An Insight Into Biochemical Basis of Root Formation on Cuttings: A Review©

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INTRODUCTION

A proper balance between auxin and nutrition is required for the production of adventitious roots on stem and the hypocotyl cuttings (Nanda et at., 1971; Nanda, 1975). The seasonal variation in rooting of cuttings has been associated with changes in levels of endogenous growth regulators and metabolites in cuttings; such regulatory processes are controlled through qualitative and quantitative changes in macromolecules, proteins, and enzymes. These macromolecular changes in the rooting zone of the cuttings may provide some of the answers of assessing cellular differentiation into roots. In the present review, I have described some of my past research results with emphasis on the biochemical and molecular changes that have been associated with adventitious rooting in hypocotyl cuttings of plants.

METHODOLOGY

Uniform seeds of *Vigna mungo* (syn. *Phaseolus mungo*) and *Impatiens balsamina* were germinated on sterilized cotton pads in Petri dishes, either under fluorescent light or in the dark. Cuttings were made by excising the roots and 3 cm of the hypocotyl. These cuttings were cultured in test solutions containing auxin, glucose/sucrose as basic media adding 30 μM chloramphenicol to prevent microbial growth. Rooting data was recorded periodically. Sampling for enzymes, proteins, and nucleic acids were also taken periodically. Proteins, RNA, and enzymes were separated electrophoretically (Ornstein, 1964; Davis, 1964).

ROLE OF CARBOHYDRATES AND AUXIN IN ROOTING

Roots were produced on etiolated cuttings cultured in water. Rooting was enhanced further in glucose or IAA + glucose. In contrast, IAA or IAA + glucose enhanced rooting on cuttings grown in the light. The enhancement in root formation by glucose in cuttings without apex and cotyledons made from dark-grown seedlings is indicative that endogenous auxin level was adequate to balance with the exogenously applied nutrition. These results suggest that a proper balance of auxin and carbohydrates is necessary for optimum production of roots (Nanda and Dhaliwal, 1974). Ethanol, methanol, and acetone are used for the extraction of growth substances from plant tissue. The significance of ethanol and other aliphatic alcohols on growth, development and senescence has been reported by various researchers (Gudjonsdottir and Burstrom, 1962). We investigated the effect of varying concentrations of ethanol, methanol, and acetone and their interaction with sucrose and IAA on root formation of etiolated stem cuttings of *Vigna radiata* L. The root formation on etiolated cuttings of *V. radiata* L. was greatly enhanced in the presence of ethanol, methanol, and acetone.

The results indicated that while low concentrations of these organic solvents promoted rooting, high concentrations inhibited it. The results suggest that these solvents act as a carbon source or as solubilizing agent for endogenous auxin in the cuttings (Bhattacharya et al., 1985).

Elevated carbon dioxide concentrations stimulated the production of roots in the presence of IAA and this effect was more pronounced at 675 ppm than at 1000 ppm carbon dioxide (Bhattacharya et al., 1985). The stimulation of rooting in this case indicates that the level of carbohydrates produced in the leaves was adequate to balance with applied auxins.

EFFECT OF METABOLIC INHIBITORS ON ROOTING

It was demonstrated in our laboratory that auxin caused increased rooting and the process involves the synthesis of proteins and the effect is mediated through nucleic acids — DNA and RNA. One of the methods for the elucidation of mode of auxin action is the use of inhibitors of protein and nucleic acid synthesis.

The blockage of the synthesis of macromolecules at any level results in the inhibition of rooting. Studies were performed to decipher the effects of protein and nucleic acid inhibitors on root formation and the associated metabolic changes in the macromolecules as well as in the transients of the molecular forms of RNAs and proteins.

PARADOXICAL EFFECTS OF SOME METABOLIC INHIBITORS

It was repeatedly reported by our research group that some of these metabolic inhibitors stimulated rooting in low concentrations, for example; hypocotyl cuttings of *V. mungo* rooted in water and more profusely in IAA. However, 1 mg•liter^{-1} actinonycin-D + IAA together stimulated rooting considerably (Bhattacharya et al., 1977). The changes in isozyme patterns of peroxidase and IAA oxidase were associated with the initiation and development of roots (Dhaliwal et al.,1974). While some isozymes disappeared, others appeared in etiolated stem segments when the cuttings were cultured in media containing cycloheximide (protein synthesis inhibitor) and actinomycin-D (RNA synthesis inhibitor), both of which inhibited rooting.

MACROMOLECULAR CHANGES ASSOCIATED WITH ROOTING

The DNA, RNA, and proteins were extracted and quantitatively analyzed. Disc electrophoresis was performed following the method of Ornstein (1964) and Davis (1964). The results demonstrated that 5-FUDR (5-fluorodeoxy-uridine), 5-fluroracil (FU), actinomycin-D (Act-D), and cycloheximide (CYCL), all inhibited rooting in high concentrations in hypocotyl cuttings. DNA, RNA and protein contents increased in cuttings that produced roots (water, IAA, glucose, glucose + IAA, glucose + IAA + low concentration of antimetabolites at 24 h and 72 h, decreased at 48 h and 96 h. These results indicate that metabolic changes occur in two phases during rooting. Phase I (0-24 h) being correlated with root initiation and Phase II (48 to 96 h) with root development.

Biochemical analysis indicated that rooting potential is closely associated with the protein pool that is available during Phase I and thus, lends support to the postulate that the magnitude of rooting is determined by the size of the protein pool that is available in the tissue at the time of root initiation. The metabolic inhibitors, namely FUDR, Act-D, CYCL and FU inhibited rooting and that protein synthesis is involved in the formation of adventitious roots and this effect of IAA is mediated through multiplication of DNA or production of m-RNA or both.

TRANSIENTS IN PROTEINS AND NUCLEIC ACIDS DURING ROOTING

Disc-electrophoretic studies reveal that nine bands of RNA could be observed initially in the cuttings. Two distinct bands of RNA (A, B) developed in water, IAA and glucose within 24 to 48 h but did not develop at all in IAA + glucose + Act-D cultures. Two other RNA species (C and D) appeared in the same cultures during 48 to 72 h, which corresponded with the development of root initials. The fact that these new bands were located at the lower potion of the gels indicated that these were low molecular weight RNAs, either messenger or tRNA. Another RNA of ribosomal type appeared only in cultures containing Act-D, which is a potent inhibitor of DNA-dependent mRNA synthesis.

The disc electrophoretic studies of proteins reveal that six new protein bands (A, Z, L, M, Q, and J) appeared in water, IAA, glucose, and IAA + glucose. All these proteins appeared in glucose at 24 h, in IAA + glucose A, Z, and L appeared at 24 h and M, Q, and J at 48 h. While some more proteins appeared in glucose at 24 h in IAA treated cuttings, others appeared at 72 h. It is interesting that one or more of these proteins failed to develop in media containing metabolic inhibitors. Two new bands (Q and M) developed in CYCL at 24 and 96 h. Another new band appeared in media containing FU, FUDR (Bhattacharya et al., 1991).

These results conclusively demonstrate that qualitative and quantitative relationships exist between the transients in proteins and nucleic acids and the magnitude of rooting. The nucleic acid and proteins associated with root initiation are different from those associated with their development and also that some of the nucleic acids and proteins are associated with suppression rather than in the formation of roots (Bhattacharya et al., 1991).

ROLE OF NUCLEIC ACIDS IN ROOTING

Exogenously supplied nitrogenous bases in combination with IAA + sucrose hastened and enhanced rooting on hypocotyl cuttings of *P. mungo*. Bases alone promoted rooting only slightly. These results suggest that the effectiveness of exogenously supplied nitrogenous bases is determined by the available auxin and carbohydrates. The enhancement of root formation may be caused by increased incorporation of these bases into nucleic acids, increasing the rate of turnover of proteins needed for rooting (Bhattacharya et al., 1978).

Ribose and 2-deoxyribose are sugar moieties (pentose sugars) of nucleic acids. We wanted to study the effect of ribose and 2-deoxyribose on rooting. These results indicated that there was a pronounced stimulation in rooting in combination with IAA and sucrose on hypocotyl cuttings of *P. mungo*. The enhancement of roots with pentose sugar in presence of IAA and sucrose indicates that its effect was mediated through multiplication of DNA, RNA, and proteins. These were novel findings and were reported for the first time (Bhattacharya et al., 1976).

These results were further supported by the findings that exogenously supplied DNA and RNA hastened root initiation and also increased the formation of roots on hypocotyl cuttings of *Impatiens balsamina*. Nucleic acid-caused enhancement in root formation was appreciably increased by IAA. In combination with lower concentrations of nucleic acid, it even stimulated the growth of roots as well as of hypocotyls (Bhattacharya et al., 1976).

PROPOSED MODEL

Rooting is mediated through a chain of biochemical reactions localized in the nucleus and cytoplasm of the cell. It appears that genetic programming which controls rooting depends on the interaction effect of external and internal factors. Thus, variations in rooting of cuttings have been associated with changes in levels of endogenous growth regulators and metabolites in cuttings and sometimes the stock plants. Such regulating processes are controlled through qualitative and quantitative changes in enzymes, proteins and other macromolecules. Qualitative and quantitative changes in macromolecules prior to rooting have been determined in many plant species. We postulate that a specific protein pool size and de-novo synthesis of mRNA is necessary for rooting. IAA acts as a trigger at the transcriptional level, carbohydrates as C-source/nutrition to regulate translation, pentose sugars and nucleic acid bases as moieties needed for the synthesis of RNA and DNA; and amino acids and phenolic precursors for synthesis of specific proteins. The fact that the enhancement of root production by either pentose sugars or nucleic acid bases is caused only in the presence of IAA and sugar clearly indicates that the effect of these precursors is through the multiplication of DNA, RNA, and proteins. This was confirmed when DNA, RNA, and protein content increased significantly in the tissues in hypocotyl cuttings cultured in IAA + sucrose + nucleic acid bases during 0 to 12 h and 18 to 24 h. The mechanism for converting all bases and nucleosides, produced by hydrolysis of nucleic acids, back into nucleosides is termed as "Salvage Pathway" (Kornberg, 1974).

Salvage of purine and pyrimidine bases and nucleosides produced from hydrolysis of nucleic acids become vital for the plant when there is inadequate de novo synthesis of nucleotides. The utilization of precursor pools for nucleic acid anabolism

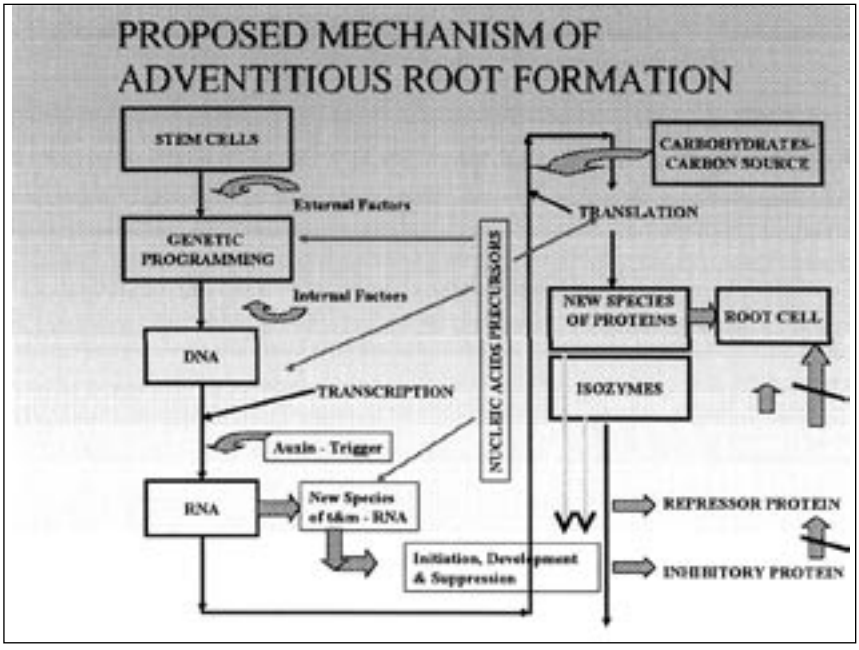

Figure 1. Proposed mechanism of adventitious root formation.

during rooting is indicated in our studies (Bhattacharya et al., 1976a, 1976b). In the latter tests, applied DNA and RNA may have been hydrolysed to their respective purine and pyrimidine bases and pentose sugars, which contributed to precursor pools for subsequent nucleic acid anabolism.

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