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GROWTH REQUIREMENTS OF HEVEA STEM CALLUS

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Degree of Master of Science

by

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A B S T R A C T

Callus initiation and growth was observed to be dependant on the maturity of the Hevea stem explant. The explant grew well on Murashige and Skoog (M & S) medium which was superior to Hildebrandt Riker and Duggar (HRD), Ewens (Y₃) White and Nitsch and Nitsch (N & N) media even when the organic constituents were replaced by those of M & S. Culture schedule for calli, grown on M & S medium in tubes (10 ml medium) was of a 3-4 week period, whereas for those grown in flasks (50 ml medium) was found to be 15 - 17 weeks. Callus cultures could be maintained by repeated subculturing for more than 2 years but the regeneration potential of the 2 year old callus was found to be inferior to that of the 3 months.

A rapid rate of increase in cell number per field of microtome sections of calli, grown in tubes (10 ml medium) was observed within the first two weeks, with that of the second week being the most prominent. The rate of dry matter production was maximum on the third week. The growth of callus after the third week was due to absorption of water.

Temperature was found to be critical for the growth of Hevea stem callus. The best temperature for both initiation and growth of callus was 26°C - 27°C. Callus grew well in complete darkness. Light was inhibitory for callus growth; but callus could be maintained in day light for a much longer period than in complete darkness. The pH, though not critical for callus initiation, the best growth was observed to occur at 5.7.

Auxin: 2,4-D, NAA, IBA, or IAA; Kinin: Kinetin, Zeatin or BAP when used alone caused a poor growth of callus. Gibberellic acid was not effective. A significant interaction of auxin with kinetin was found; the combination of 2,4-D (2 mg/l) and kinetin (0.5 mg/l) being the best for both initiation and growth of callus. Higher concentrations of vitamin than that of normal M & S medium were found to cause poor initiation and growth of callus.

The presence of lactalbuminhydrolysate or caseinhydrolysate upto 1000 mg/l and 1500 mg/l respectively was found to promote the initiation and growth of callus. As a carbon source, sucrose was better than fructose, glucose or maltose; 3% sucrose was found to be the best for both initiation and growth of callus. Sorbitol when used alone caused a poor growth but the explants remained fresh throughout the observation period in its presence. A high concentration of inorganic nitrogen, in the form of both NH_4^+ and NO_3^- was also required for the initiation and growth of Hevea stem callus. Plant tissue extracts (potato, carrot, rubber seed, banana and coconut milk) were found to cause a poor callus growth. However, the cell number per field in microtome sections of calli, treated with coconut milk was significantly higher than that of the control.

Callus derived from a Hevea seedling plant grew quicker than that from its budded plant. Different growth responses were observed with calli derived from different Hevea clones.

The alkaloid: caffeine, colchicine and nicotin stimulated both initiation and growth of callus. Tracheid formation in callus was also found to be stimulated by these substances. Cells similar to sieve elements and companion cells were also observed when the calli were treated with a high concentration of sucrose.

Root formation was observed when the calli were treated with low levels of kinetin and very low or absolute levels of 2,4-D. In this respect, the Y_3 medium was observed to be much better than the M & S medium. However, M & S medium with NAA (2 mg/l) or IBA (2 mg/l) and kinetin (0.5 mg/l) also induced good root systems. An acidic medium (pH 5.5) favoured root formation. Biotin, sucrose, cinnamic acid and amino acid complexes (lactalbuminhydrolysate and caseinhydrolysate) caused early development of roots. The alkaloid caffeine appeared to suppress root formation, whereas no effect on root formation was recorded with Ca-Pentathenate. The root regeneration potential was found to decrease with the age of the callus.

Cell suspension cultures could be formed using friable type of Hevea stem callus. Capacity of these cell suspensions to grow on solid media was visible but with no embryoid formation.

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