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A preliminary report to a new method to fuse selectively two protoplasts from different parents in higher plants

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One of the major barriers in somatic hybridisation by protoplast fusion in higher plants is considered to be the problem of the selection of heterokaryon/hybrid cell out of protoplast population after fusion treatment. In order to tide over the problem a number of selection systems have been developed so far. It is impossible, however. to apply unconditionally those selection systems for species or genera in plan of somatic hybridisation, because complementation selection systems or another systems that need special materials have not always been established in the species or genera. Such a lack of generally applicable selection systems is probably one of the causes that limit the utility of the fusion methods and make it unattractive (1). The development of fusion system that, for example, heterokaryons/hybrid cells are exclusively produced by protoplast fusion is very usefull if possible. From such a point of view, the present study is to aim at the development of fusion system that is basically explained as a method to fuse selectively two protoplasts from different parents by putting the protoplasts into a cup-shaped pit(hereinafter referred to as pit)on the surface of a thin plate. This report describes a method to put two protoplasts from two parents in a pit on a membrane filter paper.

Preparation of protoplast

Cotyledons of tomato plants about ten days after sowing were chosen as materials for the isolation of protoplast, in which an enzyme mixture containing 2.5% Meiselase P, 0.25% Macerozyme R10, 0.25% Driselase and 9% mannitol was used. Other details were as described previously (2). Protoplasts with an average size of ca. 45μm or ca. 49μm were prepared by passing them through two stainless steel sieves of 53μm and 37μm or 53μm and 44μm in pore size.

Making small pits on a membrane filter paper

About one hundred of small pits, each of which had a size of ca. 50μm in diameter and ca. 80μm in depth, were made on a membrane filter paper (TM-3, Tōyō Roshi KK, Japan) by gentle handling of a fine axle devised as shown in Fig. 1.

Putting protoplasts in pits on a membrane filter paper

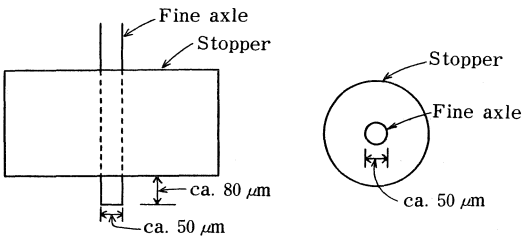

Fig. 1. A fine axle devised to make cup-shaped pits on a membrane filter paper

A petri dish with a diameter of 6cm was used as a device, where a membrane filter paper with a number of pits was set on a membrane filter support, as shown in Fig. 2. After plasmolysing solution was filled up in the petri dish and the membrane filter paper, a protoplast suspension was gently dropped into the plasmolysing solution in the membrane filter paper. Subsequently, all the protoplasts, except for those put in pits, were removed by repeatedly pippetting in and off plasmolysing solution. The number of protoplasts in a pit is a concern in this study. The

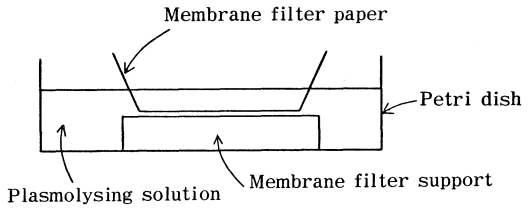

Fig. 2. A device to put protoplasts in cup-shaped pits on a membrane filter paper

Table. 1. Number of protoplasts put into a cup-shaped pit on a membrane filter paper

No. of experiments	No. of protoplasts in a pit					Size of protoplast
	0	1	2	3	Total	
1	3 (5.9)	30 (58.8)	15 (29.4)	3 (5.9)	51 (100)	ca. 45μm
2	3 (6.5)	25 (54.3)	16 (34.8)	2 (4.4)	46 (100)	do.
3	9 (11.3)	61 (76.4)	9 (11.3)	1 (1.0)	80 (100)	do.
4	8 (10.1)	56 (70.9)	15 (19.0)	0 (0.0)	79 (100)	do.
5	4 (8.3)	43 (89.6)	1 (2.1)	0 (0.0)	48 (100)	ca. 49μm

Note. : Numbers in parentheses show percentage.

results shown in Table 1 lead to a conclusion that the probability that only one protoplast can be put in a pit is 50–70 percent if protoplasts with ca. 45μm in size are used and about 90 percent if those with ca. 49μm are used. This suggests that the balance between the sizes of protoplast and pit is very important for ensuring a higher percentage of one protoplast in a pit.

In the second step the adhesion of two protoplasts from the two parents is to aim by putting one protoplast onto the other protoplast already put in a pit. The percentage of heterotypic adhesion expected in this case will be approximately 90 percent if protoplasts with ca. 49μm in size are used. This estimate of percentage is much higher than the corresponding values expected from mixing together protoplasts of two parents and inducing random adhesion each other. Then, fusion treatment will produce a higher proportion of heterokaryon/hybrid cell to the protoplast population than the proportion expected from the fusion methods developed so far.

Further problems are questioned as follows :

(1) Is it really possible to obtain the heterotypic adhesion of two protoplasts in a pit with the expected percentage (90 percent) when counterparts of protoplasts are added in the second step?

(2) Is it really possible to induce the fusion of two protoplasts in a pit with no less than percentages of the fused protoplasts obtained from routine procedures of protoplast fusion?

(3) Protoplasts may be more or less injured in the process of putting them in pits. Is it possible to induce cell division with the same extent as routine procedures?

(4) Is it possible to provide a membrane filter paper with many pits to stand practical use?

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Reference

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高等植物プロトプラストの選択的融合法の開発 (予報)

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摘 要

薄板にあけた微小な穴に2種類のプロトプラストを1個づつ順次挿入し，2種のプロトプラストを1対1で大量に接着させる方法を開発するため，今回は，メンブランフィルターに直径約50 μm，深さ約80 μm の穴を約100個あけ，1個の穴にトマト葉プロトプラストを1個だけ挿入する場合の方法を検討した.