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ASSESSMENT OF AUXIN PRODUCTION FROM RHIZOBACTERIA ISOLATED FROM DIFFERENT VARIETIES OF RAPESEED

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Different varieties of rapeseed (Canola, Raya, Toria. Gobi Sarsoon and Sarsoon) were selected for is~lation of rhizobactcria The potential of these isolates for auxin production was measured in terms of indole acetic acid equivalents by colorimeter method both in the presence and absence of an auxin precursor L-tryptophan (L-TRP). The isolates varied in their potential for auxin production. The highest auxin production (11.4 ug ml.') in the absence ofL-l'RP was measured in case ot rhizobactcria (S70) isolated from Raya variety of rape seed. L-Tryptophan application increased the auxin production by these isolates and the highest auxin production in the presence ofL-TRP was also recorded in case of rhizobacteria from the same variety (Raya) but by a different isolate (S78).

Key words: auxin. L-tryptophan, rapeseed, rhizobacteria

INTRODUCTION

Plant growth regulators (PGRs) are organic substances that influence physiological processes of plant at very low concentrations. It has been well established 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 releases these compounds (Frankenberger and Arshad, 1995: Arsbad and Frankenberger, IlJ98). Studies~j.lay·e'shown that microbial production of phytohormoues can be increased several fold by providing their suitable precursors. These precursors, due to the activities of rhizosphere microbiota, may provide a continuous source of active substances for plant uptake which is better than one time application of synthetic compounds (Arshad and Frankenberger, IlJ90).

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 a suitable precursor is one of the primary factors affecting microbial secretion of these secondary metabolites. The exogenous application of precursors resulted in increasing several fold the magnitude of phytohormone production in culture and soil (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 about physiological response of radish *(Raphanus sativuss,* 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. (1999) observed significant effect of an auxin indole acetamide and its precursor L-TRP on growth and yield of rice in a field experiment.

Zahir et al. (2000) isolated ten *Azotobacter* cultures from the maize rhizosphere and their auxin producing ability was measured colorimetrically. The auxin production b~ three efficient *Azotohacter* cultures (Zl , Z::, Z~) was also measured in the presence of filter sterilized L-tryptophan (Ca). 10'\ 10'4 and W"' M). L-Tryptophan application was found to increase auxin production compared with that measured without L-TRP and *Azotobacter* culture Z~ gave 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 contenl of rapeseed" funded by the Third World Academy of Sciences,

MATERIALS AND MEmODS

Isolation of Rhizobacteria: Rhizobacteria were isolated by dilution plate technique using glucose peptone agar medium (Wollum 11, 1982) from the rhizosphere of Cauola *iBrassica napus* L.), Var. Toria *(Brassica compestrtst.* Raya *tBrassica juncea).* Sarsoon *(Brassica compestris)* and Gobi Sarsoon *(Brassica carinata).* Colonies showing prolific growth were selected and purified by further streaking on fresh plates. The same medium was used for preparation of slants. Rhizobacteria were named as SI,. S2. S~ -------S)(IO (Table 1), and stored in a refrigerator to) be 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 (5mL) L-TRP (0.5%) solution and incubated at 28±1 QC 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 were taken in test tubes and 2 mL of Salkowski reagent (2 mL 0.5M FeCI) + 98 mL 35% $HCIO_4$) 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).

RESULTS

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

Toria: Table 2 revealed that maximum auxin production was measured from S83 (10. 9 ug ml,") that was isolated from the rhizosphere of Toria. But with the addition of L-TRP to the medium, S71 produced maximum auxin (19.13 ug ml,;') and it was fourfold higher than without L-TRP. Minimum auxin production with addition of L-TRP was 2.41 ug ml," and it was double than that without L-TRP.

G.Sarsoon: Data presented in Table 2 from the rhizosphere of G.Sarsoon. proved that all the strains have ability to produce auxin in both cases i.e. treated with L'-TRP or not. Isolate S22 produced maximum auxin (I 1.07 ug ml,;') in L-TRP free medium. But when L-TRP was added to the medium then S27 performed better than other strains, and produced maximum auxin (21.43 ug ml,;') which was threefold higher than when not supplemented with L-TRP. Minimum auxin production in the presence of L-TRP was reported by Sl9 and S20.

Canola: It is clear from Table 2 that all isolates from Canola, produced auxins in the presence or absence of L-TRP. Isolate S5 produced maximum auxin (9.2 ~g ml,;') without L-TRP. But isolate S7 performed better when L-TRP was used. The auxin production by this strain was 15.27 ug ml,;'. It was many times more than that by the same isolate incubated in the absence of L-TRP. Minimum auxin production in the presence of L-TRP was 11.47 I-l;gml," by SI isolated from the same variety.

Sarsoon: Data presented in Table 3 revealed auxin production from different rhizobacteria isolated from rhizosphere ?f Sarsoon. Isolate S60 produced maximum auxin (5.95 ug mL-I) when it was incubated in L-TRP free medium. When L-TRP was added to the growth medium the maximum auxin production was 24.43 ug mL-' from S51 isolated from rhizosphere of the same variety and it was fivefold higher when compared with auxin produced by the same isolate in me absence of L-TRP.

Raya: Data given in Table 3 revealed that ail rhizobacteria isolated from rhizosphere of Ray a produced auxins, which increased when L-TRP was added to the medium. Maxi mum auxin (11.4 ug ml,;') without L-TRP was produced by bacterial strain S70. whereas it was the minimum from strain S43. But when L-TRPwas introduced into the medium. the maximum auxin production (24.6 ug ml,") was obtained from bacterial strain S78, being fivefold higher than that produced by this strain without L-TRP. With addition of L-TRP. minimum auxin was produced by S lOO (5.16 ug rnl,;'), still it was 15 times more than that produced by the same strain in the absence of L-TRP.

DISCUSSION

In this study all rhizobacterial strains produced auxin in the presence and absence ofL-TRP although theirpotential varied a great deal. Auxin production by all strains increased when culture medium. was supplemented with an auxin precursorL-TRP. L-Tryptophan derived auxia was increased up to 24.6 ug ml,; which was 5 times more than WIthout L-TRP. Wide variation in auxin production ability of ditTerent bacterial strains may be attributed to different types of exudates from different varieties of rapeseed. A variation in auxin production ability of different soi Is was found by Sarwar et a1, (1992). They also reported that auxin biosynthesis in soil was substantially increased up to 61 times upon the addition of 5.3 g L-TRP kg:' of soil. Mordukhora et a1, (1991) also reported similar findings. They screened 216 strains of genus *Pseudomonas* for their ability to produce IAA and observed that *Pseudomonos* were stimulated to synthesize IAA 1TpQn the addition of tryptophan to the medium. Auxin production is more likely to be active in rhizosphere or at micrositcs where substrates and microorganisms are abundant. \!.t is supported by Rossi et a1, (1984) who observed ~reefold higher IAA in rhizosphere compared to non-rhizospherc environment.

L-Tryptophan is an essential amino acid and acts as R physiological precursor of auxins in higher plants as well as for microbial biosynthesis of auxins (Frankcnbcrger and Arshad, 1995). Arshad and Frankenberger (1l)l)3) concluded that L-TRP 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.

Table 1. Rhizobacteria isolated from different varieties of rapeseed

Isolate	Variety	Isolate	Variety	Isolate	VarietY	Isolate	Variety
81	Canola	S26	G.Sarsoon	S51	Sarsoon	S76	Raya
82	Canola	S27	G.Sarsoon	S52	Sarsoon	S77	Sarsoon
83	Canola	S28	G.Sarsoon	S53	Sarsoon	S78	Raya
S4	Canola	S29	G.Sarsoon	S54	Sarsoon	879	Toria
85	Canola	S30	G.Sarsoon	855	8arsoon	880	Raya
86	Canola	S31	G.Sarsoon	856	Sarsoon	881	Toria
S7	Canola	S32	G.Sarsoon	857	Sarsoon	882	Sarsoon
88	Canola	S33	G.Sarsoon	S58	Sarsoon	883	Toria
89	Canola	S34	Raya	859	Sarsoon	884	Raya
S10	Toria	S35	Raya	S60	Sarsoon	885	Raya
811	Toria	S36	Raya	S61	Raya	886	Raya
S12	Toria	S37	Raya	862	Toria	887	Raya
S13	Toria	S38	Raya	S63	Raya	S88	Raya
S14	Toria	S39	Raya	S64	Toria	S89	Sarsoon
S15	Toria	840	Raya	865	Toria	890	Sarsoon
S16	Toria	841	Raya	866	Toria	891	Sarsooh
S17	G.Sarsoon	S42	Raya	S67	Toria	S92	Raya
S18	G.Sarsoon	S43	Raya	868	Sarsoon	893	G.8arsoon
S19	G.8arsoon	S44	JYlya	869	Toria	S94	Sarsoon
S20	G.Sarsoon	S45	sarsoon	S70	Raya	895	Raya
S21	G.Sarsoon	S46	Sarsoon	S71	Toria	896	Raya
822	G.Sarsoon	S47	Sarsoon	872	8arsoon	S97	Sarsoon
S23	G.Sarsoon	848	Sarsoon	873	Sarsoon	898	Raya
824	G.Sarsoon	S49	Sarsoon	S74	Sarsoon	899	Toria
825	G.8arsoon	850	Sarsoon	875	Sarsoon	S100	Raya

Table 2. Auxin production by rhizobacteria isolated from different varieties of rapeseed

(Average of 3 years)

Isolate	IAA equivalents (J.LgmL"l)		Isolate	IAA equivalents (J.LgmL"l)		Isolate	IAA equivalents (J.Lgml;l)	
	Without L-TRP	With L-TRP		Without L-TRP	With L-TRP		Without L-TRP	With L-TRP
	Toria			G.Sanoon			Cuola	
S10	4.07	11,63	S17	7.23	12,33	SI	5.77	11,47
S11	3.33	11,60	S18	2,37	5.87	82	7,47	11,53
812	2,33	8.17	S19	6.73	5,53	83	5.67	13,47
S13	2.80	8.17	820	2.97	5,53	84	6,37	13.80
S14	5.93	6,77	S21	8.07	14.60	S5	9.20	14.97
815	6.67	6.07	S22	11,07	21,07	86	7,53	14.10
S16	7.07	7.87	S23	8,33	20.03	87	0,36	15.27
862	4,46	7.20	824	6.27	17.20	88	7.13	14.80
S64	5.86	9,36	825	5,47	20.60	S9	5.43	13,53
865	3.83	5.85	S26	8,33	17.23			
866	2.83	5.00	827	8.10	21,43			
867	4.86	10.03	828	7.93	20.03			
S69	2.68	12.90	S29	6.73	13,40			
S7.1	4.91	19.13	S30	6,47	17.63			
879	7.08	10.83	831	5.97	17.93			
S81	7,40	11,60	S32	4.27	17,40			
883	10.90	11,33	833	5.73	15.97			
899	1,65	2,41	S93	7.60	18.26			

Table 3. Auxin production by rhizobacteria isolated from differeDt varieties of rapeseed (Average of 3 ytlars)

Isolate	!AA equivalents (~ mL"l)		Isolate	!AA equivalents (J.Lg mL"I)	
	Without L-TRP	WithL-TRP		Without L-TRP	WithL-TRP
Sarsoon			Raya		
845	1,20	13.47	S34	9,47	17.20
S46	1,27	13.13	S35	8.20	17.23
S47	0.80	23.47	S36	8.97	13.27
S48	1,23	24.10	S37	5.73	14.27
S49	2.67	23.93	S38	7.30	12.0n
.850	1,87	24.23	S39	5,43	13.35
S51	5.13	24.43	S40	6.20	12.53
S52	3.53	14.30	841	1,47	24.00
S53	5.33	23.27	842	4.97	17.53
854	4.87	24.23	S43	0.13	23.87
S55	1,73	23.63	844	2.80	23.13
856	1,80	13.60	861	4.90	1136
S57	2.27	24.03	S63	6.30	12.0n
S58	2.20	17.07	S70	11,40	17.90
859	4.00	8.06	S76	4.73	17.30
S60	5.95	8.10	878	4.60	24.60
868	4.60	5,46	S80	9.60	11,10
S72	4.60	12.70	S84	8,48	11.66
S73	2045	22043	S85	8,48	13,36
S74	4.23	21.80	S86	10.00	23.26
S75	3046	18.33	S87	7,30	9,43
S77	5.13	19,33	S88	10.40	111,50
882	5.75	11,53	S92	0.95	12.06
S89	1Al	6.66	S95	0.90	11,48
S90	1,58	16.90	S96	0.86	9.23
S91	0.35	11,21	898	2.08	7.58
894	1,06	4.93	8100	0.35	5.16
'897	0.33	9.63			

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