Study of callus tissues from different parts of *Nigella sativa* (Ranunculaceae)

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Summary. Callus tissues were produced from leaf, stem and seed of *Nigella sativa* in modified Murashige and Skoog's medium. Depending on the origin of explants, the time of callus initiation and also the properties of the callus tissue were found to be different, especially regarding chromosome instability.

Plant tissue cultures have become important as an experimental tool to study many fundamental and applied problems in morphogenesis, cytology and genetics. Large numbers of plant materials of both monocotyledons and dicotyledons have been tried for the initiation of callus cultures in different media by different authors[2-4]. The establishment of actively growing cultures depends mainly on the selection of the explants and on the culture medium. It has also been found that the quantitative and qualitative nutritive requirements in vitro may vary with different plant materials and in some instances with different tissues of the same species. Another important point is that cultured cells of both plant and animal tissues are characterized by the instability of chromosome number and structure. According to Partanen[5,6] this chromosomal instability in culture depends upon the type of explant from which the callus originated. The present investigation was undertaken to study the morphological and cytological behaviour of callus tissues of *Nigella sativa* derived from different explants. This species was chosen because of its low chromosome number (2n = 12) and large chromosomes.

Seeds of *Nigella sativa* were surface sterilized in 0.1% mercuric chloride for 20 min and rinsed several times in sterile medium containing only 0.8% agar, and were kept in the dark. After 2-3 weeks seedlings were formed. Leaves obtained from these seedlings were cultured in a medium N-1 which is a modification of Murashige and Skoog's[3] medium. Cultures were maintained in 16 h/8 h light-dark photoperiod at 22 – 24 °C. Callus was also obtained directly from seeds, whereby sterilized seeds were placed in N-1 medium. These seeds were kept in complete darkness.

For cytological observations, callus tissues of stem, leaf and seed were taken just after placing in light conditions for 2 h and were fixed in Carnoy's fluid overnight. They were then slightly warmed in a 2% aceto-orcein-(N)HCl mixture of 9:1 and kept for 1 h before squashing in 45% acetic acid.

Results and discussion: The 3 different types of explants were placed in the same medium to compare their growth responses as well as their morphological and cytological behaviour. Callus tissues were initiated from the leaf segments very quickly whereas the callus tissues from stem and seed appeared more slowly (table 1). These leaf segments and stem pieces were cultured from seedlings grown in the agar medium, where the growth of the seedlings again took at least 2 – 3 weeks. In the case of seed the total time of growth for callus was less. The rate of growth in the case of leaf segments was slightly better than that of seed callus. Callus proliferation in stem explants was very slow as compared to other segments. Callus from leaf segments showed initiation of a vigorous, proliferating, soft and green-coloured tissue. A greenish-yellow and a yellow colouration were characteristic of tissues grown from stem pieces and seeds respectively.

During the proliferation of calluses from different parts of the plant, a number of changes in structure and number of chromosomes were noted. A high frequency of the normal number occurred mainly in calluses from leaf segments (table 2). The maximum chromosome number in leaf calluses was found to be 36, while up to 72 chromosomes were observed in stem calluses. The maximum frequency of tetraploid mitoses occurred in seed calluses. Fragmentation of chromosomes and micronuclei were observed in all cases. Chromosomal instability thus appeared to be higher in stem and seed calluses than in leaf calluses. From this point of view, it may be suggested that the leaf callus is better suited for tissue culture investigations than other types of callus. Similar changes in ploidy level have been reported in differentiated tissues of many species[7,8].

Table 1. Growth pattern and nature of callus in *Nigella sativa*

Plant parts used for initiation of callus:	Leaf	Stem	Seed
Time taken for initiation of callus (days):	5-6	8-10	22-30
Time taken for full development of callus (days):	22-28	30-35	35-60
Colour of callus:	Green	Yellowish-green	Yellow
Growth rate of callus after 3 weeks:	+ + +	+	+ +
Nature of the callus:	Soft	Soft	More compact

Table 2. Percentage of metaphases with various chromosome numbers (2 n = 12) in initial passage of leaf, stem and seed callus tissue from *Nigella sativa*

Origin of callus	Chromosome numbers 12	24	36	48	72	aneuploids
Leaf	55.56	38.89	5.56	-	-	-
	From 1 to 6 micronuclei in many cells; fragments present.					
Stem	33.33	29.16	25.00	8.33	4.16	-
	From 1 to 5 fragments in some polyploid cells.					
Seed	24.49	51.02	18.37	4.08	-	2.04
	From 1 to 6 fragments in many polyploid cells.					

The differential response of the different explants in the same medium may be due to changes in the physiological conditions of the material. Although there is evidence that polyploid cells may arise from endoreduplicated nuclei in the original explant, the range of chromosome number and structure observed in established cultures strongly points to the origin of these changes during culture. In the present investigation tetraploid cells occur at maximum frequency in seed calluses, while a tendency toward diploidy was found in leaf calluses. Although Partanen[5,6] reported that chromosomal instability in culture depends upon the type of plant parts from which the callus originated, the present investigations show variation in chromosome number in all the 3 different explants cultured in the same medium.

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Preservation of *Bordetella pertussis*

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Summary. Bordetella pertussis has been shown to remain viable for a period of 13 years on freeze-drying. The revived cultures had unaltered antigenic composition and biological activity.

Frequent subculture of a microbial species is liable to result in change of antigenic and other characters. Therefore, maintaining viability of an organism for longer periods without resorting to repeated subcultures is highly desirable. Freezing at −70 °C with 15% glycerol was found to be a satisfactory means of preservation of *Treponema pallidum* and other bacteria[2]. The factors affecting the death rate of *Escherichia coli* at temperatures between −1.5 °C and −195 °C have been studied[3]. Reports of the preservation of *B. pertussis* for 45 months in 15% glycerol at −70 °C and of freeze-dried cultures for 10 years are available[4,5]. The present communication reports the preservation of *B. pertussis* for a period of 13 years.

Materials and methods. Strains 134 and 509 routinely used by us for the manufacture of pertussis vaccine were suspended separately either in Lomodex (10% dextran in 5% dextrose, Rallis India Ltd) or aqueous gum acacia solution. 0.2 ml of the culture suspension containing approximately 200×10^9 organisms/ml were freeze-dried in 100×8 mm sterile glass tubes and sealed under vacuum. To study the effect of an inert gas on the viability of cultures, gaseous nitrogen was passed into some of the tubes before sealing. All tubes containing freeze-dried cells were stored at 4–10 °C until used. The viability of the cultures was tested on duplicate tubes of Bordet-Gengou (BG) medium. Tubes were incubated at 35 °C for a maximum of 96 h. Antigenic composition of BG-grown cultures was checked with *B. pertussis* monospecific factor 1, 2, and 3 sera by slide agglutination method[6]. Test for dermonecrotic activity was performed in rabbits. 0.1 ml of a suspension containing 1.0×10^9 organisms/ml was inoculated intradermally. Results were recorded at 48 and 72 h. The maximum reaction obtained has been reported.

Result and discussion. The findings have been summarized in the table. A freeze-dried culture under vacuum showed viability upto 13 years – the maximum period of observation in this study. Revived cultures of strain 134 agglutinated with factors 1 and 3 and those of strain 509 with factor 1, 2, and 3 sera. This is in agreement with the known antigenic composition of the 2 strains. The cultures from the

Viability and other characteristics of *B. pertussis* cultures after various periods of freeze-drying

Strain	Date of freeze-drying	Conditions of freeze-drying		Growth up to 96 h at 35 °C		Agglutination with factor sera			Dermonecrotic reaction (mm)
		Suspending fluid	Gaseous condition	BG Tube I	BG Tube II	1	2	3	
509	March, 66	Gum acacia	Vacuum	+	+	+	+	+	10×10
509	July, 72	Gum acacia	Vacuum	+	+	+	+	+	15×10
509	July, 72	Lomodex	Nitrogen	−	−				
509	April, 76	Lomodex	Vacuum	+	+	+	+	+	10×10
509	April, 76	Lomodex	Nitrogen	−	−				
509	April, 79	Lomodex	Vacuum	+	+	+	+	+	15×10
134	July, 66	Gum acacia	Vacuum	+	+	+	−	+	15×10
134	July, 72	Gum acacia	Vacuum	+	+	+	−	+	15×10
134	July, 72	Lomodex	Nitrogen	−	−				
134	April, 76	Lomodex	Vacuum	+	+	+	−	+	20×10
134	July, 76	Lomodex	Nitrogen	−	−				
134	April, 79	Lomodex	Vacuum	+	+	+	−	+	15×10

+ = Growth, agglutination; − = no growth, no agglutination.