

A SIMPLE PROCEDURE FOR THE MANUAL ISOLATION AND IDENTIFICATION OF PLANT HETEROKARYONS

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SUMMARY

A simple procedure is described for the aseptic manual isolation of individual heterokaryons. Heterokaryons were identified with bright field illumination using an inverted microscope and isolated by means of a micro-manipulator and capillary pipette coupled to a specially constructed syringe. When cell suspension protoplasts were labelled with fluorescein isothiocyanate and fused with mesophyll protoplasts, the heterokaryons exhibited an apple green cytoplasmic fluorescence (from cell suspension protoplasts) and a red chloroplast fluorescence (from mesophyll protoplasts). By this double fluorescence procedure, these manually isolated heterokaryons, identified initially using brightfield could be confirmed as heterokaryons.

INTRODUCTION

A general scheme, capable of selecting somatic hybrids between two wild types species is desirable for plant breeding programmes. One of the major problems presently encountered is a lack of suitable mutants for complementation selection.

Fusion products of soybean suspension protoplasts have been identified after labelling them separately, prior to fusion with fluorescein isothiocyanate and rhodamine B [1]. Similarly, mesophyll protoplasts of *Nicotiana* species have been separately labelled with fluorescein isothiocyanate and rhodamine isothiocyanate. Fusion products showing the presence of both probes were detected [2]. In the case of mammalian cells, one cell type has been labelled with the laser dye, rhodamine 123 (a mitochondrial stain) and

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Abbreviation: FITC, fluorescein isothiocyanate.

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