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An experiment to demonstrate growth promotion by brinjal bacterial isolate

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Abstract
The present study was designed with the main aim to demonstrate the growth promotion by bacteria from rhizosphere, phylloplane and caulosphere of brinjal (*Solanum melongena* L.). Brinjal (*Solanum melongena* L.) plants of different varieties were collected from seven locations around Bangalore *viz.*, Hessaraghatta, Yelahanka, Kengeri, Madi vala, Hebbal, Tirumalapura and Attibele were also screened for the presence of phosphate solubilizing bacteria. Three cultivars of Brinjal *(Solanum melongena* L) *viz*., *Arka keshav*, *Arka shirish* and *Arka kusumaker* were used in a tray and pot experiment to study the response of inoculation with the BBI. The tray trials revealed that plants inoculated with BBI showed a significant increase in germination count, root length, shoot length, plant fresh weight and dry weight, over the uninoculated controls. Among the cultivars used in the tray trials *Arka keshav* responded significantly better to inoculation than the other cultivars. Pot trial revealed that bacterial inoculation with BBI increased shoot dry weight in the treated plants significantly over the uninoculated controls. The brinjal (*Solanum melongena* L.), cultivar *Arka keshav* showed better response to inoculation with BBI. In conclusion, among the cultivars used in the tray trials *Arka keshav* responded significantly better to inoculation than the other cultivars. The brinjal *(Solanum melongena* L.) cultivar *Arka keshav* showed better response to inoculation with BBI in the pot trail.

Keywords: *Solanum melongena* L, growth promotion, rhizosphere, phylloplane, caulosphere

Introduction
Associative bacteria have been isolated from the rhizoplane, phylloplane and stem of many non-leguminous plants. Many studies have dealt with isolation of associative microorganisms from the roots of cereals, vegetable and fruit crops such as sweet potato [1], arecanut, banana, coconut, cashew, citrus, custard apple, grape, guava, jackfruit, litchi, mango, papaya, pomegranate- ate, phalsa, pepper, and strawberry [2], Spartina altemifolia [3], sugarcane [4], barley [5], wheat, maize, sorghum, millet and rice [6-11]. Nitrogen fixing organisms were isolated from the roots of many non- leguminous crops such as *Spiacia oleracea*, *Brassica chinensis* and *Brassica rapa* by Ahn *et al*. (1990) [12].

In addition to nitrogen, phosphorus is a vital nutrient for plants and microorganisms. Superphosphate is one of the common forms of phosphatic fertilizers. Biofertilizers are living organisms used as fertilizers either to fix atmospheric nitrogen or to solubilize mineral nutrients like phosphorus. Microbial inoculants have attained special significance in modern agriculture, which is basically dependent upon chemicals like inorganic fertilizers, insecticides, weedicides, fungicides, growth promoting hormones etc., most of which pollute the environment and are hazardous to the health of human beings.

As early as 1970 itself Dobereiner established the fact that plants are usually associated with nitrogen fixing microorganisms [13]. In addition to fixing nitrogen some associated bacteria are found to be phosphate solubilizing as well, hence restoring the fertility of the soil in an ecologically balanced way without altering the natural environment. The phosphate solubilizers invariably increase the uptake of phosphorus and nitrogen thereby increasing the yield of the crop. Some phosphate solubilizing bacteria are *Bacillus circulans. Bacillus subtilis* etc. Further the ability of the microorganisms to produce chemical substances like plant growth hormones, vitamins and antibiotics are well- documented [14], and could bring about significant increase in growth and yield in crop plants. Wani and Konde (1988) established that many non-leguminous plants harbour associative nitrogen fixing bacteria on their roots [15]. Studies conducted by Sukhada (1987, 1988) on tomato revealed the presence of nitrogen fixing bacteria colonizing the root system, which on isolation, multiplication and inoculation to the same plant significantly increased yield in crop plants [16, 17].

Brinjal *(Solanum melongena* L.) is a member of the family Solanaceae and a native of India. It is an important vegetable crop of south India. The fruit is rich in vitamin A, vitamin C and is employed in Ayurveda system. In the present study the presence of growth promoting bacteria from the rhizoplane, phylloplane and stem of brinjal (*Solanum melongena* L.) plants were studied.

Materials and methods

Brinjal (*Solanum melongena* L.) plants of different varieties were collected from seven locations around Bangalore *viz*., Hessaraghatta, Yelahanka, Kengeri, Madi vala, Hebbal, Tirumalapura and Attibele were also screened for the presence of associative bacteria. The standard laboratory chemicals were used and Bacteriological media used were from Himedia. The procedure given by Patriquin and Dobereiner (1978, 1983) was followed to isolate the associative bacteria from the root, stem and leaf of brinjal [10, 11]. The bacterial growth from the sterile roots was isolated and sub cultured in nitrogen free Burk's media. They were multiplied in TYMB media [18]. Both sterile and unsterile root, leaf and stem bits of brinjal *(Solanum melongena* L.) plants were used for the initial screening of associative bacteria. The dominant colonies of bacteria present in the rhizoplane, phylloplane and stem were isolated and subcultured. The pure cultures of the bacterial isolate were screened for growth promoting potential.

Tray experiment

Three cultivars of Brinjal *(Solanum melongena* L) *viz*., *Arka keshav, Arka shirish* and *Arka kusumaker* were used in a tray experiment to study the response of inoculation with the BBI. The seeds of brinjal *(Solanum melongena* L) were obtained from the Division of Vegetable crops, IIHR., Bangalore. Eighteen plastic trays (12-inch x 15-inch) were filled with sandy loam soil and topped with Farm yard manure (FYM). 50 seeds of each brinjal cultivar were sown in trays and irrigated with tap water. The experiment was conducted in natural light and the average temperature was 28 °C. Average maximum and minimum temperature recorded was 32 °C and 24 °C respectively. Each cultivar had three replicates of control and treatment. The treatment set received 500mL of 24-hour pure culture of BBI cultured in nitrogen free liquid Burk's media (pH 7.0). Biometric observations and physiological parameters such as germination count were recorded.

Pot trial

The three cultivars of brinjal *(Solanum melongena* L.) *viz*., *Arka keshav, Arka shirish* and *Arka kusumaker* were cultivated in clay pots. Clay pots of 10-inch diameter filled with sandy loam soil were topped with FYM. The experiment was replicated four times in a complete randomized design. Plant height, root length, plant dry, leaf area was recorded using Skye leaf area analyzer. The plant height and root lengths were recorded in centimeters. The fresh weight of the sample was recorded at room temperature using a balance. Fresh weight and dry weight of root and stem were measured in grams. The dry weight of the plant was recorded after the plant was dried in a hot air oven. Analysis of soil nitrogen was done by total carbon method of Walkley and Black titration [19]. The analysis of soil Phosphorus was done by Bray method as described by Jackson (1973). Soil nitrogen and soil phosphorus was analyzed at intervals of 30, 45, 60 and 75 days [20].

Results

Tray experiment

The tray trials revealed that plants inoculated with BBI showed a significant increase in germination count, root length, shoot length, plant fresh weight and dry weight, over the uninoculated controls (Table 1). Among the cultivars used in the tray trials *Arka keshav* responded significantly better to inoculation than the other cultivars. (Fig. 1)

Table 1: Growth parameters recorded in 30 day old seedlings of brinjal (*Solanum melongena* L.) cv. Arka keshav plants treated with BBI

Growth Parameters*	*Arka keshav*		*Arka kusumakar*		*Arka shirish*	
	Control	Treatment**	Control	Treatment**	Control	Treatment**
Germination count (%)	36	69	45	71	39	67
Shoot height (cms)	4.1	12.2	3.9	19.1	3.0	15.3
Root depth (cms)	1.7	3.1	2.1	3.5	2.1	4.1
Fresh weight (gms)	0.39	3.78	0.47	4.81	0.41	4.83
Dry weight (mg)	0.03	0.19	0.05	0.25	0.04	0.21

*Mean of five replications

** Significantly different from the control at 1% level

Fig 1: Seedlings of brinjal *(solanum melongena* L.) cv. *Arka keshav* grown in trays inoculated with BBI left tray contains control plants.

Pot trial

Pot trial revealed that bacterial inoculation with BBI increased shoot dry weight in the treated plants significantly over the uninoculated controls. The percent increase of shoot dry weight of plants treated with BBI was 30.68% as compared with the uninoculated controls. The MPN (Most probable number) of diazotrophic bacteria in the rhizosphere and rhizoplane of brinjal (*Solanum melongena* L.) varied between 3.4 x 10^{10} and 4.1 x $l0^{10}$ respectively. There was wide variation in MPN among the different regions of the same plant. In pot trials, the plants treated with BBI showed a significant higher plant height than the uninoculated controls (Fig.2a &2b). The maximum increase in plant height in treated plants was 75% as compared with uninoculated controls. Plants treated with BBI showed an improvement of 23% phosphorus over the uninoculated control in 90 day old plants (Table 2). The brinjal *(Solanum melongena* L.) cultivar *Arka keshav* showed better response to inoculation with BBI.

Fig 2a: Seedling of Brinjal (*Solanum melongena* L.) cv. Arka keshav frown in pots inoculated with BBI showing higher growth parameters than the uninoculated controls. Control plants on the left pot

Fig 2b: Single seedling of Brinjal (*Solanum melongena* L.) cv *Arka keshav* inoculated with BBI showing better growth parameters than the uninoculated control. Control plants on the right pot.

Table 2: Growth parameters recorded in 70 day old seedlings of brinjal (*Solanum melongena* L.) cv. Arka keshav plants treated with BBI in pots

Growth Parameters*	*Arka keshav*		*Arka kusumakar*		*Arka shirish*	
	Control	Treatment**	Control	Treatment**	Control	Treatment**
Germination count (%)	41	71	45	67	39	67
Shoot height (cms)	24.1	54.2	22.9	59.1	33.0	55.3
Root depth (cms)	11.7	23.1	12.1	23.5	12.1	24.1
Fresh weight (gms)	10.39	13.78	10.47	14.81	9.41	11.83
Dry weight (mg)	0.13	0.29	0.17	0.25	0.14	0.21

*Mean of five replications

** Significantly different from the control at 1% level

Discussion

Growth promoting bacteria have been isolated in vegetables like tomato, cabbage, spinach, winged bean, capsicum and sweet potato. Bashan and Holguin and Bashan *et al.* observed aggregates of bacteria on the surface and endosphere of root hair, root cap and elongation zones of tomato using scanning rhizobacteria [21, 22]. Similar isolations have been reported from roots of cereals, grasses and plantation crops [23-25]. Growth promoting bacteria isolated from brinjal is highly beneficial to the crop. This growth promoting inoculates could be harnessed to reduce the input of inorganic fertilizers.

Brinjal plants inoculated with the bacteria grown in trays and pots showed significant higher germination count compared with that of uninoculated controls. Even linear growth, fresh and dry matter accumulation was better in inoculated plants. The fact that these bacteria improved seed germination, plant growth and dry matter indicates its potential to be a good biofertilizer. Fages and Mulard (1986) [26], Sukhada (1987) [16], Holl and Chanway (1997) [27], and Quimio and Coroza (1986) reported similar plant growth responses [28]. Quimio and Coroza (1986) isolated rhizobacteria from the rhizosphere of cabbage and used the same for plant growth promotion on tomatoes, white potatoes and com. They found significant increase in the plant growth parameters and yield of treated plants over uninoculated controls. The isolated rhizobacteria was characterized to be *Azo pirillwn brasilense.* They also reported enhanced growth of wheat and soybean plants inoculated with the isolated *Azospirillum brasilense* [28]. Fages and Mulard (1986) isolated rhizosphere bacteria from the roots of *Zea mays* and tested the effect of the same on the crop in a pot trial. They reported significant increase in the growth of treated plants over the uninoculated controls [29]. Pati 1992, Pati and Chandra 1993, recorded significant increase in crop yield by spraying suspensions of isolated phyllosphere bacteria of mustard, banana and jute and rice on the same crop [30, 31]. Similar study by 27-Holl and Chanway (1992) showed that *Bacillus polymyxa* isolate L6-16R exerted growth promotion on pine seedlings after four weeks of inoculation [27]. Spring wheat and white clover also responded well to inoculation with *Bacillus polymyxa* [32]. These above observations were in conformity with the results of the present study.

Conclusion

The present study clearly indicates the potential of growth promoting isolates from rhizosphere, phylloplane and caulosphere of *Solanum melongena* L. Among the cultivars used in the tray trials *Arka keshav* responded significantly better to inoculation than the other cultivars. The brinjal *(Solanum melongena* L.) cultivar *Arka keshav* showed better response to inoculation with BBI in the pot trail. The use of growth promoting bacterial isolate as biofertilizer could be an efficient approach to replace chemical fertilizers for sustainable cultivation. Further studies are required involving detailed characterization of molecular and functional properties of these growth promoting bacteria for their applications in the field.

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