This investigation was undertaken to study the effect of nutrition, auxins and antimetabolites on rooting of etiolated stem segments of *Populus nigra* and *Salix tetrasperma* under varying light and temperature conditions.

Stem segments of desired size and type were taken from etiolated branches that developed on 15.0 cm long stem cuttings planted in the dark. These were cultured either in liquid medium in Petri-dishes (10.0 cm dia.) or in agar in culture tubes with known ingredients under controlled light and temperature conditions. Epiphyllous buds of *Bryophyllum tubiflorum* in experiments 12 to 14 were floated in 10.0 ml of test solution in Petri-dishes (5.0 cm dia.).
Observations on the number of segments rooted and the number and length of roots were recorded at periodic intervals till 21 days. Observations on the changes in the anthocyanin content, on the size, shape and characteristic of callus and on the growth of secondary roots and the length of the first internode of epiphyllous buds were also recorded in some experiments. Besides these, the biochemical changes in the contents of water soluble and acid hydrolysable carbohydrates, total amino acids and proteins and the contents of DNA and RNAs were also estimated at different stages of root initiation.

In all 22 experiments are described. While experiments 1 to 7 were conducted to study the role of segment size, leaves and apex as well as the effect of glucose and auxins in rooting stem segments under varying light and temperature conditions and experiment 8, the effect of different carbohydrates; experiment 9 was undertaken to estimate starch content in relation to hydrolysing enzymes. Experiment 10 was designed to study the effect of different nitrogenous compounds and experiment 11, the effect of GA₃ on rooting. In experiments 12 to 20, studies on the effect of different metabolic inhibitors such as FUDE, FU, actinomycin-D and cycloheximide in rooting, were made. The metabolic changes
involved in the initiation and development of adventitious roots were studied in experiments 21-22, using both *Populus nigra* and *Salix tetrasperma* as plant materials.

The results are as follows:

(1) The rooting ability increases with the size of the segment: More and longer roots being produced on 10.0 cm long segments (experiment 1).

(2) Leaves and apex increase rooting on stem segments: 2.5 cm long segments of *Populus nigra* with excised leaves and apex did not root but a few with intact leaves rooted in water. The number of rooted segments and roots with intact leaves increased in other test solutions and more so when both leaves and apex were left intact (experiment 2).

(3) The effectiveness of auxins varies with nutritional level and a correct balance between nutrition and auxin is essential for optimal rooting: Rooting did not take place on 2.5 cm leafless segments in water or auxin alone but occurred in glucose. The number of rooted segments and the number and length of roots increased with the concentration of glucose up to 0.5%, but decreased beyond it. An addition of 1.0 mg/l IAA inhibited rooting at 0.01% glucose, was ineffective at 0.1% and stimulated it
at higher concentrations of glucose which were inhibitory when used alone (experiment 2).

(4) The optimal response of auxin with a given level of nutrition varies not only with its concentration but also with its nature: With 0.5% glucose rooting of *Populus nigra* segments was maximum with 1.0 mg/l IAA or 0.5 mg/l IBA, IBA being stronger than IAA but 1.0 mg/l IBA added to 0.5% glucose inhibited rooting completely (experiment 3). Roots were produced on a few segments with 1.0% glucose. The number and length of roots increased with the addition of auxin, the stimulation increasing with concentration and was stronger with IBA than with IAA. With 10.0% glucose, rooting occurred only with as high a concentration of IBA as 1.0 mg/l (experiments 4 to 6).

With 0.1% glucose rooting of etiolated stem segments of *Salix tetrasperma* was maximum with 0.1 mg/l IAA or IBA; it decreased when the concentration of auxin was increased to 1.0 mg/l. With 1.0% glucose the most effective concentration of auxin was 1.0 mg/l, 5.0 mg/l IBA being inhibitory (experiment 7).

(5) Callus formation and root initiation are independent phenomena and the auxin-nutrition balance required for optimal growth of the two varies: The maximum number of
of roots were produced on segments cultured in 1.0% glucose + 1.0 mg/l IBA while the maximum amount of callus was produced with 5.0% glucose + 5.0 mg/l IBA.

(6) Light and temperature influence rooting by affecting the balance between auxin and nutrition: More profuse rooting occurred in the dark than in the light (experiments 1 to 5). 5.0 mg/l IAA balanced with 1.0% glucose at 30°C but with as low a concentration as 0.1% glucose at 15°C for optimum production of roots (experiment 6).

(7) Rooting response varies with the type of carbon source: Ribose and sucrose in addition to glucose also caused rooting of etiolated stem segments, sucrose being more effective. The addition of IAA or IBA in the medium increased rooting further. Starch also induced rooting especially in combination with auxins (experiment 8). Starch was hydrolysed by enzymes that leached out of the segments and auxins enhanced their activity (experiment 9).

(8) Nitrogenous compounds are other nutritional factors that should be provided in adequate quantities together with carbohydrates for optimal production of roots: Serine and tryptophane induced rooting of etiolated stem segments and the number of segments rooted and roots increased when
glucose was added in the medium (experiment 10).

(9) GA₃ antagonises auxin-induced stimulation of rooting and the effect increases with concentration (experiment 11).

(10) Auxin-induced root stimulation and GA₃-caused shoot growth is regulated by certain specific type of proteins: While darkness and GA₃ stimulated the elongation of first internode but inhibited the production of roots, IAA inhibited elongation but promoted the production of roots on epiphyllous buds of *Bryophyllum tubiflorum*. Cycloheximide applied even for 4 hrs inhibited both the production of roots as well as internodal elongation. Even pre-treatments of epiphyllous buds with IAA or GA₃ for 8 hrs failed to alleviate the inhibitory effects of cycloheximide (experi­ments 12 to 14).

(11) Fresh nucleic acid and protein syntheses are required for the initiation of adventitious roots on segments: FUDE, FU, actinomycin-D and cycloheximide all inhibited rooting on stem segments of *Populus nigra* and *Salix tetrasperma* (experiments 15 to 20) except FU which was in effective in inhibiting rooting on stem segments of *Salix tetrasperma* (experiment 16).

(12). The effectiveness of antimetabolites varies with the level of auxin and nutrition in the tissue: The
inhibitory effect of antimetabolites on rooting was more marked in glucose alone than in glucose + IAA/or + IBA.

(13) There exist a relationship between root initiation and changes in the contents of carbohydrates, amino acids, proteins and nucleic acids. The production of adventitious roots is dependent upon the size of the protein pool in the tissue. The water soluble sugar content was high up to 48 hrs to decrease rapidly by 72 hrs in segments cultured in glucose + IBA where rooting occurred but did not change much in segments cultured in cycloheximide alone or together with glucose + IBA where rooting did not take place (experiment 21). The total amino acid, protein, DNA and RNA contents of segments grown in glucose + IAA/or IBA where rooting occurred were markedly higher than in those cultured in antimetabolites in the medium where rooting did not occur (experiments 21 and 22).

The results have been discussed in the light of recent literature available on the subject.