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Isolation of Rhizobacteria from Different Rapeseed Varieties and Their Potential for Auxin Production

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Abstract: Rhizobacteria were isolated from rhizosphere of different varieties of rapeseed (Canola, Raya, Toria, Gobi Sarsoon and Sarsoon) selected from different sites of Punjab. These rhizobacteria were tested for auxin production in terms of indoleacetic acid equivalents by colorimeter method both in the presence and absence of an auxin precursor L-tryptophan (L-TRP). This study suggested that auxin production ability varied with variety and site ranging from 0.13 to 11.4 μg mL^{-1} without L-TRP and from 2.41 to 24.6 μg mL^{-1} with L-TRP. L-Tryptophan application increased the auxin production upto 184 fold compared with that produced without L-TRP. The significance of auxin production by rhizobacteria is discussed in relation to their effects on plant growth.

Key words: Auxin, L-tryptophan, rapeseed, plant growth promoting rhizobacteria

Introduction

Plant growth regulators (PGRs) are organic substances that influence physiological processes of plant at very low concentrations. 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 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 seedling stage. Zahir *et al*. (2000) isolated ten *Azotobacter* cultures 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}, 0^{-4} and 10^{-5} M). L-Tryptophan application was found to increase auxin production compared with that measured without L-TRP and Azotobacter culture Z4 giving relatively higher auxin production.

Keeping this in view, rhizobacteria were isolated from the rhizosphere of different rapeseed varieties and their auxin production was measured as a part of a research project entitled "Isolation and identification of plant growth promoting rhizobacteria for improving yield and oil content of rapeseed".

Materials and Methods

Isolation of rhizobacteria: Rhizobacteria were isolated by dilution plate technique using glucose peptone agar media (Wollum, 1982) from the rhizosphere of Canola (*Brassica napus* L.), Var.Toria (*Brassica compestris*), Raya (*Brassica juncea*), Sarsoon (*Brassica compestris*) and Gobi Sarsoon (*Brassica carinata*) growing at different sites of Punjab. Colonies showing prolific growth *were* selected and purified by further streaking on fresh plates. Same media was used for preparation of slants. Rhizobacteria were named as S1, S2, S3----------S100 (Table 1), were stored in refrigerator and used for measurement of auxin production *In vitro.*

Measurement of auxin production: Sterilized broth (25 mL) taken in glass tubes was inoculated with rhizobacterial cultures in the presence and absence of (5 mL) L-TRP (0.5%) solution and incubated at 28 ± 1°C for 24 hours with occasional shaking in three repeats. 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% $HClO_4$) were 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).

Table 1: Rhizobacteria isolated from different varieties of rapeseed at different sites

Isolate	Site	Variety	Isolate	Site	Variety
S1	PBG Farm (Faisalabad)	Canola	S51	PARS (Faisalabad)	Sarsoon
52	PARS (Faisalabad)	Canala	S52	PARS (Faisalabad)	Sarsoon
S3	PARS (Faisalabad)	Canola	S53	Chak 204 (Faisalabad)	Sarsoon
S4	PARS (Faisalabad)	Canola	S54	Chak 204 (Faisalabad)	Sarsoon
S5	PARS (Faisalabad)	Canals	S55	Chak 204 (Faisalabad)	Sarsoon
56	Chak 204 (Faisalabad)	Canala	S56	Chak 204 (Faisalabad)	Sarsoon
$7	Chak 204 (Faisalabad)	Canola	S57	Chak 204 (Faisalabad)	Sarsoon
S8	Chak 204 (Faisalabad)	Canola	S58	Chak 204 (Faisalabad)	Sarsoon
S9	Chak 204 (Faisalabad)	Canola	S59	PBG Farm (Faisalabad)	Sarsoon
$10	Chak Samana (Faisalabad)	Toria	S60	PBG Farm (Faisalabad)	Sarsoon
$11	Chak Samana (Faisalabad)	Toria	S61	Usmania Pull, 207 RB (Faisalabad)	Raya
S12	ChakSamana (Faisalabad)	Toria	S62	Usmania Pull, 207 RB (Faisalabad)	Toria
913	Chak Samna (Faisalabad)	Torte	S63	PBG Farm (Faisalabad)	Rays
S14	Chak Samana (Faisalabad)	Torte	S64	Salar Wala (Faisalabad)	Toria
516	Chak Samana (Faisalabad)	Toria	S65	*Dara* Din Shah (Muzzafar Garh)	Toria
S16	Chak Samana (Faisalabad)	Toria	S66	PBG Farm (Faisalabad)	Toria
$17	PBG Farm (Faisalabad)	G.Sarsoon	S67	Oars Din Shah (Muzzafar Garh)	Toria
918	PBG Farm (Faisalabad)	G.Sarsoon	S68	PBG Farm (Faisalabad)	Sarsoon
S19	PBG Farm (Faisalabad)	G.Sarsoon	S69	PBG Farm (Faisalabad)	Toria
S20	Chak Samana (Faisalabad)	G.Sarsoon	S70	Dare Din Shah (Muzzafar Garh)	Raya
S21	Chak Samana (Faisalabad)	G.Sarsoon	S71	Satan Wale (Faisalabad)	Toria
S22	Chak Samana (Faisalabad)	G.Sarsoon	S72	Dara Din Shah (Muzzafar Garb)	Samoan
523	Chak Samana (Faisalabad)	G.Sarsoon	S73	Dana Din Shah (Muzzafar Garh)	Sarsoon
524	Chak Samana (Faisalabad)	G.Sarsoon	S74	Usmania Pull, 207 RB (Faisalabad)	Sarsoon
S25	PARS (Faisalabad)	G.Srsoon	S75	Usmania Pull, 207 RB (Faisalabad)	Sarsoon
S26	PARS (Faisalabad)	G.Srsoon	S76	Salar Wale (Faisalabad)	Raya
527	PARS (Faisalabad)	0.Srsoon	S77	Usmania Pull, 207 F18 (Faisalabad)	Sarsoon
S28	Chak 204 (Faisalabad)	G.Sarsoon	S78	Salar Wala (Faisalabad)	Rays
529	Chak 204 (Faisalabad)	G.Sarsoon	S79	Salar Wale (Faisalabad)	Toria
530	Chak 204 (Faisalabad)	G.Sarsoon	S80	PBG Farm (Faisalabad)	Raya
$31	Chak 204 (Faisalabad)	G.Sarsoon	S81	Dare Din Shah (Muzzafar Garh)	Toria
S32	Chak 204 (Faisalabad)	G.Sarsoon	S82	Dare Din Shah (Muzzafar Garh)	Sarsoon
S33	Chak 204 (Faisalabad)	G.Sarsoon	S83	P843 Farm (Faisalabad)	Toria
534	PSG Farm (Faisalabad)	Rays	S84	Dare Din Shah (Muzzafar Garb)	Rays
$35	PBG Farm (Faisalabad)	Rays	S85	Usmania Pull, 207 RB (Faisalabad)	Raya
S36	MG Farm (Faisalabad)	Rays	S86	Saar Wale (Faisalabad)	Raya
S37	P80 Farm (Faisalabad)	Raya	S87	Dara Din Shah (Muzzafar Garb)	Raya
$38	Chak Samana (Faisalabad)	Raya	S88	Usmania Pull, 207 RB (Faisalabad)	Raya
$39	Chak Samana (Faisalabad)	Raya	S89	Salar Wale (Faisalabad)	Sarsoon
840	Chak Samana (Faisalabad)	Raya	S90	Clara Din Shah (Muzzafar Garh)	Sarsoon
841	Chak Samana (Faisalabad)	Raya	S91	Dara Din Shah (Muzzafar Garh)	Sarsoon
942	PARS (Faisalabad)	Raya	S92	Dara Din Shah (Muzzafar Garh)	Raya
$43	Chak 204 (Faisalabad)	Raya	S93	PSG Farm (Faisalabad)	G.Sarsoon
244	Chak 204 (Faisalabad)	Raya	S94	PSG Farm (Faisalabad)	Sarsoon
945	P83 Farm (Faisalabad)	Sarsoon	S96	PBG Farm (Faisalabad)	Raya
848	PSG Farm (Faisalabad)	Sarsoon	S96	Dare Din Shah (Muzzafar Garh)	Raya
847	P130 Farm (Faisalabad)	Sarsoon	S97	Usmania Pull, 207 RB (Faisalabad)	Sarsoon
249	PSG Farm (Faisalabad)	Sarsoon	S98	Usmania Pull, 207 RB (Faisalabad)	Raya
240	PARS (Faisalabad)	Sarsoon	S99	Usmania Pull, 207 FtB (Faisalabad)	Toria
850	PARS (Faisalabad)	Sarsoon	S100	Salar Wale (Faisalabad)	Raya

Results

Data revealed that rhizobacteria isolated from different varieties of rhizosphere of rapeseed at different locations had ability to produce auxins and this ability was increased many fold when supplemented with an auxin precursor L-TRP. Auxin production form different varieties at different sites is as under.

Usmania Pull 207 RB (Faisalabad): From this site, rhizobactaris were isolated from rhizosphere of three varieties (Torla, Rays and Sarsoon). Figure 1 revealed that rhizobacteria from all varieties of rapeseed produced auxins but it was increased when L-TRP was added in the medium. Maximum auxin (10.4 µg mL^{-1}) without L-TRP was produced by bacterial strain S88 isolated from rhizosphere of Raya. The minimum auxin production was found from the rhizosphere of Sarsoon by S97. But when L-TRP was introduced into the medium, the maximum auxin production was from rhizosphere of Sarsoon

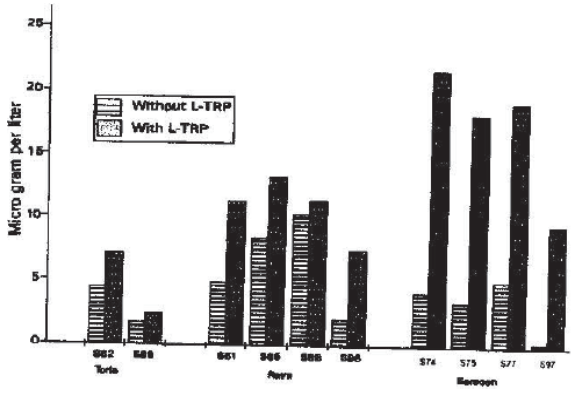

Fig. 1: Auxin production from rhizosphere of different varieties of rapessed from Usmania Pull (207 RB)

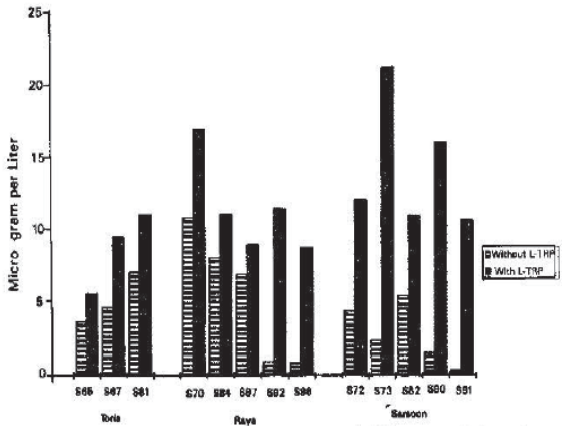

Fig. 2: Auxin production from rhizosphere of different varieties of rapessed (Dara Din Shah Muzzafar Garh)

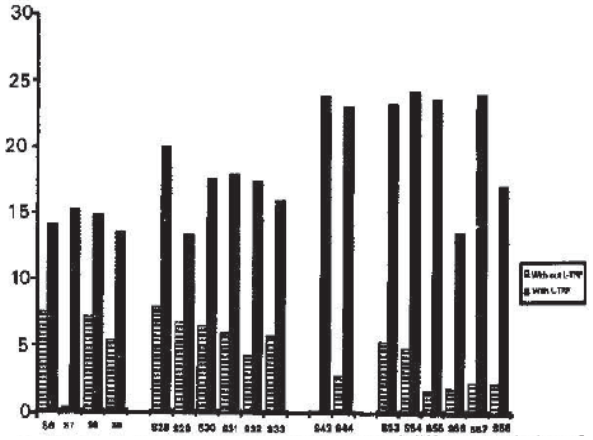

Fig. 5: Auxin production from rhizosphere of different varieties of rapessed (PARS)

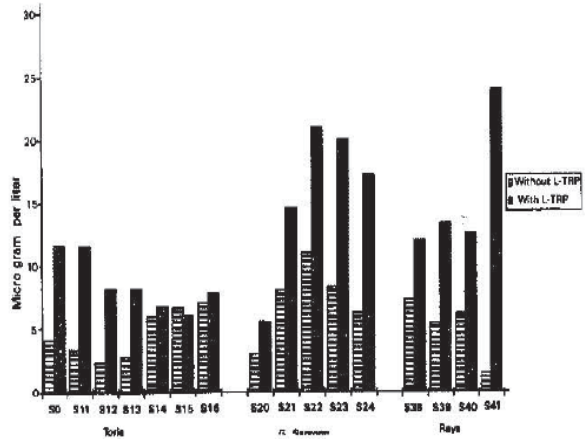

Fig. 3: Auxin production from rhizosphere of different varieties of rapessed (Chak samana)

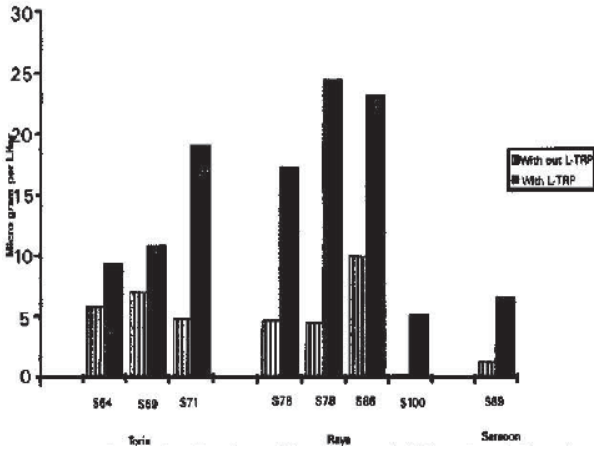

Fig. 6: Auxin production from rhizosphere of different varieties of rapessed (Salar wala)

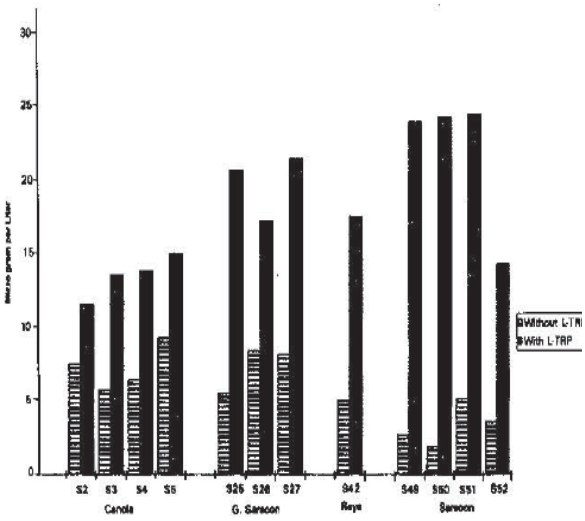

Fig. 4: Auxin production from rhizosphere of different varieties of rapessed (PARS)

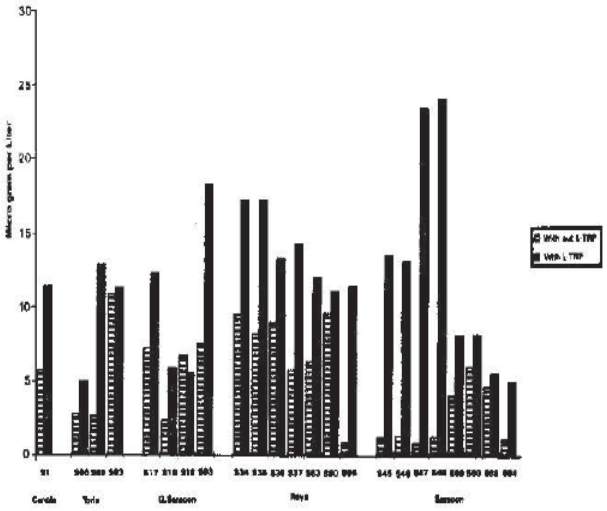

Fig. 7: Auxin production from rhizosphere of different varieties of rapessed (PBG farm)

(21.8 μg mL^{-1}) by strain S74 and it was four fold higher than that produced by this strain without L-TRP. With addition of L-TRP, minimum auxin was produced by S99 (2.41 μg mL^{-1}), it was approximately same as in the absence of L-TRP.

Data Din Shah (Muzzafar Garb): Figure 2 revealed that maximum auxin production from rhizosphere of Toria, Raya and Sarsoon was from S70 (11.4 μg mL^{-1}) strain isolated from Raya. But 573 produced maximum auxin (22.43 μg mL^{-1}) with the addition of L-TRP in the medium and it was nine fold higher than without L-TRP. Minimum auxin production with addition of L-TRP was 5.85 μg mL^{-1} and it was also two fold higher than that without L-TRP.

Chak Samana (Faisalabad): Rhizobacteria were isolated from this site, from Toria, G.Sarsoon and Raya (Fig. 3). Maximum auxin without L-TRP was produced by 522, a strain isolated from rhizosphere of G.Sarsoon. A strain S41 from Raya rhizosphere produced maximum auxin (24 μg mL^{-1}) in the presence of L-TRP and it was 16 fold higher than that produced when incubated in absence of L-TRP.Minirrium auxin production was 5.53 μg mL^{-1} by S20.

Postgraduate Agriculture Research Station, Jhang Road, Faisalabd (PARS): Data presented in Fig. 4 from the rhizosphere of Canola, G.Sarsoon, Raya and Sarsoon proved that all the strains have ability to produce auxin in both cases, when treated with L-TRP or not. An isolate S5 from Canola rhizosphere produced maximum auxin (9.2 μg mL^{-1}) in L-TRP free media. But when L-TRP was added in the media then S51 performed better than other strains. This strain was isolated from the rhizosphere of Sarsoon and produced auxin (24.43 μg mL^{-1}) which was 5 fold higher than when media was not supplemented with L-TRP. Minimum auxin production in the presence of L-TRP was reported by S2 isolate.

Chak 204 (Faisalabad): It is clear from Fig. 5 that all isolates from Canola, G.Sarsoon, Sarsoon and Raya produced auxin in the presence as well as absence of L-TRP. Isolate S26 produced maximum auxin (7.93 μg mL^{-1}) without L-TRP. This strain was isolated from variety G.Sarsoon. But isolate S54 performed better when L-TRP was introduced into the culture media. The auxin production by this strain isolated from Sarsoon field produced auxin 24.23 μg mL^{-1}. It was 5 fold more than that by the same isolate incubated in the absence of L-TRP. Minimum auxin production in the presence of L-TRP was 13.4 μg mL^{-1} by S29 isolated from field of G.Sarsoon.

Salar Wala (Faisalabad): Data presented in Fig. 6 reveals auxin production by rhizobacteria isolated from different varieties of rapeseed. Rhizobacteria from Raya produced maximum auxins (10 μg mL^{-1}) when it was incubated in L-TRP free media. When L-TRP was added in the growth medium the maximum auxin production was 24.6 μg which was produced from S78 isolated from rhizosphere of same variety and it was 5 fold higher when compared with auxin produced by same isolate in the absence of L-TRP.

PBG Farm (Faisalabad): Five varieties of rapeseed were selected for determination of auxin production ability from their rhizosphere at PBG Farm. Figure 7 shows that all isolates produced auxins both in the presence as well as absence of LTRP in the growth medium. In the absence of L-TRP, S83 isolated from Toria was most auxin producing strain (10.9 μg mL^{-1}) when L-TRP was introduced in the media an isolates S48 produced maximum auxin (24.1 μg mL^{-1}) which was 20 fold higher than auxin produced by same strain in the absence of L-TRP. Minimum auxin production (4.93 μg mL^{-1}) in the presence of L-TRP was from strain S94 isolated from Sarsoon rhizosphere.

Discussion

In this study all rhizobacterial strains produced auxin in the presence and absence of L-TRP. L-Tryptophans derived auxin was increased up to 24.6 μg mL^{-1} which was 5 times more than that without L-TRP.

Wide variation in auxin production ability of different bacterial strains may be attributed to different type of exudates from different varieties of rapeseed. A variation in auxin production ability of different soils was found by Sarwar *et al*. (1992). They also reported that auxin biosynthesis in soil was substantially increased upto 61 fold upon the addition of 5.3 g L-TRP kg^{-1} of soil. Mordukhova *et al*. (1991) also reported similar findings. They screened S16 strains of genus *Pseudemonas* for their ability to produce IAA and observed that *Pseudomonas* were stimulated to synthesize IAA upon the addition of tryptophan to medium. Auxin production is more likely to be active in rhizosphere or at microsites where substrates and microorganisms are abundant as Rossi *et al.* (1984) observed 3 fold higher IAA in rhizosphere compared to non-rhizosphere environment.

L-Tryptophan is an essential amino acid and acts as physiological precursor of auxins in higher plants as well as for microbial biosynthesis of auxins (Frankenberger and Arshad, 1995). Arshad and Frankenberger (1993) concluded that LTRP application to soil may improve the growth and yield of plants most likely via its conversion into auxins by soil indigenous microbiota. Further investigations are required to establish relation between different varieties and sites for auxin production.

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