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Assimilation of exogenous 2′-^{14}C-indole-3-acetic acid and 3′-^{14}C-tryptophan exposed to the roots of three wheat varieties

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Abstract

This study was conducted to determine if plants can assimilate indole-3-acetic acid (IAA) from rooting media and if exogenous L-tryptophan (L-TRP) can be assimilated and converted by plants into auxins. The addition of 2′−^{14}C-IAA (3.7 kBq plant^{-1}) to wheat (*Triticum aestivum* L.) seedlings of three varieties grown in nutrient solution resulted in the uptake (avg. = 7.6%) of labelled IAA. Most of the label IAA was recovered in the shoot (avg. = 7.2%) with little accumulation in the root (avg. = 0.43%). A portion of the assimilated IAA-label in the plant was identified by co-chromatography and UV spectral confirmation as IAA-glycine and IAA-aspartic acid conjugates. Little of the assimilated IAA label was found as free IAA in the wheat plants. These same assimilation patterns were observed when 2′−^{14}C-IAA was added to wheat plants grown in sterile and nonsterile soil. In contrast, the wheat varieties assimilated considerably less (avg. = 1.3 %) of the added microbial IAA precursor, 3′−^{14}C-L-TRP (3.7 kBq plant^{-1}) and thus much lower amounts of IAA conjugates were detected. Glasshouse soil experiments revealed that 2 out of 3 wheat varieties had increased growth rates and increased yields when L-TRP (10^{-5} and 10^{-7} *M*) was added to the root zone. It is surmised that this positive response is a result of microbial auxin production within the rhizosphere upon the addition of the precursor, L-TRP. The amino acid composition of the root exudates plays a critical role in microbial production of auxins in the rhizosphere. This study showed that wheat roots can assimilate IAA from their rooting media, which will supplement the endogenous IAA levels in the shoot tissue and may positively influence plant growth and subsequent yield.

Introduction

Microbial growth in the rhizosphere is stimulated by the continual input of readily assimilated organic substrates from the root (Barber and Martin, 1976). Wheat can transfer large amounts of fixed ^{14}C (up to 59%) to the root system (Whipps and Lynch, 1983; Whipps, 1984). Some of these organic compounds can be lost (up to 29%) as rhizodeposition in the soil environment (Barber and Martin, 1976; Whipps and Lynch, 1983; Whipps, 1984). These organic compounds include sugars, amino acids, and organic acids which may be lost from the root without involvement of metabolic energy. The microbial conversion of these exudates, in particular amino acids such as methionine (MET) and tryptophan (TRP), into secondary metabolites such as ethylene and indole-3-acetic acid (IAA), respectively, may play a significant role in plant development and yield (Arshad and Frankenberger, 1990). However, little is known about the role of IAA from exogenous sources such as rhizosphere microorganisms in the development of plant species.

Frankenberger and Brunner (1983) unequivocally proved by HPLC-MS that soil microorganisms produce IAA and auxin derivatives (indole-3-pyruvic acid and indole-3-acetamide) from the addition of L-TRP to soil. Other studies have revealed that the addition of L-TRP to the root ball of plant seedlings can increase growth (Frankenberger et al., 1990) and increase yield (Frankenberger and Arshad, 1991a,b) compared with no L-TRP addition. Dilute L-TRP solutions (10^{-4} to 10^{-9} *M*) promoted the greatest increase in plant growth and yield suggesting a physiological, rather than a nutritional effect. The growth response from L-TRP

additions have been attributed to microbial synthesis of IAA, assimilation of IAA by the developing roots and storage/utilization by the plant. Frankenberger and Arshad (1991b) reported that application of various rates of L-TRP to five pepper (*Capsicum annuum*) varieties resulted in different cultivar yield responses. Sensitivity of the plants to auxin application, genetic makeup and ability to assimilate and metabolize L-TRP and/or its microbial metabolites were suggested as sources of variation in the measured pepper response to L-TRP applications.

Despite the large number of investigations involving classical polar transport of auxins by use of segments of coleoptiles, stems, petioles and excised roots (Goldsmith, 1977, 1982; Jacobs, 1979), relatively little attention has been directed towards the uptake of IAA by whole roots from the rhizosphere, and transport and utilization of assimilated auxins by intact plants.

Auxin(s) that appear in the root zone of plants have several fates. They can be assimilated by the roots (intact or excised) (Meuwly and Pilet, 1991; Thurman and Street, 1962), utilized as a carbon source by soil microorganisms (Arshad and Frankenberger, 1990), or metabolized by root tissues (Beffa et al., 1990; Meuwly and Pilet, 1991). The site for IAA assimilation is the root tip (0-2 mm) and expanding cells directly behind the root tip (2-5 mm) (Martin and Pilet, 1986). The assimilated IAA has three fates: it can move acropetally as free IAA (Pernet and Pilet, 1979), it can be degraded by oxidase and peroxidases (Beffa et al., 1990) or IAA can be conjugated with carbohydrates and amino acids (Andreae and Good, 1955; Meuwly and Pilet, 1991). The time frame of these experiments involving plant uptake of IAA have been very short (2 to 48 h) with comparatively few studies published on the persistence or fate of the assimilated IAA in plant tissues.

Germinating seeds have also been reported to be a source of IAA produced from L-TRP (Davies, 1987; Monterio et al., 1988). Monteiro et al. (1988) found that the germinating seed of a woody legume (*Dalbergia dolichopetala*) converted [^{3}H]-L-TRP into [^{3}H]IAA. Metabolism of the labelled IAA resulted in production of the IAA conjugate, IAA-aspartic acid.

The yield responses noted by Frankenberger et al. (1990) and Frankenberger and Arshad (1991a) with the addition of L-TRP may be due to uptake of the applied L-TRP by plant roots and conversion into IAA rather than assimilation of microbially-produced IAA. Uptake of amino acids by plant roots has previously been demonstrated for different plant species (Petzold et al., 1989; Soldal and Nissen, 1978). Soldal and Nissen (1978) reported that excised barley roots assimilated the labelled amino acids, L-lysine, L-MET and L-proline from nonbuffered 5×10^{-4} *M* $CaCl_2$ and after 30 min, 75% of assimilated ^{3}H-lysine remained unchanged.

The objectives of this work were to determine if 2′−^{14}C-IAA present in nutrient media could be assimilated and utilized by wheat varieties to augment the endogenous auxin levels. In addition, experiments were conducted to determine if wheat seedlings could assimilate and transform 3′−^{14}C-L-TRP into auxins. The subsequent growth and yield of wheat upon the addition of L-TRP to the root zone was also monitored.

Materials and methods

Plant material and growth conditions

Triticum aestivum L. var. *INIA* 66R, *Tadina* and *ANZA* were obtained from the Foundation Seed and Plant Material Center, University of California, Davis, CA. Caryopses of three wheat varieties used in this study were surface-sterilized with 70% ethanol (5 min) prior to a 10-min rinse with sodium hypochlorite-Tween 20 solution (0.5 mL Tween 20 to 100 mL sodium hypochlorite). Under sterile conditions, the disinfectant solution was decanted and the seeds were rinsed with several 100-mL aliquots of sterile water. The disinfected seeds were then placed in sterile germination pouches and germinated at 28°C. After germination they were placed in sterile 50-mL Erlenmeyer flasks (triplicate), equipped with a rubber septum with a small hole for shoot emergence. The microcosms contained acid-washed sand, sterilized Yolo soil (Typic Xerorthent; pH, 8.4; organic C, 5 g kg^{-1} soil; total N, 1.19 g kg^{-1} soil; 575 g sand kg^{-1} soil; 225 g clay kg^{-1} soil), or nonsterile Yolo soil, treated with 0.5 *N* Hoagland's nutrient solution. The microcosms were placed into a sterilized [95% ethanol and UVB radiation (24 h)] growth chamber with a light intensity of 100 W m^{-2} at ambient temperatures with a photoperiod of 12 h. The Yolo soil used was sterilized by autoclaving at 121°C and 15 psi for 2 h.

Amino acid exudation

The amino acids present in the three varieties of wheat root tissue and root exudates were determined by the method developed by Martens and Frankenberg-

er (1992). The wheat varieties were aseptically grown for 17 days in sterile sand amended with nutrient solution. The plant material was carefully removed from the media and the free amino acids present in the sand-nutrient medium were extracted with a 20% ethanol solution, concentrated on a hot plate (40°C) to 1 mL and filtered through a 0.22 μm GS Millipore filter. The root mass was air-dried for 24 h, finely-ground and hydrolyzed in 4 *M* methanesulfonic acid (Simpson et al., 1976) at 120°C for 22 h. The amino acids were separated on an AminoPac PA1 column (Dionex, Sunnyvale, CA) by anion exchange and detected by pulsed amperometric detection (Martens and Frankenberger, 1992).

IAA and L-TRP assimilation

Radiolabelled IAA and L-TRP were obtained from Amersham Corp. (Arlington Heights, IL, USA). 2′−^{14}C-indole-3-acetic acid (specific activity, 61 mCi mM^{-1}) and 3′−^{14}C-L-tryptophan (specific activity, 53.5 mCi mM^{-1}) were added (1.85 kBq plant^{-1}; 1.45 μg IAA and 1.91 μg L-TRP plant^{-1}, respectively) to the rooting media of one-week-old wheat seedlings and a second addition was made at the end of the second week of growth. The plants were allowed to develop for two weeks after the last radiolabel addition. The plants were then carefully removed from the rooting media and, after thorough washing, pressed and dried at room temperature for 24 h. The dried plant material was placed in contact with Kodak™ X-OMAT XAR-2 film for 48 h and then dissected into shoot and root sections and macerated for auxin extraction with 2 mL 0.1 *M* KH_2PO_4 (pH 7.0) at 4°C for 24 h (Sandberg et al., 1987). The extract was filtered with a 0.22 μm filter (Millipore GS filter) and the labelled auxin(s) or derivatives were quantified by reverse phase ion-suppression high performance liquid chromatography (IS-HPLC-UV) with quantification of peaks by co-chromatography and UV spectral confirmation with authenic standards.

Auxin separation and fractionation

The IS-HPLC-UV analysis was performed on a Beckman Model 330 HPLC system (Fullerton, CA) including a Model 110A pump, a Model 210 sample injector equipped with a BioRad (BioRad Lab., Richmond, CT, USA) ODS-5μm guard column (30 × 4.6 mm) as the primary column, a R-Sil (250 × 4.6 mm) reverse phase column (Alltech Associates, Deerfield, IL, USA) as the secondary column, and detection with a Beckman Model 165 absorbance monitor set at 280 nm (0.01 absorbance units full scale). The mobile phase was 45% methanol: 55% deaired HPLC grade H_2O adjusted to pH 2.53 with H_3PO_4 (Frankenberger and Poth, 1987). Separations were conducted at ambient temperatures and the flow rate was 1 mL min^{-1}. A full description of the on-line solid-phase extraction procedure for determination of auxins present in phosphate buffer is described by Martens and Frankenberger (1991). Briefly, an aliquot of the plant extract (150-200 μL) was injected onto the primary column, rinsed free of interferences, injected onto the secondary column and fractionated (0.5 mL fractions) by an ISCO Retriever II (ISCO, Lincoln, NE, USA) into 4 mL scintillation cocktail (Complete Counting Cocktail; Research Products, Mt. Prospect, IL, USA). The fractions were then counted on a Beckman 5000 TD scintillation counter.

Glasshouse studies

Glasshouse experiments were conducted to measure the growth and yield response of the three wheat varieties to the application of L-TRP. Each of the three wheat varieties was grown in 15-cm pots containing 3 kg of a Buren soil (Haplic Durixeralf; pH, 7.3; organic C, 5.2 g kg^{-1} soil; total N, 1 g kg^{-1} soil; 575 g sand kg^{-1} soil; 275 g clay kg^{-1} soil) and treated with 500 mL of H_2O or H_2O containing 10^{-5} *M* (0.001 g pot^{-1}) or 10^{-7} *M* (1×10^{-5} g pot^{-1}) L-TRP after 1 week of growth. Plant growth was then measured weekly and harvested at the appropriate time.

Results and discussion

Microbial production of phytohormones may benefit the rhizosphere microorganisms as well as the plant. By releasing phytohormones, microbes play a role in control of their own environment by affecting the plant's metabolism and possible root exudation. Auxins produced by soil microorganisms can influence plant growth only if the released auxins are subject to plant uptake and not metabolized by other microorganisms (Arshad and Frankenberger, 1992).

The release of amino acids such as L-TRP in root exudates may result in the microbial conversion into auxins such as IAA. The presence of the IAA precursor, L-TRP in root exudates may indicate that the species has increased sensitivity for IAA present in

Table 1. Amino acid composition (free and protein-bound) of the root mass and amino acids collected as exudates of three wheat varieties[a]

Amino acid	Wheat variety		
	ANZA	INIA 66R	Tadina
	nM g^{-1} root		
Arg	28.1 (2.6)[b]	21.0 (0.1)	23.4 (0.4)
Lys	139.3 (0.4)	136.8 (0.1)	99.3 (0.4)
Gln	ND[c] (0.1)	ND (0.1)	ND (0.1)
Asn	ND (0.1)	ND (0.1)	ND (0.2)
Thr	22.5 (0.1)	19.5 (ND)	18.3 (0.1)
Ala	70.2 (0.7)	63.4 (ND)	60.6 (0.5)
Gly	149.0 (1.2)	140.7 (0.2)	207.2 (1.5)
Ser	34.7 (0.5)	27.5 (0.1)	19.6 (0.7)
Val	33.8 (0.1)	58.6 (ND)	6.5 (0.3)
Pro	22.5 (0.1)	25.8 (0.2)	23.0 (ND)
Ile	15.0 (0.8)	19.6 (ND)	18.6 (ND)
Leu	46.8 (1.1)	40.0 (ND)	21.7 (ND)
Met	4.7 (0.1)	1.5 (0.2)	2.1 (ND)
His	3.5 (ND)	0.6 (ND)	0.3 (0.1)
Phe	2.4 (0.7)	1.9 (0.1)	1.2 (0.1)
Glu	62.0 (1.9)	42.7 (0.5)	32.2 (1.0)
Asp	36.0 (0.5)	32.5 (0.2)	28.7 (ND)
Cys-Cyx	3.2 (ND)	6.0 (0.3)	4.5 (0.1)
Try	11.0 (ND)	16.0 (ND)	17.3 (ND)
Trp	22.6 (0.6)	20.3 (ND)	29.4 (0.6)
Total amino acids	707.3 (11.6)	674.4 (2.2)	613.9 (6.1)

[a]Three wheat plants of each variety were aseptically grown for 17 days. The sand-nutrient medium was extracted with 20% ethanol for amino acid analysis. The root mass was air-dried and hydrolyzed with 4 *N* methanesulfonic acid for 22 h at 120° C.
[b]Values in parentheses indicate exudate amino acid level reported as n*M* plant^{-1}.
[c]Not detected.

Table 2. Percentage of total applied label IAA and L-TRP recovered in 0.1 *M* KH_2PO_4 (pH 7.0) extracts of wheat shoot, root tissue and remaining in aseptic nutrient solution after growth for 28 d

Source	Label	Wheat variety		
		ANZA	*Tadina*	*INIA 66R*
		% Recovery		
Shoot	IAA	3.00 (±0.42)[a]	12.5 (±1.10)	6.2 (±0.75)
Root	IAA	0.15 (±0.03)	0.32 (±0.08)	0.82 (±0.10)
Solution	IAA	20.80 (±5.33) [76.1][b]	17.40 (±6.22) [69.8]	31.80 (±6.22) [62.2]
Shoot	L-TRP	0.24 (±0.06)	0.33 (±0.08)	3.2 (±0.62)
Root	L-TRP	0.35 (±0.04)	0.75 (±0.08)	2.4 (±0.21)
Solution	L-TRP	15.70 (±4.86) [83.7]	22.00 (±4.96) [76.9]	30.60 (±5.02) [63.8]

[a]Standard deviation of the mean.
[b]Values in brackets indicate percentage of radioactivity not recovered from a 3.7 kBq addition.

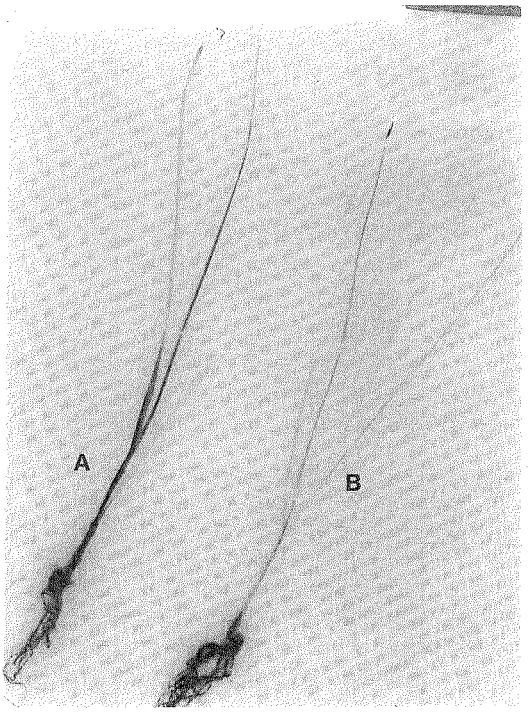

Fig. 1. Autoradiogram of aseptic *Triticum aestivum* L. var. INIA 66R treated with: A)2′−^{14}C-IAA (3.7 kBq); B) 3′−^{14}C-L-TRP (3.7 kBq), incubated for 28 d and film exposed for 48 h.

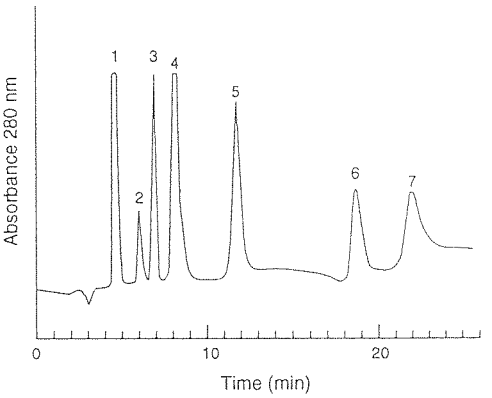

Fig. 2. Chromatogram of L-TRP and auxin derivative standards detected by IS-HPLC. 1 = 5-hydroxyindole-3-acetic acid; 2 = 3-indoleacetyl-aspartic acid; 3 = 3-indoleacetyl-glycine; 4 = indole-3-acetamide; 5 = indole-3-acetic acid; 6 = indole-3-propionic acid; 7 = tryptophan.

the rhizosphere. The presence of amino acids in the root exudates of the three wheat varieties used in this study was evaluated in an aseptic growth medium. The results showed that the amino acid exudation pattern of the wheat varieties was not similar to the amino acid composition of the root tissue (Table 1). This suggests that other processes besides cell leakage may be releasing amino acids. Two of the wheat varieties, ANZA and Tadina had detectable levels of L-TRP in the root exudates. ANZA and INIA 66R had detectable levels of MET and all three had detectable levels of phenylalanine (a precursor of phenylacetic acid) in their exudates.

The conversion of L-TRP to IAA by soil microorganisms is regulated by the enzyme, L-TRP aminotransferase (EC 2.6.1.27) (Frankenberger and Poth, 1988). Martens and Frankenberger (1993a) reported that preincubation of soil or rhizobacteria in the presence of the amino acids, alanine (ALA), arginine (ARG) or lysine (LYS) increased the conversion of L-TRP to auxins. Kutáček (1985) demonstrated that L-TRP aminotransferase had a greater specificity (2- to 3-fold) for ALA, ARG and LYS when compared to L-TRP. The varieties, ANZA and Tadina which had detectable levels of L-TRP in the root exudates also had elevated levels of ALA, ARG and LYS when compared with the INIA 66R variety. The exudation of ALA, ARG and LYS by these wheat varieties could possibly stimulate the microbial aminotransferase reaction in the rhizosphere for the conversion of L-TRP to IAA.

The addition of 2′−^{14}C-IAA and 3′−^{14}C-L-TRP to root media of the three aseptic wheat varieties resulted in assimilation (Table 2) and translocation of the label in the plant tissue (Fig.1). The aerial portion of the wheat varieties accumulated a higher percentage of the 2′−^{14}C-IAA (7.2%) than the added 3′−^{14}C-TRP (1.3%) (Table 2). Less 2′−^{14}C-IAA was recovered (avg. = 0.43%) in the root tissue indicating that the exogenous source of IAA supplied was readily translocated to the shoot. The wheat variety Tadina had 2.7-fold more IAA, IAA-aspartic acid (IAAsp), and IAA-glycine (IAAgln) counts than the ANZA variety and 4.5-fold more IAA and conjugate counts than the INIA 66R variety (Table 3) in the shoot tissue when grown with 2′−^{14}C-IAA additions.

The sterile three wheat varieties assimilated very little of the applied L-TRP label (Table 2) and extremely low levels of activity were found as auxin derivatives (Table 3) when the plant tissue was fractionated and counted. Thurman and Street (1962) have reported that there was little evidence for conversion of L-TRP to ethyl acetate soluble auxins in excised tomato and wheat roots. Of the three varieties used in this study, INIA 66R assimilated 6.8- and 3.2-fold more and translocated 13.3- and 9.7-fold more 3′−^{14}C-TRP compared with the root and shoot tissue of the

Table 3. Distribution of labelled IAA and L-TRP as auxin derivatives in shoot tissue of three wheat varieties after growth for 28 d

Auxin derivative[a]	Wheat variety		
	ANZA	*Tadina*	*INIA 66R*
	ng 100 mg^{-1} tissue (DW)[b]		
3′−^{14}C-L-TRP added			
5-OH-IAA	3.0 (±0.02)[c]	2.5 (±0.04)	4.5 (±0.12)
IAAsp	2.3 (±0.01)	2.3 (±0.04)	5.0 (±0.08)
IAAgln	2.4 (±0.04)	1.5 (±0.03)	8.5 (±0.21)
IAA	2.5 (±0.08)	1.0 (±0.09)	1.7 (±0.20)
2′-14C-IAA added			
5-OH-IAA	65.3 (±2.80)	91.0 (±4.70)	33.0 (±6.70)
IAAsp	16.6 (±2.80)	51.6 (±6.70)	10.5 (±3.00)
IAAgln	13.0 (±3.20)	27.3 (±4.10)	6.8 (±2.20)
IAA	4.2 (±1.80)	10.8 (±2.60)	2.5 (±1.30)

[a]5-OH-IAA, 5-hydroxyindole-3-acetic acid; IAAsp, 3-indoleacetyl-aspartic acid; IAAgln, 3-indoleacetyl-glycine; IAA, indole-3-acetic acid.
[b]DW, dry weight.
[c]Standard deviation of the mean.

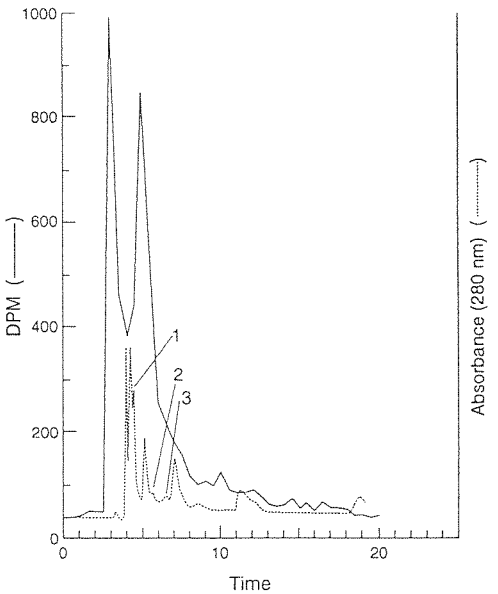

Fig. 3. Chromatogram of aseptic ^{14}C-IAA-treated *Triticum aestivum* L. var. INIA 66R (28 d) 0.1 *M* KH_2PO_4 (pH 7.0) extract and radioactivity (DPMs) present in 0.5 mL HPLC fractions. Absorbance units full scale = 0.10. See Figure 2 for reference of peaks.

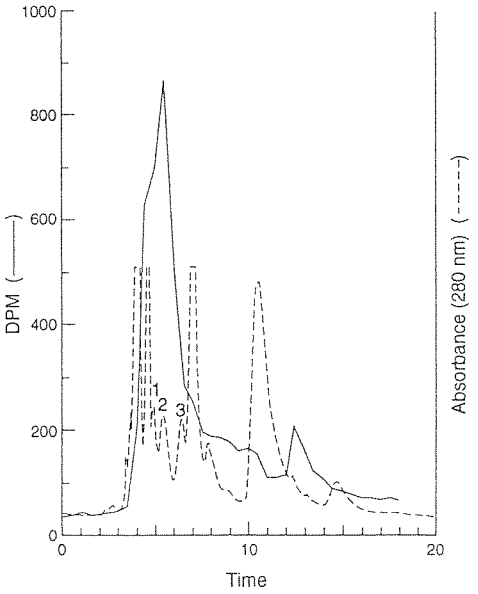

Fig. 4. Chromatogram of aseptic ^{14}C-IAA-treated *Triticum aestivum* L. var. Tadina (28 d) 0.1 *M* KH_2PO_4 (pH 7.0) extract and radioactivity (DPMs) present in 0.5 mL HPLC fractions. Absorbance units full scale = 0.01. See Figure 2 for reference of peaks.

ANZA and Tadina varieties, respectively (Table 2). This assimilation of L-TRP radioactivity resulted in a 3.2-fold increase in IAA and auxin conjugate counts than the Tadina variety and 2-fold increase in IAA and its conjugate counts when compared with the ANZA variety (Table 3). It is of interest that the INIA 66R did not have detectable levels of L-TRP in the collected root exudates compared with L-TRP released from the varieties ANZA and Tadina.

Table 5. Comparison of L-TRP concentrations on yield of three wheat varieties[a]

Variety	L-TRP added					
	0.0 *M*		10^{-5} *M*		10^{-7} *M*	
	Total yield	Weight head^{-1}	Total yield	Weight head^{-1}	Total yield	Weight head^{-1}
	g					
ANZA	6.08 (±1.10)[b]	0.49 (±0.08)	9.29 (±0.80)	0.62 (±0.10)	6.21 (±0.75)	0.52 (0.21)
Tadina	2.50 (±0.85)	0.21 (±0.04)	3.80 (±0.93)	0.76 (±0.12)	4.60 (±0.88)	0.46 (0.15)
INIA 66R	2.70 (±1.13)	0.21 (±0.03)	1.52 (±0.89)	0.30 (±0.12)	1.69 (±0.88)	0.21 (0.08)

[a]Three wheat varieties were grown in 15 cm pots (3 kg Buren soil) and treated with specified L-TRP levels and grown to maturity and grain harvested at the appropriate time.
[b]Standard deviation.

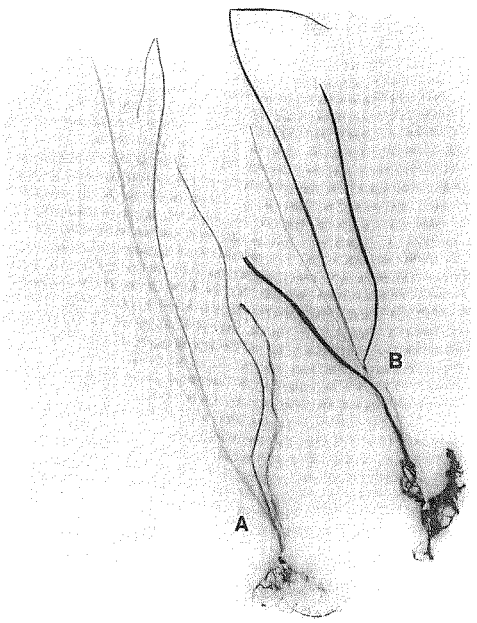

Fig. 5. Autoradiogram of *Triticum aestivum* L. var. INIA 66k treated with 2′−^{14}C-IAA (3.7 kBq) grown for 28 d in: A) nonsterile Yolo soil; B) sterile Yolo soil and film exposed for 48 h.

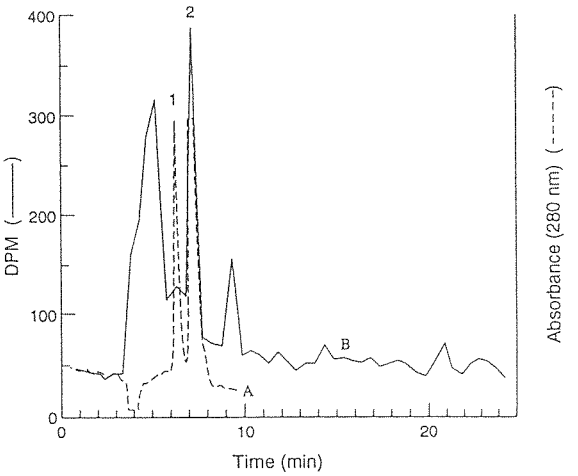

Fig. 6. Chromatogram of: *A*) auxin standards (1) 3-indoleacetyl-aspartic acid, (2) 3-indoleacetyl-glycine and *B*) the radioactivity present in 0.5 mL HPLC fractions of a 0.1 *M* KH_2PO_4 (pH 7.0) extract of ^{14}C-IAA-treated *Triticum aestivum* var. INIA 66R grown in nonsterile Yolo soil.

tionation of the soil solution indicated that the residual soil radioactivity was not IAA in the sterile and non-sterile soil. Since IAA was added to the soil surface, IAA can readily diffuse through the soil within the root zone. This study supports our previous findings (Martens and Frankenberger, 1993b) that IAA and other auxin derivatives are not highly partitioned with soil colloids but are subject to rapid degradation by soil processes.

Figure 6 shows the chromatography of the auxin standards, IAAsp and IAAgly, overlaid with the disintegrations per min (0.5 mL HPLC fractions) of a 0.1 *M* phosphate extract of the wheat variety INIA 66R grown in nonsterile Yolo soil treated with 2′−^{14}C-

Table 4. Percentage of total applied IAA radioactivity recovered in 0.1 *M* KH_2PO_4 (pH 7.0) extracts of wheat shoot and remaining in soil after growth for 28 d

Source	Conditions	Wheat variety		
		ANZA	*Tadina*	*INIA 66R*
		% Recovery		
Shoot	Sterile	1.00 (±0.02)[a]	0.49 (±0.13)	1.25 (±0.42)
Soil	Sterile	3.13 (±1.00)[95.9][b]	0.69 (±0.09)[98.8]	1.28 (±0.65)[97.5]
Shoot	Nonsterile	0.50 (±0.04)	0.33 (±0.10)	0.82 (±0.42)
Soil	Nonsterile	0.12 (±0.04)[99.1]	0.15 (±0.06)[99.5]	0.21 (±0.09)[99.0]

[a]Standard deviation.
[b]Values in brackets indicate percentage of radioactivity not recovered from a 3.7 kBq addition.

Hitchcock and Zimmerman (1953) first reported possible absorption of plant growth regulators by *Pisum sativum* (pea) roots as indicated by the response of the aerial portions of the plants. Andreae and Good (1955) and later, Andreae and van Ysselstein (1956, 1960) reported that IAA assimilated by plant tissue (*Pisum sativum*) resulted in an accumulation of IAAsp. Figure 2 is a standard chromatogram for the separation and purification of auxin derivatives. Figures 3 and 4 show HPLC-UV chromatograms of the extracts of the wheat varieties, INIA 66R and Tadina treated with $2'-^{14}$C-IAA overlaid with disintegrations per min (DPM) in the recovered 0.5 min HPLC fractions. Polar auxin compounds such as 5-hydroxyindole-3-acetic acid and the amino acid conjugates, IAAsp and 3-indoleacetyl-glycine were detected in all three varieties. Bandurski and Schulze (1977) reported that auxin-amino acid conjugates are involved in a homeostatic mechanism for maintenance of IAA concentrations. Kendall et al. (1971) identified 5-OH-IAA in *Pisum sativum* L. seedlings, in addition to IAAsp, when incubated with labelled IAA. However, in our study the INIA 66R variety was not as active in converting $2'-^{14}$C-IAA label into these storage forms compared with the ANZA and Tadina varieties (Table 3). It was also noted that very little labelled "free" IAA was detected in these wheat varieties (Table 3).

IAA assimilated by plant roots can have several fates. IAA can be degraded by peroxidases or conjugated by ester or amide compounds. The conjugation of IAA has been reported to protect IAA from peroxidative attack (Cohen and Bandurski, 1978). Meuwly and Pilet (1991) found that *Zea mays* roots rapidly assimilated and metabolized an exogenous source of IAA within 1 h of root immersion and after 4 h most of the IAA was present as IAA conjugates. Venis (1972) reported that pretreatment of *Pisum sativum* L. sections with IAA induced an enzyme which formed aspartate conjugates with the exogenous IAA.

Two pathways have been established for the oxidative degradation of IAA (Beffa et al., 1990). The first involves oxidation of the indole nucleus, whereas the second pathway consists of the oxidative decarboxylation of the side chain by peroxidases (EC 1.11.1.7). Table 2 shows that even under aseptic conditions the majority of radioactivity (62 to 76%) added to the rooting medium was not recovered from the plant tissue or rooting medium. Kendall et al. (1971) reported that $1'-^{14}$C-IAA was rapidly decarboxylated when introduced into sterile plant culture. Our work suggests that wheat varieties have different capacities to degrade auxins with the recovered radioactivity lower in the ANZA variety than the Tadina or INIA 66R varieties.

Figure 5 shows the translocation pattern of $2'-^{14}$C-IAA in the INIA 66R variety when labelled IAA was added to sterile and nonsterile Yolo soil. The autoradiogram shows that the addition of $2'-^{14}$C-IAA to the sterile soil resulted in more assimilation than addition to nonsterile soil (Table 4). This is no doubt due to the increased microbial pressures in the nonsterile soil, but it is evident that wheat varieties can assimilate IAA present in the soil rhizosphere. Most of the $2'-^{14}$C-IAA added to either soil medium (sterile vs. non- sterile) was not recovered (> 95 %) in the plant tissue or the soil solution (Table 4). This suggests that even in sterile soil, degradation processes mediated by root peroxidases and oxidases can limit the persistence of auxins and assimilation. These degradative processes may prevent levels higher than optimum IAA levels from negatively affecting plant growth. HPLC frac-

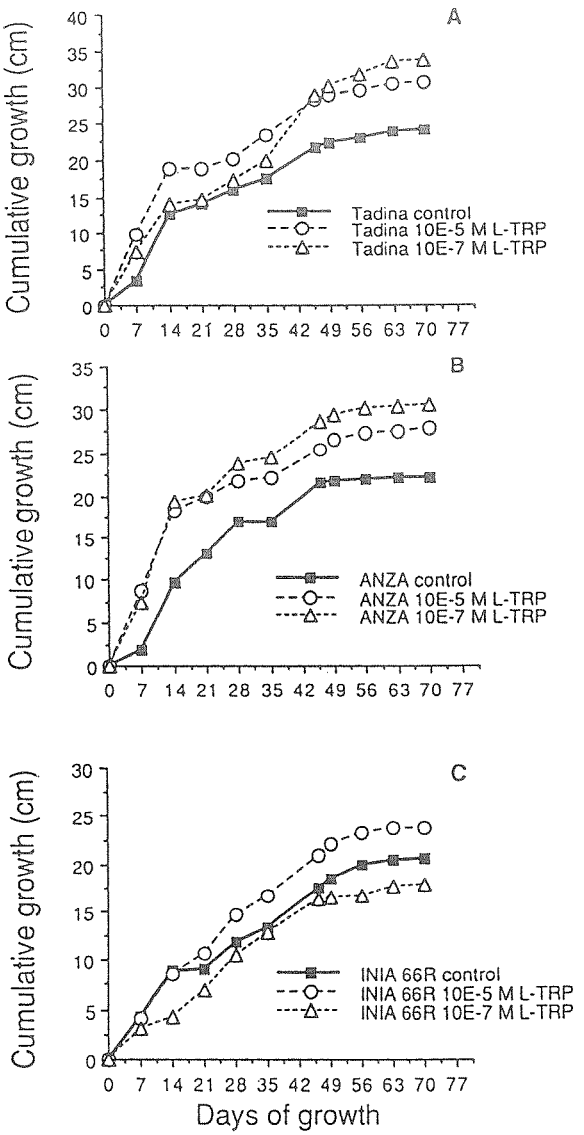

Fig. 7. Cumulative growth for the wheat varieties **A**) Tadina; **B**) ANZA; and **C**) INIA 66R during a glasshouse study.

IAA. Assimilation of IAA was not as great as when this wheat variety was grown in sterile nutrient media but these results show that IAA present in a nonsterile soil can be assimilated by the INIA 66R wheat variety to supplement endogenous IAA levels.

The growth rates of the three wheat varieties in a glasshouse study after addition of 10^{-5} and 10^{-7} *M* L-TRP are shown in Figure 7. The applications resulted in positive growth response for the ANZA variety (33% and 38% increase for 10^{-5} *M* and 10^{-7} *M* L-TRP, respectively) and the Tadina variety (28% and 41%), but only 15% (10^{-5} *M*) and a -14% (10^{-7} *M* L-TRP) growth response for the INIA 66R variety was observed. The soil used in the study is active in producing auxins including IAA from L-TRP additions (Martens and Frankenberger, unpublished data). The total mature wheat harvested and yield per head of the three wheat varieties in response to L-TRP additions are shown in Table 5. Additions of L-TRP to the soil supporting the Tadina variety substantially increased yields of the total harvest and per head compared with no L-TRP additions. This yield enhancement was less pronounced for the ANZA variety and was not observed with INIA 66R.

The results suggest that the variation in wheat yield response as observed in peppers (Arshad and Frankenberger, 1991a) may be due to the varietal differences for assimilation of auxins from the soil rhizosphere. In addition, these differences may be related to the amino acid exudation pattern noted from the wheat roots. The presence of ARG, LYS and ALA in addition to L-TRP may suggest that the plant root system of the Tadina and ANZA varieties may be more sensitive to production of auxins by the soil rhizosphere and may possibly stimulate microbial conversion of amino acids to plant growth regulators. The wheat varieties differ in their amino acid exudation pattern and their ability to absorb an exogenous source of IAA through the root system. Plant roots can assimilate IAA from soil and translocate conjugates to the aerial portions of the plant. Assimilation of L-TRP present in the rooting zone and conversion into auxins by plant tissue was less evident. The assimilated IAA can supplement the endogenous levels of IAA and positively affect plant growth and development.

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