Adventitious Root Initiation—Future Research on the Site of Auxin Action

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INTRODUCTION

It has been shown many times under controlled conditions that auxins are the applied phytohormones which consistently enhance adventitious root production. Indeed, research has shown that division of the root initial cells is dependent upon either applied or endogenous auxin (Hartmann et al. 1990.) In a recent review, Jarvis (1986) summarized the evidence supporting the idea that auxins have a central role in the initiation and development of rooting. However, even though auxin involvement in adventitious root initiation is well established, knowledge of the mechanism of auxin action in adventitious root initiation remains unclear (Blakesley et al., 1990). It is important to understand the mechanism of auxin action for the propagator as well as the basic scientist. This paper deals with experiments performed in our laboratory on the regulation of adventitious root initiation in mung bean (*Vigna radiata* [L.]R. Wilcz).

MATERIALS AND METHODS

Plant Material and General Procedures. Mung bean seeds were surfaced sterilized in 10% Clorox (v/v) for 10 min and rinsed in tap water. After aeration for 24 h in tap water, they were sown 1 cm deep in plastic trays containing perlite. The growth room was maintained at 26±1C. A 16-h photoperiod was supplied at a quantum flux density of approximately 205 $\mu E/m^{-2}/s^{-1}$.

Uniform cuttings made from 9-day-old seedlings were placed in sterilized distilled water prior to use. Each cutting consisted of a 3-cm hypocotyl, the epicotyl, two primary leaves, and the apical meristem. Ten cuttings were placed in a 19 × 65 mm shell vial containing 1 ml of the treatment solution. After uptake of the various solutions (approx. 2 h), distilled water was added to the cotyledonary node and maintained at this level for the duration of each experiment.

DISCUSSION

Inhibition of Adventitious Root Formation by Transcriptional and Translational Inhibitors in Mung Bean. As mentioned above auxin appears to be the phytohormone that consistently stimulates rooting. In mung bean, the synthetic auxins IBA and NAA are more effective and promote adventitious root formation between a concentration range of 10^{-7} and 10^{-3} molar (Geneve and Heuser, 1982) (Fig. 1); 2,4-D is less active than the other auxins tested while IAA, the native auxin, is not as active as NAA and IBA possibly because IAA is metabolized (Hess, 1965) or converted to various conjugated forms (Norcini and Heuser, 1985).

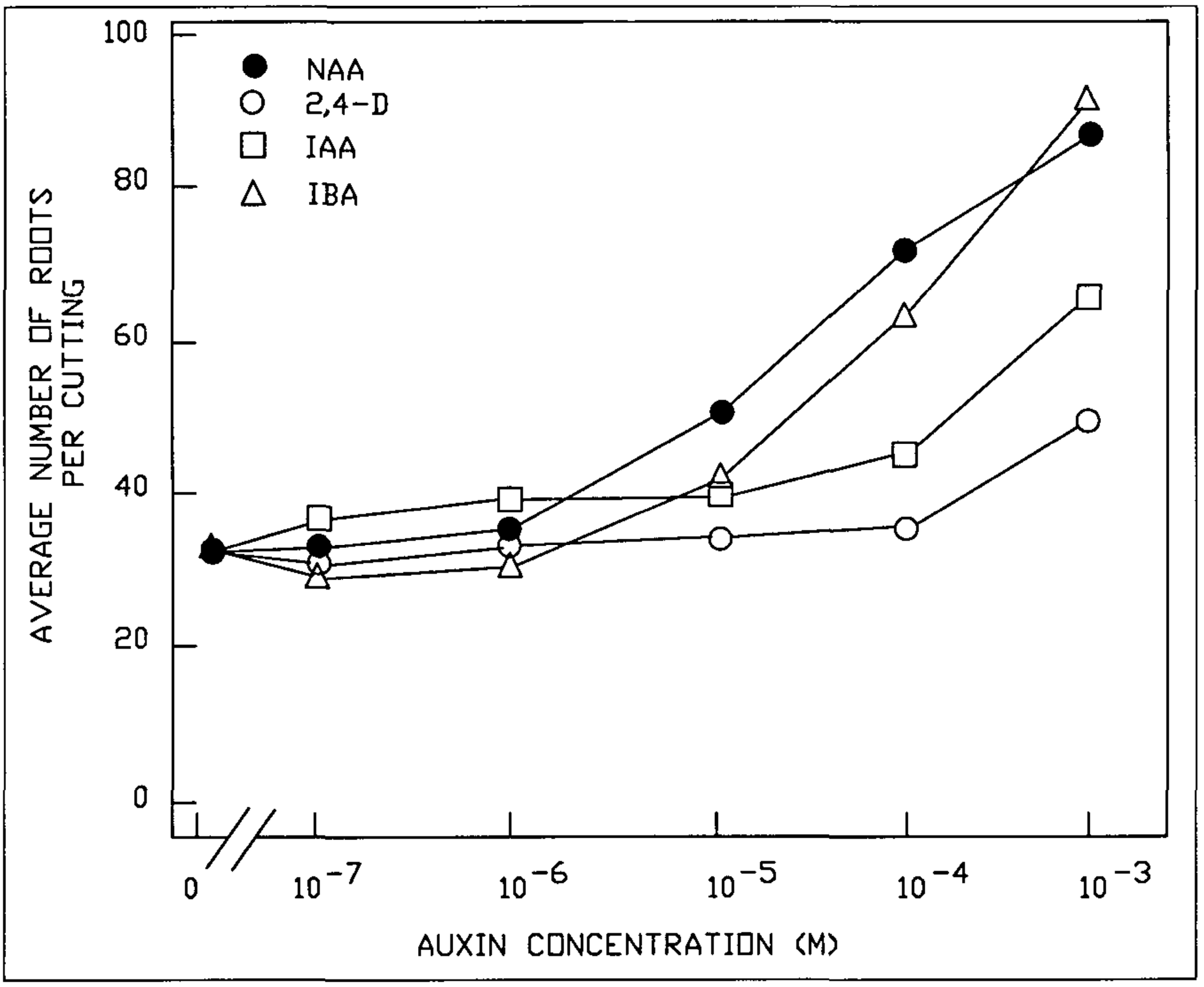

Figure 1. The relative effectiveness of IAA, IBA, NAA, and 2,4-D on adventitious root initiation in mung bean. (From Geneve and Heuser, 1982)

The process of root formation involves both cell division and enlargement, and therefore appears to be dependent on synthesis of nucleic acids and proteins. Because it is likely that the process of root formation is dependent upon the synthesis of nucleic acids and proteins, chemicals which interfere with or block nucleic acid (transcriptional) and/or protein (translational) synthesis should inhibit rooting. Indeed, in mung bean, the exogenous application of 6-methylpurine inhibits root formation presumably due to the production of defective mRNA or due to the inhibition of mRNA synthesis (Blazich and Heuser, 1981). At a concentration of 10^{-5} M or higher 6-methylpurine inhibited rooting with complete inhibition and no obsevable injury to the cuttings occurring at 6×10^{-5} to 10^{-4} (Fig. 2). Cycloheximide, a putative inhibitor of ribonucleic acid (RNA) synthesis, first inhibited rooting at 10^{-6}M which increased with concentration, the cuttings being killed at 10^{-4}M (Fig. 3).

In *Phaseolus vulgaris* (green bean)a study of early biochemical changes during root initiation showed that an increase in total RNAs and poly(A)+RNA synthesis were detected only 2 h after transfer of IBA-pretreated hypocotyls to fresh basal medium (Kantharaj et al., 1979).

In mung bean hypocotyls, the phloem parenchyma cells are the site of adventitious root initials. These "rooting-zone parenchyma" [R-ZP] were shown by Tripepi et al. (1983), who used ^{3}H-uridine and ^{3}H-thymidine as indicators of nucleic acid synthesis that nuclei in the R-ZP first became labeled with ^{3}H-uridine (2 h, nucleolus) and then with ^{3}H-thymidine (14 to 16 h). By 8 h, ^{3}H-uridine was found

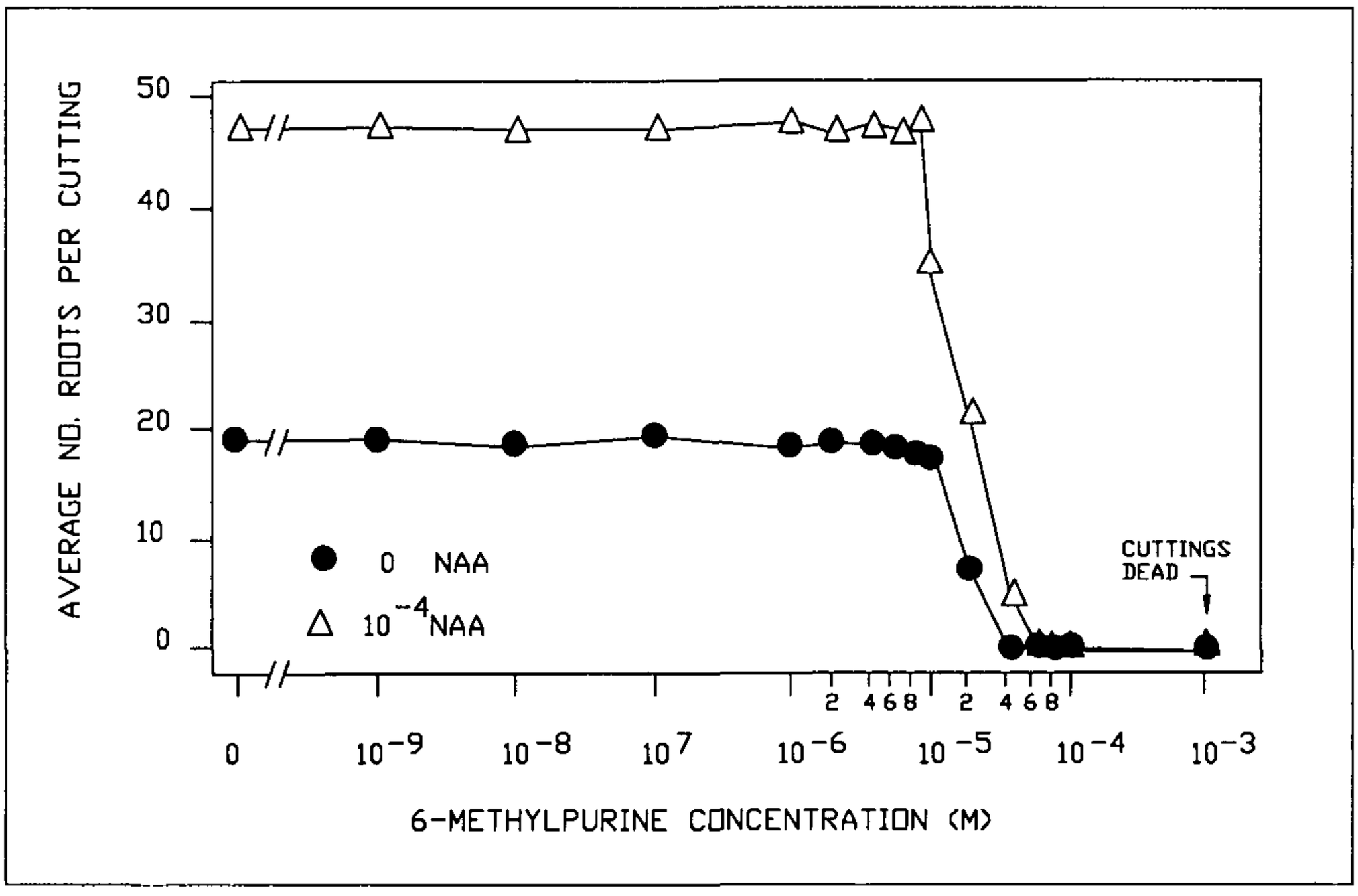

Figure 2. Effect of 6-methylpurine on adventitious root initiation in mung bean cuttings. Each point is the mean for 30 cuttings. (Redrawn from Blazich and Heuser, 1981).

Figure 3. Effect of cycloheximide on adventitious root initiation in mung bean cuttings. Each point is the mean for 30 cuttings. (Redrawn from Blazich and Heuser, 1981).

in the cytoplasm and cell wall. ^{3}H-thymidine incorporation was in close agreement with the first cell divisions (23 to 26 h). It therefore appears that the effect of auxins on adventitious root initiation may be associated with early transcriptional and translational events during the initiation phase.

Future Studies in Root Initiation. The above examples are suggestive that auxin works through its effects at the transcriptional and translational levels. Gene expression in plants (Theologis, 1986; Guifolyle, 1986; Key, 1989) have been shown to be regulated by auxin. However, there are no research reports on the isolation of auxin-responsive genes from any adventitious rooting systems, although auxin-induced or auxin-repressed genes have been isolated and characterized for responses such as cell division, cell elongation, and fruit ripening.

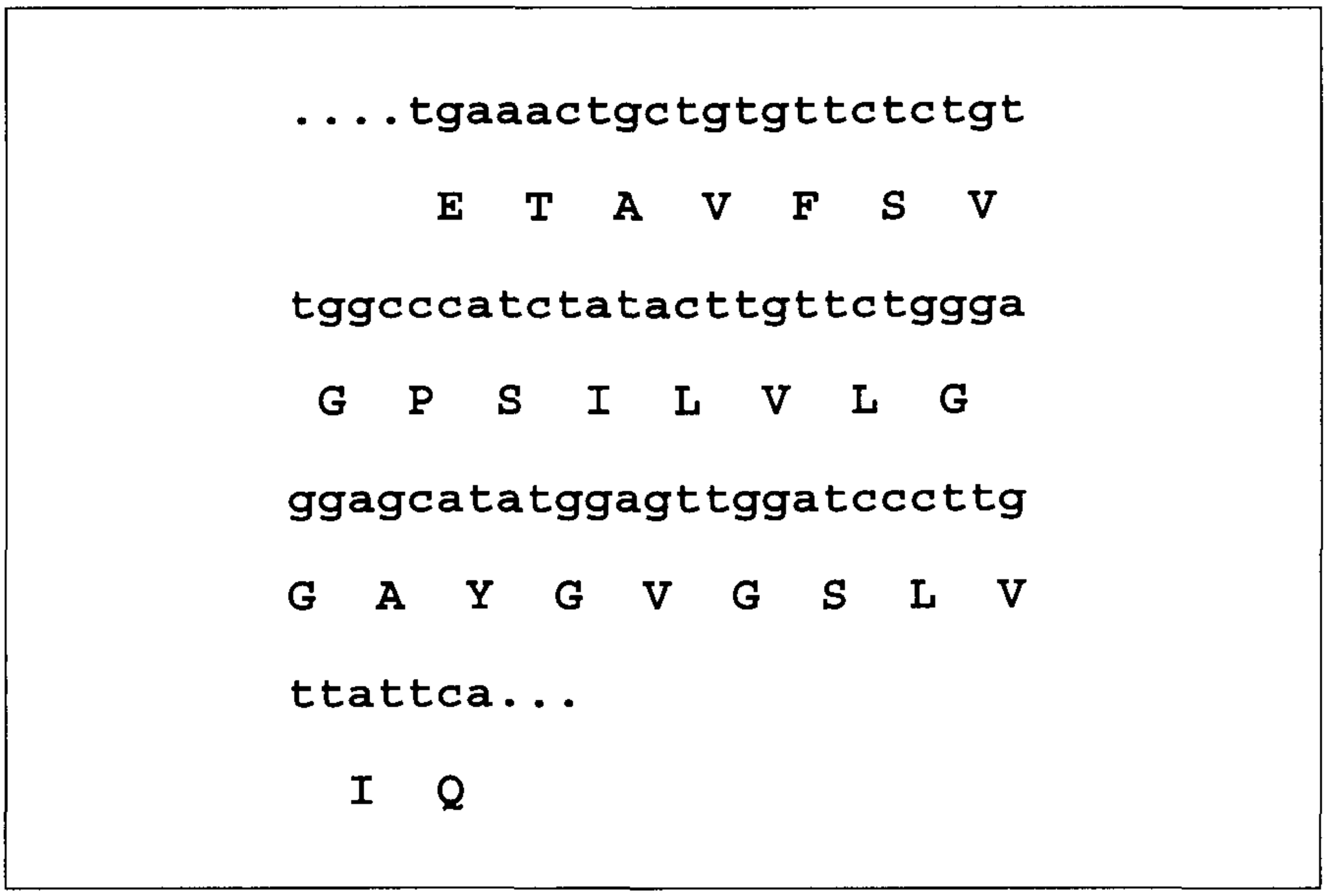

Figure 4. An example of a short nucleotide sequence and deduced amino acid sequences for cDNA clone.

Future molecular studies on the mechanism(s) of auxin action in root initiation will focus on the accumulation of specific mRNAs (messenger ribonucleic acids) in the early phases of rooting. To fully understand the rooting process, the isolation and characterization of specific genes which determine root initiation will be required. Such molecular studies will entail the construction of a cDNA library from auxin-induced adventitious rooting systems and the isolation of specific auxin-regulated cDNA clones by screening and subsequent sequence analysis. Such studies will lead to the construction of nucleotide sequences and deduced amino acid sequences. An example of part of such a sequence is shown in Fig. 4.

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