Dhaka Univ. J. Bio. Sci. **14**(2): 155-160, 2005 (July)

INDUCTION OF MUTATION IN *NEUROSPORA CRASSA* WITH DIATHANE-M45 AND GENETICAL STUDIES OF SOME SELECTED MUTANTS

T. I. M. A. MOZMADER, UDAYA SHIREEN KEYA AND TAHSINA HAQUE

Department of Botany, University of Dhaka, Dhaka-1000, Bangladesh

Key words : Diathane-M45, Mutants, *Neurospora crassa*

Abstract

Diathane-M45 at 0.125 and 0.062% concentration induced nine different groups of mutants in *Neurospora crassa*. The mutants were cauliflower, fissure, mat, ropy, check, fluffy, conidial band, vigorous and buff which showed difference with Ema in their morphology. Complementation test of different morphological mutants showed positive results but complementation of similar morphological mutants were negative. Linkage study revealed clf (D_6-34) was linked with pyr-1A of linkage group IV.

Introduction

The most technically advantageous method of studying the organization and mode of action of gene or the genic inheritance in any organism is achieved by inducing artificial mutation in the organism at different loci.

Nowadays a good number of fungicides are used in various purposes such as mutagens, biological control etc. Diathane-M45 is a fungicide which contains 8000 g Mankojeb per kg (zinc, manganese ethylenebisdithiocarbamate). Bari and Mian[1] used diathane-M45 on peanut as biological control. Sultana[2] and Rahman[3] observed the mutagenic effect of pesticide (Fenom) on *N. crassa* and nine groups of mutants were obtained. Sandhu *et al.*[4] and Bignami *et al.*[5] studied the mutagenic activities of different fungicides, Haque and Shamsi[6,7] worked with diathane-M45 against *Colletotrichum corchori* and *Macrophomina phaseolina*. In case of strong inhibition of the pathogen by the application of fungicides, deformation of the colony and mycelial aggregation were observed.[8]

Therefore, it was decided: (i) to induce mutation in *Neurospora crassa* by using diathane-M45, (ii) to study in detail a few selected and interesting mutants for their morphological changes by studying the linear growth, mycelial weight, time required for germination, fertility, mating type, heterocaryosis in comparison with the wild type Ema and (iii) to prepare extracts from the foreign marker in order to study in detail the segregation and linkage of selected mutants.

Materials and Methods

The experimental materials were *Neurospora crassa* Emerson 'a'/Ema (5297) and Emerson 'A'/EmA (5296). Ema was used for treating conidia with mutagen. Both Ema and EmA were used for crossing and checking the mating types of the mutants. Seven markers of linkage groups I-VII with 'A' mating types were used for the study of linkage of the mutants. These were leu-3(R156)A of linkage group I, trp-3(C83)A-Ext-2 of linkage group-II, ade-2(STL2)A of linkage group-III, pyr-1(H263)A of linkage group IV, ilv-1(16117)A of linkage group V, trp-2(75001)A of linkage group VI and arg-10(13317)A of linkage group VII. Diathane-M45 is a fungicide (Rohm & Hass company, France, Distributor Rhone-Polenc Bangladesh Ltd.). Active ingredient: 8000 g Mankozeb (zinc, manganese, ethylenebisdithiocarbamate) per kg was used as mutagen.

Vogel's minimal medium (VM)[9] was used for the maintenance of cultures, linear growth and for the study of mycelial growth. Sorbose minimal media (SM) was used for single colony and spore isolation. Westergaard and Mitchell's crossing medium[10] was used for crossing.

For preparation of different solutions and 5% survival of conidial colonies procedure of Mozmader *et al.*[11] was followed. Diathane-M45 solutions of 0.5, 0.25, 0.125, 0.0262, 0.03 per cent concentration were prepared which was denoted as D_3, D_4, D_5, D_6, D_7, respectively.

Complementation test: The selected morphological mutants were subcultured three times to obtain fresh cultures. Small tubes were used for preparation of VM. Observations were taken after 3, 5, 7, 15 and 21 days for the growth of heterocaryons. For further confirmation, they were tested in liquid media. In every case there were duplication test with duplicate controls.

Results and Discussion

Diathane-M45 of 0.125% (D_5) and 0.062% (D_6) concentration produced 5.08 and 7.90% viable conidia respectively (Table 1). It showed significant mutagenic activity. Nine different groups of morphological mutants were obtained cauliflower (clf), fissue (fis), mat, ropy (ro), check, fluffy (fl), conidial band (con-band), vigorous (vg) and buff. The highest frequency (43.90%) was found for check mutant and the lowest frequency (2.43%) was found in cauliflower, vigorous and buff. The types of mutants obtained with diathane-M45 solution are slightly different from those found earlier with phenol[11] and formaldehyde vapour.[12] Checked growth, vigorous growth, band and cauliflower types were obtained when formaldehyde vapour was used and in case of phenol, vigorous, mat checked, fluffy, buff and band types were obtained. These discrepancies could be due to the difference in mutagenic specificity of formaldehyde vapour, formaldehyde solution and phenol.

In case of diathane-M45 induced mutants the mycelial dry weight of mat (D_6-31), buff (D_6-33) and clf (D_6-34) was found less than Ema and lowest weight (0.0935g) was obtained in case of mat D_6-14 (Table 2). Of the diathane-M45 induced mutants after 72 hours the lowest linear growth found in mat D_6-14 (3.25 cm) and highest for mutant cauliflower D_6-43 (6.75 cm) whereas Ema showed growth of 19.45 cm (Table 3).

Table 1. The percentage of viable conidia at different concentration of Diathane-M45.

Concentration of diathane-M45	No. of germinated conidia	Mean	Percentage of viable conidia
0% (Normal)	91 86	88.5	100
0.5(D_3)	0 0	-	0
0.25(D_4)	2 1	1.5	1.695
0.125(D_5)	4 5	4.5	5.08
0.02626(D_6)	7 7	7	7.90
0.03(D_7)	12 17	14.5	16.38

Table 2. Mycelial weight of some selected mutant and Ema.

Name of the culture	Weight of the empty filter paper (g)	Weight of the filter paper with dried mycelia (g)	Weight of dried mycelia (g)	Mean
Mat (D_6-14)	0.921	1.019	0.098	0.0935
	0.921	0.1811	0.089	
Fissure (D_6-31)	0.895	1.009	0.114	0.106
	0.922	1.02	0.098	
Buff (D_6-33)	0.934	1.093	0.159	0.1785
	0.999	1.197	0.198	
Cauliflower (D_6-34)	0.892	1.002	0.110	0.1155
	0.890	1.011	0.121	
Ema	0.921	1.287	0.366	0.388
	0.922	1.332	0.410	

Mating types and fertility of mutants were checked by crossing with Mat, check, ropy, fissure, buff, cauliflower and vigorous both Ema and EmA separately. Perithecia in large number were formed only with EmA in all cases. Spores were shed in all the crosses but the mutants vary in their fertility (Table 4). Single spore of fertile crosses was isolated and classified into mutant and wild types. The mutant

and wild progenies were segregated in the ratio of 1 : 1. This finding suggests that the obtained mutants might be the results of changes in the nuclear genes (Table 5).

Table 3. Linear growth of Ema and four selected mutant.

Name of the culture	Growth in cm after the following hours											
	24	Mean	36	Mean	48	Mean	60	Mean	72	Mean	84	Mean
Mat (D_6-14)	0.1	0.15	0.3	0.4	0.95	0.9	1.90	1.95	3.1	3.25	4.2	4.3
	0.2		0.5		1.00		2.00		3.4		4.4	
Fissure (D_6-31)	0.5	0.4	1.2	1.05	3.4	3.25	5.1	5.15	6.00	6.00	6.9	6.95
	0.3		0.9		3.1		5.2		6.00		7.0	
Buff (D_6-33)	0.3	0.35	0.8	0.95	2.90	2.95	4.9	4.95	5.7	5.75	6.4	6.6
	0.4		1.1		3.00		5.0		5.8		6.8	
Cauliflower (D_6-34)	1.2	1.3	1.8	1.9	3.9	3.95	5.9	5.85	6.8	6.75	7.8	7.85
	1.4		2.0		4.0		5.8		6.7		7.9	
Ema	2.1	2.2	4.9	4.95	11.0	10.0	15.8	15.75	19.5	19.45	24.1	24.1
	2.3		5.0		10.9		15.7		19.4		24.1	

Table 4. Mating type and fertility of some mutant.

Cross	Days to initiation of perithecia	Mating type	Shedding days	Fertility
D_6-14 × Ema	-		-	Excellent
D_6-14 × EmA (mat)	7	a	15	
D_6-21 × Ema	-		-	Excellent
D_6-21 × EmA (check)	15	a	21	
D_6-28 × Ema	-		-	Poor
D_6-28 × EmA (ropy)	15	a	27	
D_6-31 × Ema	-		-	Poor
D_6-31 × EmA (fissure)	10	a	27	
D_6-32 × Ema	-		-	Poor
D_6-32 × EmA (fissure)	10	a	27	
D_6-33 × Ema	-		-	Excellent
D_6-33 × EmA (buff)	10	a	21	
D_6-34 × Ema	-		-	Excellent
D_6-34 × EmA (cauliflower)	7	a	15	
D_6-43 × Ema	-		-	Poor
D_6-43 × EmA (vigorous)	7	a	27	
D_6-50 × Ema	-		-	Poor
D_6-50 × EmA (ropy)	7	a	27	

Complementation test revealed that complementation did not take place in case of mutants of a particular group. Each group therefore, probably was produced as a result of mutation at a single locus. But complementation took place among the different morphological mutants indicating that the mutants of different groups were produced as a result of mutation at separate loci.

Table 5. Segregation ratio of four mutants.

Cross	No. of spores isolated	No. of wild types isolated	No. of mutant isolated	Segregation ratio wild : mutant
D_6-21 × EmA (check)	198	96	102	1 : 1.052
D_6-28 × EmA (ropy)	208	102	106	1 : 1.039
D_6-43 × EmA (vigorous)	230	110	120	1 : 1.090

Table 6. Linkage of cauliflower (D_6-34a) mutant.

Marker used	Linkage group	Crossing name	Total isolates	Progenies	% of the progenies	Inference on linkage
pyr-1 (H 263)A	IV	D_6-34 × EmA	146	Wild = 4 pyr-1 = 62 clf = 62 clf+pyr = 18	Wild = 2.75 pyr-1 = 42.46 clf = 42.46 clf+pyr = 12.32	Linked with pyr-1 of linkage group IV

The selected mutants induced with diathane-M45 were crossed with markers of seven linkage groups. Then linkage of the fertile crossed were studied and the distances of the mutants were calculated and obtained that mutants clf (D_6-34) linked with pyr-1, linkage group-IV (Table 6).

References

1. Bari MA and MA Wadud Mian 1979. Effect of Diathane M45 in reducing intensity of leaf spot of peanut. Bangladesh J. Bot. **8** (1&2): 6-13.
2. Sultana R 1997. Mutation, characterization, segregation and linkage study of mutants in *Neurospora crassa*, M.Sc Thesis, Dhaka University.
3. Rahman MA 1997. Mutagenic effect of organochlorin pesticide, M.Sc Thesis, Dhaka University.
4. Sandhu SS, Waters MD, Simmon VF, Mortelmans KE, Mitechell AD, Jorgenson T, Jones DC, Valencia R and Stack F 1985. Evaluation of the genotoxic potential of certain pesticides used in Pakistan. Basic Life Sci. **34**: 184-219.

5. Bignami M, Aulicino F, Veleich A, Carer A and Morpugo G 1977. Mutagenic and recombinogenic action of pesticides in *Aspergillus nidulans* Mutant. Res. **46**(6): 395-402.

6. Haque T and S Shamsi 1995. Dose effect of fungicide on the growth of *Macrophomina phaseolina* causing stem rot of jute. Dhaka Univ. J. Bio. Sci. **4**(1): 85-86.

7. Haque T and S Shamsi 1996. Effect of some fungicides on *Collectotrichum corchor*i, The causal organism of anthracnose of jute. J. Asiat. Soc. Bangladesh, Sci. **22**(1): 117-118.

8. Goltlieb D 1961. The fungus spore (Madelin M F Ed), Butterworth and Co. Ltd. London. pp. 217-235.

9. Vogel HJ 1956. A convenient growth medium for *Neurospora* (medium N). Microbial Genetics Bull. **13**: 42-43.

10. Westergaard M and HL Mitchell 1947. *Neurospora* VA synthetic medium favouring sexual reproduction. Am. J. Bot. **34**: 573-574.

11. Mozmader TIMA, Tahsina Haque and H Begum 1986. Morphological mutants in *Neurospora crassa* induced by phenol. Dhaka Univ Studies Part E **1**(1): 77-78.

12. Mozmader TIMA and Tahsina Haque 1995. Induction of mutation in *Neurospora crassa* with vapour of formaldehyde solution. Dhaka Univ. J. Biol. Sci. **4**(2): 199-202.

(Manuscript received on 11 October, 2004; revised on 24 April, 2005)