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SUBSTRATE-DEPENDENT MICROBIAL PRODUCTION OF PHYTOHORMONES: A REVIEW

M. Arshad
Department of Soil Science,
University of Agriculture, Faisalabad

This mini-review focuses on the microbial production of phytohormones including auxins, cytokinins and ethylene derived from exogenously applied precursors. The L-tryptophan, adenine plus isopentyl alcohol and L-methionine have been found to promote the production of microbially-derived phytohormones in both pure culture studies and in soils. We have evaluated the applications of these precursors as agrochemicals to promote microbially-derived phytohormones in modifying plant growth and development. Soil amendments with these precursors to the rhizosphere of young seedlings has affected plant growth, development and yield of Douglas fir, radish peppers, melons, corn, tomatoes and black ciris.

INTRODUCTION

Numerous studies have demonstrated that soil microorganisms are active in production of phytohormones. This has led scientists to speculate that phytohormones released by various inocula may be an active mechanism in affecting plant growth, development and yields (Arshad and Frankenberger, 1991 a; 1992 a, b; Brown, 1982; Brown *et al.*, 1968; Frankenberger and Arshad, 1991 c; Hubbell *et al.*, 1979; Tien *et al.*, 1979). Several studies have shown similar plant responses upon treatment with phytohormones and inoculation with specific microorganisms. Brown *et al.* (1968) found that a combination of indole-3-acetic acid (IAA) and gibberellic acid (GA_3) applied to tomato seedlings produced effects on plant growth similar to those of a pure culture of *Azotobacter chroococcum*. Azcon *et al.* (1978) reported that cell-free supernatants of *Rhizobium*, *Azotobacter* and *Pseudomonas* cultures affected plant growth similarly as did the combined application of IAA, GA_3 and kinetin. Combined applications of an auxin-gibberellin-kinetin preparation led to a change in root morphology of sorghum and pearl millet similar to that produced by inoculation with *Azospirillum brasilense* (Hubbell *et al.*, 1979; Tien *et al.*, 1979). Similarly, Kucey (1988) reported that many of the wheat shoot and root growth-altering effects of *A. brasilense* could be simulated by the application of phytohormones, most notably IAA and GA_3. The exogenous application of IAA elicited the same response as inoculation with *A. brasilense* in an assay where an increase in dry weight of intact wheat roots was demonstrated after inoculation within 10 days (Zimmer *et al.*, 1988). These studies indicate that microbially-produced phytohormones in the rhizosphere may have a significant ecological effect when subjected to direct uptake by plant roots with the intimate contact between microbial and plant cells.

Although microbial biosynthesis of phytohormones can occur even in the absence of exogenously supplied precursors, the addition of specific substrates can significantly simulate their production. We have used this approach to promote microbial production of phytohormones by adding

Table 1. Influence of L-tryptophan (L-TRP) as an auxin precursor on growth of Douglas fir (Source: Frankenberger & Poth, 1987)

Treatment	Douglas fir				
	Shoot height (cm)	Root length (cm)	Shoot weight (g)	Root weight (g)	Stem diameter (mm)
Control	2.3 a	42.6 b	3.6 b	3.5 b	0.79 b
Pisolithus tinctorius (Pt)	2.3 a	40.5 b	3.6 b	3.7 b	0.80 b
Pt + 10^{-3} *M* TRP	3.5 b	32.2 a	2.8 a	2.9 a	0.53 a
Pt + 10^{-4} *M* TRP	3.4 b	38.8 ab	3.7 b	3.8 b	0.75 b
Pt + 10^{-5} *M* TRP	4.5 bc	40.6 b	3.9 bc	4.9 b	0.87 b
Pt + 10^{-6} *M* TRP	3.8 b	53.0 c	4.3 bc	5.1 bc	1.02 c
Pt + 10^{-7} *M* TRP	4.2 bc	54.6 c	4.8 c	5.5 c	1.05 c
Pt + 10^{-8} *M* TRP	5.1 c	54.3 c	5.1 c	5.9 c	1.12 c

Values followed by different letter(s) differ significantly at $P<0.05$.

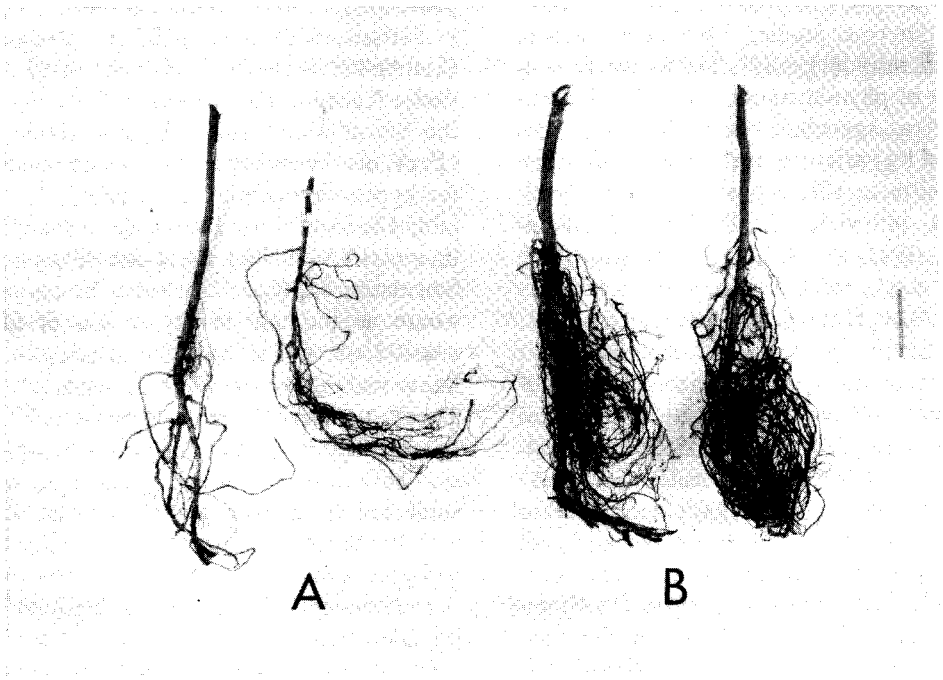

Fig. 1. Established ectomycorrhizae formed by *Pisolithus tinctorius* in the presence of 10^{-7} *M*. L-tryptophan (L-TRP) (3.4 μg kg^{-1} of soil); A = With L-TRP; B = 3.4 μg TRP kg^{-1} of soil.

Table 2. Response of radish to L-TRP (3.0 mg kg^{-1} soil) and various auxins (3.0 mg kg^{-1} soil) applied to soil (Source: Frankenberger *et al.*, 1990)

Treatment	Root dry weight	Shoot dry weight
	.....(g $plant^{-1}$).....	
Control	1.02 a	0.60 a
L-tryptophan	1.29 b	0.56 a
Nutrient solutin (NS)	1.72 d	1.16 bc
L-tryptophan + NS	2.28 ef	1.51 ef
Indole-3-acetic acid + NS	1.88 d	1.08 b
Indole-3-acetamide + NS	2.34 f	1.38 de
Indole-3-pyruvic acid + NS	1.49 c	1.33 cd
Indol-3-lactic acid + NS	2.11 e	1.68 f
Indole-3-butyric acid + NS	1.68 cd	1.42 de

Values followed by different letter(s) differ significantly at $P<0.05$.

Table 3. Response of watermelon and cantaloupe to L-TRP applied to soil (Source: Frankenberger and Arshad, 1991 a)

L-TRP (mg kg^{-1} soil)	Royal windsor watermelon		Royal sweet watermelon		Top score cantaloupe	
	Number of fruit $plant^{-1}$	Total fresh weight of fruit $plant^{-1}$ (kg)	Number of fruit $plant^{-1}$	Total fresh weight of fruit $plant^{-1}$ (kg)	Number of fruit $plant^{-1}$	Total fresh weight of fruit $plant^{-1}$ (kg)
Control	3.82*	20.5	3.69	26.9	11.94	11.8
60	4.64	28.2	5.16	37.6	14.93	15.1
6.0	4.07	31.2	4.53	34.8	12.12	14.2
6.0×10^{-1}	4.43	30.2	4.81	36.2	13.68	15.0
6.0×10^{-2}	5.31	33.7	3.81	33.2	13.62	16.6
6.0×10^{-3}	5.00	31.5	4.41	40.6*	13.37	16.7*
6.0×10^{-4}	5.06	34.5*	5.38	39.6*	12.25	16.5*
6.0×10^{-5}	5.21	36.9*	5.45*	42.6*	13.87	16.6*

*Means significantly different from control at $P<0.05$.

specific precursor(s) which is the main focus of this review. Although, numerous soil microorganisms have been shown capable of producing gibberellins (Arshad and Frankenberger, 1992 a), this topic will not be discussed in this review. Most of the literature concerning microbial production of phytohormones in soils is on auxins, cytokinins and ethylene.

Auxins: Tryptophan (TRP) is a well-established precursor of auxins in higher plants. It also serves as a precursor for microbially-derived auxins in pure culture and in soils (Arshad and Frankenberger, 1992 a). *In vitro* studies have demonstrated that some microbial cultures can produce small amounts of IAA in the absence of a physiological precursor. However, in the presence of L-TRP, the microbiota often release much greater quantities of IAA and IAA derivatives. Frankenberger and Poth (1988) separated and purified a soluble α-ketogultarate-dependent L-TRP transaminase enzyme from a cell free extract of a rhizobacterial isolate associated with *Festuca octoflora* Walt. This enzyme catalyzed the conversion of L-TRP to indole-3-pyruvic acid, an intermediate of IAA synthesis. By using HPLC, ELISA and GC-MS, they also confirmed the ability of a mycorrhizal fungus, *Pisolithus tinctorius* to produce IAA from exogenously supplied L-TRP (Frankenberger and Poth, 1987). In microcosm studies with soil, Purushothaman *et al.* (1973; 1974) reported active synthesis of auxins when amended with TRP. Frankenberger and Brunner (1983) confirmed this observation and provided unequivocal proof that IAA was produced in soil when incubated with L-TRP.

Since TRP is known to promote auxin synthesis in soils, this biotransformation may have a physiological effect on plant growth via microbially-derived auxins. We have carried out several experiments to evaluate this hypothesis. After confirming L-TRP-dependent IAA synthesis by an ectomycorrhizal fungus, *P. tinctorius,* Frankenberger and Poth (1987) investigated the influence of L-TRP and the fungus as an inoculum on Douglas fir *(Pseudotsuga menziesii).* It was found that *P. tinctoriu* stimulated the growth of potted seedlings of Douglas fir only when supplied with low concentrations of L-TRP (ng to μg quantities) (Table 1). There was no difference in plant growth between inoculated and uninoculated treatments in the absence of L-TRP, however, the addition of dilute solutions of L-TRP (10^{-8} to 10^{-6} *M*, 0.34 to 34 μg kg^{-1} soil) with *P. tinctorius* caused a significant increase in growth indicating a physiological rather than nutritional effect. The higher concentration of L-TRP applied as 10^{-3} *M* caused reduction in dry weights of seedlings which is a typical auxin response. Root examination revealed that the mycelial inoculum of *P. tinctorius* was highly effective in forming ectomycorrhizae on seedlings. However, the effectiveness of this inoculum on plant growth was not much different from that of the non-inoculated stock seedlings unless L-TRP was added as a precursor of IAA (Fig. 1).

Considering the ability of soil indigenous microbiota to produce IAA and its derivatives from L-TRP, the effects of L-TRP directly applied to soil on radish *(Raphanus sativus)* (Frankenberger *et al.,* 1990), watermelon *(Citrullus lanatus)* and muskmelon *(Cucumis melo)* (Frankenberger and Arshad, 1991 a) and pepper *(Capsicum annum)* (Frankenberger and Arshad, 1991 b) were studied with optimum nutrition. With the radish experiments, selected auxins were tested along with L-TRP to assess their effect on yield. A positive effect of L-TRP on growth parameters of radish was observed when low concentrations of L-TRP (3.0 mg kg^{-1}) were applied at the seedling stage, being comparable to that of selected

Table 4. Response of bell pepper to L-TRP applied to soil (Source: Frankenberger & Arshad, 1991 b)

L-TRP (mg kg^{-1} soil)	Gator bell pepper		California Wonder pepper	
	Number of fruits $plant^{-1}$	Total fresh weight of fruit $plant^{-1}$ (kg)	Number fruit $plant^{-1}$	Total fresh weight of fruit $plant^{-1}$ (kg)
Control	5.31	203	6.88	373
60	6.47	330*	8.11	515*
6.0	7.23	326*	8.44	510*
6.0×10^{-1}	7.75	305*	8.18	446
6.0×10^{-2}	6.00	262	8.55	470
6.0×10^{-3}	7.80	250	8.29	482
6.0×10^{-4}	7.17	292	9.00	475
6.0×10^{-5}	9.60*	267	10.05*	483

*Means significantly different from control at $P<0.05$.

auxins like indole-3-acetamide and indole lactic-3-acid (Table 2). Higher concentrations of L-TRP had negative effects, a response usually observed in the presence of high auxin levels. The response was very pronounced under high fertility status, which excluded any nutritional effect of L-TRP. Moreover, foliar application of L-TRP did not have any significant effect on the growth parameters of radish indicating that the site of entry of L-TRP and/or its microbial transformed metabolites appeared to be through the rootzone (Frankenberger *et al.*, 1990). Similarly, a positive effect of L-TRP on melon yields (number and weight of fruit) was apparent at low concentrations (ng to μg amounts) of L-TRP added at the seedling stage (Frankenberger and Arshad, 1991 a). In testing the response of peppers, the cultivars reacted differently in terms of number and weight of fruits upon exogenously applied L-TRP amendments (Frankenberger and Arshad, 1991 b). Hussain *et al.* (1989 b) reported a significant positive effect of dilute concentrations of L-TRP applied to soil on the growth, nodulation and chemical composition of *Albizia lebbeck*.

The possible mechanisms of action of exogenously applied L-TRP to soil on plant growth include:

1. substrate-dependent auxin production in soil by the indigenous microflora;
2. uptake directly by plant roots followed by metabolism within their tissues; and/or
3. a change in the balance of rhizosphere microbiota affecting plant growth (Frankenberger and Arshad, 1991 a, b; Frankenberger *et al.*, 1990).

Cytokinins: Several studies have reported the biosynthesis of cytokinins and cytokinin-like substances by soil microorganisms particularly by microsymbionts forming

Table 5. Influence of adenine (ADE) and isopentyl alcohol (IA) as cytokinin precursors on plant growth (From: Nieto & Frankenberger, 1990, 1991)

Treatment	Axenic/inoculated experiment		Glasshouse-soil experiment		Radish glass-house field experiment		Field experiment	
	Root dry mass	Shoot dry mass	Root dry mass	Shoot dry mass	Root dry mass	Shoot dry mass	Root dry mass	Shoot dry mass
	plant^{-1}							
Control	1.07 a**	0.63 a	0.45 a	0.43 a	0.46 a	0.36 a	1.54 ab	1.39 a
IA	1.16 ab	0.64 ab	0.61 b	0.44 a	0.36 a	0.36 a	1.39 a	1.40 a
IA + *Azotobacter chroococcum* (*A.c*)	1.74 c	0.71 bc	0.71 c	0.49 a	0.46 a	0.34 a	1.60 b	1.41 a
ADE + IA	1.90 cd	0.78 c	0.86 d	0.70 b	0.52 b	0.52 b	1.82 c	1.56 b
ADE + IA + *A.c.*	2.37 e	0.97 d	1.13 e	0.79 c	0.93 c	0.56 b	1.98 d	1.62 b

Corn

	Root dry mass (g)	Shoot dry mass (g)	Shoot mass (cm)	Internodal distance (cm)	Internodal width (mm)	Stem leaf width (mm)
Control	1.82 a**	3.8 a	61.3 a	5.3 a	17.1 a	3.08 a
A.c.	2.77 c	6.5 b	77.5 b	7.2 b	16.7 a	3.55 b
IA + *A.c.*	1.91 ab	8.8 c	85.1 b	8.3 c	17.4 a	8.83 b
ADE	6.02 d	10.3 cd	83.4 b	9.8 d	16.8 a	6.10 cd
ADE + A.c.	6.99 d	13.3 d	97.5 c	13.6 e	20.0 b	5.73 c
ADE + IA	4.39 d	11.4 cd	100.9 c	12.9 e	21.2 b	5.80 c
ADE + IA + A.c.	10.10 e	18.8 e	127.1 d	14.9 f	25.0 c	6.50 d

* In the radish experiment, ADE and IA were applied at 0.2 mg kg-1 soil and 13 mg kg-1 soil, respectively. In the corn experiment, ADE and IA were added at 2.0 mg kg-1 and 13 mg kg-1 soil, respectively.

** Values sharing same letter(s) do not differ significant at $P<0.05$.

symbiosis with plants (legumes and non-legume nodulation and mycorrhiza), phytopathogenic parasites (pathogens) and free living diazotrophs (Arshad and Frankenberger, 1992 a; Greene, 1980; Mahadevan, 1984; Nieto and Frankenberger, 1990 a). However, precursors that might stimulate microbial biosynthesis of cytokinins has not been thoroughly investigated until recently by Nieto and Frankenberger 1989 a, b; 1990 b; 1991). They screened a number of compounds including adenine, adenosine-5-

Table 6. Influence of L-methionine-derived C_2H_4 produced by indigenous soil microflora on etiolated pea seedlings (Source: Arshad and Frakenberger, 1988)

Treatment	Seedling length (cm)	Seedling diameter (mm)
Control	6.56 b	1.87 a
$AgNO_3$ (240 mg l^{-1})	13.50 d	1.93 ab
L-methionine (5 mM)	5.14 ab	2.49 c
L-methionine (5 mM) + $AgNO_3$ (240 mg l^{-1})	11.10 c	2.06 ab
L-methionine (10 mM)	3.90 a	2.75 d
L-methionine (10 mM) + $AgNO_3$ (240 mg l^{-1})	10.10 c	2.11 b

Values followed by the same letters are not significantly different at $P<0.05$.

monophosphate, hypoxanthine, inosine, inosine-5-monophosphate, isopentyl alcohol, isoprene, 3-methyl-butane-1-ol, and 3-methyl-1-2-butane-1-ol, as possible substrates for biosynthesis of cytokinins by three species of *Azotobacter (A. chroococcum, A. vinelandii, A. beijerickii)* and two *Pseudomonas* spp. *(P. fluorescens, P. putida)* (Nieto and Frankenberger, 1989 a). The addition of adenine (ADE) plus isopentyl alcohol (IA) enhanced cytokinin bioactivity to the greatest degree in the culture filtrates of the bacteria tested. Among the biosynthetic pathways which have been proposed, ADE is considered the common precursor for cytokinin production in both plant tissues and microorganisms. *Azotobacter chroococcum* is the most profilic producer of cytokinins among soil bacteria as of this date. Nieto and Frankenberger (1989 a) utilized HLPC and UV spectrometry for identification and quantification of the cytokinins produced by *A. chroococcum* and found that zeatin riboside and t-zeatin were the major metabolites. In another study, Nieto and Frankenberger (1989 b) investigated the effect of precursors (adenine and isopentyl alcohol) with the inoculation of *A. chroococcum* on cytokinin biosynthesis in soil. It was reported that an application of ADE plus IA and *A. chroococcum* enhanced production of zeatin riboside and t-zeatin in soil up to 1.5 and 1.39-fold, respectively in comparison with controls without the added precursors. The production of zeatin riboside and t-zeatin in the presence of ADE and IA was 1.35- and 2.44-fold greater with inoculation by *A. chroococcum,* when compared with the indigenous micriobiota alone. This study is the first to reveal that microbial biosynthesis of cytokinins can be enhanced by applying physiological precursors to soil. Later, the effects of ADE and IA on the growth of radish *(Raphanus sativus)* and corn *(Zea mays)* were evaluated (Nieto and Frankenberger, 1991), both in the presence and absence of the inoculum, *A. chroococcum.* The greatest enhancement in plant growth was observed from the combined application of ADE, IA and *A. chroococcum* (Table 5) which may be attributed primarily to an increase in microbial production of cytokinins in the rhizosphere. However, under axenic conditions, plant growth was slightly increased when the precursors (ADE and IA) were added alone, compared with the

Table 7. Influence of L-methionine (MET) as an ethylene precursor on corn (Source: Arshad and Frankenberger, 1990 a)

L-MET (mg kg^{-1} soil)	Shoot height (cm)		Shoot fresh weight (g)		Shoot dry weight (g)		Stem diameter (mm)		Resistance to stem breaking (relative unit)	
	Kandy	Miracle	Kandy	Miracle	Kandy	Miracle	Kandy	Miracle	Kandy	Miracle
Control	134 a	121 a	159 a	221 a	26.1 a	33.2 a	15.4 a	18.6 a	3.41 a	5.30 b
0.0185	159 b	134 b	185 a	226 a	28.7 ab	30.4 a	16.7 ab	18.4 a	3.67 ab	3.41 a
0.185	160 b	137 b	206 b	230 a	31.8 ab	30.5 a	17.0 ab	19.1 a	3.66 ab	3.43 a
1.85	173 b	140 b	231 b	258 b	34.5 b	32.3 a	17.4 b	20.1 b	4.35 b	4.35 a

Values sharing same letter(s) do not differ significantly at $P<0.05$.

control (without precursors and inoculation) indicating the plant's ability to assimilate and utilize ADE and IA for growth and metabolism. The precursor-inoculum interaction caused an increase in dry weight of roots and shoot tissues of corn up to 5.57- and 5.00-fold, respectively over the control. These two studies demonstrate the possibility of improving plant growth and development through increased microbial production of cytokinins in response to an exogenous application of cytokinin precursors.

Ethylene: Among the phytohormones, microbial production of ethylene (C_2H_4) has been investigated more than any other class of hormones. A diverse group of microbiota including both pathogens and non-pathogens are active in producing C_2H_4 (Arshad and Frankenberger, 1992 a, b). A wide range of difficult compounds have been reported as possible precursors for C_2H_4 generation by different microbial isolates. However, L-methionine (L-MET), the sole precursor for C_2H_4 biosynthesis in higher plants (Adams and Yang, 1979), has been thoroughly evaluated as an C_2H_4 precursor in pure culture and soil studies. In many cases, bacteria and fungi only produce C_2H_4 in the presence of L-MET (Arshad and Frankenberger, 1989; Billington *et al.*, 1979; Fukuda *et al.*, 1989; Ince and Knowles, 1985, 1986; Lynch, 1972; Primrose, 1976). Arshad and Frankenberger (1989) observed that corn rhizosphere contained an appreciable number of microflora capable of producing C_2H_4 from L-MET, most likely via pathway different from that of higher plants. After screening 63 compounds, Arshad and Frankenberger (1990 b) reported that many amino acids (including L-MET), organic acids and carbohydrates, typically found in root exudates, stimulated C_2H_4 biosynthesis in soil under field capacity. This indicates that an excellent site for microbial biosynthesis of C_2H_4 is the rhizosphere where substrate levels and

Table 8. Influence of L-ethionine (L-ETH) as an ethylene precursor on tomato growth (Source: Arshad and Frankenberger, 1990 a)

L-ETH (mg kg^{-1} soil)	Fresh fruit yield (g)	Average weight of fresh fruit (g)	Total number of ripe fruit	Epinastic movement (°) 72 h after treatment
Control	261 a	37.3 a	11 a	4.8 a
0.2	477 b	55.0 ab	15 ab	9.0 b
2.0	445 b	62.1 b	16 ab	9.8 b
20.0	351 ab	50.1 ab	19 b	12.3 c

Values sharing same letter(s) do not differ significantly at $P < 0.05$.

microbiota populations are comparatively very high. In another study, the production and stability of C_2H_4 in amended (L-MET and D-glucose) and non-amended soils under waterlogged and unsaturated conditions (field capacity) was investigated by Arshad and Frankenberger (1990 c). It was concluded that the effectiveness of the amendments to stimulate C_2H_4 generation varied under these conditions and was controlled by the nature of amendments as well as environmental factors and soil conditions. The stability of C_2H_4 under waterlogged conditions was higher than under aerobic conditions. This implies that under waterlogged conditions, C_2H_4 persists longer and because of its high solubility in water, C_2H_4 is trapped within the surrounding roots. It is most likely that the physiological action of C_2H_4 is metabolized at a faster rate. Arshad and Frankenberger (1991 b) also reported that the presence of trace elements [Ag (I), Cu (II), Fe (II, III), Mn (II), Ni (II), Zn (II), Co (II), Hg (II), Al (III), Mo (VI)] had a concentration-dependent effect on C_2H_4 production in L-MET-amended soil. Each of these studies indicates that precursor-dependent C_2H_4 accumulation in soil may be controlled by varying environmental conditions.

Accumulation of C_2H_4 in soils is of great ecological significance because very minute amounts (ppb range) of C_2H_4 in the vicinity of plant roots can have tremendous effects on plant growth and development (Arshad and Frankenberger, 1991 a; 1992 a, b; Primrose, 1979; Smith, 1976; Smith and Russell, 1969). However, the ecological significance of microbially produced C_2H_4 under non-waterlogged conditions has not been evaluated until recently. By using an C_2H_4 bioassay, the classical "triple" response in etiolated pea seedlings, Arshad and Frankenberger (1988) demonstrated that C_2H_4 of microbial origin can effect plant growth. They showed that L-MET-dependent C_2H_4 produced by an inoculum, *Acremonium falciforme* or by soil indigenous microflora (Table 6) caused elongation, swelling of the hypocotyl and change in the direction of growth (horizontal). A similar response was observed by direct application of the C_2H_4 gas. The specific anti-C_2H_4 properties of Ag (I) confirmed that C_2H_4 was the fungal metabolite responsible for the observed effects. In another study, Arshad and Frankenberger (1990 a) found that soil application of L-MET affected the vegetative growth and resistance to stem breaking (lodging) of two corn cultivars (Table 7). Similarly, soil treatment with

ethionine (as a precursor of C_2H_4) resulted in a significant epinastric response of tomato plants (a typical response of C_2H_4) (Arshad and Frankenberger, 1990 a). At varying concentrations of the soil amendment (L-ETH), we noted enhanced fruit yield with early fruit formation and ripening of tomatoes (Table 8). Hussain *et al.* (1989 a) investigated the effect of soil applied L-MET on the growth, nodulation and chemical composition of *Albizia lebbeck* (Black ciris). They found that plant height, girth, dry weight of shoots and roots, number and weight of nodules and N, P, K, Ca and Mg concentrations and their uptake were significantly increased in response to soil applied L-MET at low concentrations (μg to mg kg^{-1} range). One proposed mechanism of action in this study was substrate-dependent C_2H_4 production by the indigenous soil microflora.

DISCUSSION

There is now ample evidence that soil microbiota are active in producing phytohormones (Arshad and Frankenberger, 1992 a, b). Numerous studies have shown an improvement in plant growth and development in response to seed or root inoculation with various microbial inoculants and it has been proposed that phytohormones released as secondary metabolites may contribute to these growth-altering effects. Strong evidence to support this hypothesis has been provided by many other scientists (Azcon *et al.*, 1978; Barbieri *et al.*, 1986, 1988; Barea and Brown, 1974; Brown *et al.*, 1968; Grapelli and Rossi, 1981; Grayston *et al.*, 1991; Holl *et al.*, 1988; Hussain *et al.*, 1987; Inbal and Feldman, 1982; Kucey *et al.*, 1988; Loper and Schorth, 1986; Mahmoud *et al.*, 1984; Muller *et al.*, 1989; Tien *et al.*, 1979; Triplett *et al.*, 1981; William and deMallorca, 1982; Zimmer *et al.*, 1988). Despite the fact that plants are capable of synthesizing phytohormones, they may not have the capacity to synthesize sufficient amounts for optimal growth and development under suboptimal climatic and environmental conditions. Hence, plants may respond to exogenously supplied phytohormones during certain growth phases and under certain cultivation conditions. This view is supported by the plants responses to exogenous sources of phytohormones applied to the roots. The exogenous application of phytohormones may affect the endogenous hormonal pattern of plants, either by supplementation of suboptimal levels or interaction with the synthesis, translocation and/or inactivation of the plant hormones. The plants responses to phytohormones within the rhizosphere may be governed by the rate of hormone uptake, the active concentration in the rhizosphere and modification of the plant's own pool of hormones as a result of the exogenous supply (Frankenberger *et al.*, 1984).

The rhizosphere is an ideal site for microbial biosynthesis of phytohormones because of the dense microbial population. Because of the intimate contact between plant roots and the microbiota in the rhizosphere, the phytohormones released as secondary microbial metabolites are more likely to be subjected to direct assimilation by plant roots. Production of phytohormones as microbial metabolites is often directly related to substrate(s) availability. The studies conducted by our group have provided unequivocal proof that microbial production of phytohormones could be increased severalfold by providing suitable precursor(s). We have also demonstrated that by adding precursor(s) of specific hormones, plant growth and development can be modified most likely by the hormones released by the inocula or by the soil indigenous microflora.

Although various phytohormone preparations are commercially available, they are either expensive, unstable or require labour intensive practice to deliver these preparations to plants. The high cost of applying naturally occurring phytohormones to soil precludes their use in agriculture. The stimulation in microbial biosynthesis of phytohormone(s) within the rhizosphere with low cost precursor may be an effective approach in modifying plant growth. A microbial source of phytohormone(s) is not only the most economical but also convenient in a practical sense. Moreover, phytohormone(s) released as microbial metabolites from the added substrate(s) provide a continuous release of active substances for plant uptake compared to a one-time application of synthetic compounds. Since precursor-inoculum interactions have often been found to be much better in creating favourable plant responses than the application of precursor alone, treatment with both precursors and a particular inoculum may be the most effective approach to increase yield. This work has provided a new innovative approach in soil science for improving crop yields. However, more research is needed to determine the practical significance of this approach in today's agriculture management systems.

Conclusions: Many soil microorganisms which significantly affect plant growth are also capable of producing phytohormone(s). Moreover, the biosynthesis of phytohormones in soils have been unequivocally demonstrated. These studies reveal that phytohormones of microbial origin could be of great ecological significance in the agriculture industry. To use microbially-derived phytohormones to improve crop yields, future research should be focused on the following projects:

i. isolation of prolific microbial producers of phytohormone(s) within the rhizosphere,
ii. determination of substrate-dependent biosynthesis of phytohormones by specific inocula and the soil indigenous microflora,
iii. evaluation of environmental factors affecting substrate-dependent phytohormone production by the rhizosphere microflora and inocula,
iv. monitoring the stability and bioavailability of various phytohormones in soils,
v. determination of the concentration optima of various phytohormones to promote growth of different crops,
vi. studying the effects of phytohormones on nutritional aspects (availability and uptake of nutrients) of the plants,
vii. studying the effects of various agricultural practices (e.g. fertilization and pesticides) on phytohormones biosynthesis in soil,
viii. evaluation of the effects of root exudates of various plants in providing substrates for microbially-derived phytohormones, and
ix. investigating the role of phytohormones on plant-microbe associations (nodulation and mycorrhizal development) beneficial for plant growth.

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REFERENCES

Adams, D.O. and S.F. Yang. 1979. Ethylene biosynthesis: identification of 1-aminocyclopropane-1-carboxylic acid as an intermediate in the conversion of methionine to ethylene. Proc. Natl. Acad. Sci. (USA) 76: 170-174.

Arshad, M. and W.T. Frankenberger, Jr. 1988. Influence of ethylene produced by soil microorganisms on etiolated pea seedlings. App. Environ. Microbiol. 54: 2728-2732.

Arshad, M. and W.T. Frankenberger, Jr. 1989. Biosynthesis of ethylene by *Acremonium falciforme*. Soil Biol. Biochem. 21: 633-638.

Arshad, M. and W.T. Frankenberger, Jr. 1990 a. Response of *Zea mays* and *Lycopersicon esculentum* to the ethylene precursors, L-methionine and ethionine applied to soil. Plant Soil, 122: 219-227.

Arshad, M. and W.T. Frankenberger, Jr. 1990 b. Ethylene accumulation in soil in response to organic amendments. Soil Sci. Soc. Amer. J. 54: 1026-1031.

Arshad, M. and W.T. Frankenberger, Jr. 1990 c. Production and stability of ethylene in soil. Biol. Fert. Soils, 10: 29-34.

Arshad, M. and W.T. Frankenberger, Jr. 1991 a. Microbial production of plant hormones. Plant and Soil, 133: 1-8.

Arshad, M. and W.T. Frankenberger, Jr. 1991 b. Effect of soil properties and trace elements on ethylene production in soil. Soil Sci. 151: 377-386.

Arshad, M. and W.T. Frankenberger, Jr. 1992 a. Microbial production of plant growth regulators. pp: 307-347. In: (Metting, B.F., ed.). Soil Microbial Ecology. Marcel Dekker Inc., NY, USA.

Arshad, M. and W.T. Frankenberger, Jr. 1992 b. Microbial biosynthesis of ethylene and its influence on plant growth. Adv. Microbial Ecol. 12 (Marshall, K.C., ed.), Plenum Pub. Co., NY, USA.

Azcon, R., C.A.G.D. Aguilar and J.M. Barea. 1978. Effects of plant hormones present in bacterial cultures on the formation and response to VA mycorrhiza. New Phtopathol. 80: 359-364.

Barbieri, P., T. Zanelli, E. Galli and G. Zanetti. 1986. Wheat inoculation with *Azospirillum brasilense* sp. and some mutants altered in nitrogen fixation and indole-3-acetic acid production. FEMS Microbiol. 36: 87-90.

Barbieri, P., A. Bernardi, E. Galli and G. Zanetti. 1988. Effects of inoculation with different strains of *Azospirillum brasilense* on wheat roots development. In: (Klingmuller, W., ed.). *Azospirillum*. IV. Genetics, Physiology and Ecology. pp: 181-188. Springer-Verlag, Berlin, Heidelberg.

Barea, J.M. and M.E. Brown. 1974. Effects on plant growth produced by *Azotobacter paspali* related to synthesis of plant growth regulating substances. J. App. Bacteriol. 37: 583-593.

Billington, D.C., B.T. Goling and S.B. Primrose. 1979. Biosynthesis of ethylene from methionine. Biochem. J. 182: 827-836.

Brown, M.E. 1982. Nitrogen fixation by free-living bacteria associated with plants - fact or fiction? In: (Rhodes-Roberts, M.E. and F.A. Skinner, eds.). Bacteria and Plants. pp: 25-41. Academic Press, London, UK.

Brown, M.E., R.M. Jackson and S.R. Burlingham. 1968. Effects produced on tomato plants, *Lycopersicum esculentum* by seed or root treatment with gibberellic acid and indole-3-acetic acid. J. Exp. Bot. 19: 544-552.

Frankenberger, W.T. Jr. and M. Arshad. 1991 a. Yield response of watermelon and muskmelon to L-tryptophan applied to soil. Hort. Sci. 26: 35-37.

Frankenberger, W.T. Jr. and M. Arshad. 1991 b. Yield response of *Capsicum annuum* to the auxin precursor, L-tryptophan applied to soil. PGRSA Quarterly, 19: 231-240.

Frankenberger, W.T. Jr. and M. Arshad. 1991 c. Microbial production of plant growth regulating substances in soil. In: (Keel, C., B. Koiler and G. Defago, eds.). Plant Growth-Promoting Rhizobacteria - Progress and Prospects. Intl. Org. Biol. Control, Switzerland.

Frankenberger, W.T. Jr. and W. Brunner. 1983. Method of detection of auxin-indole-3-acetic acid in soil by high performance liquid chromatography. Soil Sci. Soc. Amer. J. 47: 237-241.

Frankenberger, W.T. Jr., A.C. Chang and M. Arshad. 1990. Response of *Raphanus sativus* to the auxin precursor, L-tryptophan applied to soil. Plant and Soil, 129: 235-241.

Frankenberger, W.T. Jr. and K.L. Fitzpatrick. 1984. Exogenous hormone production in soil-root systems. In: Improving Efficiencies in Crop Production Systems. pp: 58-61. Proc. California Plant Soil Conf., Amer. Soc. Agron.

Frankenberger, W.T. Jr. and M. Poth. 1987. Biosynthesis of indole-3-acetic acid by the pine ectomycorrhizal fungus *Pisolithus tinctorius*. App. environ. Microbiol. 53: 2908-2913.

Frankenberger, W.T. Jr. and M. Poth. 1988. L-tryptophan transminase of bacterium isolated from the rhizosphere of *Festucaa octoflora* (Graminae). Soil Biol. Biochem. 20: 299-304.

Fukuda, H., M. Takahashi, T. Fuji and T. Ogawa. 1989. Ethylene production from L-methionine by *Cryptococcus albidus*. J. Ferment. Bioengg. 67: 173-175.

Grapelli, A. and W. Rossi. 1981. The effect of phytohormones produced by *Anthrobacter* sp. on the phosphatase activity in plant roots. Folia Microbiol. 26: 137.

Grayston, S.J., J.H. Stephens and L.M. Nelson. 1991. Field and greenhouse studies on growth promotion of spring wheat inoculated with coefficient rhizobacteria. In: (Keel, C., B. Koiler and G. Defago, eds.). Plant Growth Promoting Rhizobacteria - Progress and Prospects. pp: 11-16. IOBC, Switzerland.

Greene, E.M. 1980. Cytokinin production by microorganisms. Bot. Rev. 46: 25-74.

Holl, F.B., C.P. Chanway, R. Turkington and Radley. 1988. Response of crested wheatgrass *(Agropyron cristatum* L.), perennial ryegrass *(Iolium perenne)* and white clover *(Trifolium repens* L.) to inoculation with *Bacillus polumyxa*. Soil Biol. Biochem. 20: 19-24.

Hubbell, D.H., T.M. Tien, M.H. Gaskins and J. Leel. 1979. Physiological interaction in *Azospirillum*-grass root association. In: (Vose, P.B. and A.P. Ruschel, eds.). CRC Associative Symbiosis. 1: 1-6.

Hussain, A., M. Arshad, A. Hussain and F. Hussain. 1987. Response of maize *(Zea mays)* to *Azotobacter* inoculation under fertilized and unfertilized conditions. Biol. Fert. Soils, 4: 73-77.

Hussain, A., A. Hussain, M.A. Hayee and G. Yaseen. 1989 a. Effect of L-methionine on growth, nodulation and nutrient uptake of *Albizia lebbeck*. Nitrogen Fixing Tree Res. Rep. 7: 65-68.

Hussain, A., M. Khalid, A. Latif and K. Hussain. 1989 b. Effect of L-tryptophan on growth, nodulation and nitrogen content of *Albizia lebbeck*. Nitrogen Fixing Tree Res. Rep. 7: 69-72.

Inbal, E. and M. Feldman. 1982. The response of a hormonal mutant of common wheat to the bacteria of genus *Azospirillum*. Israel J. Bot. 31: 257-263.

Ince, J.E. and C.J. Knowles. 1985. Ethylene formation by cultures of *Escherichia coli*. Arch. Microbiol. 141: 209-213.

Ince, J.E. and C.J. Knowles. 1986. Ethylene formation by cell-free extracts of *Escherichia coli*. Arch. Microbiol. 146: 151-158.

Kucey, R.M.N. 1988. Plant growth-altering effects of *Azospirillum brasilense* and *Bacillus* C-11-25 on two wheat cultivars. J. App. Bacteriol. 64: 187-196.

Loper, J.E. and M.N. Schroth. 1986. Influence of bacterial sources of indole-3-acetic acid on root elongation of sugar beet. Phytopathol. 76: 386-389.

Lynch, J.M. 1972. Identification of substrate and isolation of microorganisms responsible for ethylene production in soil. Nature, 240: 45-46.

Mahadevan, A. (ed.). 1984. Growth Regulators, Micrrorganisms and Diseased Plants. 486 p. Oxford and IBH Publishing Co., New Delhi, India.

Mahmoud, S.A.Z., E.M. Ramadan, F.M. Thabet and T. Khater. 1984. Production of plant growth substances by rhizosphere microorganisms. Zbl. Mikrobiol. 139: 227-232.

Muller, M., C. Deigele and H. Ziegler. 1989. Hormonal interactions in the rhizosphere of maize *(Zea mays* L.) and their effects on plant development. Z. Pflanzen. Bodenk. 152: 247-254.

Nieto, K.F. and W.T. Frankenberger, Jr. 1989 a. Biosynthesis of cytokinins by *Azotobacter chroococcum*. Soil Biol. Biochem. 21: 967-972.

Nieto, K.F. and W.T. Frankenberger, Jr. 1989 b. Biosynthesis of cytokinins in soil. Soil Sci. Soc. Amer. J. 53: 735-740.

Nieto, K.F. and W.T. Frankenberger, Jr. 1990 a. Microbial production of cytokinins. In: (Bollag, J.M. and G. Stotzky, eds.). Soil Biochem. 6: 1191-248. Marcel Dekker Inc., NY, USA.

Nieto, K.F. and W.T. Frankenberger, Jr. 1990 b. Influence of adenine, isopentyl alcohol and *Azotobacter chroococcum* on the growth of *Raphanus sativa* (radish). Plant Soil, 127: 147-156.

Nieto, K.F. and W.T. Frankenberger, Jr. 1991. Influence of adenine, isopentyl alcohol and *Azotobacter chroococcum* on the vegetative growth of *Zea mays*. Plant and Soil, 135: 213-221.

Primrose, S.B. 1976. Ethylene forming bacteria from soil and water. J. Gen. Microbiol. 97: 343-346.

Primrose, S.B. 1979. A review, ethylene and agriculture: the role of microbes. J. App. Bacteriol. 46: 1-25.

Purushothaman, D., K. Balaraman and G. Oblisami. 1973. Indole acetic acid metabolism in soil as influenced by pesticide application. Cuurent Sci. 42: 365-366.

Purushothaman, D., T. Marimuthu, C.V. Venkataraman and R. Kesavan. 1974. Role of actinomycetes in the biosynthesis of indole acetic acid in soil. Current Sci. 43: 413-414.

Smith, A.M. 1976. Ethylene in soil biology. Ann. Rev. Phytopathol. 14: 53-73.

Smith, K.A. and Russell. 1969. Occurrence of ethylene and its significance in anaerobic soil. Nature, 222: 769-771.

Tien, T.M., M.H. Gaskins and D.H. Hubbell. 1979. Plant growth substances produced by *Azospirillum brasilense* and their effects on the growth of pearl millet *(Pennisetum americanum* L.). App. Environ. Microbiol. 37: 1016-1024.

Triplett, E.W., J.J. Heitholt, K.B. Evensen and D.G. Blevins. 1981. Increase in internode length of *Phaseolus lunatus* L. caused by inoculation with a nitrate reductase-deficient strain of *Rhizobium* sp. Plant Physiol. 67: 1-4.

William, P.M. and M.S. deMallorca. 1982. Ascorbic acid and gibberellin-like substances in roots and root nodules of Glycine max. Plant and Soil, 65: 19-26.

Zimmer, W., K. Roeben and H. Bothe. 1988. An alternative explanation for plant growth promotion by bacteria of the genus *Azospirillum*. Planta, 176: 333-342.