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Substrate Dependent Microbially Derived Plant Hormones for Improving Growth of Maize Seedlings

Zahir Ahmed Zahir, Syed Anjum Abbas, Muhammad Khalid and Muhammad Arshad
Department of Soil Science, University of Agriculture, Faisalabad-38040, Pakistan

Abstract: Ten *Azotobacter* cultures were isolated from the maize rhizosphere and their auxin producing ability was measured colourimetrically. The auxin production by three efficient *Azotobacter* cultures (Z1, Z3, Z4) was also measured in the presence of filter sterilized L-tryptophan (at 10^{-3}, 10^{-4} and 10^{-5} M). *Azotobacter* culture Z4 gave relatively higher auxin production and was selected for further experiments. *Azotobacter* inoculation in combination with 10^{-4} M L-TRP gave maximum length and weight of maize roots, which was 117 and 60 percent higher than control, respectively. Leonard Jar experiments were conducted to study the response of shoot growth to *Azotobacter* inoculation and L-TRP application at 10^{-4} M separately and in combination with each other. Results showed that maximum length and weight of shoots were recorded by applying *Azotobacter* in combination with 10^{-4} M L-TRP which was 45.3 and 36.5 percent higher than control, respectively. The possible mechanisms of action are discussed.

Key words: *Azotobacter*, L-tryptophan, Maize

Introduction

It has been established and is now well accepted that normal plant growth and development throughout ontogeny is controlled by these compounds produced by the plant itself (Davies, 1987). However, plants may not have the capacity to synthesize sufficient endogenous plant hormones for optimal growth and development under sub-optimal growth and environmental conditions. Exogenously supplied plant hormones may affect plant growth by changing the balance of endogenous levels of hormones, allowing a modification of growth and development in desired direction and to the desired extent (Nickell, 1982).

Another potential and economical source of these phytohormones is the soil microbiota. A vast majority of soil microorganisms release these compounds (Frankenberger and Arshad, 1995; Arshad and Frankenberger, 1997). Studies have shown that microbial production of phytohormones can be increased several folds by providing their suitable precursors. These precursors may provide a continuous source of active substances due to the activities of rhizosphere microbiota for plant uptake and affect the plant growth because of the intimate contact between rhizosphere microbiota and plant roots which is better than one time application of synthetic compounds (Arshad and Frankenberger, 1990).

Many studies have shown the ability of inocula to produce plant hormones as one of the most plausible explanations for microbe-plant interactions (Hussain *et al*. 1987; Arshad and Frankenberger, 1991). The availability of suitable precursor is one of the primary factors affecting microbial secretion of these secondary metabolites. The exogenous application of precursors resulted in increasing the magnitude of phytohormone production in culture and soil by several folds (Frankenberger and Arshad, 1995).

L-Tryptophan (L-TRP) is considered an efficient physiological precursor of auxins in higher plants as well as for microbial biosynthesis of auxins (Arshad and Frankenberger, 1991). Frankenberger *et al*. (1990) reported the physiological response of radish (*Raphanus sativus*) to L-TRP applied to soil under optimal nutritional conditions. They observed a significant positive effect of L-TRP on growth parameters of radish when applied at low concentration at the seedling stage.

Zahir *et al*. (1997b) conducted a pot experiment to evaluate the effect of an auxin precursor L-TRP and *Azotobacter* inoculation on potato yield under fertilized conditions. They reported that *Azotobacter* inoculation when supplemented with L-TRP was more effective than their application alone in increasing tubers and straw yield (up to 69.9 and 47.8 percent, respectively) in comparison with control. Khalid *et al.* (1999) also reported similar results from a field experiment on wheat crop. According to them, combined application of *Azotobacter* and L-TRP increased the grain yield, straw yield and 1000-grain weight by 21.3, 20.7 and 6.4 percent, respectively, compared with untreated control.

Materials and Methods

A series of Plate and Leonard Jar experiments were conducted in a controlled temperature Growth Room (at 28 ± 1°C) under axenic conditions to study the effect of *Azotobacter* and L-TRP on growth of maize seedlings.

isolation of *Azotobacter*: *Azotobacter* cultures were isolated from maize rhizosphere by dilution plate technique using modified mannitol agar medium (Society of American Bacteriologists, 1957). Ten fast growing colonies were isolated, purified and numbered as Z1, Z2----Z10.

Measurement of auxin production: Sterilized modified mannitol broth (25 mL) taken in glass tubes was inoculated with ten *Azotobacter* cultures and incubated at 28 ± 1°C for 24 hours with occasional shaking. The contents of the tubes were filtered through whatman filter paper No.2 before measuring auxin production as indole acetic acid (IAA) equivalents. While measuring IAA equivalents, 3 mL of filtrate was taken in test tubes and 2 mL of Salkowski reagent (2 mL 0.5 M $FeCl_3$ + 98 mL 35 percent $HClO_4$) was added to it. The mixture in the tubes was allowed to stand for 30 minutes for colour development. Intensity of the colour was measured at 535 nm by using spectronic -20. Similarly, colour was also developed in standard solutions of IAA and a standard curve was drawn by measuring the intensity of this colour (Sarwar *et al*., 1992). The auxin production by three efficient auxin producing *Azotobacter* cultures was also measured in the presence of filter sterilized L-tryptophan (5 mL of 5 percent solution) using above mentioned procedure. *Azotobacter* (Z4) culture giving best auxin production was selected for plate experiments.

Preparation of inoculum: Sterilized modified mannitol broth taken in conical flasks (250 mL flask) was inoculated with *Azotobacter* culture Z4 and incubated at 28 ± 1°C for 4 days with occasional shaking (4-5 times a day). Fresh inoculum was prepared for each experiment.

Plate Experiments: Two plate experiments were conducted to study the effect of *Azotobacter* inoculation on the growth of maize roots both in the presence (10^{-3}, 10^{-4} and 10^{-5} M) and absence of L-tryptophan. Inoculated seeds (dipping in broth for half an hour) were grown (4 seeds plate^{-1}) for ten days on sterilized moist filter paper. L-Tryptophan was applied at 2 mL plate^{-1}. The precursor-inoculum combination giving best results was selected for subsequent Leonard jar experiments. Data regarding the length and weight of maize roots was recorded.

Leonard Jar Experiments: Plate experiments were repeated in Leonard jar to study the effect of *Azotobacter* inoculation with and without L-tryptophan on the growth of maize shoots. In these experiments, Hoagland solution was taken in glass jars and sand in plastic glasses with a wick passed through glass bottom and dipped in the treatment solution. The apparatus was autoclaved prior to the transfer of germinated seeds. L-Tryptophan and broth inocula of selected *Azotobacter* culture were applied at 5 ml, respectiiitily, in sand. The duration of experiment was 2 weeks. Plate and Leonard jar experiments were conducted in growth room at 28 ±1°C. Data regarding the length and weight of maize shoots were recorded.
Statistical procedures were applied to analyse the data (Steel and Torrie, 1980) using completely randomized design and means were compared by Duncan's Multiple Range Test (Duncan, 1955).

Results

Effect of microbially derived auxins on the growth of maize seedlings was studied. The results are presented as below.

Microbial (*Azotobacter*) production of auxins: Data (Fig. 1) revealed auxin production by ten *Azotobacter* culture which ranged from 1.5 to 3.1 mg L^{-1} . *Azotobacter* culture Z4 showed the highest auxin production (3.1 mg L^{-1}). Auxin production by all other *Azotobacter* cultures was followed in descending order by Z1, Z3, Z5, Z7, Z2, Z10, Z6, Z9 and Z8, respectively. Three efficient auxin producers (Z4, Z1 and Z3) were selected for further experimentation.
The selected *Azotobacter* cultures were used for measuring substrate (L-TRP) dependent auxin production. Data (Fig. 2) revealed that supplementation with L-TRP at 10^{-3} M resulted in maximum increase (171 percent) in auxin production by *Azotobacter* culture Z4, while Z1 gave maximum auxin production at L-TRP concentration of 10^{-4} M, which was 148 percent greater than the respective control. Being the most efficient auxin producer, *Azotobacter* culture Z4 was selected for further experimentation.

Table 1: Effect of *Azotobacter* and L-tryptophan on growth of maize (var. Golden) roots under normal conditions (Plate experiments)

(Average of 4 replicates)

Treatment	Root length (cm)		Root weight (g)	
	Exp.1	Exp.2	Exp.1	Exp.2
Untreated	17.1 d	16.4 e	0.70 e	0.68 f
Azotobacter (A)	17.3 d	27.6 d	0.77 e	0.91
10^{-3} M L-TRP	20.1cd	27.8 d	0.83 d	0.90 e
10^{-4} M L-TRP	22.3bcd	27.9 cd	0.85 cd	1.01 de
10^{-5} M L-TRP	24.5 bc	30.0 bcd	0.89 bc	1.06 cde
A + 10^{-3} M L-TRP	25.0 bc	31.9 abc	0.87 cd	1.23 abc
A + 10^{-4} M L-TRP	37.1 a	34.0 ab	1.12 a	1.43 a
A + 10^{-5} M L-TRP	27.8 b	33.2 ab	0.90 b	1.36 ab

Means sharing the same letter(s) do not differ significantly at p = 0.05

Table 2: Effect of *Azotobacter* and L-tryptophan on shoot growth of maize (var. Golden) under normal conditions (Leonard jar experiment)

(Average of 4 replicates)

Treatment	Shoot length (cm)	Shoot weight (g)
Untreated	32.0 c	1.70 d
Azotobacter (A)	40.0 bc	1.88 c
10^{-4} M L-TRP	39.1 b	2.03 b
A + 10^{-4} M L-TRP	46.5 a	2.32 a

Means sharing the same letter (s) do not differ significantly at 1) = 0.05

Root growth: Data (Table 1) revealed that *Azotobacter* inoculation significantly increased the root length by 68.3 percent in experiment 2, while had non-significant effect in experiment 1. Similarly, all levels of L-TRP significantly increased the root length in case of experiment 2 and highest root length (30.0 cm) was observed under the treatment of 10^{-5} M L-TRP, which was 82.9 percent higher than control. All the Combinations of *Azotobacter* and L-TRP significantly increased the root length in both of the experiments and maximum increases (117 and 107.3 percent, respectively) in root length were observed by applying *Azotobacter* plus 10^{-4} ML-TRP.
In case of root weight, similar kind of response was observed and root weight was increased (by 33.8 percent) significantly by *Azotobacter* inoculation iri experiment-2, while it had non-significant effect in experiment-1. L-Tryptophpn application at 10^{-3}, 10^{-4} and 10^{-5} M significantly increased the root weight and maximum root weights were observed by applying 10^{-5} M L-TRP which were 0.89 and 1.06 g in experiment-1 and 2, respectively.

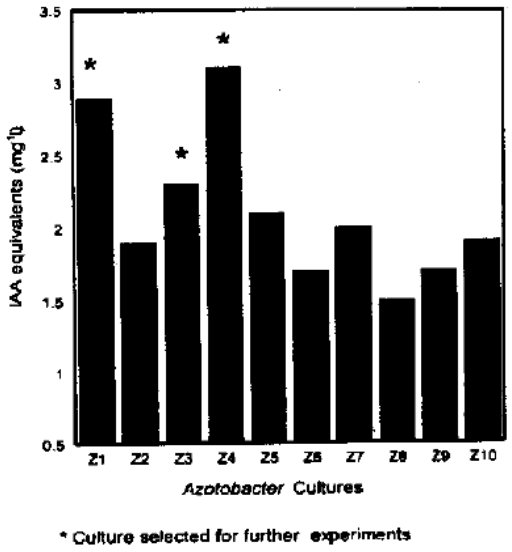

Fig. 1: Measurement of auxin production (IAA equivalents) by different *Azotobacter* cultures

Combined application of *Azotobacter* and L-TRP at 10^{-3}, 10^{-4} and 10^{-5} M also significantly increased root weight and maximum increases (60 and 110.3 percent) were observed by applying *Azotobacter* plus 10^{-4} M L-TRP in both experiments, respectively.

Shoot growth: It is evident from data (Table 2) that *Azotobacter* inoculation and L-TRP application at 10^{-4} M significantly increased the shoot length by 25.0 and 22.2 percent, respectively,

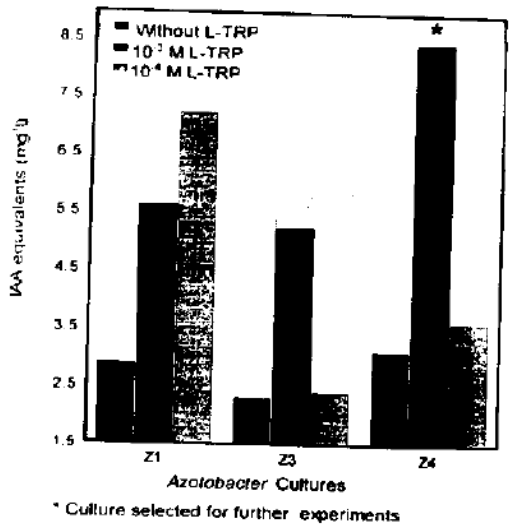

Fig. 2: Effect of L-TRP on auxin production (IAA equivalents) by three *Azotobacter* cultures

compared to uninoculated control. Similarly, compared to uninoculated control. Similarly, combined application of *Azotobacter* and 10^{-4} M L-TRP also enhanced the shoot length significantly by 45.3 percent compared to control.

In case of shoot weight, similar kind of response was observed and increased (by 10.6 *and* 19.4 percent) significantly by *Azotobacter* inoculation and L-TRP application at 10^{-4} M, respectively. Combined application of *Azotobacter* and 10^{-4} M L-TRP also promoted shoot weight by 36.5 percent significantly compared to uninoculated/untreated control.

Discussion

The current studies revealed that inoculation with *Azotobacter* alone or, in combination with L-TRP (an auxin precursor) had significantly positive effects on growth of maize seedlings under axenic conditions. However, the combined application of inoculation and precursor was more effective in improving growth of maize roots and shoots than their alone application.

The beneficial effects of *Azotobacter* on growth and yield of plants have been explained in terms of various mechanisms such as N_2-fixation, production of plant growth regulating substances, alteration in microbial balance of soil, improved mineral uptake, production of siderophores and suppression of pathogenic microorganisms (Arshad and Frankenberger, 1993; Zahir *et al*., 1997a). The ability of *Azotobacter* to produce auxins had been reported by Mahmoud *et al*. (1984) and is considered the most plausible mechanisms of action to explain the beneficial effects of *Azotobacter*. As L-TRP is considered the most common precursor of auxins, so, supplementation of L-TRP increased the production of auxins by *Azotobacter* (Frankenberger and Arshad, 1995). The positive effects of L-TRP observed on the growth of maize may be due to direct uptake of L-TRP by plant roots with subsequent catabolism to auxins within the plant tissues. Zelena *et al.* (1988) reported similar results while working on maize. In our studies, higher plant growth in response to combined application of *Azotobacter* and L-TRP than their separate application could be attributed to microbially derived metabolites such as auxins. Similar to our findings, Khalid *et al.* (1999) also reported combined application of *Azotobacter* and 10^{-4} M L-TRP to be more effective than their separate application in a field trial on wheat. However, more intensive work is still needed to further verify the validity of this approach.

References

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

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

Arshad, M. and W.T. Frankenberger Jr., 1993. Microbial Production of Plant Growth Regulators. In: Soil Microbial Ecology: Application in Agricultural and Environmental Management, Metting, Jr. F.B. (Ed.). Marcel Dekker Inc., New York, USA., ISBN-13: 9780824787370, pp: 307-347.

Arshad, M. and W.T. Frankenberger Jr., 1997. Plant growth-regulating substances in the rhizosphere: Microbial production and functions. Adv. Agron., 62: 45-151.

Davies, P.J., 1987. Plant Hormones and Their Role in Plant Growth and Development. Kluwer Academic Publishers, The Netherlands, Pages: 681.

Duncan, D.B., 1955. Multiple range and multiple F tests. Biometrics, 11: 1-42.

Frankenberger, Jr. W.T. and M. Arshad, 1995. Phytohormones in Soil: Microbial Production and Function. Marcel Dekker Inc., New York, USA., ISBN: 0824794427, Pages: 503.

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

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

Khalid, M., Z.A. Zahir, A. Waseem and M. Arshad, 1999. Azotobacter and L-tryptophan application for improving wheat yield. Pak. J. Biol. Sci., 2: 739-742.

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

Nickell, L.G., 1982. Plant Growth Regulators: Agriculture Uses. Springer Verlag, New York, pp: 173..

Sarwar, M., M. Arshad, D.A. Martens and W.T. Frankenberger Jr., 1992. Tryptophan-dependent biosynthesis of auxins in soil. Plant Soil, 147: 207-215.

Society of American Bacteriologist, 1957. Manual of Microbial Methods. McGraw Hill Book Co., New York, Pages: 315.

Steel, R.G.D. and J.H. Torrie, 1980. Principles and Procedures of Statistics: A Biometrical Approach. 2nd Edn., McGraw Hill Book Co., New York, USA., ISBN-13: 9780070609266, Pages: 633.

Zahir, Z.A., M. Arshad, A. Khaliq and S.A. Abbas, 1997a. Improving maize (*Zea mays* L.) yield by inoculation with some auxin producing *Azotobacter* isolates under fertilized conditions. J. Anim. Plant Sci., 7: 41-44.

Zahir, Z.A., M. Arshad, M. Azam and A. Hussain, 1997b. Effect of an auxin precursor tryptophan and *Azotobacter* inoculation on yield and chemical composition of potato under fertilized conditions. J. Plant Nutr., 20: 745-752.

Zelena, E., M. Kutacek and V. Cermak, 1988. Fate of Root Applied Indolyl Acetic Acid and its Influence on Growth of Intact Plants. In: Physiology and Biochemistry of Auxins in Plants, Kutacek, M., R.S. Bandurski and J. Krekule (Eds.). SPB Academic Publishing, The Hague, pp: 371-376.