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An efficient isolation method for meiotic mutants causing meiotic nondisjunction or elevated recombination frequency in Neurospora crassa.

Among several inositol-requiring mutants, inl (37401 and inl (83201(t)) complement to each other. This fact gave us an antiselection method of aneuploid ascospores in linkaqe qroup V, which contain meiotic mutants causing chromosomal nondisjunction. Ascospores haploid in linkage group V will be led to inositol-less death on minimal plates at 37°C. Meiotic mutants which cause elevated recombination frequencies can also be selected by this method. Conidia from strains KH204a (lys-1 (33933)), inl (37401, his-6 (Y152M105; nuc-2 (T28M2)) and 2309A (al-3 (RP1OO), in1 (83201(t)) were irradiated with UV for 0, 30 and 60 sec (10% survival) and intercrossed. The resulting ascospores were plated in Fries minimal plates (5.1 x 104 ascospores/plate with non-irradiated sample) and incubated at 37°C for 3 days. From 0, 30 and 60 sec irradiated samples 571, 1100 and 528 of colonies were isolated (40 colonies/plate with non-irradiated sample). Most of the strains isolated had pale orange conidia which were intermediate in color between those of al-3 and wild type. Conidial isolation of these cultures revealed segregation of al-3. They were crossed with strain 2125A and 2998a in 12 x 105 mm test tubes. As shown in Fig. 1. strains discharqing more tha 20% of abortive ascospores were screened and 60, 167 and 56 strains were obtained from each sample.

From each of these crosses of the 60 strains obtained as spontaneous mutants, 50 or 100 ascospores were isolated. White ascospore production by these isolates was analyzed by crossing with wild type, with the isolates as protoperithecial parents. When more than 30% of isolates showed more than 30% of abortive ascospores, these strains were reserved for further analysis. Isolation of single conidia of appropriate isolates which showed meiotic aberration was performed and those which produced more than 30% of abortive white ascospores when crossed as protoperithecial parents were used for further analysis. This process was essential to avoid heterogenous genetic background caused by meiotic nondisjunction. These strains thus obtained were reciprocally crossed and resulting ascospores were analyzed. As shown in Table 1, these mutants were grouped into two classes. Class I includes those strains which produced 30 to 90% of abortive white ascospores only when they were crossed as protoperithecial parents. With homozygous crosses, higher frequencies of abortion were

TABLE I

White abortive ascospore production in isolated meiotic mutants.

Genotype (Protoperithecia /conidia)	Ascospore shot (% of white spore)			
	Class I		Class II	
	N2AM152	N2AM6	N2AM195	N2AM83
+/+	0-5	0-5	0-5	0-5
+/+	0-5	0-5	50-90	70-90
Mei / +	40-70	40-60	90	80-90
Mei / Mei	90	70-90	No dis.*	90-95

*Perithecia were formed but no discharge of ascospore was observed.

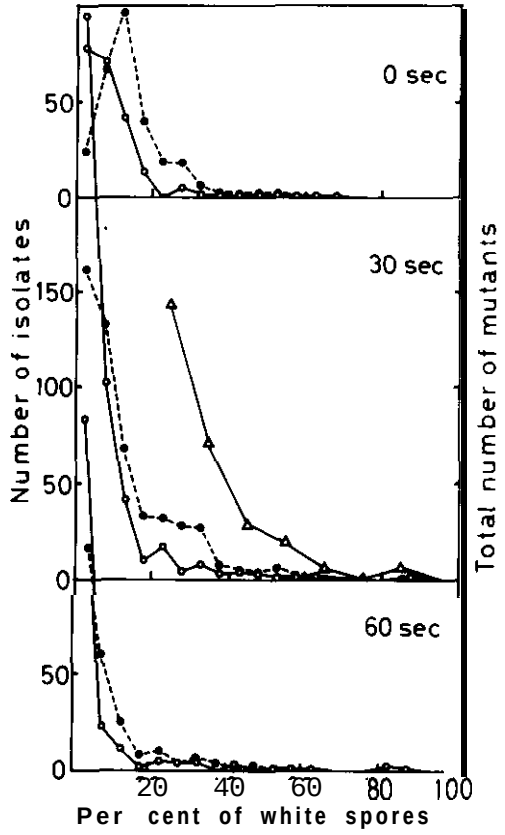

Figure 1. -- Distribution of per cent Of white abortive ascospores produced from crosses between nutrition nonrequiring isolates and wild type, 2125A or 2998a. Experimental details were as described in the text. Crossed with 2125A (--●--) or 2998a (—o—). Accumulation of 0, 30, and 60 sec irradiated samples (-A-).

observed. Finally 12 meiotic mutants belonging to class I were obtained. Seven mutants belonging to class II, which produced 30 to 90% of abortive white ascospores irrespective of conidial or protoperithecial parents, were also isolated.

Among class I mutants, N2AMI52 and N2AM189, were considered to cause chromosomal nondisjunction and three mutants, N2AN6, N2AM213 and N2AM229, were suggested to cause chromosomal degradation. Among class II mutants, N2AM195 was estimated to cause chromosomal nondisjunction, and N2AM83 was found to cause chromosomal degradation of linkage group V and elevation of recombination frequency in linkage group V. This mutation was mapped near al-3 The frequency of spontaneous mutation to meiatic aberration was approximately 2.7 x 10^{-5}.

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