PROTOPLAST CULTURE

GENERAL INTRODUCTION

- Protoplast is the biologically active and most significant material of cells.
- Since the isolated protoplast grown in culture often perform than single whole cells
- They may serve as an excellent starting material for cell cloning and development of mutant lines.
- It is used in somatic hybridization technique.

TECHNIQUE OF PROTOPLAST CULTURE

- Protoplast culture involves the following steps:-

1. Isolation of protoplast.
2. Purification of protoplast.
3. Protoplast culture and regeneration

Isolation of Protoplast

- The protoplast of plant cell is bounded by cell wall and cell membrane. The cell wall acts as a barrier and protects protoplasm from microbial invasion and environmental stresses.

1. Collection and surface sterilization of leaf samples:-
2. Rinsing of suitable osmoticum:-
3. Isolation of protoplast:-

a) Mechanical method:-

b) Enzymatic method:-

Purification of protoplast

- Leaf debris are removed with forceps and enzyme solution containing protoplast is filtered with a nylon mesh.
- Filtrate is centrifuged at 7000 rpm for 5 minutes and supernatant is decanted.
- Repeated washing with nutrients medium , centrifugation and decantation are done for about three times.

Protoplast culture on suitable culture media

- There are different methods of protoplast culture such as:-

i. Liquid culture
ii. Agar culture
iii. Feeder layer technique
iv. Co-culture
v. Droplet culture
vi. Hanging droplet culture
vii. Immobilized /bead culture