Indian J. Genet., 52 (2): 132–138 (1992)

USE OF GRISEOFULVIN FOR MITOTIC MAPPING OF PARA-FLUOROPHENYLALANINE RESISTANCE MARKER IN *ASPERGILLUS NIDULANS*

S. N. JHA* AND U. SINHA

Department of Botany, Patna University, Patna 800006

(Received: June 5, 1990; accepted: October 7, 1991)

ABSTRACT

Griseofulvin decreased survival of conidia, inhibited colonial growth, increased sectoring and induced haploid segregants in the heterozygous diploids of *A. nidulans*. It was also efficiently used for the mitotic mapping of para-fluorophenylalanine resistance maker.

Key words: Griseofulvin, haploidisation, mitotic mapping, *Aspergillus*, para-fluorophenylalanine (FPA).

Diploids of *Aspergillus nidulans* are not completely stable and breakdown spontaneously to produce haploid sectors at a frequency approximating less than 0.02%. Increased level of haploids are recovered from diploid conidia treated with D-L-p-fluorophenylalanine (FPA), arsenate, radiation, benlate, methyl benzimidazole-2-yl-carbamate (MBC), acridine yellow and chloral hydrate [1]. Treatments of these chemicals at times failed to yield certain types of haploids. For example, the widely used haploidising agent, FPA, did not allow the expression of phenylalanine requiring auxotrophic haploids. Benlate was also found to be of little use in genetically mapping the newly isolated FPA-resistant mutants. Benlate, MBC, took 7–12 days for the emergence of identifiable haploid sectors, whereas radiation as well as chloral hydrate [2] gave rise to infinite number of segregants. Although, many of the chemicals noted above increased the frequency of haploids, yet it was difficult and time consuming to obtain haploids free from diploids. Moreover, the number of haploids was not sufficient for linkage study. The differential effect of nystatin and polyene antibiotic, N-glycosyl polifungin considerably improved the method for selection of haploids in *A. nidulans*. But this too suffers from many defects [3].

Griseofulvin, an antifungal antibiotic, isolated from *Penicillium griseofulvum* was selected for inducing haploids because of its antimitotic action [4], spindle disruption in

*Present address: Bela Garden, Darbhanga, Bihar 846004.

fungi [5, 6] and its property like that of spindle poisons [7]. It has also been reported to cause haploidisation in *Coprinus lagopus* [8] and somatic segregation in diploid *A. nidulans* [9]. It induces curling of hyphae [10], missegregation of chromosomes into irregular masses of chromatin in mitotically active tissues of rats (*Mus musculus*) and beans [4] and spindle breakdown in *Pectinaria* oocytes [11] and interferes with the mitotic cycle of *Basidiobolus ranarum* [12]. In the present communication, we report the suitability of griseofulvin as a haploidising agent for mitotic mapping of FPA resistance marker in *A. nidulans*.

MATERIALS AND METHODS

A heterozygous diploid strain was synthesized, using phenylalanine requiring MSD and FPA resistant strains following Roper's technique [13]. The genotype of the strain used is shown in Fig. 1.

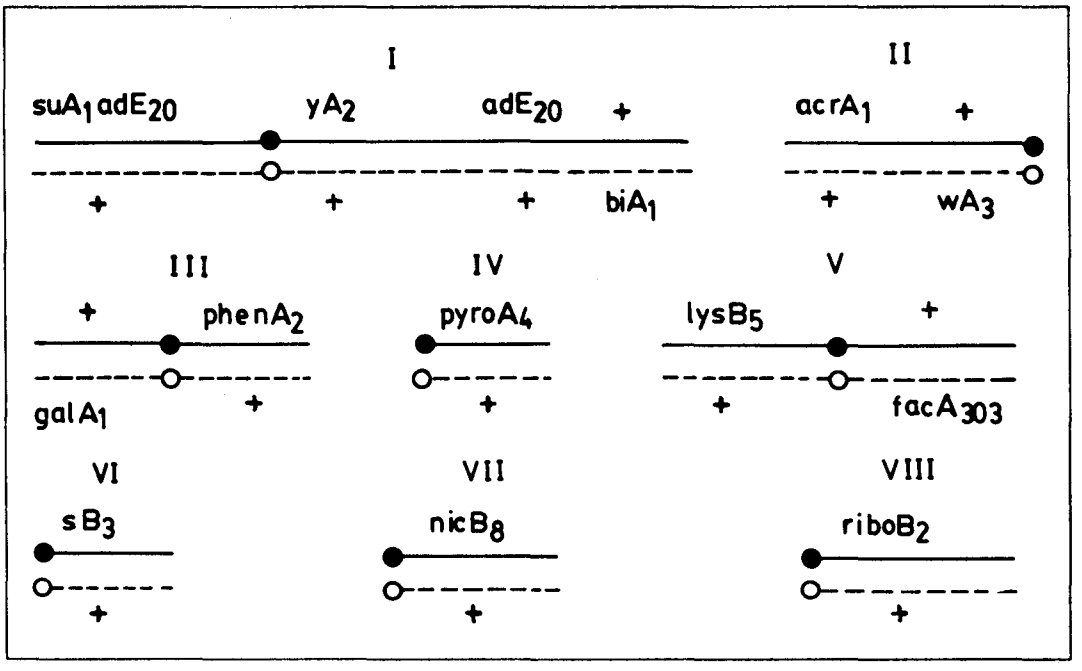

Fig 1. The genotype of the diploid *Aspergillus* strain.

Gene symbols. yA2—yellow conidial colour, acrA1—acriflavin resistance, fpaM72—para-fluorophenylalanine resistance, phenA2—phenylalanine requiring, pyroA4—2pyridoxine requiring, lysB5—lysine requiring, nicB8—nicotinamide requiring, riboA1 and riboB2—riboflavin requiring, and sB3—unable to utilise sulphate as the sole source of sulphur.

The strain has at least one marker on each linkage group to facilitate the detection of segregation of eight different linkage groups in resultant haploids. There are markers on both the arms of linkage group I to distinguish diploid cross-overs from diploid nondisjunctional types. The general techniques employed were those as described by [14–16]. The strains used were obtained from A. J. Clutterbuck (Department of Genetics, University of Glasgow, U.K.). All the chemicals used were of analytical grade.

Treatment. Griseofulvin (Sigma) was first dissolved in acetone and then added to complete medium before pouring. These plates were inoculated with diploid conidia and incubated at 37°C for 5 days to get haploids. The control plates were without griseofulvin.

RESULTS

It was observed that the plates containing griseofulvin less than 500 μg/ml showed a few or no sectors. Therefore, griseofulvin was added in concentrations ranging from 500–1250 μg/ml of the medium. The effect of the drug was measured in terms of survival and growth inhibition. It was found that the viable counts as well as the colonial diameter were drastically reduced with increasing concentrations of the drug (Table 1).

Periodic examination of the plates revealed few visible sectors per plate at 500 and 750 μg of griseofulvin/ml.

Table 1. Survival and colony diameter of diploid *Aspergillus* on griseofulvin containing media after 5 days of incubation

Concentration (μg/ml)	Survival (%)	Colony diameter (mm)*
00	100	64.0 ± 2.5
500	65	38.4 ± 1.8
750	56	33.9 ± 1.9
1000	48	28.8 ± 1.7
1250	32	24.3 ± 1.9

*Average of 10 plates with only one inoculum at the centre of the Petri dish.

However, the best sectoring and conidiation of each sector was observed on complete medium having 1250 μg of griseofulvin/ml. At concentrations higher than 1250 μg/ml, the sectors were few, tiny and indistinct. Conidiation was also poor making the isolation cumbersome. The sectors obtained at concentration equal to or more than 1500 μg of griseofulvin/ml were not tested for segregation of visual and biochemical markers, since they needed streaking and purification. It was found that increase in the frequency of coloured sectors per plate as well as per inoculum was proportional to the rising concentration of the drug (Table 2).

The sectors were isolated and tested for their genotypes on various types of media. The colour of the mycelium, the density of conidiation, the conidial diameter and the mode of segregation of markers were taken into consideration for the determination of ploidy levels. The pattern of segregation of visual, biochemical and resistance markers showed that the

non-disjunctional diploids were predominant at lower concentrations and mainly haploid segregates were induced at higher doses, i.e., the proportion of diploids declined, as the amount of griseofulvin was increased in the media (Table 3). At 500 μg/ml, only 33% of the sectors analysed turned out to be haploids and it touched 90% at 1250 μg/ml. At this concentration no mitotic crossover or aneuploid was recovered. Thus the haploids were found to be induced in a dose dependent manner. The haploid/diploid ratio, as obtained after griseofulvin treatment showed progressive rise with further increase in griseofulvin concentrations.

Table 2. The frequency of griseofulvin induced coloured sectors of *Aspergillus* per plate and their range per colony

Concentration (μg/ml)	No. of visible sectors per plate			Range of sectors per colony
	green	yellow	total	
0	0	0	0	0
500	1	5	6	0—1
750	4	9	13	0—2
1000	13	35	48	1—3
1250	23	67	90	2—8

Table 3. Griseofulvin induced mitotic segregation in *Aspergillus*

Concentration (μg/ml)	No. of sectors analysed			No. of haploid segregates	No. of diploid segregates	Haploid/ diploid ratio
	green	yellow	total			
500	4	20	24	8	16	0.50
750	6	22	28	16	12	1.33
1000	11	45	56	42	14	3.00
1250	18	94	112	102	10	10.00

Table 4 shows the segregation pattern of all possible pairs of markers in the 102 haploids induced by griseofulvin. It is already known that riboA_1, biA_1 and yA_2 markers are located on linkage group I (Fig. 1) and do not recombine among themselves during haploidisation but they do recombine with each and every marker located on all the other linkage groups (i.e., linkage groups II to VIII). Similarly, fpaM_{72} (the marker, whose linkage group is to be assigned) recombines with each and every marker of the strain MSD except yA_2 (linkage group I). Therefore, it is inferred that fpaM_{72} is located on linkage group I.

DISCUSSION

Griseofulvin causes C-mitosis [7], loss of typical metaphase tubulin structures and aberrant polymerisation of tubulin structures [17]. Hence, it is expected to induce abnormal segregation of chromosomes and polyploidisation in *A. nidulans*. In the former case, the direct genetic effect shall be the induction of transient unstable aneuploids and a proportion of haploids and nondisjunctional diploids, which will ultimately give rise to stable haploids

Table 4. Segregation pattern of markers in griseofulvin induced haploids

Pairwise segregation of markers (trans)			Parentals		Recombinants		Recombination fraction
			− +	+ −	− −	+ +	
*y*A2	;	*ribo*A1	56	46	00	00	00/102
*y*A2	;	*bi*A1	56	46	00	00	00/102
*y*A2	;	*fpa*M72	56	46	00	00	00/102
*acr*A1	;	*fpa*M72	06	15	31	50	81/102
*phen*A2	;	*fpa*M72	38	40	06	18	24/102
*pyro*A4	;	*fpa*M72	42	41	05	14	19/102
*lys*B5	;	*fpa*M72	50	46	00	06	06/102
*s*B3	;	*fpa*M72	32	39	07	24	31/102
*nic*B8	;	*fpa*M72	17	31	15	39	54/102
*ribo*B2	;	*fpa*M72	50	13	33	06	39/102
	(cis)		++	− −	+ −	− +	
*acr*A1	;	*phen*A2	26	05	39	32	71/102
*acr*A1	;	*pyro*A4	24	06	41	31	72/102
*acr*A1	;	*lys*B5	18	03	47	34	81/102
*acr*A1	;	*s*B3	28	02	37	35	72/102
*acr*A1	;	*nic*B8	37	04	28	33	61/102
*acr*A1	;	*ribo*B2	10	28	55	09	64/102
*phen*A2	;	*pyro*A4	24	13	34	31	65/102
*phen*A2	;	*lys*B5	50	42	08	02	10/102
*phen*A2	;	*s*B3	47	28	11	16	27/102
*phen*A2	;	*nic*B8	41	15	17	29	46/102
*phen*A2	;	*ribo*B2	16	41	42	03	45/102
*pyro*A4	;	*lys*B5	32	27	23	20	43/102
*pyro*A4	;	*s*B3	37	21	18	26	44/102
*pyro*A4	;	*nic*B8	43	20	12	27	39/102
*pyro*A4	;	*ribo*B2	13	41	42	06	48/102
*lys*B5	;	*s*B3	41	28	11	22	33/102
*lys*B5	;	*nic*B8	40	20	12	30	42/102
*lys*B5	;	*ribo*B2	09	40	43	10	53/102
*s*B3	;	*nic*B8	40	09	23	30	53/102
*s*B3	;	*ribo*B2	12	32	51	07	58/102
*nic*B8	;	*ribo*B2	10	23	60	09	69/102
*ribo*A1	;	*fpa*M72	56	46	00	00	00/102
*bi*A1	;	*fpa*M72	56	46	00	00	00/102

in a dose dependent manner. The transition from diploid to haploid will, as a rule, display whole chromosome segregation, as the chemical does not interfere with chromosomal integrity [1]. In the latter case, griseofulvin will produce polyploid nuclei like higher plants, but unlike higher plants these polyploid nuclei are highly unstable in *A. nidulans* and in subsequent divisions, they produce haploids, diploids and transient aneuploids. The transient aneuploids lose their extra chromosome to become stable haploid or diploid. Thus, the polyploidising agent of higher plants behaves as an efficient haploidising agent in *A. nidulans* due to natural selection against the aneuploids, which are the first genetic outcome of griseofulvin. As expected, griseofulvin predominantly produced haploids at higher concentrations on account of gross chromosomal misdistribution (Table 3).

Like earlier known haploidising chemicals, griseofulvin too inhibits growth of *A. nidulans* (Table 1), by disturbing the spindle formation and thereby impeding mitosis at 1250 μg/ml, many coloured sectors per inoculum with profuse conidiation show the induction of genetical segregation by the chemical (Table 2). Table 3 shows the optimum concentration of the chemical for quick and efficient haploidisation. In addition, the rise in haploid as well as diploid segregants in presence of griseofulvin suggests that the process of non-disjunction is responsible for the simultaneous increase of haploids and nondisjunctional diploids.

The marker $fpaM_{72}$ is assigned to linkage group I (Table 4) on the basis of free recombination with all the markers of MSD except those located on linkage group I and complete linkage with $riboA_1$, biA_1 (cis) and yA_2 (trans). The phenylalanine requiring auxotrophs and FPA resistant haploids were also obtained. The manifold increase in the frequency of coloured sectors (Table 2) and mitotic segregants (Table 3) over the control indicates the inducibility of the genetic event.

Griseofulvin has an edge over other haploidising agents. For example, phenylalanine requiring recombinants are not obtained in the presence of FPA, as phenylalanine auxotroph is hypersensitive to it. Chloral hydrate affects cytoplasmic pigmentation making at times the sectors indistinguishable. Benlate and MBC failed to haploidise FPA resistant heterozygous diploids. Radiation produces many kinds of undesired sectors making the isolation of haploids cumbersome. But all possible types of easily distinguishable haploid segregants are produced on griseofulvin containing media and there is no inhibition of any class of haploid segregants expected out of diploids. Therefore, it is concluded that griseofulvin is best suited for mitotically mapping FPA resistant marker in *A. nidulans*.

REFERENCES

1. U. Sinha and S. N. Jha. 1986. *Aspergillus nidulans* as a genetic test system. *In*: Frontiers in Applied Microbiology, Vol. I (eds. K. G. Mukherjee and V. P. Singh). Print House, Lucknow: 137–159.

2. U. Sinha and S. N. Jha. 1983. Chloral hydrate induced haploidisation through aneuploidy in *Aspergillus nidulans*. XV Intern. Genet. Congr., II : 796.

3. J. Bal, E. Bartnik, B. Goryluk and N. J. Pieniazek. 1975. An easy way of obtaining *Aspergillus nidulans* haploids in the parasexual cycle using N glycosyl polifungin. Genet. Res., **25**: 249–272.

4. G. E. Paget and A. L. Walpole. 1958. Some cytological effects of griseofulvin. Nature, **182**: 1320–1321.

5. K. Gull and A. P. J. Trinci. 1973. Griseofulvin inhibits fungal mitosis. Nature, **244**: 292–294.

6. S. H. B. Crackower. 1972. The effect of griseofulvin on mitosis in *Aspergillus nidulans*. Can. J. Microbiol., **18**: 683–687.

7. L. Wilson and J. Bryan. 1973. Biochemical and pharmacological properties of microtubules. Adv. Cell Mol. Biol., **3**: 21–72.

8. J. North. 1977. The effect of griseofulvin on diploid strains of *Coprinus lagopus*. J. Gen. Microbiol., **98**: 529–534.

9. A. Kappas and S. G. Georgopoulos. 1974. Interference of griseofulvin with the segregation of chromosomes at mitosis in diploid *Aspergillus nidulan*. J. Bacteriol., **119**: 334–335.

10. P. W. Brian. 1960. Presidential address. Tr. Br. Mycol. Soc., **43**: 1–13.

11. S. E. Malawista, K. G. Bensch and H. Sato. 1968. Vinblastine and griseofulvin reversibly disrupt the living mitotic spindle. Science, **160**: 770–772.

12. K. Gull and A. P. J. Trinci. 1974. Effect of griseofulvin on the mitotic cycle of the fungus *Basidiobolus ranarum*. Arch. Microbiol., **95**: 57–65.

13. J. A. Roper. 1952. Production of heterozygous diploid in filamentous fungi. Experientia, **8**: 14–15.

14. G. Pontecorvo, J. A. Roper, L. M. Hemmons, K. D. Macdonald and A. W. J. Bufton. 1953. The genetics of *Aspergillus nidulans*. Adv. Genet., **5**: 141–238.

15. K. S. McCully and E. Forbes. 1965. The use of FPA with 'Master strains' of *Aspergillus nidulans* for assigning genes to linkage groups. Genet. Res., 6: 352–359.

16. A. J. Clutterbuck. 1981. Loci and linkage map of *Aspergillus nidulans*. Aspergillus Newsl. **15**: 58–72.

17. K. Weber, J. Wehland and W. Herzog. 1976. Griseofulvin interacts with microtubules both in vivo and in vitro. J. Mol. Biol., **102**: 817–829.