

A method to improve the fertility of interallelic crosses

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Abstract

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To elucidate the genetic fine structure at a locus, the first barrier to be overcome in most cases may be the sterility of the interallelic crosses. In fact, interallelic

crosses at the ad-8 locus usually produce a considerable number of perithecia but no ascospores are formed, or when ascospores are formed, almost all ascospores are white or brownish and sterile (fertility, less than 1%). Various attempts have been made to improve the fertility of ad-8 interallelic crosses using variations of crossing media and culture conditions.

A fruitful finding was the fact that lower concentrations of sucrose (0.05 - 0.01%) in the Westergaard and Mitchell's crossing media containing an optimum amount of the supplement (400 μ /ml) adenine) was definitely effective in increasing the fertility (up to 30%). With a low concentration of sucrose, the possibility of improving further the fertility has been investigated by supplementing varying amounts of amino acids, purines, pyrimidines, and inorganic salts. None of these supplements seemed to have any effect in improving the fertility. The optimum temperature for interallelic crosses appears to be about 25°C, light has essentially no effect and pH variation shows no significant effect on the fertility within the range 4.0 - 8.0. In place of sucrose, various carbon sources were tested as well. Most carbon sources tested - acetate, citrate, fumarate, lactate, malate, various hexoses, various disaccharides, starch, etc. - were active in supporting crossing as well as growth. Among these substances, acetate gave a higher fertility than other carbon sources (about 40%).

It was noted, however, that a low concentration of carbon also resulted in a reduction in the number of perithecia (less than 10 perithecia in a 20 x 150 mm test tube). In the course of these experiments, it was found by chance that the addition of filter paper as a sole carbon source was effective in producing large numbers of perithecia (more than 20 perithecia in a tube) which shot a considerable number of fertile ascospores. Powdered cellulose and several derivatives of cellulose (e.g., methylcellulose, N,N-diethylaminoethylcellulose, etc.) showed the same effect as filter paper. The addition of a small amount of acetate (0.01 - 0.005%) as another carbon source to the filter paper media seems to give better germination. One third of a circular filter paper (Balstone No. 1; diameter 110 mm) was torn into several pieces and put into the crossing medium in a 125 ml Erlenmeyer flask before autoclaving. A cross made in such a flask gave a significant number of fertile ascospores for an analysis of the fine structure within the ad-8 locus. It may be, however, commented that the fertility problem should be investigated for various possibilities at each locus, since this method was not effective for the ad-4 and ad-5 interallelic crosses.

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