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ROLE OF PHYSIOLOGICAL PRECURSOR AND RHIZOBIUM-WHEAT ASSOCIATIONS IN FIELD CONDITIONS

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ABSTRACT

Sole dependence on mineral fertilizers results high input cost for raising crops. Plant nutritionists are in search of substitutes to mineral fertilizers or the way that enhance the efficiency of mineral fertilizers or to compensate / supplement the mineral fertilizers. *Rhizobium* is very well recognized due to its symbiotic relationship with legumes has now been used in non-legumes due to its great root colonization ability, growth hormone production potential, improving the nutrient use efficiency, P-solubilization and inducing systemic resistance. Precursor-inoculum interaction provide constant source of hormones to plants and improved the growth and yield of cereals. Field studies were planned to ascertain the role of microbial biosynthesis of auxins through L-TRP on auxins production potential and wheat growth. Different *Rhizobium* species were isolated and screened out for their auxin production potential and root / shoot elongation assay was carried out. Two N levels i.e. 80 and 120 kg ha^{-1}as urea was used while uniform rate of P (as SSP)and K (as SOP) i.e. 115 and 60 kg ha^{-1}were used and L-TRP @ 10^{-5} M was applied as seed soaking for three hours. Results revealed that precursor-inoculum interaction has affected the yield parameters of wheat as compared to their separate application. Precursor-inoculum interaction produced highest grain yield of wheat at Soil Bacteriology Section, Soil Chemistry Section (Institute of Soil Chemistry & Environmental Sciences, ISC&ES) Faisalabad i.e. 5689, 5827 and 5042, 5292 kg ha^{-1} at 80 and 120 kg N ha^{-1}, respectively. Other growth parameters, soil-plant analyses were also improved by the approach of precursor-inoculum interaction. Precursor-inoculum interaction exhibited higher IAA equivalents in the rhizosphere soil of wheat determined at 15 and 30 days after germination.

Keywords: L-TRP, *Rhizobium*, precursor-inoculum, IAA equivalents, wheat.

INTRODUCTION

Role of plant hormones like auxins, gibberellins and cytokinins etc has been well established for controlling plant growth and development throughout the life cycle of the plant. Plant and microbes are the natural source of these hormones in soil. Numerous microbes have been reported to produce auxins in solution or in soil (Dobbelaere *et al.,* 2003; Asghar *et al.,* 2004; Zahir *et al.,* 2004). Under sub-optimal conditions, plants may not have the potential to synthesize adequate hormones endogenously for optimal plant growth. Application of plant hormones exogenously may affect plant growth by altering the endogenous levels of hormones and improved the plant growth. Plants produce these hormones in particular cells and translocate to other parts where they exert their effect. Plants also respond to exogenously applied hormones (Khalid *et al.,* 2001; Zahir *et al.,* 2005; Qureshi *et al.,* 2013).

L-tryptophan (L-TRP), the physiological precursor of auxins is directly involved in microbial biosynthesis of auxins. Exogenous application of L-TRP influenced the plant growth has been reported by number of workers (Sarwar *et al.,* 1992; Zahir *et al.,* 2000a; Zahir *et al.,* 2005). Microbe interactions with the precursors in the rhizosphere provide a continuous source of growth hormones to plants (Arshad and Frankenberger, 1998; Egamberdieva and Kucharova, 2009; Habig *et al.,* 2015; Kumar and Jagadeesh, 2016). Application of precursors instead of growth hormones is a classical approach because they are water soluble, inexpensive, lack photosensitivity and provide continuous source of hormones to plants. Microbial biosynthesis of auxins increased many times with the application of precursors. Precursors act like substrate and microbial activities results in accumulation of biological active substances to plants and thus promote the growth. Precursors provide better source than growth hormones because growth hormone triggers the plant growth once and boosted the growth and then undergoes bio-degeneration. Microbial biosynthesis of auxins was stimulated due to application of L-TRP results in enhanced plant growth (Zahir *et al.,* 2004; Khalid *et al.,* 2006; Parthiban *et al.,* 2016).

The best known and most exploited symbiotic N_2-fixing bacteria are belonging to the family Rhizobiaceae (Graham and Vance, 2000). The beneficial effect of rhizobia in legumes is very well known and recently many researchers reported its positive effects on non-legumes and could act as plant growth promoting

rhizobacteria (PGPR) (Chabot *et al.,* 1996; Yanni *et al.,* 1997, Biswas *et al.,* 2000 a, b; Egamberdieva *et al.,* 2008; Cassán *et al.,* 2009; Mehboob *et al.,* 2009; Gopalakrishnan *et al.,* 2015).

Like other rhizobacteria, *Rhizobium* has great colonizing ability and thus promotes the plant growth. *Rhizobium* can influence the cereals by producing growth hormones, antibiotics, vitamins, siderophores and also involved in solubilizing / mineralizing of inorganic / organic phosphates, improving the nutrient uptake, decrease stress and inducing systemic resistance (Antoun *et al.,* 1998; Antoun and Prevost, 2005; Mehboob *et al.,* 2008; Noreen *et al.,* 2012). Recently, literature showed that species of *Rhizobium* have also been involved in the disease suppression (Huang and Erickson, 2007; Mia and Shamsuddin, 2010; Egamberdieva, 2011; Qureshi *et al.,* 2013). Precursor-inoculum interaction offered continuous source of hormones to plants and enhanced the plant-microbe interactions results in better plant growth. It was reported that precursor-inoculum interaction affected the yield of crops (Zahir *et al.,* 2005) than the separate application. Present studies were planned to assess the precursor (L-TRP)-inoculum (*Rhizobium*) interaction for the yield promotion of wheat.

MATERIALS AND METHODS

Isolation of Rhizobium Species: *Rhizobium* species were isolated from chickpea nodules and rhizosphere. Plants of these legumes were uprooted from the Soil Bacteriology Section, AARI Faisalabad and roots were placed in tap water to remove the soil particles. Nodules were separated, placed in Petri dishes and surface-sterilized as reported by Russell *et al.* (1982). After surface sterilization, nodules were crushed with the sterilized forceps to obtain suspension. The suspension was streaked out with inoculating needle on the yeast extract mannitol agar medium (YMA) [mannitol 10.0 g, yeast extract 1.0 g, K_2HPO_4 0.5 g, $MgSO_4.7H_2O$ 0.2 g, NaCl 0.1 g, distilled water 1000 mL, pH 6.8-7.0] (Vincent, 1970). The growth of *Rhizobium* species was undergone purification on YMA. Biochemical tests were carried out to screen out the isolates for different characteristics. Isolates were tested on different dyes like Congo red and Bromothymol blue (BTB) in the YMA and stained for its gram reaction. The yeast mannitol agar (YMA) medium having 25 mg Bromothymol blue L^{-1} was tested for organic acid production (Keneni *et al.,* 2010). *Rhizobium* species did not attain the colour of Congo red and conversion of BTB colour from green to yellow indicated that isolates were organic acid producer. The well isolated colonies were re-streaked again and again to obtain pure cultures. Thus, three isolates of *Rhizobium* specie were selected, purified and multiplied in yeast extract mannitol broth. The purified isolates were stored at 4 ± 1°C on slants and maintained for further screening / characterization. Isolates of *Rhizobium* sp were further screened out for biochemical tests like Gram, Catalase, urease, starch hydrolysis and citrate utilization.

Determination of Auxin Biosynthesis: Three isolates of *Rhizobium* species (Cp_1, Cp_2 and Cp_3) were screened for their auxin biosynthesis potential with and without L-TRP. For this, the broth of general purpose medium (GPM) was inoculated and incubated for 72 hours at 28 ± 2. Auxin biosynthesis potential was determined as IAA equivalents using Salkowski's reagent (2 mL of 0.5 M $FeCl_3$ + 98 mL of 35% $HClO_4$) as reported by Sarwar *et al.* (1992). *Rhizobium* isolates having the highest auxin biosynthesis potential were selected for other biochemical tests and experimentation.

Plate Experiment: To test the response of *Rhizobium* specie (chickpea) on wheat, isolate having the highest auxin biosynthesis potential (Cp_3) was used in plate experiment (root-shoot elongation assay). Plate experiment was conducted under controlled conditions. Different parameters after one week of incubation, like root / shoot length, mass and IAA equivalents were determined from root and shoot. One gram of root / shoot was surface sterilized (Russell *et al.,* 1982) and placedin the sterilized GPM, crushed with glass rod and shake repeatedly. After one week of incubation, GPM was centrifuged and the supernatant was used for IAA equivalents determination as reported by Sarwar *et al.* (1992).

Inoculum preparation: After preliminary investigations, isolate Cp_3 was multiplied in broth of yeast extract mannitol. Broth was inoculated in and incubated at 28 ± 2 °C under shaking at 100 rpm for 4 days to give an optical density of 0.5 at 535 nm. Carrier of inoculum was leaf mould collected from the 'Changa Manga' forest, sieved to get a powdery shape. Inoculum was prepared by mixing 50 mL of 15% sterilized sugar solution, 20 mL broth and 200 g of sterilized peat and incubated for one week before application.

Field Studies: Field studies were conducted at multi sites (Soil Bacteriology Section and Soil Chemistry Section, Institute of Soil Chemistry & Environmental Sciences (ISC&ES)) Ayub Agricultural Research Institute (AARI), Faisalabad. At Soil Bacteriology Section, soil having pH 7.84, EC 1.40 dSm^{-1}, N 0.036 %, available P 7.87 mg kg^{-1} and organic matter 0.76%. At Soil Chemistry Section, soil having pH 7.89, EC 2.03 dSm^{-1}, N 0.035%, available P 6.18 mg kg^{-1} and organic matter 0.68%. Phosphorus was applied at 115 kg ha^{-1} as single super phosphate (SSP) and K was 60 kg ha^{-1} as Sulphate of Potash (SOP)while two levels of N i.e. 80 (F_1) and 120 (F_2) kg ha^{-1} as urea was applied in two splits. The experiment was laid out in Randomized complete block design (RCBD) with three replications. Seeds of wheat (cv.

Seher-2006) were surface sterilized as reported by Russell *et al.* (1982).

Wheat seeds were soaked in water (treated as control) and in L-TRP (10^{-5} M) for three hours and inoculum of *Rhizobium* sp was applied as seed coating. For the treatment of precursor-inoculum, wheat seeds were dipped in equal amount of L-TRP and inoculum suspension. IAA equivalents from the rhizosphere soil of wheat trials from the both sites was determined after 15 and 30 days of germination and incubated in the general purpose medium after inoculated one gram soil as reported by Sarwar *et al.* (1992)

Data regarding yield components of wheat, N-P content in grains and straw were determined. Post-harvest soil N and available P was also determined. Phosphorus was determined by modified Olsen method (Olsen and Sommers, 1982) while nitrogen according to Kjeldhal method (Bremner and Mulvany, 1982). The statistical analysis of mean of two years data was carried out following RCBD using standard procedures (Steel *et al.,* 1997). The differences among the means were compared by applying the Duncan's multiple range tests (DMR) (Duncan, 1955).

RESULTS

Results revealed that *Rhizobium* inoculation and L-TRP application enhanced the yield parameters of wheat significantly. Separate application of L-TRP and *Rhizobium* affected the wheat yield components positively at both the sites but the effect was more pronounced with their interaction. Results clearly demonstrated that precursor-inoculum interaction enhanced the yield components marvelously.

The lab screening showed that all the three isolates of *Rhizobium* sp produced IAA equivalents in the absence of L-TRP and the effect was more affirmative with L-TRP application (Table 1). Highest IAA equivalents was produced by Cp_3 i.e. 4.34 µg mL^{-1} in the absence of L-TRP and this increased to 5.49 µg mL^{-1} with L-TRP application. Isolate Cp_3 exhibited positive Congo red, BTB test and urea, starch hydrolysis and citrate utilization. Isolates showed variable response to these biochemical tests.

Results of plate experiment demonstrated that precursor and *Rhizobium* inoculations improved the root / shoot length and mass as compared to control (Table 2). The maximum enhancement in root / shoot length and mass was observed with the interaction of precursor and inoculum. Similar trend was observed IAA equivalents in shoot and root with precursor-inoculum interaction. The highest IAA equivalents in shoot and root were observed 4.17,4.24 µg g^{-1} with precursor-inoculum interaction, respectively.

Results of wheat yield parameters at Soil Bacteriology Section (Table 3) clearly showed that precursor-inoculum interaction enhanced the yield parameters significantly. Precursor-inoculum interaction enhanced yield parameters like plant height, tillers m^{-2}, grains $spike^{-1}$, grain weight $spike^{-1}$, 1000-grain weight and grain yield at both N levels i.e. 80 and 120 kg N ha^{-1}. Maximum plant height i.e. 104.7 cm was observed with precursor-inoculum interaction at F_1that was at par with *Rhizobium* inoculation alone at F_2. Precursor-inoculum interaction produced maximum tillers m^{-2} i.e. 365, grains $spike^{-1}$ (56), grain weight $spike^{-1}$(2.87 g), 1000-grain weight (55.7 g) and grain yield (5827 kg ha^{-1}) at F_2.Percent increase in tiller m^{-2} (20 and 23%) and grain yield was (13.9 and 10.2%) at F_1 and F_2, respectively. Similar trend was observed with other yield parameters.

Results regarding grain and straw N-P content and post-harvest soil N and available P (Table 4) demonstrated positive trends with precursor-inoculum interaction. Although, application of *Rhizobium* inoculation and L-TRP separately increased the plant and grain N-P content than control yet their interaction has more sound effect on the grain, straw and soil parameters at harvest. Precursor-inoculum interaction showed the maximum grain N (1.690 and 1.703%), grain P (0.351 and 0.381%), straw N (0.297 and 0.302%), straw P (0.163 and 0.163%), soil N (0.039 and 0.040) and available P (11.10 and 12.01) at F_1 and F_2, respectively.

Results regarding wheat yield at Soil Chemistry Section (Table 5) clearly indicated that precursor-inoculum interaction enhanced the yield components significantly than their separate application. Precursor-inoculum interaction enhanced the wheat yield parameters at both N levels i.e. 80 and 120 kg N ha^{-1}.Precursor-inoculum interaction exhibited maximum plant height i.e. 104.3 cm, tillers m^{-2} i.e. 305, grains $spike^{-1}$ (52), grain weight $spike^{-1}$(2.427 g), 1000-grain weight (46.43 g) and grain yield (5292 kg ha^{-1}) at F_2. Percent increase in tiller m^{-2} (20 and 23%) and grain yield was (13.9and 10.2%) at F_1 and F_2, respectively.

Results presented in (Table 6) clearly verified the approach of precursor-inoculum interaction than the alone application of L-TRP and *Rhizobium* inoculation. *Rhizobium* inoculation enhanced the grain N (1.657 and 1.670%) while L-TRP (1.647 and 1.653%) than control (1.620 and 1.633%) at F_1 and F_2, respectively. Precursor-inoculum interaction enhanced the grain N (1.680 and 1.690%), grain P (0.313 and 0.334%), straw N (0.282 and 0.293%) and straw P (0.147 and 0.156%) at F_1 and F_2, respectively. Similarly, the highest soil N (0.039 and 0.040%) i.e. 12.5and 13.3% higher than control and available P (9.24 and 9.55 mg kg^{-1}) i.e. 29.7 and 28.7% higher than control at F_1 and F_2, respectively.

Results about IAA equivalents of both the sites was determined after 15 and 30 days of germination (Table 7) indicated that separate application of L-TRP and *Rhizobium* inoculation enhanced the IAA equivalents at both sites than control at F_1 and F_2, respectively.

Precursor-inoculum interaction produced maximum IAA equivalents after 15 and 30 days of germination at F_1 and F_2 i.e. (3.25 and 3.74 µg g^{-1}), (3.88 and 4.01 µg g^{-1}) and (3.84 and 4.39 µg g^{-1}), (4.41 and 4.87 µg g^{-1}) at Soil Bacteriology and at Soil Chemistry Section, respectively.

Table 1. Some important traits of isolates screened out for the lab and field studies.

Isolates	IAA equivalents(µg mL^{-1})		BTB test	Congo red test	Urease Test	Starch hydrolysis	Citrate utilization
	L-TRP[-]	L-TRP [+]					
Cp1	3.50	4.55	+ve	+ve	-ve	-ve	-ve
Cp2	3.15	4.30	+ve	+ve	-ve	+ve	-ve
Cp3	4.34	5.49	+ve	+ve	+ve	+ve	+ve

Table 2.Root-shoot elongation assay and IAA equivalents in root / shoot of wheat.

Treatments	Shoot length (cm)	Root length (cm)	Shoot mass (g)	Root mass (g)	IAA in shoot (µg g^{-1})	IAA in root (µg g^{-1})
Control	18.0	18.2	0.62	0.32	1.93	1.80
L-TRP @10^{-5} M	27.6	23.1	0.69	0.36	3.13	2.99
Rhizobium	23.8	21.4	0.57	0.32	2.55	2.42
L-TRP+ Rhizobium	30.5	25.5	0.84	0.54	4.17	4.24
LSD	2.32	2.55	0.01	0.01	0.02	0.035

Table 3.Precursor-inoculum interaction effect on the yield parameters of wheat at Soil Bacteriology Section, Faisalabad.

Treatments	Plant height (cm)		Tiller m^{-2}		Grains $spike^{-1}$		Grain weight $spike^{-1}$		1000-grain weight (g)		Grain yield (kg ha^{-1})	
	F_1*	F_2*	F_1	F_2	F_1	F_2	F_1	F_2	F_1	F_2	F_1	F_2
Control	103.4 bc	103.5 abc	284 f	296 ef	47.7 f	53.7 bc	2.14 f	2.34 e	45.7 e	47.4 d	4993 e	5288 d
L-TRP @10^{-5} M	103.2 c	104.3 abc	305 def	320 cde	50.0 e	54.7 b	2.52 d	2.65 c	50.2 c	52.1 b	5352 cd	5537 bc
Rhizobium	103.8 abc	104.7 a	322 cd	349 ab	52.3 cd	56.3 a	2.65 c	2.72 bc	49.1 c	52.3 b	5493 bcd	5654 ab
L-TRP+ Rhizobium	104.7 a	104.5 ab	340 bc	365 a	51.7 d	56.3 a	2.77 b	2.87 a	51.7 b	55.7 a	5689 ab	5827 a
LSD	1.19		23.6		1.59		0.084		1.428		232.9	

*F_1= 80 kg N ha^{-1}; F_2= 120 kg N ha^{-1}

**Means sharing the same letter(s) in a column do not differ significantly at p<0.05 according to Duncan's Multiple Range Test.*

Table 4.Precursor-inoculum interaction effect on the NP content and post harvest status at Soil Bacteriology Section, Faisalabad.

Treatments	Grain N (%)		Grain P (%)		Straw N (%)		Straw P (%)		Soil N (%)		Avail. P (mg kg^{-1})	
	F_1*	F_2*	F_1	F_2	F_1	F_2	F_1	F_2	F_1	F_2	F_1	F_2
Control	1.650 f	1.660 ef	0.306 f	0.321 e	0.267 f	0.273 ef	0.115 d	0.129 c	0.035 e	0.037d	7.90 c	9.73 bc
L-TRP @10^{-5} M	1.667 de	1.677 cd	0.318 ef	0.340 cd	0.278 de	0.286 cd	0.129 c	0.144 b	0.036 de	0.038 bcd	10.19 ab	10.64 ab
Rhizobium	1.667 de	1.687 bc	0.329 de	0.355 b	0.285 cd	0.292 bc	0.148 b	0.159 a	0.037 d	0.039 ab	9.27 bc	10.64 ab
L-TRP+ Rhizobium	1.690 b	1.703 a	0.351 bc	0.381 a	0.297 ab	0.302 a	0.163 a	0.163 a	0.039 bc	0.040 a	11.10 ab	12.01 a
LSD	0.01		0.013		0.001		0.01		0.0001		1.86	

*F_1= 80 kg N ha^{-1}; F_2= 120 kg N ha^{-1}

**Means sharing the same letter(s) in a column do not differ significantly at p<0.05 according to Duncan's Multiple Range Test.*

Table 5.Precursor-inoculum interaction effect on the yield parameters of wheat at Soil Chemistry Section, Faisalabad.

Treatments	Plant height (cm)		Tiller m^{-2}		Grains $spike^{-1}$		Grain weight $spike^{-1}$		1000-grain weight (g)		Grain yield (kg ha^{-1})	
	F_1*	F_2*	F_1	F_2	F_1	F_2	F_1	F_2	F_1	F_2	F_1	F_2
Control	97.8 e	100.0 d	250 e	268 d	41 e	43 d	1.907 e	2.090 d	41.47 d	42.50 d	4125 f	4542 de
L-TRP @10^{-5} M	100.8 d	102.7 bc	264 d	276 cd	39 e	48 bc	1.977 e	2.147 cd	42.73 d	45.00 abc	4375 e	4875 bc
Rhizobium	102.3 c	103.3 b	276 cd	293 ab	43 d	49 b	2.163 cd	2.250 bc	42.87 cd	45.23 ab	4708 cd	4875 bc
L-TRP+ Rhizobium	103.2 bc	104.3 a	283 bc	305 a	47 c	52 a	2.330 ab	2.427 a	43.20 bcd	46.43 a	5042 b	5292 a
LSD	0.904		12.84		2.00		0.109		2.266		238.2	

*F_1= 80 kg N ha^{-1}; F_2= 120 kg N ha^{-1}

**Means sharing the same letter(s) in a column do not differ significantly at p<0.05 according to Duncan's Multiple Range Test.*

Table 6.Precursor-inoculum interaction effect on the N-P content and post harvest parameters at Soil Chemistry Section, Faisalabad.

Treatments	Grain N (%)		Grain P (%)		Straw N (%)		Straw P (%)		Soil N (%)		Avail. P (mg kg^{-1})	
	F_1*	F_2*	F_1	F_2	F_1	F_2	F_1	F_2	F_1	F_2	F_1	F_2
Control	1.620 e	1.633 d	0.273 e	0.285de	0.252 g	0.259 f	0.117 c	0.134 b	0.034 f	0.035 e	7.12 d	7.42 cd
L-TRP @10^{-5} M	1.647 c	1.653 c	0.281de	0.297cd	0.265 e	0.272 d	0.128bc	0.147 a	0.036 e	0.037cd	8.33 bc	8.64 ab
Rhizobium	1.657 c	1.670 b	0.310bc	0.318ab	0.277 c	0.286 b	0.133 b	0.153 a	0.036de	0.038bc	8.94 ab	8.94 ab
L-TRP+ Rhizobium	1.680ab	1.690 a	0.313bc	0.334 a	0.282bc	0.293 a	0.147 a	0.156 a	0.039 b	0.040 a	9.24 ab	9.55 a
LSD	0.013		0.018		0.0005		0.012		0.0001		0.930	

*F_1= 80 kg N ha^{-1}; F_2= 120 kg N ha^{-1}

**Means sharing the same letter(s) in a column do not differ significantly at p<0.05 according to Duncan's Multiple Range Test.*

Table 7.Precursor-inoculum interaction effect on the IAA equivalents at Soil Bacteriology and Soil Chemistry Section, Faisalabad.

Treatments	Soil Bacteriology Section				Soil Chemistry Section			
	IAA Equivalents (µg g^{-1}) after 15 days*		IAA Equivalents (µg g^{-1}) after 30 days*		IAA Equivalents (µg g^{-1}) after 15 days*		IAA Equivalents (µg g^{-1}) after 30 days*	
	F_1*	F_2*	F_1	F_2	F_1	F_2	F_1	F_2
Control	1.71 f	1.89 ef	1.89 e	2.33 de	1.62 c	1.79 c	1.787 e	2.64 d
L-TRP @10^{-5} M	2.11de	2.59 c	2.85 cd	3.26 c	2.69 b	2.75 b	3.78 c	4.33 abc
Rhizobium	2.19d	2.68 c	2.88 cd	3.32 bc	2.50 b	2.77 b	3.85 bc	4.10 bc
L-TRP+ Rhizobium	3.25 b	3.74 a	3.84 ab	4.39 a	3.88 a	4.01 a	4.41 ab	4.87 a
LSD	0.254		0.552		0.3459		0.591	

F_1= 80 kg N ha^{-1}; F_2= 120 kg N ha^{-1};

*Days after germination.

**Means sharing the same letter(s) in a column do not differ significantly at p<0.05 according to Duncan's Multiple Range Test.*

DISCUSSION

Exogenous application of L-TRP and use of *Rhizobium* in wheat affected the growth and yield components of wheat. However, their interaction affected the growth, yield components of wheat, N-P content in straw and grains and post harvest soil N and available P at both the sites significantly than their separate application.

Microbial biosynthesis of auxins by *Rhizobium* sp was increased to a great extent by introducing the L-TRP (Zahir *et al.,* 2005, 2010). *Rhizobium* sp of chickpea (three isolates) were screened with and without L-TRP (Table 1) and characterized for different biochemical tests. Isolates tested during this examination produced IAA equivalents in the absence of L-TRP and produced much higher levels of IAA equivalents in the presence of L-TRP. Isolate Cp_3 produced higher values of IAA equivalents in the absence / presence of L-TRP. The biosynthesis of auxins by *Rhizobium* sp was reported by numerous workers (Sarwar *et al.,* 1992; Zahir *et al.,* 2004; Qureshi *et al.,* 2013; Verbon and Liberman, 2016).

Lab study (plate experiment) was conducted to check the role of L-TRP, isolate Cp_3 and their combined application (precursor-inoculum interaction). Rroot-shoot elongation assay clearly demonstrated that interaction of precursor and *Rhizobium* enhanced the root /hoot length, mass and IAA equivalents in root and shoot. Results clearly indicated that under axenic conditions, precursor-inoculum interaction accumulated more IAA equivalents in root and shoot (Zahir *et al.,* 2004).

Field studies demonstrated improved plant growth and yield components, N-P content in grains and straw due to the interaction of precursor-inoculum might be attributed to microbial biosynthesis of auxins resulted in improved root system architecture and thus growth and yield of crops (Fatima *et al.,* 2006; Mehboob *et al.,* 2011; Hussain *et al.,* 2014). Being the physiological precursor of auxins, L-TRP introduction enhanced the biosynthesis of auxins. The production of IAA equivalents in lab study confirmed the findings of mentioned researchers (Arshad and Frankenberger, 1998; Khalid *et al.,* 2001; Mehboob *et al.,* 2011; Hussain *et al.,* 2014).

Application of L-TRP separately produced significant response might be attributed to its uptake of plants or biosynthesis of L-TRP to auxins altered the endogenous levels of auxins resulted in biotransformation's of L-TRP to auxins by indigenous microflora and influenced plant growth. Root-shoot elongation assay (plate experiment) confirmed this evidence that L-TRP accumulation in root / shoot. Accumulation of IAA equivalents are little bit contradictory to the findings of Martens and Frankenberger, (1994), they reported very low uptake of L-TRP by wheat seedlings. Martens and Frankenberger, (1994) and Mehboob *et al.,* (2008; 2011) proposed that exogenous application of L-TRP or IAA resulted in accumulation of amino acid conjugates. *Rhizobium* inoculation enhanced the yield components of wheat and N-P content in plants as compared to control (Höflich, 2000; Fatima *et al.,* 2006; Pandey and Maheshwari, 2007; Mehboob *et al.,* 2011; Noreen *et al.,* 2012).

The yield enhancement in wheat parameters by *Rhizobium* inoculation might be attributed to the various means viz. production of plant hormones, improved nutrient uptake, production of siderophores and organic acids and suppression of plant pathogens (Arshad and Frankenberger, 1998; Meyer, 2000; Mehboob *et al.,* 2008; Hmissi *et al.,* 2011; Mehboob *et al.,* 2011; Parthiban *et al.,* 2016).

Precursor-inoculum interaction promoted the yield components by producing hormones by the known *Rhizobium* specie in the presence of L-TRP and enhanced biosynthesis of auxins in the rhizosphere reported by many researchers (Hossain *et al.,* 2008; Hussain *et al.,* 2009; Qureshi *et al.,* 2013; Hussain *et al.,* 2014). As elevated levels of IAA equivalents were found by *Rhizobium* inoculation, by application of L-TRP and their combined application due to biosynthesis of auxins (Sarwar *et al.,* 1992; Zahir *et al.,* 2005; Zahir *et al.,* 2010; Gopalakrishnan *et al.,* 2015).

More N-P content in grains and straw with *Rhizobium* inoculation, LTRP or their combination might be attributed to more developed root system and enhanced root growth due to presence of IAA equivalents in the rhizosphere. Also enhanced N-P content at both N levels might be specific to rate of N or N rate dependent. Increased soil N and available P might be attributed to enhance microbial activities, production of organic acids, enhanced root proliferations for acquisition of nutrients verified by numerous researchers (Zahir *et al.,* 2005; Fatima *et al.,* 2006; Pandey and Maheshwari, 2007; Mehboob *et al.,* 2011; Hussain *et al.,* 2014).

Studies concluded that precursor-inoculum interaction is an efficient approach to enhance the growth and yield of wheat and plant functions can be better regulated by applying the suitable precursor with specific rate. More studies in different ecologies are required to validate this approach.

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