METABOLISM DURING ADVENTITIOUS ROOT PRIMORDIUM INITIATION AND DEVELOPMENT

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ABSTRACT

The review compiles and discusses literature concerning the metabolism of carbohydrates, nitrogen, nucleic acids, and proteins during adventitious root initiation and development. In addition, the review includes discussion of approaches to the study of metabolism during adventitious root initiation, and proposes potentially productive areas for future research.

INTRODUCTION

The present review concerns metabolism associated with the initiation and development of adventitious root primordia.[1] Influences of hormones, particularly auxins, on metabolism in propagules have been included because root primordium initiation and development are, in part, hormonal responses, and because metabolic studies have frequently included hormonal treatment effects. In general, the scope and depth of the review are limited because many topics that concern the metabolism of inorganic and organic compounds during root primordium initiation and development have not been investigated.

Enzymes catalyze the reactions of metabolism, and shifts in the type and activities of enzymes thus vary metabolism and development. Development mirrors metabolism, which supplies essential organic molecules (including enzymes) and energy. Development does not occur apart from metabolism, and, as a corollary, developmental stages probably result from particular, experimentally demonstrable patterns of metabolism.

The controls of metabolism also control development. Therefore, developmental studies have increasingly explored metabolism for applicable trigger, modulating, and braking mechanisms. As yet, however, it remains obscure whether some aspect of metabolism directly senses primary developmental stimuli, or whether varied metabolism occurs secondarily, as a response to a lower order stimulus (Kahl, 1973). Such lower order stimuli presumably occur in the cascade of physiological and biochemical responses to the elusive primary stimulus or stimuli.

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[1] See Haissig (1974) for definition of "adventitious root primordium initiation", "adventitious root primordium development", and related terms.

There are many direct approaches to the investigation of metabolism, such as applying radioactive substrates to tissue and tracing the pathways and rates by which radioactive atoms become incorporated into products. Keying such radiotracer data to developmental anatomy directly links metabolism and development. Less direct studies have attempted to evaluate the metabolic status by treating tissues with various metabolites or inhibitors, and noting the developmental response. Such studies often yield ambiguous results but the present review includes some of the pertinent results.

CARBOHYDRATES

Starch Hydrolysis and Free Sugars

Root initiation requires energy. Degradation of carbohydrates via the EMP-TCA[2] or PP pathways constitutes the likely source of the energy in cuttings, simply because other energy sources, such as lipids, prevail less generally and abundantly in stems. Many investigators have, in fact, positively related carbohydrate levels in cuttings with root primordium initiating ability (Reid, 1924a, 1924b; Schrader, 1924; Starring, 1923; Winkler, 1927), and similar observations have been made concerning bud initiation (Thorpe and Murashigi, 1968). However, cuttings with high carbohydrate levels may not initiate root primordia (Brandon, 1939) because of overriding physiological factors. Nanda and coworkers (Nanda, Anand, Kochhar and Jain, 1971) reported, for example, that *Hibiscus rosa-sinensis* cuttings exhibited the highest natural rooting ability in June, when the cuttings contained less starch than in other months of the year. IBA treatment of cuttings erased the inverse seasonal relation between root primordium initiating ability and starch content. Thus, seasonal variation in endogenous auxin levels, or in factors related to auxin metabolism, apparently overrode the often noted positive relation between starch content of cuttings and primordium initiating ability (cf. Nanda and Anand, 1970).

Most commonly, the starch content of the stems of cuttings rapidly declines during root primordium initiation (Nanda, Anand, and Kumar, 1970; Negisi and Satoo, 1956; Schrader, 1924; Smith, Nash and Davis, 1940; Stuart, 1938) and may then increase (Stuart, 1938; Yusufov, Tylik and Akhlakova, 1965).

The free sugar content in the lower stem of cuttings usually increases during the early stages of root primordium initiation and development because of starch hydrolysis and increased basipetal translocation (Smith, Nash and Davis, 1940; Stuart, 1938; Stuart and Marth, 1937). However, starch, where present, apparently acts as the prime and possibly sole carbohydrate source for root primordium initiation and development. The total free sugar content of starch-containing stems, although redistributed, remains relatively constant throughout the rooting period (Stuart, 1938) but starch levels do not, as noted above. Initial starch hydrolosis apparently maintains or nearly maintains the

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[2] The following abbreviations are used throughout: EMP—Embden-Meyerhof-Parnas "glycolytic" pathway; PP—pentose phosphate pathway, also known as the hexose shunt; TCA—ribose-5-phosphate; G-3-P—glyceraldehyde-3-phosphate; Pi—inorganic phosphate; ATP—adenosine triphosphate; NAD—nicotinamide-adenine dinucleotide; NADP—nicotinamide-adenine dinucleotide phosphate; RNA—ribonucleic acid; DNA—deoxyribonucleic acid; IAA—indole-3-acetic acid; IBA—indole-3-butyric acid; NAA—naphthaleneacetic acid; 2,4-D—2,4-dichlorophenoxyacetic acid.

free sugar levels in the early, high carbohydrate demand stage of primordium initiation and development. Starch synthesis prevents increase in the size of the free sugar pool as excess starch hydrolysis, translocation and photosynthesis supply metabolically unneeded free sugar. Altered patterns of sugar translocation and starch deposition appear to occur as a normal wound response that accompanies all cell proliferation (Kupila-Ahvenniemi, 1966).

The free sugar pool declines if cuttings do not contain starch (Stuart and Marth, 1937), but applied free sugars supplant starch hydrolysis only in the absence of starch (Bausor, 1942b), or under conditions of generally insufficient carbohydrate (Bausor, 1942a; Nanda and Jain, 1971a; Nanda and Jain, 1972b; Nanda, Jain and Malhotra, 1971; Nanda, Anand, Kochlar and Jain, 1971). Applied simple sugars have no or an inhibitory effect under conditions of endogenous carbohydrate sufficiency (Lovell, Cobb and Moore, 1971; Lovell, Illsley and Moore, 1972; Moore, Cobb and Lovell, 1972).

Enhanced starch metabolism in cuttings probably results from the direct or indirect effects of basipetally transported IAA. Auxin treatment of tissues markedly enhances starch depletion when starch is present (Alexander, 1938; Bausor, 1942b; Beal, 1940; Borthwick, Hamner and Parker, 1937; Mitchell and Whitehead, 1940; Mitchell, Kraus and Whitehead, 1940). According to Hilton (1966) IAA treatment promotes starch depletion, where starch constitutes a storage carbohydrate, but does not appreciably change disaccharide levels. Sucrose levels decline under the influence of IAA treatment in the absence of starch. Experimental evidence obtained with cuttings generally concurs with Hilton's summary (Bausor, 1942b; Schrader, 1924; Stuart and Marth, 1937).

Unfortunately, very little is known about the mechanism of starch depletion in cuttings or other auxin-treated tissues, even though the influence of IAA on starch hydrolysis was noted 35 years ago (Borthwick, Hamner and Parker, 1937). Stem tissues of rooting cuttings, but apparently not the root primordia themselves, produce starch hydrolyzing enzymes (Molnar and La Croix, 1972a). Auxin-treated cuttings exude starch hydrolyzing enzymes to the extent that exogenous starch can replace glucose as a carbohydrate source for etiolated cuttings (Nanda and Jain, 1972b). However, the hydrolytic enzymes have not been identified.

Generation of Glucose-6-Phosphate

Both the EMP and PP pathways employ G-6-P as the primary substrate. Thus, starch or sugars must undergo preliminary enzymatic conversion. The enzymatic conversions ultimately expend energy in the phosphorylation of glucose. However, the source of the energy differs for various initial substrates and participating enzymes. In two of the energetically most favorable transformations, starch phosphorylase (E.C. 2.4.1.1.) or sucrose phosphorylase (E.C. 2.4.1.7.) produce G-1-P from their respective substrates plus Pi. G-1-P may be readily converted to G-6-P. The phosphorylations involving phosyhorylases do not require ATP, and, therefore, do not lower cellular energy levels.

Glucose may also be liberated from starch by amylases (E.C. 3.2.1.1 through 3.2.1.3) followed by the phosphorylation of glucose by hexokinase (E.C. 2.3.1.1.) or glucokinase (E.C. 2.7.1.2). Kinases phosphorylate glucose at the expense of ATP, which initially lowers the energy currency of cells. Both phosphorylases, and amylases and kinases, may yield the G-6-P needed to support root primordium initiation and development but their individual roles need much investigation because pathways of carbohydrate metabolism seem to hold a key controlling mechanism for root primordium development, and,

possibly, initiation. For example, regulation of TCA cycle activity, as discussed later, may be involved.

The route of G-6-P metabolism deserves consideration because the partial degradation of G-6-P not only liberates energy but also supplies the necessary carbon skeletons for root primordia. The success or failure of root primordium initiation and development depends upon the availability and type of carbon skeletons, in addition to the type and availability of energy.

Metabolism of Glucose-6-Phosphate

Inhibition of aerobic metabolism reduces primordium initiation and development. Primordium initiation and development require oxygen (Turetskaya and Kof, 1965; Winkler, 1927; Zimmerman, 1930) and are prevented by uncouplers or inhibitors of oxidative phosphorylation (Krul, 1968; Wirth, 1960). In addition, both succinate dehydrogenase (E.C. 1.3.99.1) and cytochrome oxidase (E.C. 1.9.3.1) exhibit high activity in developing root primordia (Molnar and La Croix, 1972a). Thus, G-6-P presumably becomes degraded either by the aerobic EMP pathway, leading to the production of pyruvate; or part or all of the G-6-P may enter the PP pathway, which also functions under anaerobic conditions, and may yield pyruvate. Pyruvate, of course, enters the TCA cycle after decarboxylation. Enhanced primordium initiation and development that result from oxygen treatment apparently preclude anaerobic operation of the EMP pathway as the means of G-6-P degradation.

The TCA cycle generates ATP, and, when ATP levels exceed the physiological optimum, activity of the cycle diminishes on account of ATP-induced inhibition of citrate synthase (E.C. 4.1.3.7.), the first enzyme of the cycle (Krebs, 1970). Conversely, declining ATP levels trigger increased TCA activity. Therefore, the mode of supplying G-6-P may control the level of TCA activity and aerobic respiratory patterns (Turetskaya and Kof, 1965) during root primordium initiation and development. For example, phosphorylations involving phosphorylases would not lower ATP levels, nor promote TCA activity; phosphorylations involving kinases would initially both lower ATP levels and promote TCA activity .The foregoing relation may explain why IAA treatment so markedly stimulates aerobic respiration, and how the stimulation arises, but other possibilities exist which include regulation of activity of other TCA cycle enzymes (Kahl, 1973).

The pathway of G-6-P degradation apparently switches during development from predominantly EMP (growing regions) to predominantly PP (differentiating regions) (Gibbs and Beevers, 1955; Gibbs and Earl, 1959). During the last 15 years a number of investigations have attempted to confirm the "Gibbs Hypothesis", and to determine whether auxins operate the switch between the EMP and PP pathways (Black and Humphreys, 1962; Bourke, Butts and Fang, 1964; Carlier and van Hove, 1964; Humphreys and Dugger, 1957a, 1957b, 1959; Scott, Daly and Smith, 1964; and references therein). The literature defies overall interpretation because of the variety of tissues, auxins, and experimental methods employed, coupled with the inherent difficulties encountered in the accurate determination of EMP and PP activity (Carlier and van Hove, 1964; Humphreys and Dugger, 1959). A few studies, which are discussed below, have employed suitable tissues and auxins so that results may bear upon metabolic changes during root primordium initiation and development. In my opinion, suitable tissues must readily initiate root primordia and, where used, an applied auxin must

increase the primordium-initiating response of the tissue under the conditions used to determine participation of EMP and PP pathways. Most previous studies have either employed tissues or organs that do not readily initiate root primordia (such as root tips), or they employed auxins (such as 2, 4-D) that induce root primordium initiation weakly if at all in comparison with IAA, IBA, or NAA.

According to Kaminek and Stemberova (1967), "relative" PP pathway activity increased for the first 64 hrs. during the formation of "root-meristematic foci", in non-auxin-treated stem cuttings of *Pisum sativum* var. Lincoln, and then declined. Their results apparently conflict with the Gibbs Hypothesis, which would predict a rise in EMP pathway activity relative to PP pathway activity as the result of renewed meristematic activity in the cuttings. However, Kaminek and Stemberova (1967) may or may not (Carlier and van Hove, 1964; Humphreys and Dugger, 1959) have properly evaluated EMP and PP contributions to glucose degradation. Thus, their interesting experiments need confirmation.

Other studies (Haissig, 1971a and Haissig unpublished) suggest that EMP pathway activity increases markedly during adventitious root primordium initiation (0 to 72 hours) in bean (*Phaseolus vulgaris* cv. Top Crop) hypocotyl cuttings. Large increases occur in the specific activity of glyceraldehyde-3-phosphate dehydrogenase (NAD) (E.C. 1.2.1.12) in leafy cuttings, in comparison with leafless, non-rooting controls. IAA treatment of leafy cuttings induces further marked increases in G-3-PD (NAD) activity in comparison with leafy, rooting, and leafless, non-rooting controls. IAA treatment of leafless cuttings both induces primordium initiation and enhances G-3-PD (NAD) activity.

These data alone are inconclusive because G-3-PD (NAD), though commonly considered an EMP pathway enzyme, may also function when R-5-P produced via the PP pathway is converted to G-3-P. As noted above, the PP pathway may convert G-6-P to pyruvate, and that conversion results from the shunt of G-3-P derived from R-5-P into the later portion of the EMP pathway.

However, the specific activity of glucose-6-phosphate dehydrogenase (E.C. 1.1.1.49), the first and an exclusive enzyme of the PP pathway, increases steadily for the first 72 hours, whether or not the cuttngs initiate root primordia or are auxin-treated. In rooting cuttings, particularly those treated with IAA, the activity increases for G-3-PD (NAD) far exceed those of G-6-PD. No changes occurred in the specific activities of the photosynthetic enzyme G-3-PD (NADP) (E.C. 1.2.1.13) or of citrate synthase. The later enzymes were used as checks because G-3-PD (NADP) does not play a role in carbohydrate degradation, and because levels of citrate synthase do not fluctuate with TCA activity (Krebs, 1970). Thus, activities of those enzymes should not and did not vary during root primordium initiation.

These results strongly suggest that, whereas PP pathway activity increases during root primordium initiation, the significant increase occurs in the EMP pathway, and that IAA treatment leads to the increase in EMP activity. Other of my experiments suggest that in non-auxin-treated cuttings the EMP activity increases relate to basipetal transport of endogenous auxin, and, therefore, applied auxin simply enhances a natural process. The above results, however, require confirmation with further enzymological and, in particular, radiotracer experiments. Some confirming evidence was supplied by an earlier investigation concerning the influence of NAA on *Phaseolus aureus* stem segments.

Carlier and van Hove (1964) used specifically labeled ^{14}C-glucose to demonstrate that NAA induced EMP pathway activity in comparison with PP pathway activity. Unfortunately, the authors were not studying root regeneration, and, as a result, their experiments did not include a time-course study of changes in pathway activity; nor did the authors supply supporting enzymological data.

NITROGEN

Metabolism of nitrogen within cuttings is not as well understood as that of carbon. Nitrogen becomes redistributed to the bases of cuttings during root primordium initiation and development, and auxin treatment markedly enhances the amount and rate of redistribution and use (Borthwick, Hamner and Parker, 1937; Stuart, 1938). Sircar and Chatterjee (1973) noted that total nitrogen, soluble nitrogen, and free amino acid levels increased in the basal regions of cuttings until root primordia initiated, and then declined. However, elevated nitrogen levels apparently do not trigger primordium initiation because nitrogen levels also increase, and subsequently decline, in the basal regions of non-rooting cuttings (Sircar and Chatterjee, 1973).

A number of studies have evaluated "nitrogen relations" (influences of applied or endogenous nitrogen on rooting ability). Nitrogen relations have most frequently been considered along with carybohydrate relations, and the results reduced to carbohydrate/nitrogen ratios, somewhat misleadingly termed C/N ratios.

A body of evidence suggests that high endogenous C/N ratio favour root primordium initiation and development and that low C/N ratios favour shoot growth (Haun and Cornell, 1951; Pearse, 1943; Preston, Shanks and Cornell, 1953; Reid, 1924a, 1924b; Schrader, 1924; Starring, 1923). Actually, the C/N ratio gains complexity because the root- and shoot-forming responses depend as much upon the absolute magnitude of carbohydrate and nitrogen as upon their ratios. Thus, high C/high N may lead to abundant root primordium initiation and development, whereas low C/low N may yield none (Starring, 1923). In addition, a low total nitrogen level in cuttings may, in fact, yield a high nitrogen level in the basal regions due to redistribution of nitrogen. The form of nitrogen may also influence the validity of C/N ratios (Hyun, 1967; Reid, 1924b) but usually total or nitrate nitrogen have been estimated. Finally, the required nitrogen level depends upon the degree of lignification of a cutting (Preston, Shanks and Cornell, 1953).

High endogenous nitrogen levels in cuttings seem to enhance shoot growth if the level of nitrogen exceeds the optimum for root primordium initiation and development, but the optimum nitrogen level for root primordium initiation and development varies with endogenous and environmental factors. Nitrogen-induced shoot growth then diverts available carbohydrate, and probably other metabolites, from root primordium initiation and development (Reid, 1924a, 1924b; Schrader, 1924; Starring, 1923) because the developing shoot establishes a sink with which root primordium-regenerating zones cannot compete for substrates. The observed effects of nitrogen on shoot growth in cuttings seem reasonable because of the influences of nitrogen on the mechanism of apical dominance (McIntyre, 1971).

However, according to refences cited later, root primordium initiation and development requires nitrogen to a high degree for nucleic acid and protein synthesis. Thus, there exists a critical threshhold nitrogen level below which root primordium initiation

and development do not occur, or occur at a reduced rate. Applied nitrate enhances root primordium initiation and development under conditions of suboptimum nitrogen (Reid, 1924a; Schrader, 1924). Enhanced primordium initiation and development by applied nitrate has been attributed to the need for nitrogen in the metabolism of carbohydrates (Schrader, 1924). Undeniably, carbohydrate metabolism cannot occur without nitrogen-containing molecules and macromolecules. However, there may exist a specific nitrogen effect on carbohydrate metabolism that, if in excess, precludes root primordium initiation.

Cartwright (1972) has recently demonstrated a marked depression of EMP enzyme activity by applied nitrogen, and a possible enhancement of PP enzyme activity. EMP, as noted earlier seems to be an essential pathway of carbohydrate metabolism during root primordium initiation. Thus, excessive nitrogen levels in cuttings may, in addition to other effects, limit EMP activity that supports root primordium initiation. Nitrogen may reverse the metabolic switch from the PP pathway to the EMP pathway that endogenous and applied IAA seem to induce. A test of that hypothesis seems essential to a better understanding of nitrogen relations in cuttings.

Much of the mobilized nitrogen in cuttings probably becomes fixed in protein and nucleic acids. Hyun (1967) could not positively correlate qualitative differences in free amino acids with the rooting potential of cuttings, probably because amino acids are so readily interconverted within cuttings. Kaminek (1968) found that the levels of free amino acids, with the exceptions of asparagine and glutamine, fell with time in both rooting and non-rooting cuttings, and that levels of free amino acids did not correlate well with root primordium initiation (cf. Sircar and Chatterjee, 1973). Rather, higher rates of conversion of glucose to amino acids characterized primordium-initiating as opposed to non-primordium-initiating cuttings. Most of the label from ^{14}C-labeled glucose entered amino acids via glutamate, which originates from alpha-ketoglutarate that glutamate dehydrogenase (E.C. 1.4.1.4) diverts from the TCA cycle. Thus, Kaminek's result suggests metabolism of glucose to supply by *de novo* synthesis at least part of the amino acids required during root primordium initiation. However, Kaminek also found the concomitant interconversion of homoserine to aspartic acid, which precludes *de novo* synthesis as the exclusive supply of necessary amino acids.

Hyun (1967) noted that "poor-rooting" cuttings contained higher levels of arginine, histidine, lysine, and, especially, gamma-aminobutyric acid than did "good-rooting" cuttings. Apparently these amino acids contributed a substantial part of the nitrogen level that yielded low C/N ratios characteristic of trees that supplied poor-rooting cuttings. Kaminek (1968) found, in part, that kinetin treatment of cuttings inhibited root primordium initiation, increased levels of gamma-aminobutyric acid, and decreased levels of glutamate, arginine, and lysine. Comparison of Hyun's and Kaminek's data suggests that neither arginine nor lysine levels influence root primordium-initiating ability. However, gamma-aminobutyric acid arises by the decarboxylation of glutamate (by E.C. 4.1.1.15), and the glutamate level declined after kinetin treatment. Hyun's and Kaminek's results indicate a link between root primordium-initiating ability and the conversion of aplha-ketoglutarate to' 1) glutamate and amino acids that support root primordium initiation, or 2) gamma-aminobutyric acid that inhibits root primordium

initiation directly, or indirectly by diverting glutamate from pathways of metabolism that synthesize essential amino acids.

Werner and Gogolin (1970) related increased glutamate dehydrogenase activity to root primordium initiation. They found a 3-fold increase in glutamate dehydrogenase activity in carrot callus tissue that initiated root primordia, in comparison with non-rooting cultures. Increased glutamate dehydrogenase activity occurred before morphological indications of primordium initiation, and the increases exceeded those of aspartate amino-transferase (E.C. 2.6.1.1.), isocitrate dehydrogenase (E.C. 1.1.1.42), and acid phosphatase (E.C. 3.1.3.2).

NUCLEIC ACIDS AND PROTEINS

Cells of root primordia synthesize RNA (Haissig, 1971b), DNA, and proteins (Molnar and LaCroix, 1972b). Thus, root primordium initiation and development can be blocked by substances which interfere with or modify DNA, RNA, or protein synthesis (Anzai, Shibaoka and Shimokoriyama, 1971; Fellenberg, 1965, 1966; Guillot, 1965; Höhn, 1955; Jain and Nanda, 1972; Kaminek, 1967; Knypl, 1966; Melichar, 1964; Mitsuhashi, Shibaoka and Shimokoriyama, 1969; Nanda and Jain, 1972c; Nanda, Anand, Kochhar and Jain, 1971; Ruge, 1971) or by uncouplers or inhibitors of oxidative phosphorylation (Krul, 1968; Wirth, 1960), which restrict the energy supply for synthesis of macromolecules.

If RNA synthesis is blocked with Actinomycin D (Fellenberg, 1966; Knypl, 1966) or protein synthesis is inhibited by chloramphenicol or puromycin (Fellenberg, 1966; Kaminek, 1967; Knypl, 1966; Nanda and Jain, 1972c); the number of roots per cutting is drastically reduced, even in the present of IAA. Fellenberg (1966) found that, although the number of roots produced per pea cutting fell markedly after either Actinomycin D or chloramphenicol treatment, the number of cells in remaining root primordia was not decreased. These results are contrary to those of Kaminek (1967) who, also with pea, found that chloramphenicol treatment reduced rooting by inhibiting development of root primordia.

Purine and pyrimidine analogues that supposedly promote the formation of defective DNA and RNA, thus causing synthesis of abnormal proteins, may inhibit or enhance root primordium initiation and development. Fellenberg (1966) found that 8-azaguanine irreversibly (with guanine) reduced root production by cuttings, while enhancing root primordium initiation. Ginzburg (1966) reported a stimulation of root primordium initiation by both 8-azaguranine and 8-azaadenine. Apparently not the entire effect of these purine analogues on root primordium initiation and development is attributable to their influence on nucleic acid and protein metabolism, though what side effects they have remains uncertain (Fellenberg, 1966; Ginzburg, 1966).

Several investigators have studied the effect of 2-thiouracil on root primordium initiation and development (Fellenberg, 1966; Guillot, 1965; Höhn, 1955; Knypl, 1966; Melichar, 1964; Mitsuhashi, Shibaoka, and Shimokoriyama, 1969). With two exceptions (Höhn, 1955; Mitsuhashi, Shibaoka and Shimokoriyama, 1969), the inhibitory effects were not overcome with uracil. Guillot (1965) concluded that the deleterious effects of 2-thiouracil could probably be accounted for by its ability to chelate copper ions. Fellenberg (1966) found that 2-thiouracil depressed root primordium initiation

in a manner similar to 2, 4-dinitrophenol and cysteine, which indicated it had a pronounced side effect on "oxidative" processes.

In general, utilization of purine and pyrimidine analogues to assess the importance of nucleic acid and protein metabolism during root primordium initiation has proven unreliable because the analogues have secondary effects. Exceptions exist; for example, work done with 5-bromouracil and an appropriate species of plant (Guillot, 1971). This mutagen reversibly (with thymine) inhibited initiation and development of root primordia, even in the presence of IAA, but was not always inhibitory if applied at the start of an experiment or 36 to 120 hours later (Fellenberg, 1967). Application of 5-bromouracil at the early or late time often stimulated root primordium initiation and development. Melichar (1964) has also shown that 5-bromouracil stimulates root primordium initiation and development. Fellenberg's experiments with 5-bromouracil led him to conclude that IAA-induced root primordium initiation was dependent on RNA and protein synthesis that occurred 11 to 48 (Fellenberg, 1965) or 12 to 24 (Fellenberg, 1966) hours after application of auxin. His results varied, but they seem to support that general conclusion. At the very least, it appears that the influence of IAA on root primordium initiation and development is manifest through quantitative or qualitative changes in protein synthesis during an early part of the regeneration period ((Fellenberg, 1965; Kaminek, 1967) after an initial lag (Mitsuhashai, Shibaoka and Shimokoriyama, 1969; Moore and Lovell, 1972; Nanda and Jain, 1972c). Inhibitors of nucleic acid synthesis may lengthen the lag phase, and thereby enhance root primordium initiation and development in some species (Anzai, Shibaoka and Shimokoriyama, 1971).

The influence of IAA on RNA synthesis in cells of root primordia in different stages of development has recently been demonstrated by autoradiographic investigation of uridine-2-^{14}C incorporation (Haissig, 1971b). Limiting the supply of IAA to root primordia reduced RNA synthesis only during the initiation stage. Applied IAA increased RNA synthesis most, not during initiation, but during early development of primordia. The results suggest that some factor in addition to IAA apparently triggers maximum RNA synthesis during initiation of primordia.

Fellenberg's (1967; 1969a; 1969b) more recent work indicates that IAA and synthetic auxins enhance root primordium initiation by stimulating RNA synthesis through derepression of genes (cf. Jain and Nanda, 1972). Histones applied to cuttings were shown to depress root primordium initiation, in a manner similar to 5-bromouracil, which indicated that the applied histones were somehow masking or repressing essential genetic information. In more direct experiments, Fellenberg showd that auxin treatment of cuttings reduced the melting point of chromatin and DNA extracted from the zone of root regeneration. Apparently the auxin promoted uncoupling of histone from DNA and of complementary DNA strands, either of which might relate to derepression of genes. Bajaj and Fellenberg (1972) also reported IAA induced quantitative changes in histone fractions. Most of Fellenberg's work requires confirmation, though it suggests that auxins enhance RNA synthesis, and thus root primordium initiation, through some action at the chromosomal level, possibly on the transcription of messenger RNA (mRNA). It has been previously shown at the cellular level (but not for root primordium cells) that the radioactive label from applied auxins accumulates in chromosomal material (Liao and Hamilton, 1966) and nucleoli (Zwar and Brown, 1968). This nicely supports Fellenberg's results, but considerable experimentation may be required to verify them,

especially since Sarkissian and Spelsberg (1967) have shown that IAA treatment of bean hypocotyls resulted not in changes in histones but in the appearance of new acidic nuclear proteins.

Böttger and Lüdemann (1964) showed that root primordium initiation and development may require mRNA synthesis. They found that ^{32}P supplied to cuttings during root regeneration rapidly accumulated in a fraction that was probably mRNA. The specific activity of the ^{32}P-mRNA fraction increased rapidly from the start of the experiment until roots broke forth from the cuttings, then it decreased as root growth proceeded (cf. Moore and Lovell, 1972). No similar results are available, although it is known that IAA treatment can result in increased mRNA synthesis in non-regenerating systems (Key, 1969).

If auxins do enhance root primordium initiation and development by promoting the synthesis of specific RNAs (and proteins), it seems probable that applied cytokinins and gibberellins may inhibit root primordium initiation and development by interfering with this process as a consequence of their own normal function in regulating nucleic acid and protein synthesis (Key, 1969; Srivastava, 1967). These hormones also seem capable of modifying gene expression. Thus, application of gibberellins and cytokinins to root primordia may confound the nucleic acid and protein synthesis that directs the specific course of differentiation leading to root primordia and roots (cf. Bajaj and Fellenberg, 1972).

Production of specific mRNAs should lead to the *de novo* synthesis of specific enzymes (cf. Jain and Nanda, 1972). The activities of several enzymes such as peroxidase (E.C. 1.11.1.7) (Chandra, Gregory and Worley, 1971; Haissig, unpublished), cytochrome oxidase (Molnar and LaCroix, 1972a), succinate dehydrogenase (Molnar and LaCroix, 1972a), glucose-6-phosphate dehydrogenase (Haissig, 1971a; Haissig, unpublished), glyceraldehyde-3-phosphate dehydrogenase (Haissig, 1971a; Haissig, unpublished), and starch-hydrolyzing enzymes (Molnar and LaCroix, 1972a; Nanda and Jain, 1972b; Nanda, Anand and Kumar, 1970) increase at the base of cuttings or in the root primordia. In addition, isozyme patterns may vary during root primordium initiation and development (Chandra, Gregory and Worley, 1971). However, only further experimentation of suitable rigour (Filner, Wray and Varner, 1969) will establish whether these enzymes increase in activity, or whether new isozymes appear, due to the *de novo* synthesis of enzyme, or to activation or release of existing molecules (Hammes and Wu, 1971). It would also be interesting to know whether the enzyme activity changes result from or direct root primordium initiation and development.

CONCLUSIONS

The results of direct or indirect quantitative biochemical studies of root primordium initiation and development do not necessarily describe the actual events within or in the near vicinity of the root primordium cells because of limitations imposed by experimental methods, for example, the study of whole or partial organs. Results from biochemical as opposed to cytochemical studies probably represent net changes that resulted from simultaneous, differential variations in several tissues, of which the root primordium-initiating regions comprise a very, very small part of the total mass and metabolic activity.

All cuttings are wounded, and the regeneration of roots is only a partial manifestation

of the wounding response. Thus, changes shown to be characteristic of regenerating tissues should be confirmed for root primordium cells, and adjacent cells. Greater use could be made of combined biochemical-cytochemical-anatomical approaches in individual studies of root primordium initiation and development. In such studies, the cytochemical-anatomical experiments make it possible to key gross biochemical data to specific stages of primordium initiation and development, and also to determine whether gross biochemical changes within the basal regions of cuttings accurately reflect happenings within the primordium and adjacent cells. Employing the biochemical approach appears necessary because all cytochemical methods are not reliable enough, or readily used on numerous samples, or with diverse enough material, to support quantitative biological studies. In addition, difficulties encountered in applying some cytochemical methods to the study of root primordia sometimes preclude their use, at least until an investigator can suitably refine the techniques.

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