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Early growth promotion and charcoal rot suppression in sorghum by plant growth promoting rhizobacteria

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ABSTRACT: **Thirty rhizobacterial isolates from sorghum were evaluated for potential for seedling growth promotion and charcoal rot suppression in sorghum. Seven isolates repeatedly promoted early stage plant growth on the sorghum cultivar M35-1. Few isolates increased seed germination up to 43 per cent and improved biomass up to 66 per cent on 30-day-old seedlings. Two isolates of fluorescent pseudomonads showed combined efficacy for seedling growth promotion and disease suppression and reduced charcoal rot incidence considerably. *In vitro* properties of these isolates such as growth hormone, siderophore, HCN and ammonia production, P-solubilization, antibiosis and production of volatile growth inhibitor have been discussed in relation to plant growth promotion and disease suppression.**

KEY WORDS: Charcoal rot, fluorescent pseudomonads, *Macrophomina phaseolina*, PGPR, sorghum

INTRODUCTION

Sorghum is one of the most important cereal in India after rice, wheat and maize. Rabi sorghum, which is mainly grown under dryland conditions, occupies around 5.6 million hectares of area in Maharashtra, Karnataka and Andhra Pradesh and is highly valued for grain and fodder. Occurrence of drought during flowering or grain filling stage (terminal drought) is quite common. Terminal drought stress causes premature leaf senescence and weakening of plant leading to increased incidence of charcoal rot (c.o. *Macrophomina phaseolina* (Tassi) Goid), lodging and significant yield loss (Rosenow and Clark, 1995).

Charcoal rot incidence in sorghum is strongly correlated with physiological and environmental stresses. Maintenance of good health and stalk freshness greatly reduces disease incidence. As the crop frequently encounter drought during flowering, initial boost up in vegetative growth should provide the crop the much needed competitive advantage which in turn would help to maintain good health and less disease. For this situation plant growth-promoting rhizobacteria (PGPR), which can enhance plant growth as well as improve disease resistance by antagonistic effect and induction of systemic resistance (van Loon, 1997) would be a good option. Sorghum is gaining importance as health food. Under such conditions organically produced sorghum is expected to draw more attention than before. Past researches indicated that microbial isolates originated from stressed environment proved superior when used

as inoculants. Moreover, sorghum being cyanogenic plant produces antimicrobial hydrogen cyanide in root. Therefore, microorganisms isolated from sorghum rhizosphere would have better adaptability than the one introduced from other crop system. In the present study, we explored the potential of sorghum rhizobacteria for early stage growth promotion and charcoal rot suppression.

MATERIALS AND METHODS

Isolation of sorghum rhizobacteria

Fresh sorghum roots were collected at flowering stage from 10 different fields in and around Sholapur district in Maharashtra. All the samples were mixed together, roots cut into small pieces and pulverized to make a pooled sample. The pooled sample was transferred to a 500 ml conical flask containing 200 ml sterile distilled water, stirred vigorously on a rotary shaker for two hours and strained through muslin to obtain the extract. Rhizobacteria were isolated from the extract on nutrient agar plates. Initially 125 bacterial colonies were selected randomly. Finally 30 bacterial colonies were short-listed based on different size, shape, color and luster and were used for further studies.

Initial screening of rhizobacteria

Thirty rhizobacterial isolates were tested for seedling growth promoting efficacy in polythene bag experiment (15 cm diam). Surface sterilized sorghum seeds (cv M35-1) were bacterized with 24h -old-bacterial culture (1×10^7 cfu/ml), air-dried under shade and sown in four perforated polythene bags (five seeds in each) previously filled with field soil and sand (2:1). During seed bacterization 1 per cent carboxymethyl cellulose (CMC) was added in the suspension as sticker. Four replications were maintained for each treatment (isolate). Dry root weight, shoot weight and biomass were recorded on 15day-old seedlings.

In vivo plant growth promoting efficacy of selected isolates

Seven isolates (SRB6, 20, 22, 25, 26, 27 and 28) that increased seedling biomass as a result of increase in both root and shoot weight during initial screening (Fig. 1) were selected for further studies. The isolates were characterized for morphological and cultural properties (Table 1) and used for further testing of plant growth promoting efficacy using 25 cm diam plastic pots. Ten bacterized (1×10^7 cfu/ml) seeds (cv M35-1) were sown in each pot previously filled with field soil and sand (2:1). Ten days after sowing, seed germination was counted and excess seedlings were thinned out leaving only 4 per pot. Root length, dry root and shoot weight and plant biomass were recorded on 30-days-old seedlings and the data were analysed.

Factors involved in plant growth promotion and antagonism

The above isolates were characterized for their plant growth promoting and antagonistic properties such as indole acetic acid (IAA), siderophore, hydrogen cyanide (HCN) and ammonia production, P-solubilization, antibiosis and production of volatile growth inhibitor.

Production of siderophore was studied qualitatively on chrome azurol-S agar (CAS) as described by Schwyn and Neilands, 1987. Production of HCN was tested on succinate agar (SA) plates as described by Castric and Castric, 1983 ammonia on peptone water as described by Dye (1962). Production of IAA was tested by colorimetric methods. Bacteria were shaking cultured in Luria Broth for 24h. Two-three drops of Ortho-phosphoric acid and 4 ml of reagent (1 ml of 0.5 M $FeCl_3$ in 50 ml of 35% $HClO_4$) were added to 2 ml of bacterial supernatant and incubated at room temperature for 25 minutes. Production of IAA was indicated by development of pink color. Intensity of color was graded as – (no color change), + (very light pink) to ++++ (deep pink) indicating no production, very light production and strong production of IAA. Phosphate solubilization ability was tested on Pikovskaya's medium amended with tricalcium phosphate. Formation of clear zone around the bacterial growth was considered positive for phosphate solubilization.

Table 1. Morphological and cultural characteristics of some sorghum rhizobacteria

Isolate	Gram reaction	Size & shape	Motility	Pigment production on KB medium	Colony character
SRB6	+	Short rod	Motile	None	Dull white, umbonate, smooth (later rough), & filiform
SRB20	–	rod	Motile	None	Creamy white, smooth, glistening
SRB22	–	rod	Non-motile	None	Warm buff, mucoid, raised, glistening
SRB25 mucoid	–	Long rod	Motile	None	Light yellow, circular, smooth,
SRB26	–	Long rod	Motile	Yellowish green fluorescent	Colony straw-yellow to greenish yellow, smooth, entire and later flatten
SRB27	–	Long rod	Motile	Yellowish green fluorescent	Colony straw-yellow, later turn greenish, smooth, entire, spreading type
SRB28	+	Short rod	Motile	None	Dull white, umbonate, smooth (later rough), & filiform

In vitro growth inhibiting ability of rhizobacteria on the charcoal rot pathogen *M. phaseolina* was tested following dual culture technique. A 5 mm culture disc from 5- days-old fungal growth was placed at one end of a PDA (1/ 4th strength) plate. On the other end of the plate 24 h -old - test bacteria was streaked in a straight line perpendicular to the fungal disc. The per cent growth inhibition was calculated. Growth inhibition of *M. phaseolina* by volatile compounds released by rhizobacteria was tested by using two-compartment Petri plate method as described by Gagne *et al.* (1991). The per cent of growth inhibition was calculated. Sclerotia density in different treatments was measured visually by observing under light microscope and expressed on a 0 - 4 scale, where 0 = no production and 4 = normal production of sclerotia.

Testing disease suppressive capabilities of rhizobacterial isolates

In vivo disease suppressive capabilities of the seven selected isolates were tested in pot experiment (45 cm diam). Pathogen inoculum was mixed in soil of the pots 15 days before seed sowing. Mycelial mat of 6-7 days-old *M. phaseolina* culture was filtered through filter paper, vortexed in sterile water and applied in pot soil. Bacterized seeds (ten in each pot) (cv CSV8R) were sown in cement pots previously filled with potting mixture of black soil: sand: farmyard manure (3: 2: 2). Pots with non-bacterized seeds served as control. Thirty days after sowing extra plants were thinned out and four plants with uniform growth and spacing were finally maintained in each pot. Three pots were used for each treatment. In order to induce moisture stress during grain development stage water supply was withdrawn and the flag leaf was removed from each plant at flowering stage. After harvest each stalk along with roots was observed for infection of *M. phaseolina* and charcoal rot symptom. Disease incidence for each treatment was calculated and data were analysed.

RESULTS AND DISCUSSION

Early stage plant growth promotion in sorghum by rhizobacteria

Results of initial screening showed that like any other rhizosphere, sorghum rhizosphere also harboured a mixture of plant growth promoting as well as growth demoting bacteria (Fig. 1). Out of 30,

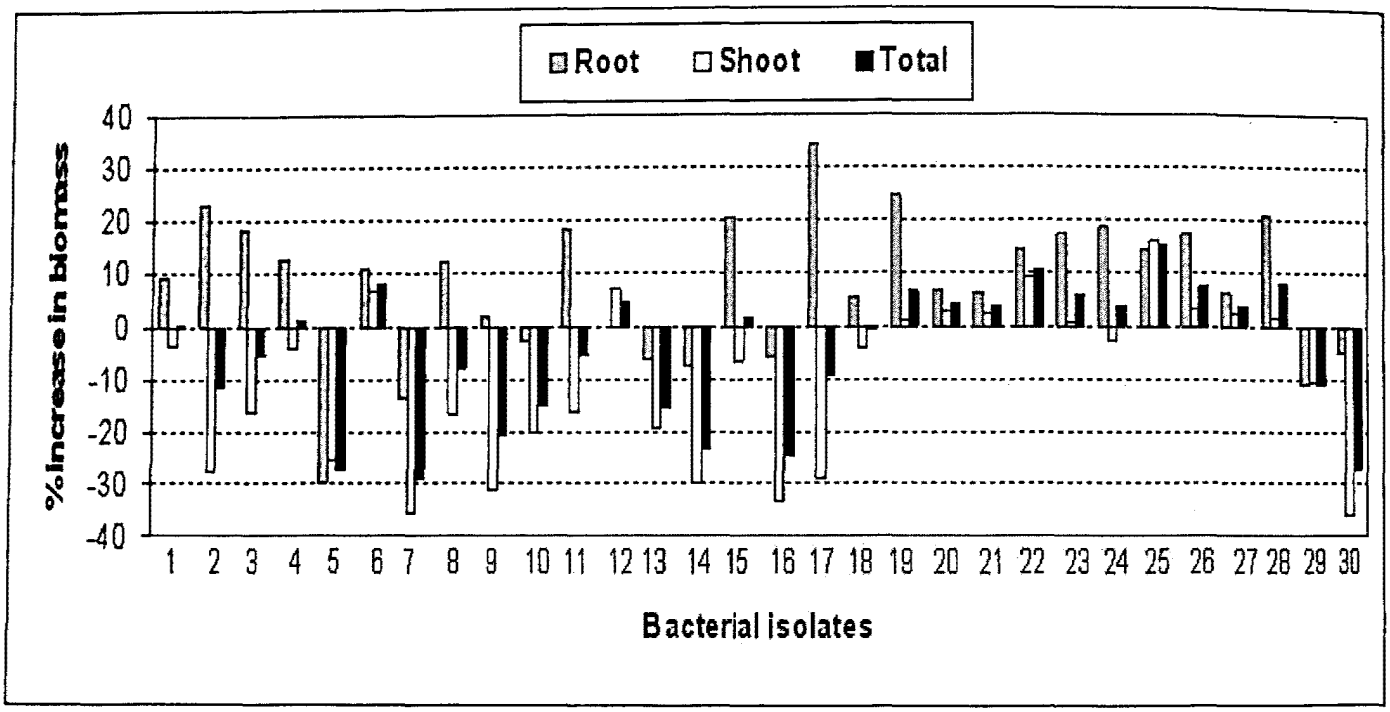

Fig. 1. Effect of some sorghum rhizobacteria (SRB1 to SRB30) on root, shoot and biomass production on 15 days-old sorghum seedlings

the number of isolates that increased root-weight, shoot-weight and biomass on 15 days-old sorghum seedlings were 20, 10 and 13, respectively. A large proportion of isolates (around 66%) increased early root growth. But all might not be beneficial for overall growth as few of them showed adverse effect on shoot growth. Root growth promoting isolates might be helpful for quick establishment of seedling especially under dryland situations. Seven isolates increased seedling biomass (ranging from 4 to 16%) as a result of increase in both root and shoot weight. These isolates did not show any negative effect on early seedling growth and might be called as early plant growth promoters for sorghum. There were 14 isolates that reduced biomass, indicating that under natural field conditions a considerable number of rhizobacteria inhibit seedling growth. These are deleterious rhizobacteria that are integral part of rhizosphere and known to inhibit plant growth (Nehl *et al.*, 1996).

Morphological and cultural characteristics of the seven selected isolates (that increased seedling biomass as a result of increase in both root and shoot mass during initial screening) showed that 2 were fluorescent pseudomonas. When these seven isolates were retested for plant growth promoting efficacy it was observed that except SRB25, all others repeatedly promoted growth and increased root, shoot and biomass production on 30 days-old plant (Table 2). Most of these isolates significantly increased the major plant growth parameters such as seed germination (SRB20, 22 and 28); shoot weight (SRB6, 20, 26 and 27) and biomass production (SRB6, 26, 27 and 28). Maximum root growth was induced by SRB28 followed by SRB27 and SRB20. Five isolates out of 7 repeatedly improved plant biomass ranging from 45 to 66% in 30 days old plants. These isolates also increased biomass on 15 days-old seedlings (7 to 16%). As the aim of the present study was to assess

Table 2. Growth promoting effects of native rhizobacteria on 30 days old sorghum plant

Isolate	Seed germination (%)	Root length (mm)	Dry root wt. (mg/plant)	Dry shoot wt. (mg/plant)	Biomass (mg/plant)
SRB6	72 (07)[a]	180 (00)	405 (20)	725 (80)	1130 (53)
SRB20	96 (43)	193 (07)	425 (27)	648 (61)	1073 (45)
SRB22	87 (30)	198 (10)	352 (04)	585 (45)	937 (27)
SRB25	80 (19)	203 (13)	270 (-20)	283 (-30)	553 (-25)
SRB26	78 (16)	197 (09)	350 (04)	757 (88)	1107 (50)
SRB27	78 (16)	213 (19)	433 (29)	797 (98)	1230 (66)
SRB28	87 (30)	187 (04)	460 (36)	630 (56)	1090 (47)
Control	67 (00)	180 (00)	337 (00)	403 (00)	740 (00)
CD (P=0.05)	13.1	NS	NS	246.1	351.7
CV (%)	17.3	8.9	23.6	25.5	22.4

[a] Figures in the parentheses are per cent change over control; NS= non-significant

early growth promotion, observations on the effect on biomass (yield) in mature plants were not looked into. However, increase in grain yield can also be expected in fully grownup plants because in sorghum, seedling dry weight at 15 days was found to correlate with the final plant height and total dry weight (Maiti, 1996).

In vitro properties of test bacteria and their relation with plant growth promotion

The mechanisms by which plant growth promoting rhizobacteria (PGPR) promote plant growth are not fully understood, but are thought to be due to ability to produce growth hormone (Beyeler *et al.*, 1999), solubilization of mineral phosphate (De Freitas *et al.*, 1997), antagonism against major and minor plant pathogens by production of siderophore, cyanide, chitinase, and antibiotics (Shanahan *et al.*, 1992). Analysis of *in vitro* plant growth promoting properties of the isolates showed that all of them were capable of producing the growth hormone, indole acetic acid (IAA) and siderophore though quantities varied among isolates (Table 3). SRB26, 27 and 28 produced more IAA and siderophore than other isolates, while SRB27, 22, and 28 were capable of solubilizing phosphate. SRB26 and 27 were also very strong in production of cyanide and ammonia. Maximum increase in biomass (66%) was brought about by SRB27, which was positive for all the 5 growth related tests performed followed by SRB6 (53%), which was positive only for siderophore, and IAA. Therefore, it was not clear which factor contributed most in sorghum growth promotion. However, except SRB6, other 3 prominent growth promoters produced at least 3 of the 5 factors such as siderophore, IAA and ammonia more in quantity than others. Siderophores are thought to sequester iron available in the rhizosphere making it unavailable to deleterious microorganisms, thereby restricting their growth (O'Sullivan and O'Gara, 1992). In the present study we have seen that sorghum roots (rhizosphere) harboured around 40 per cent deleterious rhizobacteria. Therefore, one of the mechanisms of plant growth promotion by the isolates (SRB26, 27 and 28) might be greater production of siderophore. Rhizobacteria are also known to influence plant growth by contributing to host plant's endogenous pool of phytohormones such as IAA. Main function of IAA is elongation of primary roots and proliferation of lateral and

Table 3. Plant growth promoting and disease suppressive properties of isolates of sorghum rhizobacteria

Isolates	Siderophore production (diam in mm)[a]	Growth related parameters				Disease suppressive properties[a]			
		IAA production	Phosphate solubilization	Ammonia production	HCN production	Growth inhibition on dual plating (%)	Growth inhibition by volatile compounds (%)	Inhibition of sclerotia production by volatiles(0-4 scale)[b]	Charcoal rot (%)
SRB6	8.8±0.6	++	–	–	–	23±3.0[d]	5.2±2.8	3.3±1.2	50.0
SRB20	0.0±0.0	+	–	–	–	29±4.4	2.6±1.7	4.0±0.0	50.0
SRB22	10.3±0.6	+	+	–	–	29±1.0	4.4±2.2	3.7±0.6	33.2
SRB25	9.7±0.6	+	–	–	–	31±3.2	0.0±0.0	4.0±0.0	41.7
SRB26	16.7±1.5	++++	–	+	+++	44±2.3	27.4±5.6	1.0±1.0	25.0
SRB27	16.0±1.0	++++	+	+	+++	53±2.6	57.0±3.4	0.7±0.6	25.0
SRB28	20.0±2.0	+++	+	+	–	36±2.0	7.8±4.8	1.0±0.0	33.3
Control	-	-	-	-	-	-	-	4.0±0.0	50.0
CD (p = 0.05)								-	14.8
CV (%)								-	24.0

[a]average of 3 replications; [b]0= no production and 4=normal production of sclerotia; [c]– = no production and ++++ = strong production; + positive; - negative; [d]Mean ± standard deviation of means.

adventitious roots (Mordukhova *et al.*, 1991). Therefore, the IAA producing isolates might be helpful for quick establishment of seedling especially under dryland situations.

In vitro antagonism and *in vivo* disease suppression

Analysis of *in vitro* antagonistic properties of the isolates showed that all of them were able to inhibit growth of *M. phaseolina*. Maximum inhibition was recorded by SRB27 (53%) followed by SRB26 (44%) and SRB28 (36%) (Table 3). All the isolates, except SRB25, produced volatile compounds inhibitory to *M. Phaseolina.* Maximum inhibition of fungal growth by volatile compounds was recorded by SRB27 (57%) followed by SRB26 (27%) and SRB28 (8%). In addition to growth inhibition, the volatile compounds from SRB27, 26 and 28 also significantly reduced sclerotia producing capability of *M. phaseolina*. As the charcoal rot pathogen perpetuate by sclerotia in soil, this might have an adverse effect on the building up of inoculum in soil. Out of the 7 isolates, 4 reduced disease incidence ranging from 34-50 per cent over control. Pal *et al.* (2001) observed that a plant growth-promoting isolate of a fluorescent *Pseudomonas* sp. EM85 and two bacilli isolates MR-11 (2) and MRF, isolated from maize rhizosphere, were strongly antagonistic to *M. phaseolina* causing charcoal rot in maize. Maximum reduction of disease (50%) was shown by the isolates SRB26 and SRB27 both of which were fluorescent pseudomonads. Fluorescent pseudomonads are well known for their plant growth promoting and disease suppressing capabilities and have emerged as the largest and potentially most promising group of PGPR involved in biological control of plant diseases (O'Sullivan and O'Gara, 1992). Bacterization of groundnut seeds with fluorescent *Pseudomonas* strain, GRC (2), reduced charcoal rot disease in *M. phaseolina*-infested soil as compared with the control by 99 per cent (Gupta *et al.*, 2002). The strain SRB27, 26 and 28 showed combined efficacy of plant growth promotion as well as charcoal rot suppression. Development of effective formulation and suitable delivery system for these bioagents in the rhizosphere might improve their efficacy further under field conditions. The study showed that, like any other rhizosphere, sorghum rhizosphere also harbours plant growth promoting as well as plant growth deleterious bacteria. Selected isolates of native rhizobacteria can promote early stage plant growth in sorghum, a property that might have additional value for dryland conditions. It also showed that sorghum rhizobacteria especially fluorescent pseudomonads were able to reduce charcoal rot incidence considerably in pot experiments.

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