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ISOLATION AND SCREENING OF DROUGHT TOLERANT PHOSPHATE SOLUBILIZING BACTERIA FROM RHIZOSPHERE OF *RABI* SORGHUM

M. H. Shete[1], D. R. Murumkar[2] and D. V. Indi[3]

[1]*Biological Nitrogen Fixation Scheme, College of Agriculture, Pune*

[2]*AICRP on Safflower, Zonal Agril. Research Station, Solapur*

[3]*National Agricultural Research Project, Zonal Agril. Research Station, Solapur*

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Abstract: *A pot culture experiment was conducted to know the drought tolerance of phospahte solubilizing bacteria isolated from rhizosphere soils of rabi sorghum. Out of ten samples, seven isolates were studied for their morphological, biochemical and cultural characteristics and all are found to be gram positive. Out of seven isolates, PSB strain-1 showed maximum zone of clearance (5 mm) and 'P' solubiliztion (156 mg/0.5gCa_3P_{O4}) followed by PSB strain-4. The seed treatment with PSB strain-1 was found to be most effective at 30 and 50 days of dry spell recording significantly highest number of leaves (9 leaves/plant), plant height (121.5 cm), dry matter weight (34.28 g/plant) and PSB count (5.3 x 104 cfu/g soil) over PSB strain-4 and commercial PSB strain. Based on phosphate solubilizing ability and drought tolerance, the PSB strain-1 may be further exploited in commercial biofertilizer production.*

Key words: *Phosphate solubilizing bacteria, Drought tolerance, Rabi sorghum.*

Introduction

Phosphorous is one of the essential nutrients in plant growth and yield. Most of the time phosphorous is fixed in the soil and not easily available to the plants. Phosphate solubilizing bacteria are well known to promote plant growth because of their ability to convert insoluble forms of P to soluble forms that can be readily taken by plant roots (Awasthi *et al.*, 2011). In major parts of Maharashtra and Karnataka, *rabi* sorghum is grown under stored and receding soil moisture conditions with increasing temperature after flowering. Drought is the major abiotic stress factor which affects the growth and yield of *rabi* sorghum. The phosphate solubilizing bacteria have attracted researchers to exploit their potential to utilize the phosphate reserves in semi arid regions and to enhance the crop yield. Several studies has revealed that inoculation of PSB enhance the crop yields by solubilizing the soil fixed 'P' and applied phosphorous (Zaidi, 1999). Considering the importance of phosphate solubilizing bacteria, the present study was undertaken to isolate and screen the drought tolerant phosphate solubilizing bacteria from rhizosphere of *rabi* sorghum from different locations of Solapur, Ahemdnagar and Satara district.

Materials and Methods

Eleven soil samples were collected from rhizosphere of *rabi* sorghum from different tahsils of Solapur, Ahemdnagar and Satara district during *rabi* season of 2011. For this purpose, the plants were uprooted carefully, the shoot portion was cut off and roots along with the rhizosphere soil aseptically collected in small plastic bags and brought to the laboratory for isolation.

Isolation of Phosphate solubilizing bacteria:

The isolations of phosphate solubilizing bacteria from soil samples collected from Solapur, Ahemdnagar and Satara district was carried out by using serial dilution pour plate technique (Aneja, 2003). The PSB population was enumerated on Pikovskay's medium at 10^4 dilutions (Pikovaskaya, 1948). The plates were incubated at 28ºC temperature for 72 hours and observed for the bacterial colonies with zone of clearance showing utilization of tricalcium phosphate. Further, pure colonies were transferred on slants.

Purification and Maintains of the Isolates:

Loopful of growth from the slants was streaked again on fresh plates of Pikovskay's medium and incubated for independent colony development at 28ºC in BOD incubator. Thereafter every isolate was transferred on fresh slants maintained in pure state and preserved for further studies.

Identification of Cultures:

Morphological characterization:

All the phosphate solubilizing bacterial isolates were examined for their colony morphology (colony growth, form, margin, elevation, appearance and pigmentation) and gram reaction as per the standard procedure (Cappuccino and Sherman, 1987). The cell motility test was performed using cavity slide by hanging drop technique.

Biochemical and physiological characterization:

The isolates were subjected to biochemical characterization by employing different biochemical tests *viz*., starch hydrolysis, H_2S production, gelatin liquefaction, catalase test and oxidase test as per the standard procedure (Cappuccino and Sherman, 1987). Further the isolates were tested for utilization of different carbon sources *viz*., fructose, glucose, maltose, mannitol and citrate.

Phosphate solubilizing ability of the bacterial isolates:

The ability of the bacterial isolates to solubilize insoluble inorganic phosphate was tested by spotting 10 µl overnight cultures on Pikovskaya's agar plates and incubating at 28°C for 2-3 days. The isolates which showed clear zone of solubilization of tricalcium phosphate (TCP) around the colony were noted as phosphate solubilizers. The diameter of the zone of TCP solubilization was measured and expressed in millimeters.

Screening of Phosphate solubilizing isolates for 'P' Solubilization:

PSB isolates were multiplied on Pikovaskia broth and amount of 'P' solubilized was estimated at the end of seven days inoculation by Olsen's method.

Screening of Phosphate solubilizing isolates by pot culture experiment:

Two PSB isolates *viz*., PSB strain-1 and PSB strain-4 showing maximum zone of clearance and 'P' solubilization were screened in pot culture by Complete Randomized Block Design with two replications and twelve treatments at Biological Nitrogen Fixation Scheme, College of Agriculture Pune. The commercial PSB strain was used as a check. The artificial drought condition was created by using PVC pipe of 90 cm height and 60 cm in diameter. The seeds of sorghum variety M-35-1 treated with PSB isolates were used for sowing. For pot culture study, six seeds per pot were sown. Two dry spells of 30 and 50 days interval were kept. The observations on soil moisture percentage at sowing and at each dry spell, crop stand at the end of dry spell, PSB count, plant height, No. of leaves/plant and dry matter weight of plant were recorded.

Results and Discussion

Morphological characterization:

The data on variation in colony morphology of PSB isolates is presented in Table 1. Out of seven PSB isolates, four isolates *viz*., The PSB-2, PSB-3, PSB-6 and PSB-7 were observed to be irregular form and three isolates *viz*., PSB-1, PSB-4 and PSB-5 as rhizoid form. The colony margins of all isolates were lobate and raised elevation. The pellicle growth was exhibited by all isolates of Phospahte solubilizing bacteria in broth culture. The gram staining reaction of Phospahte solubilizing bacteria was to be gram positive. Cappuccino and Sherman (1987) reported *Pseudomonas* spp. and *Bacillus* spp. as the most efficient phosphate solubilizing isolates identified up to generic level based on the morphological, cultural and biochemical test.

Biochemical and physiological characterization:

All the seven PSB isolates were tested for their biochemical characters *viz*., starch hydrolysis, H_2S production, gelatin liquefaction, catalase test, oxidase test and physiological characters like utilization of different carbon sources. All the seven PSB isolates were positive for starch hydrolysis, gelatin liquefaction and catalase test, but were

negative for H_2S production, KOH and oxidase test. Regarding utilization of different carbon sources; fructose, glucose and maltose were used as a sole carbon source for growth by all the PSB isolates, while mannitol and citrate showed negative result. Taking into account the morphological, cultural and biochemical characteristics, ability of the isolates to utilize various carbon sources for their growth (Claus *et. al.*, 1986), 07 isolates were tentatively identified as *Bacillus megaterium*.

Phosphate solubilizing ability of the PSB isolates:

All the 07 PSB isolates were tested for their ability to solubilize inorganic phosphate both qualitatively and quantitatively and their results are presented in Table 1. Quick analysis of P-solubilization was carried out on Pikovskaya's agar medium. All the 07 isolates were able to form zone of P-solubilization on the medium. The diameter of the zone of P-solubilization ranged from 1 mm (PSB-3 & 7) to 07 mm (PSB-1). Kumar *et al,* (2012) isolated 50 phospahte solubilizing colonies on Pikovskaya's medium. The colonies showing clear halo zones around the bacterial growth were considered as phospahte solubilizers. Hemlata *et.al.* (2013) isolated five phospahte solubilizing bacteria and the amount of phosphorous released was estimated using Pikovskaya's medium.

***In-vitro* screening of phosphate solubilizing isolates for 'P' solubilization:**

The 'P' solubilizing efficiency of different isolates was in the range of 10 to 156 mg/0.5gCa_3Po_4. The highest "P" solubilization was recorded by the isolates PSB strain-1 (156 mg/0.5gCa_3Po_4) followed by PSB strain-4 (138 mg/0.5gCa_3Po_4). These results are in agreement with the findings of Patel and Parmar (2013) who reported quantitative estimation of tri-calcium phosphate solubilization between 68 mg/l to 181 mg/l.

Screening of phosphate solubilizing isolates for 'P' solubilization in pot culture:

Out of seven isolates, two highly efficient isolates *viz*., PSB strain-1 and PSB strain-4 based on maximum zone of clearance and 'P' solubilization in *in-vtro* were screened in pot culture by creating artificial drought condition. The data on soil moisture and growth parameters of *rabi* sorghum are presented in Table 2. The soil moisture in pot culture at sowing was in the range of 34.1 to 34.7 %, further soil moisture was reduced to 21.4% to 22.9% at the end of 30 days dry spell and 12.9% to 13.05% at the end of 50 days dry spell.

100 % germination was recorded in each treatment. Cent percent crop stand i.e. 6 plant per pot was recorded at 30 days dry spell and it was reduced to 4 plant per pot as dry spell increase up to 50 days. Sharma *et al.* (2007) reported that seed inoculation with PSB was found to increase the seed germination as well as radical and plumule length.

Treatment T_3 and T_7 i.e. PSB strain 1 and 4 recorded maximum number of leaves (9 leaves/plant) at 50 days dry spell and found at par with treatment T_{11} (Commercial PSB strain) (8.5 leaves/plant). Maximum plant height (121.5 cm) was recorded in treatment T_1 (PSB strain-1 at 50 days dry spell) and found at par with treatment T_7 (108.8 cm) (PSB strain-4 at 50 days dry spell) but significantly superior over treatment T_{11} (100.5 cm) (Commercial PSB strain at 50 days dry spell). Bahadhur and Singh (1990) reported that increase in plant height and shoot length in green gram was due to presence of sufficient amount of phosphorus which plays a major role towards alteration of cell division and elongation in the meristematic regions.

The significantly highest dry matter weight (34.28 g/plant) was recorded by treatment T_1 (PSB strain-1 at 50 days dry spell) and found significantly superior over T_7 (28.73 g/plant) (PSB strain-4 at 50 days dry spell) and T_{11} (30.50 g/plant) (Commercial PSB strain at 50 days dry spell). The PSB strain-1 at 50 days dry spell recorded maximum count ($5.3x10^4$ cfu) at flowering stage of crop. Zaghloul (2002) reported that growth characteristics such as plant height, number of leaves per plant were significantly increased in sorghum inoculated with *Bacillus megaterium* var. *phosphaticum*.

Conclusion:

Among different PSB strains screened for their drought tolerance, PSB strain-1 was found to be most effective at 30 and 50 days of dry spell recording significantly highest plant height, dry matter weight and PSB count over PSB strain-4 and commercial PSB strain. Based on phosphate solubilizing ability and drought tolerance, the PSB strain-1 may be further exploited in commercial biofertilizer production.

Table 1: Morphological characteristics and phosphours solubilization by different isolates

Sr. No.	Name of strain	Form	Elevation	Margin	Available P (mg/0.5gCa_3Po_4)	Zone of 'P' solubilization on TCP (mm)
1	PSB strain-1	Rhizoid	Raised	Lobate	156	7
2	PSB strain-2	Irregular	Raised	Lobate	3	3
3	PSB strain-3	Irregular	Raised	Lobate	2	1
4	PSB strain-4	Rhizoid	Raised	Lobate	138	5
5	PSB strain-5	Rhizoid	Raised	Lobate	10	4
6	PSB strain-6	Irregular	Raised	Lobate	2	2
7	PSB strain-7	Irregular	Raised	Lobate	1	1

Table 2: Effect of drought tolerant PSB on growth parameters of *rabi* sorghum

Tr. No	Treatment details	Soil moisture at sowing (%)	Soil moisture at the end of dry spell (%)	Germi-nation (%)	Plant stand at the end of dry spell	No. of leaves/ plant at harvest	Plant Height at harvest (cm)	Dry matter weight (g/plant)	PSB count at flowering (x 10^4 cfu /g soil)
T_1	MPKV PSB Strain 1 + 30 days dry spell	34.55	21.75	100	6	10	126.5	35.10	9.1
T_2	No inoculation +30 days dry spell	34.20	22.90	100	6	8.5	90.1	27.15	1.3
T_3	**MPKV PSB Strain 1 + 50 days dry spell**	34.55	12.95	100	**6**	**9**	**121.5**	**34.28**	**5.3**
T_4	No inoculation + 50 days dry spell	34.35	12.95	100	5.5	8	86.1	24.65	1.0
T_5	MPKV PSB Strain 4 + 30 days dry spell	34.50	22.75	100	6	9.5	114.0	31.51	7.3
T_6	No inoculation +30 days dry spell	34.05	21.80	100	6	8	90.8	26.18	1.0
T_7	**MPKV PSB Strain 4 + 50 days dry spell**	34.50	12.90	100	**6**	**9**	**108.8**	**28.73**	**4.1**
T_8	No inoculation +50 days dry spell	34.40	13.05	100	4.5	7.5	86.5	24.53	0
T_9	Commercial PSB strain + 30 days dry spell	34.45	21.40	100	6	9	108.4	32.12	6.2
T_{10}	No inoculation +30 days dry spell	34.70	21.60	100	6	7.5	96.05	25.14	1.0
T_{11}	**Commercial PSB Strain + 50 days dry spell**	34.10	13.00	100	**6**	**8.5**	**100.5**	**30.50**	**3.5**
T_{12}	No inoculation +50 days dry spell	34.00	12.90	100	4	8	82.6	23.05	0
	S.E.	-	-	-	0.4	0.8	5.1	1.02	-
	C.D.at 5%	-	-	-	1.1	2.2	15.1	3.05	-
	C.V.	-	-	-	8.9	12.5	7.1	6.15	-

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