and maintained separately on Czapek's solution agar. Oxalic acid produced by different variants was determined (Bracken, 1955). The various mutagenic agents/chemicals used per litre of Czapek's solution agar included; aniline blue, 50 mg; colchicine, 300 mg; malachite green, 1 mg; methylene blue, 50 mg; mannitol, 50 g; mannitol, 50 g + sodium nitrite, 300 mg; ninhydrin 0.1%; potassium bichromate, 50 mg; potassium iodide, 200 mg; sodium nitrite, 300 mg; thiourea, 50 mg; and urea, 4 g. Suitable controls were also maintained. All these chemicals, except mannitol, were added to the Czapek's solution agar after sterilization. Mannitol was added before autoclaving the media.

RESULTS AND DISCUSSION

Twelve mutagenic agents/chemicals were tested for inducing variation in 121 isolates. According to the results (Table 1) 76 variants were observed in 38 isolates (31.4%). Forty five variants (59.2%) were stable while 31 reverted (40.8%) to the parent cultures (Fig. 1). Thiourea and urea did not induce variation. Treatment with mannitol induced 17 variants : 11 stable and 6 unstable; followed by 14 in malachite green : 8 stable and 6 unstable; 10 in colchicine : 8 stable and 2 unstable; 7 in methylene blue : 2 stable and 5 unstable; 6 in sodium nitrite : 4 stable and 2 unstable; 6 in ninhydrin : 3 stable and 3 unstable; 6 in potassium bichromate : 4 stable and 2 unstable : 4 in potassium iodide : 2 stable and 2 unstable; 3 in mannitol + sodium nitrite : 2 stable and 1 unstable; 3 in aniline blue : 1 stable and 2 unstable. The maximum number of variants (Raper and Fennell, 1965) were shown in isolate LC_{82} followed by 5 in $B_{14 \cdot 2}$; 4 each in $B_{7 \cdot 8}$, LC_{3}, PC_{1} and KC_{6} (Fig. 1) ; 3 each in $B_{19\ 2}$,LC_{10} and PC_{4}. All the remaining isolates gave out only one or two variants.

Two variants each in isolates $B_{5 \cdot 1}$ B_{12}, $B_{14\ 0}$, $B_{19 \cdot 2}$ and LC_{14} ; 3 each in B_{7-3}, LC_{3} and LC_{28}; and one each in isolate B_{6-2}, B_{34}, LA_{14}, LC_{9}, LC_{16} andSC[1] produced more oxalic acid than the parent cultures.

In 56 variants conidial production was depressed by mutagens while in 3 variants there was no conidial production at all. In 51 variants mycelial growth was either higher than or equal to the parent cultures due to the mutagens. Mutants were obtained in *A. niger* by subjecting it to various concentrations of potassium bichromate (Schiemann, 1912). Several dyes and biological stains are also reported to induce variations in *A. niger* (Schiemann, 1912; Steinberg and Thom, 1940 a, b; Thom and Steinberg, 1939).

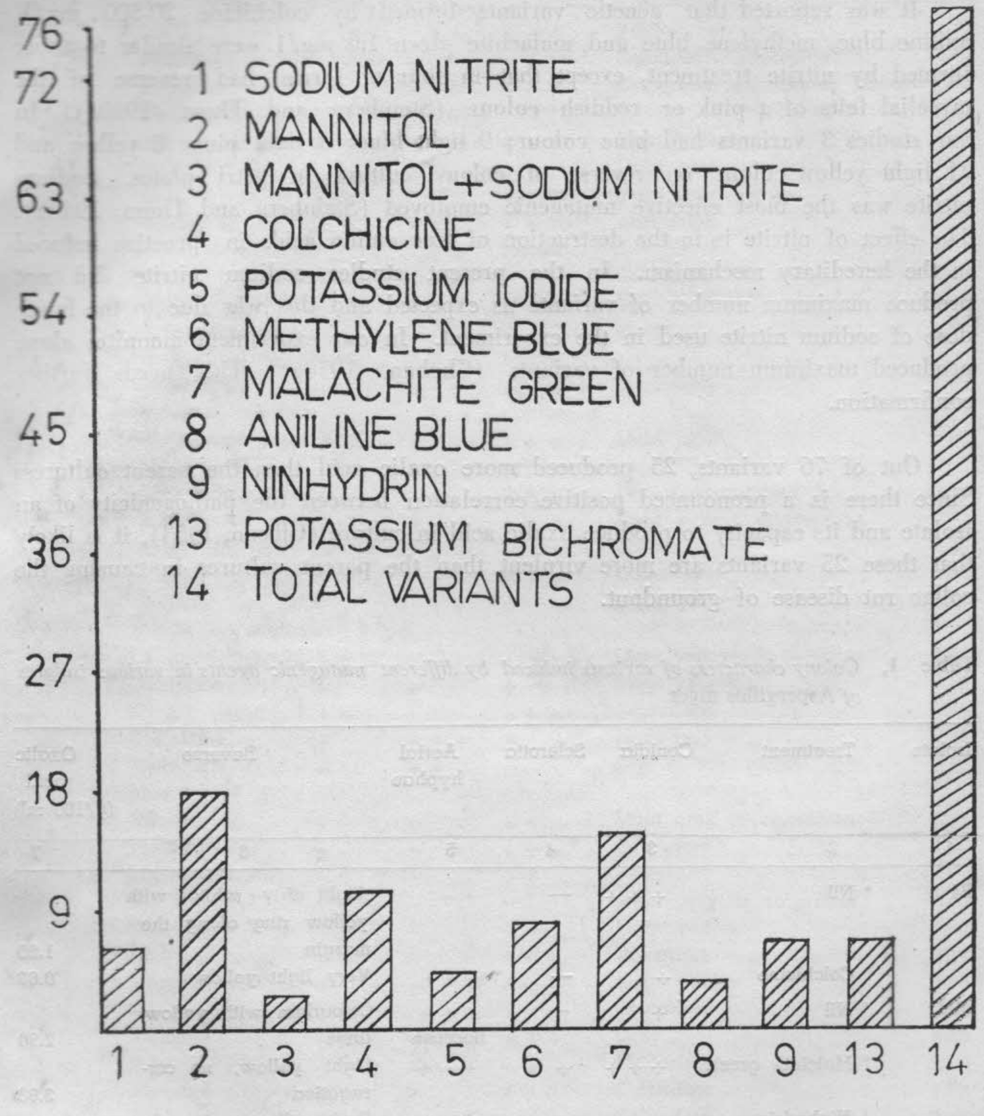

Fig. 1 Showing efficacy of different mutagenic agents in inducing variation in KC_6

9 : Ninhydrin
13 : Potassium bichromate
6 : Methylene blue
3 : Sodium nitrite + Mannitol

It was reported that genetic variants formed by colchicine 20-500 mg/1, aniline blue, methylene blue and malachite green 1.0 mg/1 were similar to those formed by nitrite treatment, except that in four of forms had reverse of the mycelial felts of a pink or reddish colour (Steinberg and Thom, 1940a). In our studies 3 variants had blue colour; 9 light blue; 4 light pink; 8 yellow and 21 light yellow colour on reverse of colony culture in petri plates. Sodium nitrite was the most eflective mutagenic employed (Steinberg and Thom, 1940a). The effect of nitrite is in the destruction of free amino acids in proetins induced in the hereditary mechanism. In the present studies sodium nitrite did not produce maximum number of variants as expected and this was due to the lower dose of sodium nitrite used in the experiment. In our experiment mannitol alone produced maximum number of variants (Chohan, 1970a). This needs further confirmation.

Out of 76 variants, 25 produced more oxalic acid than the parent cultures. Since there is a pronounced positive correlation between the pathogenicity of an isolate and its capacity to produce oxalic acid in culture (Gibson, 1953), it is likely that these 25 variants are more virulent than the parent cultures in causing the collar rot disease of groundnut.

Table 1, *Colony characters of variants induced by different mutagenic agents in various isolates of* Aspergillus niger

Isolate	Treatment	Conidia	Sclerotia	Aerial hyphae	Reverse	Oxalic acid (g/100 ml)
1	2	3	4	5	6	7
B4	* Nil	+ + +	—	+ +	Light dirty white with yellow ring along the margin	1.20
	* Colchicine	+	—	+ + +	Very light yellow	0.63
B5-1	* Nil	+	—	+ + + floccose	Colourless with yellow tinge	2.90
	* Malchite green	+ + +	+	+ + +	Light yellow, no corrugation	3.63
	* Ninhydrin	+	+ + +	+ +	Dark yellow, changes to colourless on Czapek's agar	3.71
B6-2	* Nil	+ + +	—	+ + +	Light grey	2.30
	* Malchite green	+ +	—	+	Light blue	3.80
B7-3	* Nil	+ + +	—	+ +	Colourless	0.25
	* Malchite green	+	—	+ + +	Colourless	1.80
	* Colchicine	+	—	+ + +	Colourless	0.31
	* * Methylene blue	+	—	+ + +	Light yellow	0.00
	* Mannitol	+	—	+ + +	Light yellow	0.63

1	2	3	4	5	6	7
B12	* Nil	+ +	+ + +	+ + +	Yellow, corrugated	2.70
	* Mannitol	+ black	–	+	Colourless, no corrugation	3.40
	* Potassium bichromate	+ + +	–	+ + +	Colourless, corrugation absent	2.90
B14-1	* * Nil	+ +	+ +	+ + +	Light yellow	1.23
	* Methylene blue	+	+	+ appressed	Light blue	3.27
	* Ninhydrin	+ + +	–	+ + + raised	Light grey, highly corrugated	3.15
B14-2	* * Nil	a. + + +	+ + +	+ + +	Colourless	4.53
		* * b. +	+	+ + +	Light yellow	2.00
	* Colchicine	+ + +	+	+ raised	Grey	1.90
	* Potassium iodide	+	+	+ + appressed	Colourless	1.26
	* Methylene blue	+	+	+ + + appressed	Light blue	1.90
	* * Ninhydrin	+	–	+ + +	Yellow, corrugated	3.27
	* Aniline blue	+	+	+ + +	Light yellow, corrugation absent	1.50
B19-2	* Nil	+ +	+ +	+ + +	Colourless	1.95
	* * Sodium nitrite	a. + scattered	+ + +	+	Light yellow	0.63
		* b. + + +	+	+ + +	Light yellow	3.40
	* Colchicine	+	+	+	Light yellow	2.50
B26	* Nil	+ +	+ +	+ + +	Colourless	1.19
	* Colchicine	+	+ + +	+	Light yellow	1.26
	* * Methylene blue	+	+ + +	+	Blue to light yellow	0.00
B34	* Nil	+ +	–	+ +	Light grey to colourless	0.58
	* * Potassium bichromate	+	–	+ + + + +	Colourless	1.26
B36	* Nil	+ + +	–		Light grey to colourless	0.00
	* Mannitol	+	–	+ + +	Light yellow	0.00
B41	* Nil	+ +	–	+ + +	Colourless	2.14
	* Mannitol	+	–	+ + +	Light yellow	1.69
LA3	* Nil	+ +	–	+ +	Colourless	2.54
	* * Mannitol	+ +	–	+ + +	Grey	0.63
LA4	* Nil	+ +	+ +	+ +	Colourless	2.90
	* Mannitol	a. +	+	+ + +	Colourless	1.00
		b. + +	–	+ + +	Colourless	1.40
LA5	* Nil	+ +	–	+	Colourless	1.50
	* * Colchicine	+ + +	–	+ + +	Light yellow	0.63
LA8	* Nil	+ + +	–	+ + +	Colourless	3.27
	* Sodium nitrite	+	+ +	+ + +	Colourless, appressed	1.00
LA13	* Nil	+ +	–	+	Colourless	0.94
	* Colchicine	+ + +	–	+ + +	Light yellow	0.63
	* * Malachite green	+	–	+ + +	Yellow	0.00

1	2	3	4	5	6	7
LA14	* Nil	+ +	–	+ +	Colourless	2.00
	* Mannitol	+ + +	–	+ + +	Colourless	5.7
	* * Colchichine	+ +	–	+ + +	Colourless	0.00
LA16	* Nil	+ + +	–	+ +	Colourless	2.54
	* Sodium nitrite	+	–	+ + +	Colourless, appressed	1.90
LC1	* Nil	+ + +	–	+ +	Colourless	0.00
	* Colchicine	+	–	+ + +	Colourless, appressed	0.56
	* * Malachite green	+	–	+ + +	Pink, floccose	1.26
LC3	* Nil	+ + +	–	+ +	Colourless with yellow ring all along the margin	0.00
	* Mannitol	+ +	–	+ + +	Colourless	0.63
	* Potassium iodide	+	–	+	Light grey without yellow ring	0.38
	* * Methylene blue	+ + +	–	+ +	Light blue with yellow margin	0.31
	* Malachite green	+ +	–	+ + +	Light pink with yellow margin	0.00
LC4	* Nil	+ + +	–	+ + +	Light grey to colourless with yellow ring	2.20
	* Ninhydrin	+	–	+ + +	Yellow	1.26
LC6	* Nil	+ + +	–	+	Colourless	0.94
	* Malachite green	+	–	+ + +	Colourless floccose	0.00
LC9	* * Nil	+ +	–	+	Colourless	2.37
	* Mannitol	+	–	+ + +	Colourless	0.63
LC10	* Nil	+ + +	–	+ + +	Colourless, corrugation absent	2.39
	* * Sodium nitrite	+	–	+ +	Colourless	0.75
	* * Mannitol	+ + +	–	+	Colourless	1.26
	* * Malachite green	+ + +	–	+	Light blue	0.88
LC14	* Nil	+ + +		+ + +	Light yellow	1.13
	* * Potassium iodide	+	+	+ + +	Colourles	1.80
	* * Aniline blue	+	+	+ +	Blue	2.01
LC15	* Nil	+ + +	–	+ +	Light yellow, corrugated	0.94
	* Malachite green	+ + + raised	–	+ + +	Very light blue, corrugation absent	0.50
LC16	* * Nil	+ + +	–	+	Colourless, corrugated	0.00
	* Malaçhite green	+ raised	–	+ + +	Light blue, no corrugation	0.95
LC28	* Nil	+ + +	–	+ +	Colourless	0.00
	* Colchicine	+ + +	–	+ + +	Colourless	0.65
	* Methylene * blue	+	–	+ + +	Light blue	1.26
	* * Aniline blue	+	–	+ + +	Blue	1.00
	* * Ninhydrin	+	–	+ + +	Colourless	0.00
	* Potassium bichromate	+	–	+ + +	Light blue	0.00
	* Mannitol	+	–	+ + +	Yellowish white	0.00
SA1	* * Nil	+ + +	–	+	Colourless	2.52
	* * Mannitol	+	– dark brown	+	Pale yellow	1.50
SA4	* Nil	+ + +	–	+	Colourless	1.26
	* * Mannitol	+	–	+ +	Light yellow	1.26

1	2	3	4	5	6	7
SC1	* Nil	+ + +	–	+ +	Light yellow	1.17
	* * Malachite green	+	–	+ + +	Light pink, colourless on Czapek's agar	1.90
PC1	* Nil	+ + +	–	+ + +	Colourless	3.40
	* Mannitol	+ + +	–	+ appressed	Yellow	0.63
	* * Mannitol, Sodium nitrite	+	–	+	Light yellow	0.95
	* * Potassium iodide	+	+ + +	+ + +	Cololrless	1.26
	* * Malachite green	+	+ + +	+ + +	Lightpink	0.75
PC4	* Nil	+ + +	–	+ + +	Colourless	1.90
	* Mannitol + Sodium nitrite	+	–	+ + +	Colourless	0.31
	* Malachite green	+	–	+ + +	Colourless	0.43
	* Potassium bichromate	+	–	+ + +	Light yellow	0.00
KA4	* Nil	+ +	–	+	Colourless	1.89
	* Potassium bichromate	+	–	+ +	Yellow, later on light yellow on Czapek's media	1.26
KC3	* Nil	+ + +	–	+	Colourless, corrugated	0.63
	* * Malachite green	+ +			Colourless, corrugated absent	1.26
KC4	* Nil	+ + +	–	+ +	Colourless	1.95
	* Sodium nitrite	+	–	+ + +	Colourless	1.38
	* * Mannitol	a. +	–	+	Yellow	1.13
		b. +	–	+ + +	White	1.13
KC6	* Nil	+ + +	–	+ + +	Colourless	5.16
	* Mannitol + Sodiumnitrite	–	–	+ + appressed	Colourless	4.41
	* * Methylene blue	+	–	+ + + floccose	Slightly coloured	5.04
	* * Nithydrin	–	–	+	Yellow	0.21
	* * Poteassium bichromate	–	–	+	Yellow	6.30

The mark * denotes stable; * * = unstable; – denotes nil; + = sacanty; + + = moderate; * * * = abundant.

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