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Potential of Indigenous Rhizobacteria and the Consortium for Promoting the Growth of *Sorghum bicolor* (L.)

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ABSTRACT

Background: The present scenario of agriculture is dependent on chemical fertilizer, pesticides and artificial growth promoters *etc*. The extensive uses of these chemicals fertilizers affect soil and it become less fertile. These problem requireaying an attention towards the use of native biological agents for endorsement of plant growth. Indigenous rhizobacteria are the native rhizospheric bacteria having ability to enhance the growth and yield of crop along with bulk soil bacteria.

Methods: In present study the selected area was North Maharashtra region in which mainly the sorghum is cultivated. Total 320 rhizobacteria were screen for PGP traits *viz*. IAA, GA, ACC deaminase production, phosphate, zinc, potassium solubilization. The potential PGPR used for seed germination and pot assay.

Result: The highest IAA production was 79.74 µg/mL and GA producing isolate shows 227.27 µg/mL. The quantitative analysis of Phosphate reports 455 µgm/mL. Most of the isolates showed positivity for all the traits. From among all isolates, 12 were selected for preparation of consortium. Seed germination plate assay and pot assay. Seed germination assay shows highest vigor index (820) by mixture of both biostimulators. Similar results were obtained during pot assay, vigor index was 3320 for the combine treatment of both biostimulators.

Key words: Biostimulator, Gibberlic acid phosphate solubilization, Indole acetic acid, Rhizobacteria.

INTRODUCTION

Sorghum (Jowar / Solam) is one of the most important cereal crop grown in India and is also the fifth most important cereal crop grown in the world. The weather conditions in the content suited for the plantation in Asia mainly in India. In our agro-ecosystems intensive farming practices that attain high yield require continuous application of chemical fertilizers. However, the prices and availability of these chemical fertilizers become the limiting factor for crop production especially in developing countries around the world.

Constant use of these developed harmful effect on agriculture such pollution of soil, water and air, cause irretrievable harm to the overall environment, shows effect on the growth, mineral nutrient uptake capacity and yield of crop. Some native bacterial strains, plant growth-promoting rhizobacteria (PGPR) directly affect the plant mechanisms by coping synthesis of plant growth hormones and others increase mineral nutrient accessibility in the soil as a way to improve growth and yield. (Vishwakarma *et al.,* 2017). Indigenous PGPR exhibited plant growth promoting potential such as phosphate solubilization, phytohormone production (IAA production), ammonia production, siderophore production, *etc*. (Kiruthika and Arunkumar, 2020). Also, these microbes have been shown *in vitro* to possess potent antagonistic activity against the phytopathogens (Kumari *et al.,* 2018). Mechanism of biocontrol action of PGPR include competition with phytopathogens for an ecological niche or substrate. As well as production of inhibitory compounds and hydrolytic enzymes that are often active against a broad spectrum of phytopathogens. In various stress conditions such as salinity, drought, flooding, metal and organic contamination and phytopathogens the plant hormone ethylene is produce by plant. Ethylene at low concentration responsible for an enhancement of seed germination and root expansion, while its higher concentration resulted into inhibition of root elongation. ACC is the precursor of ethylene and PGPR having ability to synthesize ACC-deaminase enzyme having ability to break ACC for own growth, so at stress condition (Chaudharyand Sindhu, 2016). PGPR controls the ethylene concentration and provide the protection to crop against different biotic and abiotic environmental conditions (Barnawal *et al.,* 2017).

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MATERIALS AND METHODS

Sample collection

Soil samples were collected by composite sampling technique from the sorghum cultivated field from North Maharashtra region (Jalgaon, Nandurbar, Dhule). Plants uprooted from the field with adherent soil samples were distributed in four different parts, bulk soil, rhizospheric soil, ectorhizosphere and endorhizosphere (Raval *et al.,* 2011).

Isolation of indigenous bacteria

Isolation of various rhizobacteria by using selective and differential media. Nutrient Agar was used for isolating heterotrophic bacteria. For primary isolation yeast extract mannitol agar, Ashbey's mannitol agar, Kings B medium, Pikovskaya medium, *etc*. were used. Determination of Indole Acetic Acid production: Indole acetic acid (IAA) production was determined by the modification quantitative analysis described by Gordon and Paleg (1961). The fresh bacterial cultures were inoculated in 50 ml Nutrient broth containing L-tryptophan (5 µg/ml). The broth cultures were incubated at room temperature (30+2°C) for 5 days. Cultures were centrifuged at 3000 rpm for 30 min and 2 ml supernatant was transferred to the empty clean tube, then 2 drops of O-phosphoric acid and 4 ml of Salkowaski reagent were added. Red colour indicates the test positive and the absorbance of the reaction mixture were measured at 535 nm.

Determination of gibberellic acid production

Gibberellic acid (GA) production was determined following the method of Borrow *et al*. (1955). The fresh bacterial cultures were inoculated in 50 ml Nutrient broth and incubated at room temperature (30±2°C) for 7 days. After incubation broth cultures were centrifuged at 8000 rpm for 5 min. Then 15 mL of supernatant was transfer in to another tube and 2 ml of zinc acetate solution was added. After 2 min add 2 ml of potassium ferrocyanide solution was added. Solution was mixed properly and then centrifuged at 8000 rpm for 10 min. Take the 2 ml supernatant 2 ml of 30% HCl was added and incubated for 75 min at room temperature. Absorbance was measured at 254 nm.

ACC deaminase production

Penrose and Glick (2001) described method was use for the ACC deaminase production The fresh bacterial cultures were inoculated in Nfb medium containing 1-aminocyclopropane-1-carboxylate (ACC). Tubes were incubated at room temperature (30±2°C) for 5-7 days. Growth appearance with change in the colour of the medium.

Nitrogen fixation

Nitrogen fixing ability the bacterial cultures were qualitatively checked as bacterial cultures were inoculated on nitrogen free malate medium containing Bromothymol blue as an indicator. The plates were then incubated at room temperature for 3-4 days and were observed for the growth and blue colour zone around the growth. Okon *et al*. (1994)

Potassium solubilization

Potassium solubilization was checked by inoculating bacterial cultures on Aleksandrove agar medium Aleksandrov *et al*. (1967) and incubating the plates at room temperature for 5 days. Appearance of zone of clearance around the bacterial growth was indicative of potassium solubilisation ability of an isolate.

Phosphate solubilization

Phosphate solublizing ability of the isolate was checked on Pikovskaya (PVK) medium incorporated with tricalcium phosphate $Ca_3(PO_4)_2$ by Fiske and Subbarow method (1925). Formation of transparent halo zone around the developing colonies indicated phosphate solublizing ability of the bacteria.

Zn solubilization

The isolates were inoculated into modified PKV medium, (Pikovskaya, 1948) containing 0.1% insoluble zinc compounds (ZnO, $ZnCO_3$ and ZnS). The test organisms were inoculated on these media and incubated at 28°C for 48 hours. Formation of transparent halo zone around the developing colonies indicated zinc solublizing ability of the bacteria.

Formulation of isolates for consortium preparation

Bacterial isolates were selected on the basis of nitrogen-fixation capability, nutrient solubilization, high IAA and GA production, *etc*. Table 1 shows the list Twelve bacterial isolates out 320 were selected to formulate biostimulators 1 and biostimulators 2 to check growth promoting abilities (Table 2) (Alam *et al.,* 2020).

In vitro studies of seed germination by plate assay

Initially jowar seeds (*Sorghum bicolor*) were surface sterilized by exposing to 95% ethanol and immersed thrice in 0.2% $HgCl_2$ solution for 3 min (Stewart and Kane, 2006). The seeds were then subjected to 5 successive washings with sterile distilled water (Benmati *et al*., 2020). An aliquot of biostimulator organisms overnight grown bacterial suspensions of each in proportion were (106 cells/ml, 0.5 OD at 540 nm) applied on each seed total 25 seeds were used for 30 min. Seeds were then transferred to filter papers were soaked with sterile distilled place in empty sterile Petri plate water for allowing seeds to germinate. The results of plate assay were reported as Percent germination, seedling length, seed vigor index at an interval of 24 hrs ((Jha and Subramanian, 2014). Number of germinated seeds was recorded per plate after three days post incubation and percentage germination calculated (Mishra and Sundari, 2015) using formula given below:

Percentage germination of seed =

$$\frac{\text{Number of seeds germinated}}{\text{Total number of seeds placed in petri plates}} \times 100$$

Further seedling length of all germinated seeds was recorded at same time for calculating seed vigor index (SVI). Seed vigor index was calculated using following formula:

SVI= Mean seedling length (cm)×Percentage germination (%)

Effect of various treatments on germination percentage and seed vigor index was analyzed by calculating percentage increase or decrease observed in specific treatment/s as compared to uninoculated control and tabulated (Tables 5 and 6).

Pot assay

The pot experiment was conducted in pots for total seven treatments (three replications for each treatment) were filled with sterile field soil and watered a day before transferring total 10 seed per pot to it, after giving treatment to each seed same as seed bacterization experiment.

After 15th day of sowing 1st treatment was given by adding to each pot 50 ml of the diluted inoculums of biostimulators consortium as per labels given (50 ml of 10^8 CFU/ml density culture was diluted to 2 lit) (Bashir *et al*., 2013). Similar treatment of consortium was given after 30th by adding consortium to the appropriate pot. Negative control/treatment means addition of only water to that pot. 15 days old seedlings were carefully removed from the pots for studying plant vigour and growth parameters. The plants were then taken to the laboratory and shoot length, root length, fresh shoot weight, fresh root weight were recorded. Similar readings were taken after 45 and 60 days. The plants and roots were then kept in an oven at 55°C for 3 days to attain constant dry weight. After which, the dry weight of shoots and roots were recorded (Bashir *et al*., 2013).

RESULTS AND DISCUSSION

In present study samples were collected from sorghum cultivated field from north Maharashtra region. Total 320 isolates were isolated from different site.All the isolates were screened for their PGP traits *viz*., Indole aceticacid (IAA), GA production phosphate, zinc, potassium solubilization and ACC deaminase production. Among three regions of North Maharashtra, Nandurbar, Dhule and Chopda many isolates shows all the trait positive.

Highest 64 isolates from chopda region shows Zinc solubilization Total 45 isolates from chopda shows Phosphate solubilizing isolates.44 isolates from Dhule are the highest one showing potassium solubilization. Also from Chopda region nitrogen fixing, ACC deaminase and IAA GA producers are highest (Fig 1).

Among all these isolates, the isolates which are having ability to produce all trait positive at highest range are selected for consortium preparation Table 3 and Table 4 shows the selected isolates for consortium *viz*. Biostimulator 1 and Biostimulator 2.The effect of consortium on the seeds were tested by using plate assay in that different 7 treatments were used for the assay. Table 5 shows the treatments for seeds. Germination of seeds shows the effect of consortium different parameters are used for assay in that total number of seeds germinated after particular time, on the basis of germination percent seed germination was calculated the seedling were appear after 48 hrs the seedling length was also measured and vigor index also calculated, Table 5 shows all these criteria.

Pot assay

Pot assay in that consortium treated seeds were sawn in sterilized soil in pot Table 6 shows after 15 days shoot length,root length, plant height and vigor index of plant also shows result after 45 days and after 60 days (Table 6).

Table 1: Selection of maltitrait isolates for consortium.

Biostimulators 1		Biostimulators 2	
A	Ecto/KBM	G	Ecto/KBM
B	Endo/AMA	H	BS/YEMA
C	Ecto/Piko	I	BS/AMA
D	RS/Piko	J	Ecto/Piko/
E	BS/Piko	K	Ecto/AMA
F	Ecto/T/NA	L	BS/CMA

Table 2: Proportion of different multitrait isolates for consortium.

Consortium	Proportion
Biostimulator 1	A:B:C:D:E:F
	1:2:2:1:2:2
Biostimulator 2	G:H:I:J:K:L
	1:2:2:1:2 :2

Table 3: Multiple traits of the selected rhizobacteria for biostimulator 1 consortium.

Isolate code	IAA (µgm/mL)	GA (µgm/mL)	ACC	N2-Fixation	Zn Solu.	P solu.[a]	K solu.
Ecto/KBM	3.42	99.66	++	++	++	316.667	+
Endo/AMA	6.18	203.23	+	+	+	520	-
Ecto/Piko	79.74	117.39	+	+	+	255.833	-
RS/Piko/	7.76	120.23	+	++	+	339.167	++
BS/Piko	7.76	63.64	-	+	++	163.333	+
Ecto/T/NA2	16.18	275.11	+	+	+	224.167	-

a- µgP/mg of protein.

Table 4: Multiple traits of the selected rhizobacteria for biostimulator 2 consortium.

Isolate code	IAA (µgm/mL)	GA (µgm/mL)	ACC	N2-Fixation	Zn Solu.	P solu.[a]	K solu.
Ecto/KBM	3.42	99.66	++	++	++	316.6667	+
BS/YEMA	3.29	102.27	+	+	+	455	-
BS/AMA	50.79	108	+	+	+	103.3333	-
Ecto/Piko	12.89	116.48	+	+	+	405.8333	-
Ecto/AMA	7.37	63.64	-	+	+	318.3333	+
BS/CMA	7.11	227.27	+	++	+	196.6667	-

a-µgP/mg of protein.

Table 5: Seed bacterization plate assay by using biostimulator consortium.

Seed	Germination of sorghum seeds in number			%	Seedling length	Seed vigor
treatment	24 hrs	36 hrs	48 hrs	germination	(cm)	index
T1	19	22		100	7.1	710
T2	24	25	25	100	6.9	690
T3	24	25	25	100	8.2	820
T4	19	20	25	100	6.5	650
T5	20	21	24	81.8	6.6	539.88
T6	14	16	18	72	4.9	352.8
T7	15	17	20	80	4.1	328

Table 6: Seed bacterization pot assay for Biostimulator.

	After 15 days				After 45 days			After 60 days		
Tretment	Mean shoot length	Mean root length	Mean plant height	Vigor index	Mean shoot length	Mean root length	Mean plant height	Mean shoot length	Mean root length	Mean plant hight
T1	26.8	4	30.8	3080	47.3	11.4	58.7	69.1	21.3	90.4
T2	27.3	3.9	31.2	3120	48	13.1	61.1	68.3	20.5	88.8
T3	28.5	4.7	33.2	3320	49.1	14	63.1	72.2	24.5	96.7
T4	28	4.2	32.2	3220	45	11	56	66.2	21.1	87.3
T5	27.6	3.9	31.5	2576.7	42	11.4	53.4	61.4	22.5	83.9
T6	26	3.3	29.3	2109.6	31	9	40	59.8	19.4	79.2
T7	25	3.8	28.8	2304	30	10.4	40.4	58.3	19.1	77.4

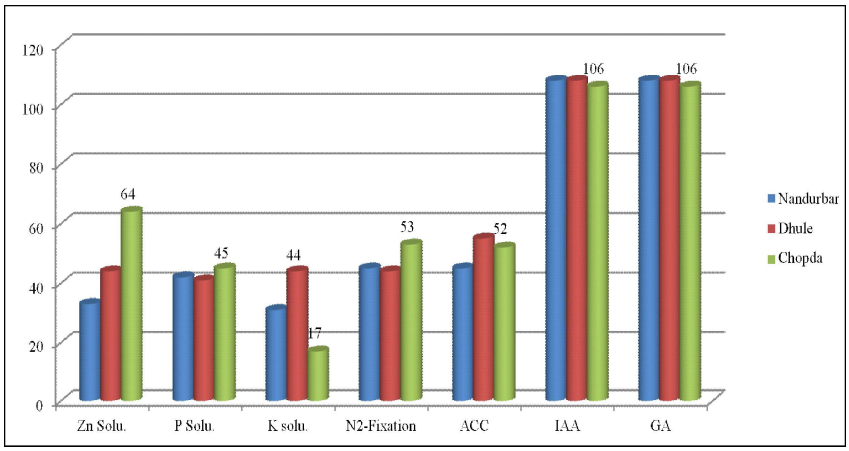

Fig 1: Total Number of isolates shows Zn, P, K solubilization, N2 fixation and IAA, GA, ACC deaminase Production.

CONCLUSION

Many researchers shows that the rhizobacteria having ability to produce PGP traits and the effect is on growth, seed germination and yield (Kumari *et al*., 2018, Vishwakarma *et al*., 2017). Selected Biostimulators were evaluated for their PGP activity on sorghum seedlings on the basis of seed germination, shoot, root length elongation and vigor index (VI). PGPR seed inoculation significantly enhanced seed germination and seedling vigor of sorghum. The highest response for seed germination and vigor index and result was endorsing the growth of crop.

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Conflict of interest

All authors declare that they have no conflict of interest.

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