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Azotobacter and L-tryptophan Application for Improving Wheat Yield

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Abstract

A field experiment was conducted to evaluate the effect of *Azotobacter* and L-tryptophan (L-TRP) application on the growth, yield and nitrogen content of wheat (*Triticum aestivum* L.) crop. Two levels of L-TRP (10^{-3} and 10^{-4} M) were tested with and without *Azotobacter* inoculation in a fertilized (NPK: 125-100-60 kg ha^{-1}, respectively) field. Results revealed that *Azotobacter* inoculation and L-TRP application significantly affected the wheat crop, however, their combined application produced more pronounced effect as compared with their separate application. Combined application of *Azotobacter* and 10^{-3} M L-TRP significantly increased the grain yield (21.3%), straw yield (20.7%), plant height (5.8%), number of tillers (15.3%), number of spikelets $spike^{-1}$, (12.3%), spike length (11.6%), 1000-grain weight (6.4%), nitrogen concentration in grains (31.4%) and straw (26.1%) and total nitrogen uptake (56.3%), compared with untreated control.

Introduction

Inoculation with specific microbial preparations for increasing crop yields is used in some parts of the world. *Azotobacter* is one of the microorganisms which has widely been used for inoculation purposes. It has been reported that seed inoculation with *Azotobacter* increased the yields of non legumes by about 10 percent, cereals by about 15-40 percent and potato by about 30 percent (Bhandari *et al*., 1989; Zahir *et al*., 1996a,b,1997). The production of plant growth regulators (PGRs) is considered one of the most plausible mechanisms to explain the beneficial effects of *Azotobacter* (Arshad and Frankenberger, 1991, 1993; Frankenberger and Arshad, 1995; Zahir *et al*., 1997). The ability of *Azotobacter* to produce PGRs has been reported and confirmed by various workers (Sarwar *et al*., 1992; Frankenberger and Arshad, 1995).

L-Tryptophan is considered an efficient physiological precursor of auxins in higher plants as well as for microbial biosynthesis of auxins and the possible pathways are well documented in literature (Arshad and Frankenberger, 1997; Frankenberger and Arshad, 1995). The presence of L-TRP increases the microbial production of PGRs by many fold (Frankenberger and Arshad, 1995). Our previous work revealed that 10 and 10^{-4} M L-TRP gave better results compared with other concentrations.

Thus this study was conducted to evaluate an *Azotobacter* L-TAP interaction for improving yield and nitrogen content of wheat.

Materials and Methods

Isolation of *Azotobacter*: *Azotobacter* was isolated from the University Farm soils by employing dilution plate technique using modified mannitol agar medium (Anonymous, 1957).

Auxin Production: Sterilized modified mannitol agar broth amended with or without filter sterilized L-TRP was taken in glass tubes and inoculated with *Azotobacter*. The tubes were incubated at 28 ± 2°C for five days with occasional manual shaking. The contents of the tubes were then filtered through Whatman No. 2 filter paper and auxin production (indole acetic acid equivalents) was measured colorimeterically at 535 nm as described by Sarwar *et al*. (1992).

Seed Inoculation: *Azotobacter* cultures were grown in 250 mL carrel culture flasks containing 150 mL modified mannitol broth and incubated at 28 ± 2°C for five days. A slurry was prepared by mixing together the sterilized peat, 10 percent sugar solution and 5-day old *Azotobacter* culture. L-Tryptophan was also added according to the treatments and mixed in the slurry. For control, seeds were treated with slurry containing no *Azotobacter* and no L-TRP. The seeds were dried in shade before sowing in the field.

Field Experiment: The treated wheat (Cv. Incilab) seeds were sown in plots (210×540 cm) with row to row distance of 30 cm at research area, Department of Soil Science, University of Agriculture, Faisalabad, Pakistan. The experiment consisted of the following treatments:

T1 = Control (Untreated)
T2 = *Azotobacter* inoculation (A)
T3 = 10^{-3} M L-TRP
T4 = 10^{-4} M L-TRP
T5 = A + 10^{-3} M L-TRP
T6 = A + 10^{-4} M L-TRP

Treatments were replicated three times in randomized complete block design. The fertilizers; urea, diammonium phosphate and sulfate of potash were applied at the rate of NPK: 125, 100 and 60 kg ha^{-1}, respectively. Full doses of PK and half of N were applied at the time of sowing and rest of the N was applied with first irrigation.

Data regarding plant height and number of tillers per m^2 were recorded at maturity. After harvesting, data regarding spike length, number of spikelets per spike, grain and straw yield and 1000-grain weight were recorded. The plant samples were oven dried at 65°C till constant weight. Plant and grain samples were analysed for nitrogen percentage and its uptake was calculated. Statistical procedures were

applied to analyse the data (Steel and Torrie, 1980) and means were compared by Duncan's Multiple Range Test (Duncan, 1955).

Results

Auxin production: Colorimeteric analysis showed that *Azotobacter* produced (*in vitro*) 12.5 μg mL^{-1} indole acetic acid equivalents in the absence of L-TRP which were increased to 30 μg mL^{-1} when incubated in the presence of L-TRP.

Field experiment: It is evident from Table 1 that the application of L-TRP (10^{-3} and 10^{-4} M) significantly enhanced the grain yield as compared with control while *Azotobacter* inoculation had no significant effect. However, supplementing *Azotobacter* inoculation with L-TRP (A + 10^{-3} M and A + 10^{-4} M) produced significantly higher grain yield than their separate application. The treatment (A + 10^{-3} M) produced the maximum grain yield which was 21.3 percent higher than control. All treatments except *Azotobacter* inoculation differed significantly from the control.

Table 1 revealed that L-TRP application (10^{-3} and 10^{-4} M) significantly increased the straw yield compared with the control. Similarly, *Azotobacter* inoculation also significantly increased the straw yield compared with uninoculated control. The combination of L-TRP application and *Azotobacter* inoculation produced even better results. The highest straw yield was recorded in the treatment where *Azotobacter* inoculation was supplemented with 10^{-3} M L-TRP and it was statistically similar with treatment combination of A + 10^{-4} M L-TRP but different from rest of the treatments.

It is clear from Table 1 that *Azotobacter* inoculation alone produced a similar result to the control for 1000 grain weight. Both the levels of L-TRP (10^{-3} and 10^{-4} M) were similar to each other. However, 10^{-4} M L-TRP produced a significantly greater 1000-grain weight than the control. *Azotobacter* inoculation supplemented with L-TRP gave a similar effect to L-TRP application alone. The maximum 1000-grain weight was recorded in the treatment (A + 10^{-3} M L-TRP), which was 6.4 percent higher than control. L-Tryptophan (10^{-3} and 10^{-4} M) and *Azotobacter* inoculation both had a significant effect on plant height (Table 1). Again, the combinations of L-TRP and *Azotobacter* inoculation were more effective than their separate application. Maximum plant height was recorded with treatment A + 10^{-3} M L-TRP. All the treatments were statistically different from control, *Azotobacter* inoculation and 10^{-3} M L-TRP produced statistically similar results while both levels of L-TRP were also similar to each other. Non-significant differences were observed in the combination of treatments (A + 10^{-3} M L-TRP and A + 10^{-4} M L-TRP).

Both levels of L-TRP (10^{-3} and 10^{-4} M) produced statistically similar results (Table 1) for tiller number. L-Tryptophan application at 10^{-4} M produced significant greater number of tillers than the control. It was statistically similar to *Azotobacter* inoculation alone which increased the number of tillers by 7.1 percent compared with conter Supplementing L-TRP with *Azotobacter* inoculation gave additional benefit. The combined application was, however better than the *Azotobacter* inoculation alone. Maximum number of tillers (566) was recorded in A + 10^{-3} M L-TRP. All treatments differed significantly from the control. The results (Table 1) showed that *Azotobacter* inoculation and L-TRP whether applied separately or in combination with each other had no significant effect on number of spikelets $spike^{-1}$ and spike length. The maximum number of spikelets $spike^{-1}$ (18.3) and spike length (12.5 cm) was produced by A + 10^{-3} M L-TRP and were 12.3 and 12.5 percent higher than the control, respectively.

It is revealed from Table 2 that L-TRP application (10^{-3} and 10^{-4} M) and *Azotobacter* inoculation significantly increase the N concentration in grains compared with control. However, N concentration was increased further when *Azotobacter* inoculation was supplemented with L-TRP application. The maximum N concentration was recorded when *Azotobacter* inoculation was supplemented wit L-TRP (10^{-3} M) and was 31.4 percent higher than control. The treatments were statistically different from each other and the control.

The results in Table 2 showed that *Azotobacter* inoculation and L-TRP application both significantly increased than concentration in straw compared with the untreated control. L-Tryptophan (10^{-3} M) gave a similar result compared with the *Azotobacter* inoculation alone.

However, L-TRP (10^{-4} M) significantly increased the concentration. The maximum N-concentration (0.53 percent) was recorded in A + 10^{-3} M L-TRP which was 2 percent higher than control.

Table 2 showed that *Azotobacter* inoculation also significantly increased total N-uptake compared with uninoculated control. Both the levels of L-TRP (10^{-3} and 10^{-4} M) gave statistically similar results but significantly increased than control and *Azotobacter* inoculation along maximum N-uptake was recorded when *Azotobacter* inoculation was supplemented with 10^{-3} M L-TRP, 56.3 percent higher than control.

Discussion

Azotobacter inoculation and L-TRP application significantly affected the wheat crop. However, the combine application of L-TRP and *Azotobacter* inoculation had maximum pronounced effect as compared with their separate application.

Azotobacter inoculation promoted yield, most of the contributing parameters, N concentration and its uptake compared with uninoculated control. This is in agreement with the findings of many other workers (Zahir *et al*., 1996a,b, 1997; Khalid *et al*., 1997). The

Table 1: Effect of *Azotobacter* inoculation and L-tryptophan application on grain yield, straw yield, 1000-grain weight, plant height, number of tillers, number of spikelets spike^{-1} and spike length of wheat

Treatment	Grain yield (kg ha^{-1})	Straw yield (kg ha^{-1})	1000-grain weight (g)	Plant height (cm)	No. of tillers (m^{-2})	No. of spikelets	Spike length spike^{-1} (cm)
Control	3042.9d	6219.2d	34.9c	93.6d	491.0c	16.3NS	11.2NS
Azotobacter (A)	3185.4d	7156.9c	35.3bc	97.8c	526.0b	17.3	11.9
10^{-3} ML-TRP	3342.5c	7246.1c	36.2ab	98.8bc	541.0ab	17.3	12.0
10^{-4} M L-TRP	3398.3bc	7359.8b	36.6a	99.6b	545.0ab	17.6	12.1
A + 10^{-3} M L-TRP	3692.3a	7508.5a	37.2a	101.9a	566.0ab	18.3	12.5
A + 10^{-4} ML-TRP	3529.3b	7502.8a	36.9a	101.6a	559.0a	18.0	12.2
S.E.	233.12	483.99	0.91	2.18	26.88	0.69	0.43

Means sharing similar letter(s) do not differ significantly at p = 0.05; NS = Non-significant

Table 2: Effect of *Azotobacter* inoculation and L-tryptophan application on N concentration, N uptake in grains and straw and total N uptake of wheat

	N concentration (%)		Total N Uptake
Treatment	Grain	Straw	(kg ha^{-1})
Control	1.40f	0.42d	69.0d
Azotobacter (A)	1.58e	0.46c	83.2c
10^{-3} ML-TRP	1.66d	0.47c	90.0b
10^{-4} ML-TRP	1.73c	0.50b	95.21b
A + 10^{-3} ML-TRP	1.84a	0.53a	107.86a
A + 10^{-4} ML-TRP	1.80b	0.50b	101.4a
S.E.	0.16	0.04	13.60

Means sharing similar letter(s) do not differ significantly at p<0.05

growth promotion increase in crop yields in response to *Azotobacter* inoculation could be explained on the basis of various mechanisms like the production of plant growth regulating substances and fixation of nitrogen. But the hypothesis of N fixation has now been rejected due to insufficient *Azotobacter* number in the rhizosphere, absence of suitable energy and carbon source, inability of *Azotobacter* to effectively utilize crop residues, observations of yield increases in N-rich environment and the beneficial effects being recorded even by inoculation with non-nitrogen fixing bacteria (Arshad and Frankenberger, 1993). Stimulation of plant growth was later attributed to the production of biologically active substances (Frankenberger and Arshad, 1995). It is now well established that various *Azotobacter* species are capable of synthesizing plant hormones and is considered the most plausible mechanism of action.

Significant yield increase in response to L-TRP application in present study is in agreement with the findings of many other workers (Martens and Frankenberger, 1994; Arshad *et al*., 1995). The increased N concentration and uptake in grain and straw in our study is also in accordance with the findings of many other workers (Arshad *et al*., 1994a,b; Zahir *et al*., 1997).

L-Tryptophan is a well established precursor of auxins in higher plants and for microbially-derived auxins in pure culture and in soil (Frankenberger and Arshad, 1995). The effect of L-TRP on growth and yield of wheat could be attributed to either auxin metabolites produced by the rhizosphere microflora which were subsequently taken up by plant roots or direct uptake of L-TRP by the plant roots with subsequent catabolism into auxins within the plant tissue and/or alteration in the balance of the rhizosphere microbial community in response to L-TRP addition which may affect growth and yield of wheat. However, Martens and Frankenberger (1994) reported very poor uptake of labelled L-TRP compared with labelled IAA by wheat seedling roots. They also demonstrated poor endogenous conversion of exogenously applied labelled tryptophan into auxins by wheat seedlings grown under axenic environments. Addition of labelled 3-^{14}C-IAA to sterile and non sterile soil resulted in assimilation and translocation of the label to the shoot tissues as amino acid conjugates of IAA (Martens and Frankenberger, 1992). This implies that auxins produced by the rhizosphere microflora derived from L-TRP could be taken up by the plant roots and may be tanslocated to the shoots resulting in a physiological response. So the physiological response could most likely be evoked by the auxins derived from microbial catabolism of L-TRP in the vicinity of rhizosphere, The application of higher levels of chemical fertilizers in this study rules out the possibility of any nutritional effect of L-TRP and supports the idea of physiological effect. Frankenberger *et al*. (1990) compared the effect of L-TRP with known auxins (indole-3-acetic acid, indole-3-acetamide and indole-3-lactic acid) on growth and yield of radish and found that L-TRP was either equally effective or better than these pure auxins in terms of yield.

In present study *Azotobacter* inoculation when supplemented with L-TRP (10^{-3} M and 10^{-4} M) significantly increased the yield and most of its contributing parameters compared with control. Similar to our findings, Zahir *et al*. (1997) reported that tuber and straw yield of potato was increased by 62.9 and 47.8 percent, respectively, when *Azotobacter* inoculation was supplemented with L-TRP application. Our results are also in accordance with those of Nieto and Frankenberger (1990, 1991). They confirmed that precursor inoculum interaction gave better results than their application alone.

Present study emphasizes the need of focusing future research on 1) screening rhizosphere microbes for their ability to produce and release PGRs in the absence and presence of physiological precursors; 2) testing inexpensive physiological precursors; 3) determining soil characteristics that favour optimum production of PGRs by the indigenous soil microbes or by inocula with added substrates; 4) evaluating the optimum PGR requirements for plant growth and development and 5) demonstrating the uptake of exogenous microbially supplied PGRs by plant roots and the subsequent plant response.

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