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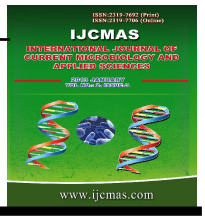

Original Research Article

Effect of plant growth promoting *Bacillus subtilis* and *Pseudomonas fluorescence* as Rhizobacteria on seed quality of sorghum

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A B S T R A C T

Sorghum (*Sorghum bicolor (*L) Moench) is vital life sustaining crop in many parts of the world ranking fifthafter wheat (*Tritium* species), rice (*Oryza* spp) maize (*Zea mays*) and barley (*Hordeum vulgare*). Nutritionationally it is superior to all other cereals. Sorghum is vulnerable to many disease including 23 different fungal diseases. The plant-growth promoting rhizobacteria (PGPR) such as *Pseudomonas fluorescens and Bacillus subtilis* were used to study the effect on seed germination and also on nutritional qualities such as total protein, carbohydrate and also peroxidase activity. Therefore, the present study showed PGPR strains, *Pseudomonas fluorescens and Bacillus subtilis* were effective in improving seed quality such as seed germination, vigour index and nutritional quality such as protein content and carbohydrate content. In addition to this peroxidase activity also observed. So these PGPR can be used to improve the quality of sorghum. The molecular weight of the protein were carried out by SDS-PAGE.

K e y w o r d s

Pseudomonas fluorescens; Bacillus subtilis; Sorghum seeds; SDS-PAGE; peroxidase activity.

Introduction

Preparations of live microorganisms (bacteria, fungi) utilized for improving plant growth and crop productivity are generally referred to as biofertilizers or microbial inoculants (Subba Rao and Dommergues, 1998). Plant growth-promoting rhizobacteria (PGPR) are free-living, soil-borne bacteria, isolated from the rhizosphere, which, when applied to seeds or crops, enhance the growth of the plant or reduce the damage from soil-borne plant pathogens (Kloepper *et al.,* 1980).

Breakthrough research in the field of PGPR occurred in the mid 1970s with studies demonstrating the ability of *Pseudomonas* strains capable of controlling soil-borne pathogens to indirectly enhance plant growth and increase the yield of potato and radish plants (Burr *et al.,* 1978; Kloepper and Schroth 1981; Kloepper *et al.,* 1980; Howie and Echandi, 1983).

Sorghum grain is used as staple food in several forms baked, cooked or fermented. Nutritionationally it is superior to all other

cereals. In India it is the third important crop after rice, wheat, produced primarily for food in the area of 11.2 mh. importance states producing this crop are Maharasastra, Karnataka, Andrapradesh and Madhya Pradesh. In Karnataka about 125.92 lakh tones (Fao,1999). Chitradurga, Bijapur, Raichur, Dharwad, Bellary and Gulbarga are the major sorghum growing districts of Karnataka.

Sorghum is vulnerable to many disease including 23 different fungal diseases (Richerdson 1990). Fungal disease of sorghum are caused by species of *Fusarium, Curvularia and Phoma* etc (Williams and Rao, 1981). Grains severely infected with grain mould fungi appear to be completely covered with pink or black mould. These seeds have poor seed quality such as germination vigour and also nutritional quality such as carbohydrates, proteins. Therefore such seeds can not be use for consumption purposes. In order to improve seed quality and nutritional growth chemical and biocontrol agents were used. Workers word wide made efforts to identify effective chemical fungicides to control fungal diseases and also improve seed germination and nutritional qualities, but no chemical fungicides can control fungal diseases completely and improve the seed germination qualities.

In addition to that continuous applications of chemical fungicides have created many environmental hazardous and residual problems. So the only alternative for this problem is biological control, based on these reasons this work was carried out. The main aim of this study was carried out the effect of PGPR on seed qualities such as seed germination and vigour index. Then the nutritional qualities such as total protein, carbohydrate and also peroxidase activity were performed and also SDS-PAGE study was carried out for determination of molecular weight of the proteins.

Materials and Methods

Collection of seed sample

Two verities of sorghum, csh-14 and proagro were collected from Davangere, Karnataka seed processing unit, INKEN respectively. Collected varities are then treated with strains of PGPR.

Plant growth promoting rhizobacteria strains

Two strains of PGPR are used in this experiment; they are *Pseudomonas fluorescence, Bacillus sublitis.* The strains were obtained from stock culture collection of department of studies in applied botany and biotechnology, university of Mysore, Mysore, Karnataka State, India.

Mass multiplication of PGPR

The bacterial culture *Pseudomonas fluorescens* and *Bacillus subtilis* are obtained from stock culture was multiplied by using different media for different bacterial strains. *Pseudomonas fluorescens* was mass multiplied by inoculating on King's B medium and *Bacillus Sublitis* on nutrient broth media.

After the preparation, medium was sterilized by autoclaving at 15 lbs/15 min.Then King's medium B is inoculated with *Pseudomonas fluorescens* and Nutrient broth with *Bacillus subtilis*.Inoculated medium was incubated at 35^0C in a incubator for 48 hours.

Seed treatment

After 48 hours of incubation, broth was centrifuged at 10,000 rpm/min for 20 minutes. Therefore bacterial cells are sedimented by centrifugations. Then the

pellet was centrifuged with distilled water at 10,000 rpm for 10 minutes. Then the pellet of bacterial cells was adjusted to $1x10^8$ cfu/ml using UV spectrophotometer, obtained by adjusting 0.45 OD at 610 nm. Seeds were treated by immersing in bacterial suspension incubated at temperature 25±2 ^{0}C for 24 hours. These seeds were air dried and were subjected to paper towel method to assess the germination and vigour index test. The effect of seed treatment on growth of the plant was also studied. Each variety of seeds was soaked in distilled water, this serves as control.

Effect of PGPR treatment on seed germination and vigour index

Sorghum seeds from each treatment and control were subjected to between paper method (ISTA, 1999) to know their effect on seed germination and seedling vigour. Seeds (400) were taken in four replicates of 100 seeds each and placed in between two wet paper towels specially made for germination test,50 seeds each were placed equidistantly and the papers were rolled and kept for incubation at 23±2 ^{0}C. After seven days of incubation, seeds were evaluated for germination percentage.The length and shoot length was measured in centimeter scale and vigour index was calculated by adding mean root length and shoot length and multiplied by percentage of germination (Abdul baki and Anderson, 1973).

Vigour Index = (Mean root length+ Mean shoot length) X Percentage of germination.

Determination protein, carbohydrate and peroxidase activity

The total protein content of the sorghum plant was estimated as per the Bradford method (1976) and Anthrone method (Hedge and Hofreiter 1962) were followed to analyse total carbohydrate content in the sorghum plant. Samples were prepared by homogenized in mortar by using pestle with 30 Mm phosphate buffer (pH 7.0). The peroxidase (POD) enzyme activity were assayed from the sorghum plant, enzyme was extracted by homogenization with phosphate buffer 0.1M (pH 7.0) as per the method of Putter (1974). Guaiacol is used as substrate for the assay of peroxidase.

Polyacylamide gel electrophoresis (SDS-PAGE) to determine the molecular weight of the Sorghum protein.

The molecular weight of the treated sorghum plant proteins were determined by using SDS-PAGE. Samples and standards of known molecular weight were used and finally determined the molecular weight of unknown sorghum protein (Figure 1).

Calculation

Since the extinction coefficient of guaiacol dehydrogenation product at 436nm under the conditions specified is 6.39 per micromole, the enzyme activity per litter of extract is calculated as below:

$$\text{Enzyme activity units/liter} = \frac{3.18 \times 0.1 \times 1000 = 500}{6.39 \times 1x \Delta t \times 0.1 \quad \Delta t}$$

Results and Discussion

The collected verities of sorghum CSH-14 and proagro were used for the studies on seed germination and vigour index by plant growth promoting rhizobacteria such as *P.fluorescence and B.subtilis*. Seed germination was represented in Table 1. Control Sorghum CSH-14 variety were showed 78% of seed germination but in case *P.fluorescence* showed 84% of seed germination *and B.subtilis* showed 81% of seed germination and another variety i.e Proagro control exhibited 80% seed germination and PGPR strains were showed 83% and 82% with respect to *P.fluorescence and B.subtilis*.

Table.1 Effect of PGPR *P.fluorescence* and *B.subtilis* on seed germination of sorghum

Cereal	Variety	Treatment	No. of seeds used in BP method	No .of seeds germinated	No .of seeds ungerminated	% of germination
Sorghum	CSH-14	Control	50	39	11	78
		P.fluorescence	100	84	16	84
		B.subtilis	100	81	19	81
Sorghum	Proagro	Control	50	40	10	80
		P.fluorescence	100	85	15	83
		B.subtilis	100	82	18	82

Table.2 Effect of PGPR *P.fluorescence and B.subtilis* on vigour Index of sorghum

Cereal	Variety	Treatment	MSL	MRL	% .of germination	VI
Sorghum	CSH-14	Control	9.60	9.65	78	1507.74
		P.fluorescence	12.2	8.6	81	1688.4
		B.subtilis	10.3	9.8	84	1684.8
Sorghum	Proagro	Control	7.3	7.7	80	1200
		P.fluorescence	9.3	8.5	82	1470.5
		B.subtilis	9.3	8.0	85	1459.6

MSL -Mean Shoot Length; MRL-Mean Root Length; VI- Vigour index

Table.3 Effect of PGPR *P.fluorescence* and *B.subtilis* on total protein content of sorghum

Cereal	Variety	Treated	Concentration of protein(ug/ml)
Sorghum	CSH-14	Control	72
		P.fluorescence	80
		B.subtilis	76
Sorghum	Proagro	Control	62
		P.fluorescence	72
		B.subtilis	66

Vigour index studies were calculated for the sorghum CSH-14 and Proagro control and also effect of PGPR of *P.fluorescence and B.subtilis.* In CSH-14 control showed 1507.74 VI (vigour index) and in proagro exhibited 1200 VI but in case of PGPR effect on *P.fluorescence and B.subtilis* vigour index showed an increased in vigour index. 1688.4 VI and 1684 VI respectively in case of CSH-14 and 1470.5 VI and 1459.5 VI in case of proagro represented in Table 2.

Treatment of sorghum seeds variety CSH-14 and proagro with pure culture of *Pseudomonas flourescens* at the rate of 1x 10^8 cfu/ml increased the germination by 6% and 5 % respectively. *Bacillus subtilis* increased the seed germination of variety CSH-4 and proagro by 3% and 2 % respectively over control.

Concentration of the proteins in untreated as control and seeds treated with *P.fluorescence* and *B.subtilis* were determinated by Bradford method. From the graph, concentration of the protein is determined (Table-3).Concentration of the proteins in seeds/gram treated with *P.fluorescence* is increased by 8µg and seeds treated with *B.subtilis* is increased by 4 ug/ml.

Concentration of the carbohydrate is determined by anthrone method. Concentration of the carbohydrate in seeds with *P.fluorescence* is increased by 26 ug/ml and seeds treated with *B.subtilis* is increased by 16 ug/ml (Table 4).

Peroxidase is a pathogenesis related enzyme which shows different activities depending on resistance exhibited by the plant. In the present study seed treated with *P.fluorescence* and *B.subtilis* were increased the peroxidase activity compared to untreated one. Peroxidase activity in the seeds treated with is more when compare to seeds treated with *B.subtilis* (Table-5).

Peroxidase is a pathogenesis related enzyme which shows different activities depending on resistance exhibited by the plant. In the present study seed treated with, *P.fluorescence* and *B.subtilis* were increased the peroxidase activity compared to untreated one. Peroxidase activity in the seeds treated with is more when compare to seeds treated with *B.subtilis* (Table-5).

Electrophoresis to check the protein variations of sorghum in Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE)

The protein banding pattern in control, *P.fluorescence* and *B.subtilis* treated seeds differed significantly. A 50KDa protein band was present in all the three samples but was more prominent in the control lane. A 40 KDa protein band was present only in *P.fluorescence* and *B.subtilis* treated seeds.35KDa protein band was present in all the seed samples.14KDa protein band was present in control and *P.fluorescence* treated seeds with more intensity in *P.fluorescence* treated seeds. It was absent in *B.subtilis* treated lane.

In the present investigation, two varieties of sorghum cultivars were treated, with PGPR such as *P. fluorescence* and *B.subtilis* and it was observed that the seed germination seedling vigour, protein and carbohydrate content increased significantly. Raju *et al.,* (1999) reported that *P.fluorescence* was increase seed germination, vigour index and field emergence.

Table.4 Effect of PGPR, *P.fluorescence* and *B.subtilis* on total carbohydrate content of sorghum

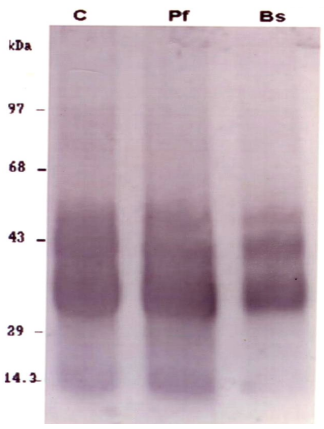
Cereal	Variety	Treated	Amount of carbohydrate in ug/ml
Sorghum	CSH-14	Control	214
		P.fluorescence	240
		B.subtilis	230
Sorghum	Proagro	Control	206
		P.fluorescence	227
		B.subtilis	225

Table.5 Effect of PGPR, *P.fluorescence* and *B.subtilis* on Peroxidase activity of sorghum

Sorghum	O.D at 430 nm (0 sec)	O.D at 430nm (60sec)	60-0 sec	Graph value (mg of protein)	Y value
Control	0.140	0.141	0.001	117	$8.5x10^{-6}$
P.fluorescence	0.278	0.304	0.026	145	$1.79x\ 10^{-4}$
B.subtilis	0.240	0.281	0.041	140	$2.93x10^{-4}$

The term biological control was first adopted into the field of plant pathology by Smith (1999). Baker and Cook (1974) defined biological control as the reduction of inoculum or disease producing activity of a pathogen accomplished by or through one or more organisms other than man.

Figure.1 Protein profiles of Sorghum seeds treated with *B. subtilis* and *P.fluorescence* analyzed by SDS-PAGE



The term biological control clearly implies control of a diseases through some biological agent means antagonist, a living organisms which may be micro or macro organisms other than the diseased or damaged plant acting as host and the pathogen causing the disease .These biological agents that provide benefit to plant can be either symbiotic or free living in soil, but are found in abundance near the roots are termed as PGPR.

In experiment two PGPR strains were used and study was conducted to know the effect of PGPR strains *P.fluorescence* and *B.subtilis* on seed germination, vigour index, protein, carbohydrates content, peroxidase activity and also protein analysis by SDS-PAGE.

Effect of these two strains on plant growth promotion has already been proved by various workers. Recent reports suggested that beneficial effect of PGPR was due to

the induction of the SAR signaling pathway in plant.

In the current study two of the tested PGPR *P.fluorescence* and *B.subtilis* significantly induced plant defense response in plants. Plant peroxidase has been reported to catalyses the last step in biosynthesis of lignin and hydrogen peroxide (Chen *et al.,* 2002). In our study, the seeds treated with *P.fluorescence* and *B.subtilis* show marked increase in peroxides' activity. *P.fluorescence* is one of the most extensively studied PGPR *P.fluorescence* was known to produce the plant growth regulators like gibberellins, cytokininsis and indole acetic acid (Dubeikovsky *et al*., 1993).The ability of the *P.fluorescence* to increase the vigour index, seed germination was attributed the plant growth promoting substances produced by the bacteria that could act to enhance various stage of plant growth.

Bacillus species have been tested for control on wide variety of crops of greatest is *Bacillus subtilis* A-13 which was isolated by Broadbent *et al.,* (1971) from lysed mycelium of *Scleroticum rolfsii*. It is inhibitory in vitro to several plant pathogens and has improved the growth of many plants in streamed and natural soils (Broadbent *et al*., 1977; Yuen *et al.,* 1985). As a seed treatment, it increased the yield of carrots by 48%, Oats by 33% (Merriman *et al.,* 1974) and pea nuts up to 37% (Turner and Backman,1986).Its plant growth yield promoting activity appears to be direct stimulation of plant growth (Broadbent *et al.,* 1977; Turner and Backman,1986).

Experiment proved that the PGPR such as *P.fluorescence* and *B.subtilis* were effective in improving seed quality such as seed germination, vigour index and nutritional quality such as protein, carbohydrates content. So these PGPR can be used to improve the quality of sorghum.

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