B-09: A study of fractionated carrot (*Daucus Carota*) somatic embroye cultures

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In mass scale somatic-embryogenesis, output of the high quality embryos is a vital aspect. Presence of 3 different embryo types ('competent', 'aberrant' and 'proliferated' embryos) at torpedo stage of embryo growth was elaborated and 'competent embryos' were identified as the superior ones to meet the embryo quality standards. Enhancement of 'competent embryo' amount depends on the alleviation of other undesired embryo types, for which the development patterns of those embryo types within the culture is important. During this experiment, embryo cultures were periodically sieved and studies were carried out to elaborate the embryo development patterns.

Callus induction was done from carrot (*D.carota*) seedling hypocotyl and the maintenance and embryo induction were done following the procedure explained by Liyanage & Kurata (1992).

Starting from 12 days of culture age, culture sieving was done at 4 day intervals until 28 days, using 2 nylon sieves of 950 μm and 435 μm. Sieved embryo fractions were categorized as Large fraction ($>$ 950 μm), Medium fraction (950 - 435 μm) and Small fraction ($<$ 435 μm), of which the large fraction contained cotyledonary stage embryos, Medium fraction contained torpedo stage embryos whereas Small fraction consisted of other early embryo growth stages and callus. After every sieving, Small fraction was recultured.

Embryo counting was done microscopically, for Large and Medium fractions, at each sieving date. Five sample repetitions were done at each counting for 3 replicates.

Sieving resulted in 10 embryo fractions (5 groups x 2; Medium & Large fractions) and were named as mini-embryo-groups (MEG). They represented different growth rates; e.g. MEGM1 (MEG Group 1 of Medium fraction) reached torpedo stage within 12 days, whereas MEGM5 took 28 days.)

Total embryo number between different MEGs were different. Proliferated embryos, which represent secondary embryogenesis, were higher in Large fraction (50%) than Medium fraction, which implies the increasing tendency of secondary embryogenesis towards cotyledonary stage of embryo growth.

In contrast, aberrant embryo percentages were always high with Medium fraction (35%) compared to Larger fraction (30%). That implies the retardation of growth rate with aberrations.