Pakistan J. Sci. Ind. Res., Vol. 15, No. 3, June 1972

STUDIES IN ROOT-PROMOTING SUBSTANCES

Part I. Rooting Activity of Poplar Stem Cuttings

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(Received August 31, 1971)

Abstract. Alcoholic extracts of poplar stem cuttings were fractioned by descending paper chromatography and assayed by mung bean seedlings. Seven zones of different colours and R_f values were detected and some of the substances were found to be active in root inition and promotion.

Apart from normal production of roots from seeds, roots may also be developed from other parts of the plant, i.e. stems, leaves and buds. Stems are generally considered an ideal rooting part of the plant as they have undifferentiated tissues in sufficient quantity to be easily differentiated into root primordia. Various workers [2–8,12] have also reported that specific root forming substances are accumulated near the basal cut surface of the stem cuttings. These substances being formed in the leaves and move towards the base of the stem. Von der Lek[13] was first to report that the existence of buds was also responsible for root-forming substances which are transported in a basal direction and stimulate pericyclic division. For this reason, stems are generally used for vegetative propagation.

The rooted stem, growing into a new plant is a valuable key for propagating economic and medicinal plants. Stem cuttings of some plants are easy rooters while others are difficult-to-root. It is also now known that root initiation is governed by specific naturally occuring rooting hormones.[1,4,5,6,7,10,11] Such substances or hormones are believed to occur in easy-to-root cuttings while scarce in difficult-rooters. It is not surprising then, that those substances or hormones extracted from easy-to-root cuttings can stimulate rooting in difficult-to-root cuttings if applied exogenously.[8]

For our present studies we have selected *Populus alba* stem which grows very vigorously and is very easy to propagate vegetatively.

Vegetative propagation is also important as by this method a great many genetically identical plants may be made from a single individual and a desired genetic pattern (as a seedless orange, a flower of new colour or a fruit with certain flavour and quality) may be preserved from generation to generation. In the case of plants produced from seeds two parents are involved and some of the inferior characters of either of the parent plant may appear in the next generation.

Another advantage which can be derived from such induced root initiation is that the plants will mature much earlier than seed-propagated individuals. This time factor will considerably reduce the cost of production of various crops. By this method it will be possible to dispense with the practice of grafting, budding etc. of those plants which are raised from seed and then grafted with better varieties (mango, etc. citrus)

Materials and Methods

Soft-wood stem cuttings of popular were collected from the experimental farm of our Laboratories during the period when root formation was very active and buds were renewed. These cuttings were placed in deep freezer for 24 hr immediately after collection. The freezed soft-wood cuttings were crushed in mortar and extracted twice in absolute methanol at room temperature. These extracts were combined, filtered and concentrated to a final volume under reduced pressure. Root-promoting substances were separated by descending paper chromatography. The concentrated extract was applied as a streak on wide strip of Whatman No. 3 MM chromatographic paper. Chromatograms were developed unidirectionally with isopropanol–water (4:1, v/v) as a solvent, at room temperature until solvent front was about 25 cm from the origin. Dried chromatograms were examined under UV lamp with ammonia fumes and then cut into 10 transverse strips, each corresponding to 0.1 R^f unit.

For qualitative determination of root-initiating and root-promoting activity of the substances extracted from popular cuttings; we employed the mung bean bioassay technique as described by Zimmerman.[9] (During biogenesis of auxins, IAA is formed through the primary precursor tryptophan, after a series of oxidative processes. In metabolically active tissues, enzyme system is involved for this conversion.[3] There are plant species which cannot utilize tryptamine in the formation of IAA due to lack of enzymes or oxidase inhibitors. The mung bean seedlings cannot produce IAA from tryptamine, therefore, it was selected for the bioassay). Mung bean seeds were washed thoroughly in absolute alcohol, and sowed in petri dishes on water-saturated filter paper. These petri dishes were placed in seed germinator at 20°C. After 6 days of 12 hr photoperiod the primary leaves were fully developed and first trifoliate leaf was in bud condition. Cutting of the seedlings were taken at 3 cm below the cotyledonary node. Five of such mung bean cuttings were placed in petri dishes, which also contained the chromatograph sections and distilled water. The number of roots on each cutting was counted after eight days and averaged.

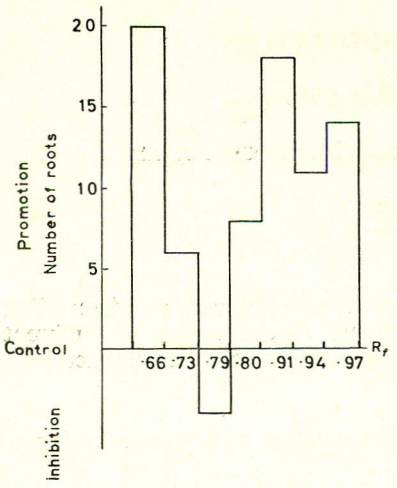

Fig. 1. Histogram showing root promotion and nhibition activity by different substances of poplar stem extract.

Results and Discussion

Seven zones of different colours appeared on chromatogram of the extracts of popular soft-wood stem cuttings as viewed under UV light. The R_f values of all these colour zones were calculated and are as follows: 0.66 faint blue, 0.73 greenish blue, 0.79, green, 0.80 blue, 0.91 dark blue, 0.94 pale yellow and 0.97 pinkish violet.

The root initiating ability of the cuttings showed that there are many substances in popular twigs to stimulate root initiation in the mung bean cuttings. A zone of inhibition was also detected. The extracts and chromatograms were repeated five times to check the consistency of the results.

The rooting activity in the mung bean bioassay of extracts from poplar cuttings is expressed by histograms (Fig. 1). The columns above the horizontal line indicate promotion of rooting while columns below the line show inhibition of rooting. In all the control experiment there were no rooting of mung bean cuttings.

The most active zone of the chromatogram, where maximum rooting occurred is faint blue R_f 0.66. Three cuttings have 4 roots each and 3–5 roots on the other two cuttings. The root initiation is at the basal portion of the mung bean cuttings and is regular. Total No. of roots is 20.

Greenish blue zone shows lesser root initiating activity. Only two cuttings out of five have small roots. The total number of roots per cutting is 3 only. Which means 6 roots on the whole.

Next zone with the green colour and of R_f 0.79 completely inhibited the rooting. There is almost no initiation.

Blue portion of the chromatogram, R_f 0.80, showed slightly active substance on it. There are 8 roots on two cuttings, 4 roots on each while three cuttings remain rootless.

The second most active substance which promote root initiation is on the dark blue portion of the chromatogram. Total number of roots is 18. There are 3–5 roots per cuttings.

The pale yellow strip of the chromatogram, R_f 0.94, also show some active substances. The total number of roots is 11, although two cuttings have no initiation at all.

The pinkish violet zone which is near the solvent front, and of R_f value 0.97, is the third best to start rooting. The substances on this strip of chromatogram produced roots on every cutting. The number of roots is 14, 3 roots per cutting except one having 2 roots.

From the above-mentioned results it is clear that there are many substances in the poplar stem which are capable of initiating roots in plants. Aqueous extracts of these substances can be applied exogenously to induce root formation in other plants. Detailed investigations regarding the chemical structure of these substances are in progress.

Acknowledgement. The authors wish to thank Dr. N.A. Malik for valuable suggestions, discussions and correction of the manuscript.

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