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Lohith Kumar N
Department of Agricultural Microbiology, University of Agricultural Sciences, Bengaluru, Karnataka, India

Muthuraju R
Department of Agricultural Microbiology, University of Agricultural Sciences, Bengaluru, Karnataka, India

Avinash M
Department of Agricultural Microbiology, University of Agricultural Sciences, Bengaluru, Karnataka, India

Neekhitha Shetty
Department of Agricultural Microbiology, University of Agricultural Sciences, Bengaluru, Karnataka, India

Ameena Sulthana JH
Department of Agricultural Microbiology, University of Agricultural Sciences, Bengaluru, Karnataka, India

Correspondence
Lohith Kumar N
Department of Agricultural Microbiology, University of Agricultural Sciences, Bengaluru, Karnataka, India

Screening of desiccation tolerant rhizobacteria for their plant growth promotion and water stress alleviation under *in vitro* osmotic conditions

Lohith Kumar N, Muthuraju R, Avinash M, Neekhitha Shetty and Ameena Sulthana JH

Abstract
Drought is the most destructive stress among abiotic stresses that increased in intensity over the past decades affecting world's food security. Drought stress may range from moderate and short to extremely severe and prolonged duration, restricting the crop yields. In recent times, the use of rhizobacteria for alleviation of drought stress in plants has gained momentum. In this study desiccation tolerant rhizobacterial isolates were subjected to quanitative analysis of phosphate solubilizing abilities of the desiccation tolerant rhizobacterial isolates results revealed that isolate KOP-2 CHI-4, and GAD1-1(1.42, 1.27 and 0.84 µg/ml) were able to solubilize higher phosphorous under stress condition. Similarly, under non-stress condition, isolate KOP-2, CHI-4, and GAD-1 (2.47, 2.07 and 1.69 µg/ml) were able to solubilize higher phosphorous compare to other isolates. The nine desiccation tolerant rhizobacterial isolates were examined for the production of IAA, GA3 and ABA with and without tryptophan under stress and non-stress condition. Among the isolates KOP-2, GAD-1 and CHI-4 showed maximum IAA, GA_3 and ABA production under stress and non-stress condition.

Keywords: Desiccation tolerant rhizobacteria, plant growth promotion, water stress alleviation

Introduction
Drought stress affects the water relations of a plant at cellular and whole plant level causing economic losses in agriculture. In recent times, the use of rhizobacteria for alleviation of drought stress in plants has gained momentum. The term 'Induced Systemic Tolerance' (IST) has been proposed for PGPR-induced physical and chemical changes that result in enhanced tolerance of plants to abiotic stress (Yang *et al*., 2009) [6]. Several mechanisms of drought resistance in plants have been proposed to be induced by PGPR, through elicitation of rhizobacterial-induced drought endurance and resilience (RIDER) process that involves various physiological and biochemical changes. It includes modifications in phytohormonal content and antioxidant defense. PGPR also produce osmolytes and bacterial exopolysaccharides (EPS) to ensure survival of plants under drought stressed conditions (Vanderlinde *et al*., 2010) [8]. Production of heat-shock proteins (HSPs), (Berjak 2006) [10], dehydrins (Timmusk and Wagner 1999) [9] and volatile organic compounds (VOCs) have been reported to impart drought tolerance to plants. The term 'Induced Systemic Tolerance' (IST) has been proposed for PGPR-induced physical and chemical changes that result in enhanced tolerance of plants to abiotic stress (Yang *et al*., 2009) [6]. A rhizobacteria qualifies as PGPR when it exhibits growth-promoting properties like production of phytohormones (indole acetic acid (IAA), Gibberellic acid (GA), and cytokinins), HCN, and ammonia and with the ability to solubilizemineral phosphate and to antagonize plant pathogens, etc. (Glick 1995). The amount of IAA is a suitable marker for bacterial effectiveness particularly under osmotic stress (Boiero *et al*. 2006) [11]. Interestingly, some of the volatile organic compounds (VOCs) that are emitted from Bacillus subtilis GB03 (Ryu *et al*. 2004) [13] are bacterial determinants involved in IST. In addition to causing general plant growth, some PGPR promote root development (Mantelin and Touraine 2004) [12] and alter root architecture by producing phytohormones such as IAA and cytokinins, which results in increased root surface area and number of root tips. Such stimulation of roots can also relate to IST.

Material and methods
Phosphorous solubilization
The isolated bacterial culture was grown in nutrient broth. The one ml of the actively grown culture was inoculated to the 100 ml of NBRIP broth with tri-calcium phosphate (with PEG and without PEG) as a phosphate source and incubated at 28°C for seven days.

After seven days of incubation the broth were checked for pH and centrifuged at 10000 rpm for 5 minutes. The 0.5ml supernatant was taken and examined for phosphate solubilization using spectrophotometer. To the supernatant 1-2 drops of p-nitrophenol (0.25%) was added as an indicator followed by addition of 5N HCl dropwise to neutralize the colour. The above solution was diluted with 40 ml of double distilled water and 8 ml of ammonium paramolybdate-ascorbic acid reagent was added to the solution and incubated at room temperature for 20 minutes. Final volume of the solution was made up to 50 ml with double distilled water. The absorbance was read at 880nm (Murphy and Riley, 1962) [3].

Extraction and estimation of IAA, GA_3 and ABA

Four different types of media like nutrient broth, nutrient broth +Tryptophan, nutrient broth + PEG and nutrient broth + PEG +Tryptophan was prepared. Inoculated the specified media with 24 hrs old grown culture and incubated at 37°C for 7 days at dark condition. After seven days of incubation, centrifuged at 6000 rpm for 10 minutes. To the supernatant, added 1N Hcl and adjusted pH at 2.8. The total acidified supernatant taken into 250 ml conical flask and added equal volume of diethyl ether to the supernatant and incubated in dark condition for 4 hrs. Then kept the samples at 4°C overnight in separating funnel. Then organic phase (down layer) discarded and solvent phase (upper layer) collected. Upper layer allowed to evaporate and 2-3 ml of HPLC grade methanol added and IAA, GA_3 and ABA were quantified by a high performance liquid chromatography (HPLC).

Results and discussion

The plant growth promotion attributes of the desiccation tolerant isolates was determined under normal and osmotically stressed conditions. Quanitative analysis of phosphate solubilization abilities of the desiccation tolerant rhizobacterial isolates was examined on NBIRP broth with tri-calcium phosphate under both stress and non-stress condition. It is presented in Table 2. Results revealed that isolate KOP-2 CHI-4, and GAD1-1(1.42, 1.27 and 0.84 µg/ml) were able to solubilize higher phosphorous under stress condition. Similarly, under non-stress condition, isolate KOP-2, CHI-4, and GAD-1 (2.47, 2.07 and 1.69 µg/ml) were able to solubilize higher phosphorous. Such observation are in agreement with the findings made earlier by Palika *et al.*, (2013), who isolated 47 bacterial isolates from different rhizospheric soils of chickpea from Punjab and studied their P-solubilizing abilities. The nine desiccation tolerant rhizobacterial isolates were examined for the production of IAA, GA_3 and ABA with and without tryptophon under stress and non-stress condition, and the results are presented in Table 3. Among the nine isolate, KOP-2 maximum produced the IAA in treatment with tryptophan and tryptophan under non-stress condition (247 and 187 µg/ml) followed by the isolates CHI-4 (235 and 142 µg/ml) and GAD-1(234 and 138 µg/ml). Under stress condition isolate CHI-4 able to produce higher IAA (185 and 112 µg/ml) followed by GAD-1 (171 and 112 µg/ml) and KOP-2 (162 and 102 µg/ml).The maximum GA_3 produced by isolate, KOP-2 in treatment with tryptophan and tryptophan under non-stress condition (298 and 126 µg/ml) followed by the isolates CHI-4 (241 and 113 µg/ml) and GAD-1 (232 and 186 µg/ml). Under stress condition isolate KOP-2 able to produce higher GA_3 (169 and 129 µg/ml) followed by GAD-1 (146 and 79 µg/ml) and CHI-4 (128 and 83 µg/ml). Similar trend were observed in production of ABA. The maximum production of ABA by isolate KOP-2 (196 and 167 µg/ml) followed by CHI-4 (142 and 161 µg/ml) and GAD-1 (135 and 162 µg/ml). Similarly under stress condition isolate KOP-2 able to produce higher ABA (217 and 253 µg/ml) followed by CHI-4 (208 and 213 µg/ml) and GAD-1 (204 and 192 µg/ml). The production of phytohormone by bacterial isolates has been reported earlier by various workers Basharat Ali *et al.*, (2009) [4] quantified the indole-3-acetic acid from plant associated *Bacillus spp*. and their phytostimulatory effect on *Vigna radiata* (L.)

Table 1: Phosphate solubilization of desiccation tolerant rhizobacterial isolates under *in vitro* osmotic conditions.

SL. No	Isolates	Soluble phosphorous (µg/ml)	
		With stress	Without stress
1	CHI-1	0.46	0.71
2	CHI-2	0.80	0.41
3	CHI-3	0.82	0.89
4	CHI-4	0.84	1.69
5	CHI-5	0.55	0.44
6	GAD-1	1.27	2.07
7	GAD-2	0.79	1.03
8	KOP-1	0.61	1.28
9	KOP-2	1.42	2.47
10	SEM±	0.02	0.02
11	CD at 5%	0.06	0.08

Note: CHI-Chitradurga, GAD- Gadag and KOP- Koppal

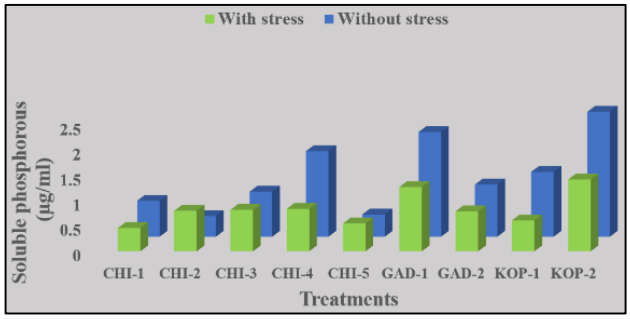

Fig 1: Phosphorous solubilization of different desiccation tolerant rhizobacterial isolates under *in vitro* osmotic condition.

Table 2: Phytohormone production of desiccation tolerant rhizobacterial isolates under *in- vitro* osmotic conditions

Isolates	IAA (µg/ml)				GA_3 (µg/ml)				ABA (µg/ml)			
	-PEG		+PEG		-PEG		+PEG		-PEG		+PEG	
	+TRY	-TRY	+TRY	-TRY	+TRY	-TRY	+TRY	-TRY	+TRY	-TRY	+TRY	-TRY
CHI-1	207	102	132	86	195	102	119	72	132	115	184	183
CHI-2	136	71	112	74	176	101	124	59	156	102	175	175
CHI-3	221	86	163	42	163	105	91	56	142	91	132	154
CHI-4	235	142	185	112	241	113	128	83	142	161	208	213
CHI-5	196	119	158	84	185	98	114	56	102	129	156	163
GAD-1	234	138	171	112	232	186	146	79	135	162	204	192
GAD-2	165	92	132	80	177	103	123	76	91	129	132	172
KOP-1	213	78	95	61	263	117	102	46	62	149	167	129
KOP-2	247	186	162	102	298	126	169	129	196	167	217	253
SEM±	4.30	2.43	3.09	1.71	4.63	2.41	2.71	1.55	2.71	2.74	4.18	3.74
CD at 5%	12.68	7.17	8.964	5.04	13.67	7.13	7.99	4.57	8.00	8.04	12.34	11.05

Note: TRY- Tryptophan, -TRY- (without tryprophan), + TRY- (with tryprophan), PEG- Polyethelyne glycol, -PEG (without stress), +PEG (with stress), CHI- Chitradurga, GAD- Gadag and KOP- Koppal. IAA- Indole acetic acid, GA_3 – Gibberlin and ABA- Abscisic aci

Among nine isolates, CHI-4, GAD-1 and KOP-2 isolates showed performances when they were subjected to screening for plant growth promotion and water stress alleviation under *in vitro* osmotic condition. These isolates were considered as elite desiccation tolerant rhizobacterial isolates. These isolates were further used for evaluation of plant growth promotion in chilli under glass house condition.

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