MATERIAL AND METHODS

Scope of experimentation and source of plant material

Seven experiments were carried out in all. Experiment 1 was designed to study the effect of polarity on rooting response of stem cuttings of *Populus nigra* L. var. Lombardy, *Salix tetrasperma* Roxb., *Ipomoea fistulosa* Mart ex Choisy, *Hibiscus notodus* Jaba., *Dalbergia sissoo* Roxb. and *Delonix regia* Raf. (Gold Mohar) and experiment 2 that of different auxins in relation to polarity. Experiments 3 and 4 were conducted to study the effectiveness of auxins in rooting in relation to polarity when the cuttings were exposed to varying light conditions and seasons respectively and experiments 5 and 6 to study the effect of morphactin.
on rooting stem cuttings. An attempt to determine and identify some factors that may be responsible for rooting stem cuttings of these species has been made in experiment 7.

**Preparation of stem cuttings**

One-year old branches of more or less uniform size cut from trees growing near the Panjab University Campus were brought to the laboratory and were made into 15 cm long cuttings after removing their apical parts and leaves.

**Preparation of stock solutions**

Stock solutions of indole-3-acetic acid (IAA), indole-3-butyric acid (IBA), 2,4-dichlorophenoxyacetic acid (2,4-D) and naphthaleneacetic acid (NAA) were prepared by dissolving the weighed quantity of each chemical in 1.0 ml alcohol and then making the volume to one litre by adding distilled water. The stock solution of morphactin (chlorflurenol; CFL) obtained from E. MERCK, Darmstadt, West Germany containing 80% methyl ester of 2-chloro-9-hydroxyfluorene-9-carboxylic acid (CFL), 20% of mixture of 9-hydroxyfluorene-9-carboxylic acid (methylester) and lesser amounts of 2,7-dichloro-9-hydroxyfluorene-9-carboxylic acid (methyl ester) was prepared by dissolving 40 mg of the chemical in about 1.0 ml acetone and then making the volume to 100 ml with hot distilled water to make 40 mg/l.
solutions of requisite concentrations in each case were prepared by diluting the stock solution with distilled water whenever desired.

**Treatment of stem cuttings**

Stem cuttings were treated with auxins or morphactin at the basal or the apical ends for 24 hours and were then planted erect or inverted in distilled water contained in thick-walled glass specimen tubes (10 x 4 cm). The water was changed on alternate days. These treatments were given either initially, or after 2, 4, 6 days as in experiment 6.

**Light treatments:** Stem cuttings after planting were exposed to natural conditions of temperature and photoperiod (Figs. 1 and 2) at Chandigarh unless otherwise stated. In experiments 3 to 5 the cuttings were exposed to continuous light and continuous dark.

**Observations**

**Morphological:** The number of cuttings rooted, number and length of roots produced, and callus formation were recorded at regular intervals in all experiments. The records of the number of buds that sprouted, their length and the number of leaves per branch and their size were also taken in experiments 6 and on the sprouting of buds in experiment 4.
Figs. 1-2. Temperature (Fig. 1) and day length (Fig. 2) conditions prevailing at Chandigarh during January-December (1969).
Developmental: Visual observations on the morphophysiological status of branches of the mother plants were taken in experiment 4 before each trial. These trials were carried out in April, August and December.

Anatomical: For observations on cambial activity and the development of pre-existing root primordia, the lower basal treated portion of stem cuttings were fixed in 1:3 aceto-alcohol. Sections 30 μ in thickness were then cut with a sliding microtome, were passed through alcohol-xylol series for dehydration and were stained with safrannin-fast green.

Activity of hydrolysing enzymes: The basal 2-3 cm of each cutting weighing approximately 1 g was thoroughly washed and crushed with 10 ml cold distilled water. The extract was centrifuged at a speed of 2000 g for 15 minutes and the supernatant was collected and its volume was made to 20 ml. This crude extract was stored in a refrigerator at 15°C till used.

Ten ml of 0.1% starch sol along with 5 ml citrate phosphate buffer at pH 7.0 was taken in 50 ml Corning test tube. Five ml of plant extract was added to it and the reaction mixture was incubated at 30°C for 24 hours. After incubation the reaction was stopped by adding 2 ml of 1N HCl. The optical density of the solution was measured.
colorimetrically using a yellow filter after adding 0.2 ml of $I_2KI$ solution (6% $KI$ and 0.6% $I_2$ v/m). The amount of starch hydrolysed was determined by comparing the optical density of the mixture against the standard curve of different concentrations of starch.

**Extraction and determination of endogenous factors**

For the determination of the levels of endogenous regulatory substances, 500 gm of fresh cuttings before planting and at the time of rooting were crushed with peroxide-free ether and stored in refrigerator at 0°C for extraction. After 24 hours of extraction, the extract was used for bioassay as such after evaporation or processed into acid, alkaline and neutral fractions by the method of Dannenberg and Liverman (1957) as modified by Vieties et al. (1964, 1966). The procedure is given as a flow sheet diagram in Table I.

**Mungbean bioassay of the extracts**

Uniform and healthy seeds of *Phaseolus mungo* var. Simla were selected and were sown on moist cotton in Petri-dishes (10 cm dia.) in continuous light (1900 lux) provided by three florescent air-tubes hung on a light bench in an air-conditioned room at a temperature of 28 ± 1°C. After a week, when the seedlings had elongated to about 7-8 cm
above the cotyledons, these were selected for uniformity of size and thickness of epicotyl and hypocotyl. The hypocotyl portion below the cotyledons was cut to 3.5 cm and the seedlings with about 7-8 cm of epicotyl portion with a pair of primary leaves left intact but with cotyledons excised, were taken and treated with different concentrations of extracts or their fractions singly or in combination with auxin or sucrose in specimen tubes (5 x 1 cm) for 24 hours. These were then transferred to distilled water contained in specimen tubes (5 x 1 cm) under continuous illumination (1900 lux) at the above temperature. Observations for the number of hypocotyls rooted, number of roots and their length were recorded after 7 days.