DEVELOPMENT OF ROOT ENDOPHYTIC PLANT GROWTH PROMOTERS AS BIO-INOCULANTS FOR PROTRAY SEEDLINGS

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Development of root endophytic plant growth-promoters as bio-inoculants for pro-tray seedlings

by

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DECLARATION

I, hereby declare that this thesis entitled **"Development of root endophytic plant growth-promoters as bio-inoculants for pro-tray seedlings"** is a bonafide record of research work done by me during the course of research and the thesis has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

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Introduction

INTRODUCTION

Vegetable transplant production is the system of raising seedling of vegetables using plug tray/pro tray. Use of transplants to establish vegetable crops in the field is an accepted practice throughout the world. Researchers have focused on ways to produce transplants that meet mechanization requirements, better field establishment, and contribute to plant health that could affect yield of plants.

Plant growth promoting rhizobacteria are universal symbionts of higher plants, which enhance the adaptative potential of their hosts through a number of mechanisms, such as the fixation of molecular nitrogen, mobilization of recalcitrant soil nutrients, synthesis of phytohormones and the control of phytopathogens. Studies have shown that plant growth-promoting rhizobacteria can be applied to a wide range of plants for the purpose of disease control and growth enhancement.

Endophytic bacteria have been isolated from a large diversity of plants. Endophytic bacteria in a single plant host are not restricted to a single species but comprise several genera and species. The growth stimulation by the endophytes can be a consequence of nitrogen fixation or the production of phytohormones, biocontrol of phytopathogens in the root zone (through production of antifungal or antibacterial agents, siderophore production, nutrient competition and induction of systematic acquired host resistance, or immunity) or by enhancing availability of mineral nutrients.

Bacterial endophytes have been recovered from all plant species examined until now and thus they represent a ubiquitous component of the terrestrial plant community. Endophytic habitat appears to provide a protective environment that helps a potentially exploitable bacterium with reduced competition from the indigenous microbial populations.

Mycorrhizal fungi and rhizosphere bacteria are, depending on formulation, permissible for use as amendments in potting media in organic production. Bacteria can interact synergistically with mycorrhizal fungi to increase root colonization by nodulation of roots and amount of nutrients available to plants.

Piriformospora indica, a member of the newly created order Sebacinales, is extremely versatile in its mycorrhizal associations and its ability to promote plant growth. *P. indica* is widely distributed as a symptomless root endophyte, and it colonizes members of bryophytes, pteridophytes, gymnosperms and angiosperms. Root colonization by *P. indica* results in an increase in plant growth, early flowering, higher seed yield, alteration in the secondary metabolites, and adaptation to abiotic and biotic stresses.

The present study aims to develop microbial root endophytic plant growth-promoters as bio-inoculants in pro-tray seedling production of major solanaceous vegetable crops chilli, tomato and brinjal, as it would help develop bioinoculants for seed treatment.

Review of Literature

2. REVIEW OF LITERATURE

2.1 SOLANACEOUS VEGETABLES

2.1.1 Tomato

Tomatoes are one of the most widely used and versatile vegetable crops. They are consumed fresh and are also used to manufacture a wide range of processed products (Madhavi and Salunkhe, 1998). Tomatoes and tomato products are rich in health-related food components as they are good sources of carotenoids in particular, lycopene, ascorbic acid (vitamin C), vitamin E, folate, flavonoids and potassium (Beecher, 1998; Leonardi *et al*., 2000). Other constituents are protein and dietary fibre (Davies and Hobson, 1981). Regular consumption of tomatoes has been correlated with a reduced risk of various types of cancer (Franceschi *et al*., 1994; Gerster, 1997; Weisburger, 1998) and heart diseases (Lavelli *et al*., 2000; Pandey *et al*., 1995). These positive effects are believed to be attributable to the antioxidants, particularly the carotenoids, flavonoids, lycopene and β-carotene (Lavelli *et al*., 2000). Flavanols and flavones are of particular interest as they are potential antioxidants and have been found to possess antioxidative and free radical scavenging activities in foods and their consumption is associated with a reduced risk of cancer (Kaur and Kapoor, 2001). The disease-preventing potential of a food is a consequence of a several such constituents which may show some synergistic interactions. While most tomatoes produced worldwide are used in the production of tomato paste, an ingredient in different processed tomato products such as ketchup, sauces, and soups (Sanchez *et al*., 2003), a significant number of tomatoes are consumed fresh.

2.1.2 Chilli

Capsicum peppers are among the oldest cultivated plants in the world . This genus is indigenous to Central and South America from pre-Colombian times and is in the nightshade family Solanaceae. Presently, this genus is believed to

consist of 27 species, five of which are domesticated and used as fresh vegetables and spices, along with approximately 3000 varieties (Ibiza *et al.,* 2012). Wide spread geographic distribution of *Capsicum annuum* and *Capsicum frutescens* from the New World to other continents occurred in the sixteenth century *via* Spanish and Portuguese traders; soon afterwards, they became an integral part of food habits of several countries, including India. The dried ripened red pod of *C. annuum* is known to offer the pepper, which is used as a spice to flavor dishes worldwide. In addition to acting as a flavouring and colouring agent, this fruit also has ethno medicinal prestige and is used to treat a variety of human ailments. Red chilli has been used as an alternative medicine for the treatment of inflammation, diabetes, low back pain and acute tonsillitis (Tolan *et al*., 2004; Spiller *et al*., 2008). Moreover, capsicum plaster containing powdered capsicum and capsicum tincture has been used in Korean hand acupuncture to reduce post-operative nausea, vomiting and sore throat . Chilli was an important plant in traditional Mayan medicine to treat various ailments, such as sore throat, earache and skin care (Kim *et al*., 2002; Park *et al*., 2004).

2.1.2 Brinjal

Eggplant (*Solanum melongena* L.), fruit commonly known as brinjal, is ranked amongst the top ten vegetables in terms of oxygen radical absorbance capacity due to the fruit phenolic constituents (Cao *et al.,* 1996). The colour, size, and shape of the eggplant fruit vary significantly with the type of the eggplant cultivar, and its fruit is commonly cooked as a vegetable in many parts of the world. The cultivated eggplant has significant economic importance in many tropical and subtropical parts of the world. the antioxidant activity of eggplant with different assays was reported by Huang *et al*. (2004). Stommel and Whitaker (2003) reported that the presence of phenolic acid content of the fruit flesh of seven commercial eggplant cultivars.

2.2 VEGETABLE TRANSPLANTS

Vegetable transplant production is the system of raising seedling of vegetables using plug tray/pro tray. In this system each seedling is grown in individual cell of a pro-tray. It allows the grower to establish near perfect stands, optimal spacing and uniform physiological plant age during transplanting (Vavrina, 1998). Pro-tray seedlings enable less transplanting shock and quicker re-establishment. They grow to maintain root-soil contact when transplanted. Pro-tray transplants are commercially used for various crops like chilli, tomato, brinjal, cauliflower, cabbage, broccoli, celery, cucumber etc. Researchers have focused on ways to produce transplants that meet mechanization requirements, good field establishment, and contribute to plant health that could affect yield of plants (Damato and Trotta, 2000; de Grazia *et al.*, 2002., Russo 2004).

2.3 PLANT GROWTH-PROMOTING RHIZOBACTERIA (PGPR)

Plant growth-promoting rhizobacteria (PGPR) are capable of improving the plant growth in many plants and they also act as biological control agents against various soil-borne plant pathogens (Kloepper *et al*., 1980). Many of them, such as *Pseudomonas fluorescens, Bacillus subtilis, Trichoderma* spp, *Azospirillum, Azotobacter* etc., are having endophytic root colonizing ability and play a key role in plant growth promotion as well as biological control of soil borne plant diseases. PGPR strains *B. licheniformis* which was isolated from the rhizosphere of *Alnus glutinosa* is known to produce high levels of indolacetic acid (IAA) and gibberellins (GAs) (Gutierrez-Man˜ ero *et al*., 2001), *P. fluorescens* able to produce IAA and siderophores, and *C. balustinum*, IAA producer, both isolated from the rhizosphere of *Lupinus albus* .

Vegetable transplants have greater adaptability, increased water cum fertilizer use efficiency of crops (Vavrina, 1998). Survival and colonization of rhizobacteria in a tomato transplant system has been reported. Application for

rhizobacteria in transplant production increases plant growth and reduce disease (Kloepper *et al*., 2004). Biological amendment with a *Sinorhizobium* sp. has been reported to have positive effect on the transplant production of bell pepper (Russo, 2006).

Many of the rhizobacterial isolates are found to have profound influence on root development and better establishment of both vegetatively propagated and seed propagated plants in the nursery (Anith and Manomohandas, 2001; Anith *et al.*, 2004; Anith, 2009).

2.4 PIRIFORMOSPORA INDICA

Recently, *Piriformospora indica*, a plant-root-colonizing basidiomycetes fungus, has been discovered in the Indian Thar desert and was shown to provide strong growth-promoting activity during its symbiosis with a broad spectrum of plants (Verma *et al.*, 1998). *Piriformospora indica* is a wide host root colonizing endophytic fungus which allows the plants to grow under extreme physical and nutrient stress condition. The fungus can be cultivated on complex or minimal substrates. It belongs to the Sebacinales in Basidiomycota (Varma *et al*., 1999). This fungus functions as a plant growth promoter and biofertilizer in nutrient deficient soils, bio-protector against biotic and abiotic stress including root and leaf fungal pathogen and insect invaders, bio-regulator for plant growth development such as early flowering, enhanced seed production etc. (Sahay and Varma, 1999).

Endophytic root colonization by the fungus, *Piriformospora indica*, Sebacinales, Basidiomycota has been reported in many plants (Varma *et al*., 1999; 2012). This fungus can be cultivated *in vitro* unlike the root-colonizing AM fungi. *P. indica* colonizes the cortex of plant roots and develops hyphal coils and pear shaped chlamydospores. Druedge *et al.* (2007) reported that *P. indica* promotes adventitious root formation in cuttings of vegetatively propagated plants like Pelargonium, Poinsettia and Petunia. Many plant species respond positively to

inoculation with this fungus and hence, the fungus has multiple biotechnological applications (Oelmüller *et al*., 2009). Anith *et al.* (2011) reported that inoculation of black pepper plants with mixture of the two biological agents, *P. indica* and *T. harzianum*, promoted the plant growth. Besides increasing the growth of plants, *P. indica* also enhances the defense capability of colonized plants against various plant pathogens (Deshmukh and Kogel, 2007; Fakhro *et al*., 2009). Inoculation with the fungus also enhances secondary metabolite production in medicinal plants (Satheesan *et al*., 2012).

2.5 ENDOPHYTES

Endophytes are those microorganisms that reside within growing plant tissues without doing substantive harm or gaining benefit other than residency. Microbial endophytes actively colonize above ground host tissues and establish long-term associations, actually lifelong natural associations, without doing substantive harm to the host. They include bacteria and fungi that can be isolated from surface-disinfected plant tissues or extracted from inside the plant which does not visibly harm the plant (Hallmann *et al*., 1997). Colonization by endophytes, both fungi and bacteria are reported to enhance the disease resistance of many crop plants (Hallmann *et al*., 1997). Endophytes are considered to be important candidates for developing into biocontrol agents against plant diseases as they are highly adapted to the host plant system and thus effectively deter the attack of the invading pathogen (Johri, 2006).

Biological agents used as a consortium, or as mixture, is advantageous than when they are used separately. However, their *in vitro* and in vivo interactions are to be studied for efficient use. Many root associated bacteria help the colonization by beneficial fungi such as Vesicular Arbuscular fungi (VAM) and are referred to as Mycorrhizal helper bacteria (MHB) (Duponnois *et al*., 1993; Bonfante and Anca, 2009). It has been reported that two biological agents, though

they interact negatively on each other, could also be used in combination, if their application is spatially and temporally separated (Anith *et al*., 2011). Bacteria can interact synergistically with mycorrhizal fungi to increase root colonization and nodulation of roots and make available nutrients to plants (Suresh and Bagyaraj, 2002)

Utilizing the root colonizing ability of the endophytic fungus, *P. indica* and that of naturally occurring root endophytes belonging to both bacteria and fungi of selected vegetable crops as bio-inoculants in the transplant system of the crops is expected to enhance the seedling vigour and establishment of seedlings in the nursery.

Materials and Methods

3. MATERIALS AND METHODS

The experiment on the "Development of root endophytic plant growth promoters as bio-inoculants for pro-tray seedlings" was carried out at the Department of Agricultural Microbiology in College of Agriculture, Vellayani during the period 2013-15.

The details of the materials used and methods followed during the course of investigation are mentioned below.

3.1 ISOLATION OF BACTERIAL ROOT ENDOPHYTES FROM TOMATO, CHILLI AND BRINJAL

3.1.1 Isolation of root endophytes from tomato

Root samples were collected from vigorously growing portray grown seedlings of tomato (var. Anagha) maintained at the Department of Olericulture, College of Agriculture, Vellayani. Soil particles were removed from the roots under running tap water until the washings were very clear. Roots were cut into pieces and four pre washes were given with sterile distilled water. Surface sterilization was carried out by soaking the root pieces in 1% sodium hypochlorite for three minutes and then they were rinsed four times in sterile distilled water (SDW) to clear them of sodium hypochlorite before obtaining bacterial isolates. Sterility checks were carried out to monitor the efficiency of the disinfestation procedure. For these checks, either 0.1 ml of the last wash was transferred to Tryptic soy agar (TSA) and spread plated or, alternatively, 0.1 ml of the final wash was transferred to 9.9 ml Tryptic soy broth (TSB), and incubated at room temperature. After 48 h, if no bacterial growth occurred in the sterility check, the recovered bacteria in the isolation processes were considered to be endophytes. The tissue was triturated in 1 ml phosphate-buffered saline solution (PBS with pH 7.4) with mortar and pestle under aseptic condition. 0.1ml of the macerated tissue was spread plated on nutrient agar, TSA and King's B agar plate. The agar plates were incubated at 28°C. Bacterial colonies that appeared

frequently and looked morphologically different were selected for further studies. After each isolate had been recultured and checked for purity, they were suspended in sterile water containing 20% glycerol (pH 7) and then frozen at -80°C for long-term storage. For short-term storage, the isolates were preserved on slants under refrigerated condition.

3.1.2 Isolation of root endophytes from chilli

The same procedure was repeated with chilli (var. Athulya) seedlings.

3.1.3 Isolation of root endophytes from brinjal

The same procedure was repeated with brinjal (var. Haritha) seedlings.

3.2 PRIMARY SCREENING OF ROOT ENDOPHYTES FOR PLANT GROWTH PROMOTION

3.2.1 Primary screening of root endophytes for plant growth promotion in tomato

3.2.1.1 Seed vigour index

Tomato seeds (var. Anagha) were surface sterilized with 1% sodium hypochlorite for five minutes followed by three washing with sterile water and blot dried with blotting paper. Single colony isolates of each of the endophytes were cross streaked on nutrient agar plates and incubated for 24 hours. The plates were drenched with 10 ml of sterile distilled water and the suspension was aseptically collected. 20 seeds each were separately soaked in fresh cultures of the 15 isolates for 30 minutes. Ten seeds are placed at the center of moist towel papers in such a way that the micropyles are oriented towards bottom to avoid root twisting. The rolled towel papers are kept in the germinator maintained at room temperature. Two replications of the treatment were maintained. After 10 days towel papers were removed, germination per cent is recorded and the seedling length (mm) was measured. Seed vigour index was calculated by multiplying germination percentage (%) and seedling length (mm).

$$\text{Seed Vigour Index} = \frac{\text{Germination percentage (\%)}}{\text{Seedling length (mm)}}$$

3.2.1.2 Plant growth promotion in protrays.

Sterilized planting medium was used for growing tomato seedlings.Vermicilite : perlite in the ratio 3:1 by volume was moistened, packed in polypropylene bags and autoclaved for three consecutive days at 121ºC (15 lbs) for one hour each. Sterilized planting medium was filled in protray cavities. Preparation of bacterial cell suspension for seed treatment and seed treatment were carried out as described in section 3.2.1 above. Single seeds were sown in each cavity of the protrays and maintained in a glass house. Plants were watered twice daily with sterile water. At 14 days after sowing 1% NPK (19:19:19) 5ml/cavity was applied to the protray seedlings. The experiment was conducted as Completely Randomised Design (CRD) and replicated thrice.

3.2.2 Primary screening of root endophytes for plant growth promotion in chilli

The same procedure was repeated with chilli, treating the seeds with endophyte isolates from chilli (var. Athulya) both for finding seed vigour index and plant growth promotion.

3.2.3 Primary screening of root endophytes for plant growth promotion in brinjal

The same procedure was repeated with brinjal, treating the seeds with endophyte isolates from brinjal (var. Haritha) both for finding seed vigour index and plant growth promotion.

3.2.4 Biometric observation

On 21 days after sowing the plants were uprooted and per plant shoot and root fresh weight (mg), height of the plant (cm) and number of leaves were recorded. Dry root and shoot weight of the plant samples were recorded after drying them in drier at 80°C for three days.

3.3 COMPATIBILITY OF SELECTED ENDOPHYTES WITH *Piriformospora indica*

Endophytes with plant growth promoting ability obtained through preliminary screening were assessed under *in vitro* conditions using dual culture plate assay for understanding the compatibility with *P. indica.* The assay was done on either potato dextrose agar (PDA) or NPDA . NPDA was a combination of nutrient agar (NA) and PDA. This was prepared by combining half strength NA and half strength PDA and autoclaved at 121ºC (15 lbs) for 20 minutes. PDA and NPDA plates were prepared and 8 mm diameter mycelial disc from *P. indica* previous grown on PDA media for 7 days was placed at the center of the plates and incubated at 28ºC for three days. Endophytes were seperately streaked as a band of 2 cm on four sides of the plates inoculated with *P. indica* and the plates were incubated for three more days. Mycelial growth inhibition by the bacterial isolates was noted and those isolates showing no inhibition of growth were selected for further studies.

3.3.1 Molecular Characterization

Molecular characterization of bacterial isolates were done by 16S rRNA cataloging using universal primers with the help of microbial identification service at the Department of Microbiology, College of Horticulture, Vellanikkara.

3.3.1.1 Genomic DNA Isolation

Genomic DNA was isolated from the tissues using NucleoSpin® Tissue Kit (Macherey-Nagel) following manufacturer's instructions.

Loop full bacterial culture was transferred to one ml sterile distilled water taken in a microcentrifuge tube. 180 µl of T1 buffer and 25 µl of proteinase K was added and incubated at 56 °C in a water bath until it was completely lysed. After lysis, 5 µl of RNase A (100 mg / ml) was added and incubated at room

temperature for 5 minutes. 200 µl of B3 buffer was added and incubated at 70 °C for ten minutes. 210 µl of 100% ethanol was added and mixed thoroughly by vortexing. The mixture was pipetted into NucleoSpin® Tissue column placed in a 2 ml collection tube and centrifuged at 11000 × g for one minute. The NucleoSpin® Tissue column was transferred to a new 2 ml tube and washed with 500 µl of BW buffer. Wash step was repeated using 600 µl of b5 buffer. After washing the NucleoSpin® Tissue column was placed in a clean 1.5 ml tube and DNA was eluted out using 50 µl of BE buffer.

3.3.1.2 Agarose Gel Electrophoresis for DNA Quality and Quantity Check

The quality of the DNA isolated was checked using agarose gel electrophoresis. 1 µl of 6X gel-loading buffer (0.25% bromophenol blue, 30% sucrose in TE buffer pH – 8.0) was added to 5 µl of DNA. The samples were loaded to 0.8% agarose gel prepared in 0.5X TBE (Tris-Borate-EDTA) buffer containing 0.5 µl / ml ethidium bromide. Electrophoresis was performed with 0.5X TBE as electrophoresis buffer at 75 V until bromophenol dye front has migrated to the bottom of the gel. The gels were observed in a UV Transilluminator GeNei®.

3.3.1.3 PCR Analysis

PCR amplification reactions were carried out in a 20 µl reaction volume which contained 1X PCR buffer (100 mM Tris HCl, pH – 8.3, 500 mM KCl), 0.2 mM each dNTP's (dATP, dGTP, dCTP and dTTP), 2.5 mM $MgCl_2$, 1 unit of AmpliTaq Gold DNA polymerase enzyme, 0.1 mg / ml BSA, 4% DMSO, 5 pM of forward and reverse primers and FTA disc as template.

Primers used :

Target	Primer Name	Direction	Sequence (5' → 3')
16S rRNA	16S-RS-F	Forward	CAGGCCTAACACATGCAAGTC
	16S-RS-R	Reverse	GGGCGGWGTGTACAAGGC

The amplification was carried out in a PCR thermal cycler (GeneAmp PCR System 9700, Applied biosystems).

PCR amplification profile :

16S rRNA

95 °C - 5.00 min

95 °C - 30 Sec

60 °C - 40 Sec } 35 cycles

72 °C - 60 Sec

72 °C - 7.00 min

4 °C - ∞

3.3.1.4 Agarose Gel Electrophoresis of PCR Products

The PCR products were checked in 1.2% agarose gels prepared in 0.5X TBE buffer containing 0.5 µg / ml ethidium bromide. 1 µl of 6X loading dye was mixed with 5 µl of PCR products and was loaded and electrophoresis was

performed at 75 V power supply with 0.5 TBE as electrophoresis buffer foe about 1-2 hours, until the bromophenol blue front had migrated to almost the bottom of the gel. The molecular standard used was a 2-log DNA ladder. The gels were visualized in a UV Transilluminator Genei®.

The PCR products obtained were send for 16S rRNA sequencing at SciGenom, Kakkanad, Kochi.

3.3.1.5 Sequence Analysis

The nucleotide sequence of 16S rRNA was compared with the sequence available in the database using the BLAST tool offered by National Centre for Biotechnology Information (NCBI). BLASTn provided by NCBI (http://blast.ncbi.nlm.nih.gov/Blast.cgi) was carried for homology search.

3.4 PLANT GROWTH PROMOTION USING *P. indica* AND COMPATIBLE ENDOPHYTES

3.4.1 Plant growth promotion in tomato

Combination of *P. indica* with selected endophytes from tomato were tested for plant growth promotion of tomato seedlings in pro-trays under green house condition. Bacterial inoculants were provided as seed treatment and the fungal inoculant as additives in the transplant medium. Planting medium was prepared by mixing vermiculite : perlite in the ratio 3:1 by volume and slightly moistened. Sterilization was done by performing autoclaving (121°C for one hour each) for three consecutive days. Bacterial inoculants was prepared as described in section 3.2.1. *P. indica* mycelia disc of 1mm size was inoculated to 250 ml of potato dextrose broth and incubated at 28°C for 10 days. The mycelia growth was filtered out using a strainer and washed twice with sterile water. Mycelia was added to the sterile planting medium at the rate of 1% (w/v), mixed properly and filled in portray cavities. Seeds of tomato variety Anagha was surface sterilized

as described in section 3.2.1. Wherever bacterial inoculation was involved, seeds were soaked in bacterial inoculum for 30 minutes prior to sowing and single seeds were planted in protray cavities. Plants were maintained in a glass house and watered twice a day with sterile water. At 14 days after sowing 1% NPK (19:19:19) 5ml/cavity was applied to the protray seedlings. The experiment was conducted as CRD and replicated thrice.

Treatment details of the experiments are given below

T_1 - T_4	: Isolate from tomato
T_5 – T_8	: Combination of isolates & *P. indica*
T_9	: *P. indica* alone
T_{10}	: Uninoculated control
No. of replications	: 3
Design	: CRD

3.4.2 Plant growth promotion in chilli using *P. indica* and compatible endophytes

Similar procedure as above was repeated with chilli variety Athulya.

Treatment details of the experiments are given below

T_1 - T_2	: Isolate from chilli
T_3 – T_4	: Combination of isolates & *P. indica*
T_5	: *P. indica* alone
T_6	: Uninoculated control
No. of replications	: 3
Design	: CRD

3.4.3 Plant growth promotion in brinjal using *P. indica* and compatible endophytes

Similar procedure as above was repeated with brinjal variety Haritha.

Treatment details of the experiments are given below

T_1 - T_2	: Isolate from brinjal
T_3 – T_4	: Combination of isolates & *P. indica*
T_5	: *P. indica* alone
T_{10}	: Uninoculated control
No. of replications	: 3
Design	: CRD

3.4.4 Root colonization by *P. indica*

Roots were collected from 21 days old seedlings treated with *P. indica* alone and combination treatments with root endophytes. Roots were carefully separated from the plant, washed thoroughly with tap water to get rid of the planting media. Roots were cut into small pieces of approximately of 1 cm. Root bits were transferred to a small beaker having 5 ml of 10 % KOH and boiled for 5 minutes. KOH solution was drained out from the beaker and three washing with tap water was given. Then the root bits were soaked in 2 % HCl for 5 minutes. Root bits were then removed from the acid and transferred to lactophenol trypan blue for 10 minutes for staining. The root bits were then allowed to destain in lactophenol solution. They were then placed on a glass slide and covered with cover slip with gentle pressing. The samples were viewed under a microscope and checked for presence of chlamydospores in each root bit. The percentage root colonization was found out by the following formula,

$$\text{Percentage root colonization} = \frac{\text{No. of root bits with clamydospores}}{\text{Total no. of root bits observed}} \text{ X } 100$$

3.7 STATISTICAL ANALYSIS

Data of each experiment were analyzed applying suitable methods of analysis (Panse and Sukhatme, 1967). Data on percentage germination was analyzed by one way analysis of variance after square root transformation.

Results

4. RESULT

The experimental data collected from the investigation on "Development of root endophytic plant growth-promoters as bio-inoculants for pro-tray seedlings." were analysed and the results presented in this chapter under following headings.

4.1 ISOLATION OF ROOT ENDOPHYTES

4.1.1 Isolation of root endophytes from tomato

Endophytic bacteria from roots of vigorously growing seedlings of tomato were isolated by trituration after surface sanitization. 15 bacterial isolates were obtained, out of which 11 were Gram positive, rod shaped and all of them grow well at 28°C (Table 1 and Plate 1). Growth pattern of all isolates on different culture media showed that all the isolates grow well on NA and TSA, where as four isolates were unable to grow on PDA. (Table 2).

4.1.2 Isolation of root endophytes from chilli

Microorganisms were isolated by triturating the roots of vigorously growing seedling of chilli after surface sanitization. 14 bacterial isolates, which showed morphological variations were obtained. Their colony color ranges from white to pale orange and had different colony morphology (Plate 1). All of them were able to grow at 28°C (Table 3). Out of the 14, 9 isolates were Gram + ve and 5 were Gram – ve. Growth pattern of all isolates on different culture media were also studied (Table 4). All the isolates were able to grow on NA and TSA where as seven isolates failed to grow on PDA.

4.1.3 Isolation of root endophytes from brinjal

Thirteen bacterial isolates were obtained from brinjal on isolation (Plate1). 11of them were Gram positive and all were rod shaped and grow well at a temperature of 28°C (Table 5). Growth pattern of all isolates on different culture

media showed that PDA could not support the growth of four isolates where as NA and TSA supported growth at all of them (Table 6).

4.2 PRIMARY SCREENING OF ROOT ENDOPHYTES FOR PLANT GROWTH PROMOTION

4.2.1 Screening of root endophytes for plant growth promotion on tomato

4.2.1.1Seed vigour index and germination percentage

There was no significant difference observed in the germination per cent of tomato on treating with different endophytes (Table 7). The highest value was observed in NAT004 (99 %), NAT009 (99 %) and KBT006 (99 %). The lowest value was observed in KBT010 (53.7 6%). However analysis of data indicated that the treatments had significant effect on the seed vigour index . The treatment KBT003 (10930) was found to be significantly superior over the rest of the treatments (Plate 2) .

4.2.1.2 Plant growth promotion in protrays

Data on influence of different isolates on the growth promotion of the tomato seedlings are given in Table 8 (Plate 2). Endophytic treatments had significant effect on the leaf number of tomato seedlings. The treatment NAT001 (3.33) was found to be superior to the other treatments and this was on par with all other treatments except the uninoculated control (2.33).

Shoot length of the tomato seedlings was also influenced by the endophyte treatment (Plate 3). The highest mean plant height of 8.09 cm was observed in plants treated with NAT004. Control plants had the least shoot length (5.30cm).

Effect of endophytes on the mean fresh shoot weight of tomato seedlings are presented in Table 8 . There was significant difference among treatments over control with respect to the fresh shoot weight. The best treatment KBT006 found

to have a mean fresh shoot weight of 277.80 mg/plant and was on par with KBT010 (273.56 mg/plant). Shoot weight was the minimum with the un inoculated control (174.34 mg/plant).

Treatments also had significant effect on the dry shoot weight of tomato seedlings. Plants treated with KBT001 yielded maximum dry shoot weight per plant (25.97 mg/plant). Similar to fresh shoot weight, the control plants had minimum shoot dry weight.

Data given in Table 8. indicated that the treatments had significant effect on the fresh root weight. Plants treated with NAT004 yielded maximum fresh root weight of 45.47 mg/plant.

Observation revealed that the treatments significantly influenced the dry root weight. The maximum mean root dry weight was observed in KBT001(4.26 mg/plant).

4.2.2 Screening of root endophytes for plant growth promotion on chilli

4.2.2.1 Seed vigour index and germination percentage

There was no significant difference observed in the percentage germination of chilli seed treated with different endophytes (Table 9 and Plate 4). The highest value was observed in NAC008 (89.06%) and NAC010 (89.06%). The lowest value was observed in control (53.76%). Data given in Table 7 indicated that the treatments had significant effect on the seed vigour index (Plate 4). The treatment NAC011 (9180) was found to be significantly superior over the rest of the treatments. All the treatments gave superior results over control (4375).

4.2.2.2 Plant growth promotion in protrays

The data on efficacy of different isolates on the growth promotion of the chilli seedlings during the primary screening are given in Table 10 (Plate 4). The treatments had significant effect on the leaf number of chilli seedlings. The

Table 1 . Morphological characteristics of isolated endophytes in tomato

Isolates	Cell shape	Arrangement	Growth pattern in NA	Colony morphology	Colony color	Growth at 28°C	Gram's staining
NAT001	Rod	Single	Fast	Round	Off white	+	G+
NAT002	Rod	Single	Fast	Round	White	+	G-
NAT004	Rod	Single	Slow	Round	White	+	G+
NAT005	Rod	Single	Slow	Irregular	White	+	G+
NAT006	Rod	Paired	Fast	Round	White	+	G+
NAT009	Rod	chain of 7 cells	Slow	Round	Orange	+	G+
NAT012	Rod	Single	Fast	Round. smooth	Yellow	+	G+
NAT015	Rod	Single	Fast	Round	Orange	+	G+
KBT001	Rod	Single	Fast	Round ,dry	White	+	G+
KBT003	Rod	Paired	Slow	Round, slimy	White	+	G-
KBT004	Rod	Single	Fast	Round	Yellow	+	G+
KBT005	Rod	Single	Fast	Round, slimy	Yellow	+	G-
KBT006	Rod	Single	Fast	Round	White	+	G+
KBT010	Rod	Single	Fast	Round	Pink	+	G-
KBT011	Rod	Single	Fast	Round	Sandal	+	G+

Table 2. Growth pattern of endophytes of tomato on different cultural media

Isolates	Nutrient agar	King's B agar	Potato dextrose agar
NAT001	+	+	+
NAT002	+	+	+
NAT004	+	+	+
NAT005	+	+	+
NAT006	+	+	-
NAT009	+	+	-
NAT012	+	+	+
NAT015	+	+	+
KBT001	+	+	+
KBT003	+	+	+
KBT004	+	+	+
KBT005	+	+	+
KBT006	+	+	+
KBT010	+	+	-
KBT011	+	+	-

Table 3. Morphological characteristics of isolated endophytes in chilli

Isolates	Cell shape	Arrangements	Growth pattern on NA	Colony morphology	Colony Color	Growth at 28°C	Gram's staining
NAC001	Rod	Single	Slow	Irregular	Pale orange	+	G-
NAC002	Rod	Single	Fast	Round	White	+	G+
NAC003	Rod	Single	Fast	Round	Light brown	+	G-
NAC004	Rod	Single	Fast	Round	White	+	G-
NAC005	Rod	Single	Fast	Round,creamy	White	+	G+
NAC006	Rod	Single	Slow	Round	Transparent	+	G+
NAC007	Rod	Single	Fast	Round	Orange	+	G+
NAC008	Rod	Single	Fast	Irregular	White	+	G+
NAC009	Rod	Single	Fast	Round	Yellow	+	G+
NAC010	Rod	Paired	Slow	Round	White	+	G+
NAC011	Rod	Paired	Fast	Round	White	+	G-
NAC012	Rod	Single	Fast	Irregular	White	+	G-
TSAC001	Rod	Single	Slow	Round	White	+	G+
TSAC002	Rod	Single	Fast	Round	White	+	G+

Table 4. Growth pattern of endophytes of chilli on different culture media

Isolates	Nutrient agar	Tryptic soy agar	Potato dextrose agar
NAC001	+	+	-
NAC002	+	+	-
NAC003	+	+	+
NAC004	+	+	+
NAC005	+	+	+
NAC006	+	+	+
NAC007	+	+	+
NAC008	+	+	+
NAC009	+	+	+
NAC010	+	+	-
NAC011	+	+	-
NAC012	+	+	-
TSAC001	+	+	-
TSAC002	+	+	-

Table 5. Morphological characteristics of isolated endophytes in brinjal

Isolates	Cell shape	Arrangements	Growth pattern in NA	Colony morphology	Colony color	Growth at 28°C	Gram's staining
NAB001	Rod	Single	Slow	Round	Orange	+	G+
NAB002	Rod	Paired	Fast	Round	Milky white	+	G+
NAB004	Rod	Single	Fast	Irregular,spreading	light orange	+	G-
NAB005	Rod	Single	Slow	Irregular.elastic	Off white	+	G+
NAB006	Rod	Single	Fast	Round.spreading	light brown	+	G+
NAB007	Rod	Single	Slow	Irregular	Off white	+	G+
TSAB001	Rod	Single	Fast	Round ,smooth	White	+	G-
TSAB002	Rod	Single	Fast	Round,raised	White	+	G+
TSAB003	Rod	Single	Slow	Round,glassy	Transparent	+	G+
TSAB004	Rod	Paired	Slow	Round	Light orange	+	G+
TSAB005	Rod	paired	Slow	Round	White	+	G+
TSAB006	Rod	Single	Fast	Round	White	+	G+
TSAB007	Rod	Single	Slow	Round	Light orange	+	G+

Table 6. Growth pattern of endophytes of brinjal on different cultural media

Isolates	Nutrient agar	Tryptic soy agar	Potato dextrose agar
NAB001	+	+	+
NAB002	+	+	-
NAB004	+	+	-
NAB005	+	+	+
NAB006	+	+	+
NAB007	+	+	+
TSAB001	+	+	+
TSAB002	+	+	+
TSAB003	+	+	-
TSAB004	+	+	-
TSAB005	+	+	+
TSAB006	+	+	+
TSAB007	+	+	+

Table 7. Seed vigour index and germination per cent of tomato

Isolates	Germination (%) *	Seed vigour index
NAT001	68.55(8.34)	8410^{e}
NAT002	93.87(9.74)	10870^{b}
NAT004	99(10.00)	6665^{gh}
NAT005	88.68(9.47)	9520^{d}
NAT006	68.55(8.34)	6525^{h}
NAT009	99(10.00)	5820^{i}
NAT012	83.27(9.18)	5525^{j}
NAT015	83.82(9.22)	5900^{i}
KBT001	83.82(9.22)	9700^{d}
KBT003	83.82(9.22)	10930^{a}
KBT004	93.86(9.74)	10175^{c}
KBT005	93.86(9.74)	6770^{gh}
KBT006	99(10.00)	7400^{f}
KBT010	83.82(9.22)	5110^{k}
KBT011	83.82(9.22)	6845^{g}
CONTROL	73.82(8.65)	5670^{ij}
CD(0.05)	NS	278

Means followed by a common letter are not significantly different at 5% level of significance

*Values in the parenthesis are square root transformed values.

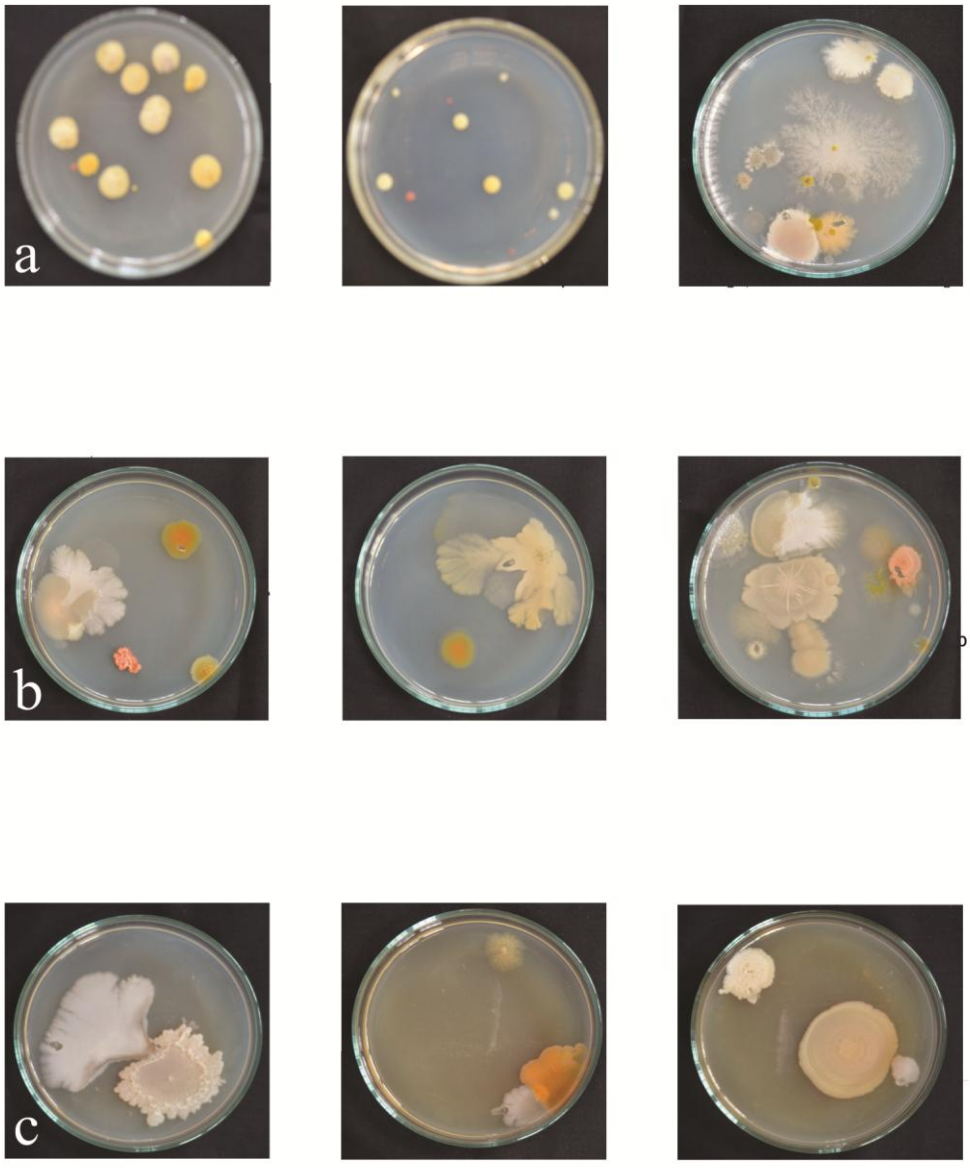
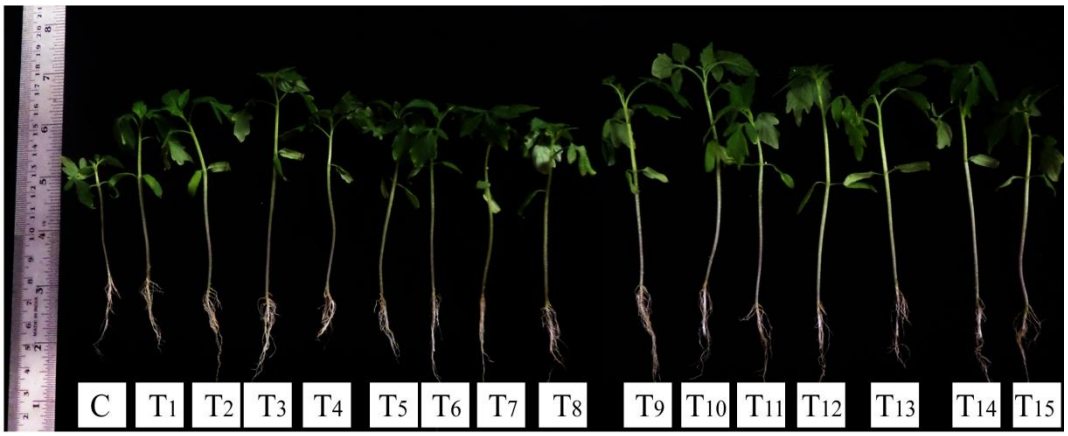


Plate 1. a, b and c are the root endophytes isolated from tomato, chilli and brinjal respectively.

Seed vigour index of tomato seedlings treated with different isolates.



Preliminary screening of endophytes for growth promotion on tomato seedlings

C- control, T_1 – NAT001 T_2- NAT002 T_3- NAT004 T_4- NAT005 T_5- NAT006 T_6- NAT009 T_7- NAT012 T_8- NAT015 T_9- KBT001 T_{10}- KBT003 T_{11}-KBT004 T_{12}- KBT005 T_{13}- KBT006 T_{14}- KBT010 T_{15}- KBT011

Plate 2. Seed vigour index and preliminary screening of endophytes in tomato seedlings.

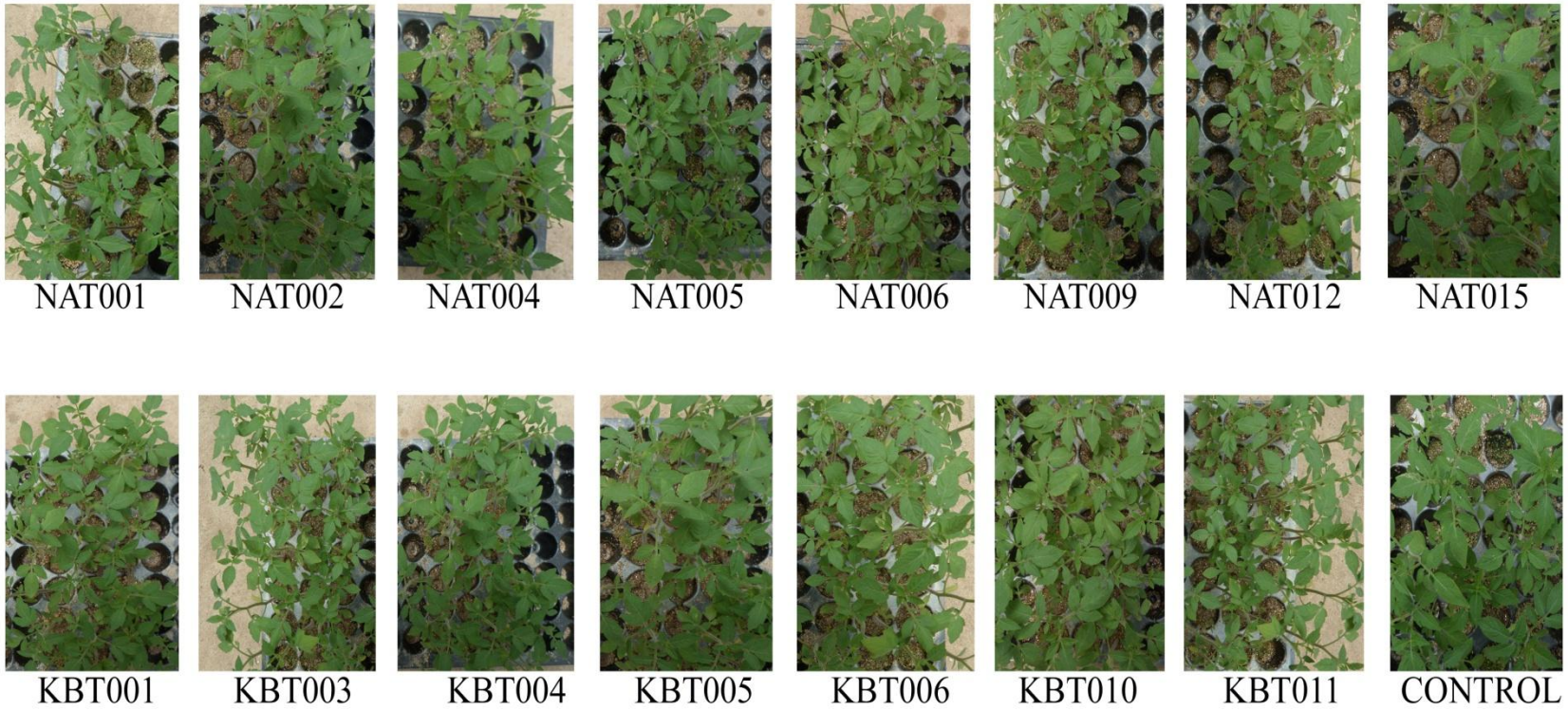


Plate 3. Preliminary screening of endophytes for growth promotion on tomato seedlings

Seed vigour index of chilli seedlings treated with different isolates.

Preliminary screening of endophytes for growth promotion on chilli seedlings

C- control, T_1 – NAC001 T_2- NAC002 T_3- NAC003 T_4- NAC004 T_5- NAC005 T_6- NAC006 T_7- NAC007 T_8- NAC008 T_9- NAC009 T_{10}- NAC010 T_{11}-NAC012 T_{12}- TSA001 T_{13}- TSA002 .

Plate 4. Seed vigour index and preliminary screening of endophytes in chilli seedlings.

treatment TSAC001 (5.27) was found to be significantly superior to the other treatments and this was on par with NAC006 (5.20), TSAC002 (5.13), NAC010 (5.07), NAC004 (4.87) and NAC008(4.87). Uninoculated control had 4.13 leaves/plant where as seedlings treated with the endophytes NAC003 and NAC009 produced less number of leaves/plant than the uninoculated control.

The maximum shoot length was obtained in seedling that received the endophytic treatment with NAC002 (10.53cm) which was significantly superior but on par with NAC006 (10.25 cm). The uninoculated control showed the minimum plant height of 6.95cm.

Endophyte treatment also had influence on the fresh shoot weight of the chilli seedlings (Table 10). All the treatments gave superior results over control. Maximum mean fresh shoot weight was observed with the treatment NAC010 (737.39 mg/plant) which differed significantly from all other treatments. The minimum value was observed by the un inoculated control (309.76 mg/plant).

Effect on the mean dry shoot weight of the chilli seedlings as influenced by endophyte treatment is shown in Table 10 (Plate 5). The seeds treated with the isolate NAC010 (66.56 mg/plant) was notably higher in the mean dry shoot weight which was on par with the treatment NAC002 (64.08 mg/plant). The least value was observed in un inoculated control (27.87 mg/plant).

There was significant difference among treatments with respect to the fresh root weight (Table 10). NAC010 (173.76 mg/plant) was found to be statistically superior. The lowest value was recorded with the un inoculated treatment (52.71mg/plant).

The maximum mean root dry weight of 14.61 mg/plant was observed in plants treated with the isolate NAC010 which was statistically superior to all other treatments (Table 10).

4.2.3 Screening of root endophytes for plant growth promotion on brinjal

4.2.3.1 Seed vigour index and germination percentage

There was no significant difference observed in the germination percentage of brinjal on treating with different endophytes (Table 11 and Plate 6). The highest value was observed in NAB005 (88.68 %). Seeds treated with the isolate NAB007 showed the lowest value of germination(48.42%) which was preceded by the control.

Data given in Table 11 indicated that the treatments had significant effect on the seed vigour index . The treatment NAB005 (7730) was found to be superior over all the other treatments.

4.2.3.2 Plant growth promotion in protrays

. The data on the biometric observation of the brinjal seedlings are given in Table 12 (Plate 6). Data given in table indicated that the treatments had significant effect on the leaf number of brinjal seedlings. The treatment NAB001(4.47) was found to be superior to the other treatments.

Treatments also had significant effect on the shoot length of the brinjal seedlings (Plate 7). The highest mean plant height of 10.38 cm was observed in NAB007.

Effect of endophytes on the mean fresh shoot weight of brinjal seedlings are presented in Table 12. There was significant difference among treatments over control with respect to the fresh shoot weight. The best treatment NAB001 found to have a mean fresh shoot weight of 1105.79 mg/plant and was statistically superior over all other treatments. The lowest value for shoot weight (607.46 mg/plant) was observed in the untreated control.

A similar trend was also observed with shoot dry weight of brinjal seedlings. Plants treated with NAB001 yielded maximum dry shoot weight per plant (99.52 mg/plant) which was closely followed NAB005 (84.94 mg/plant).

Data given in Table12 indicated that the treatments had significant effect on the fresh root weight. Plants treated with NAB004 yielded maximum fresh root weight of 201.70 mg/plant which was on par with NAB002 (201.04 mg/plant).

Analysis of data showed that the treatments significantly influenced the dry root weight of brinjal seedlings (Table 12). The maximum mean root dry weight was observed in NAB004 (18.15 mg/plant) which was closely followed by NAB002 (18.09 mg/plant).

4.3 COMPATIBILITY OF SELECTED ENDOPHYTES WITH *P. indica*

Endophytes with plant growth promoting ability selected through the preliminary screening were assessed under *in vitro* condition using dual culture plate assay for assessing the compatibility with *Piriformospora indica* (Pi) (Plate 8) . Presence of a zone of inhibition in dual culture was considered as a sign of inhibitory nature of the endophytes.

4.3.1 Between endophytes from tomato and *P. indica*

Out of 15 endophytes 11 showed antagonism and only four *viz* NAT001, KBT001, KBT004 and KBT006 were compatible with *P. indica* and mycelium grow over it (Table 13 and Plate 9).

4.3.2 Between endophytes from chilli and *P. indica*

Out of 14 endophytes 12 showed inhibition and only two *viz* NAC002 and NAC007 were compatible with *P. indica* and mycelium grow over it. (Table 14 and Plate 10).

4.3.3 Between endophytes from brinjal and *P. indica*

Out of 13 endophytes 11 showed inhibition only two *viz* NAB002 and TSAB007 were compatible with *P. indica* (Table 15 and Plate 10).

Table 8. Biometric observations of tomato seedling treated with the endophytes

Isolate	No.of leaves	Shoot length (cm)	Fresh shoot weight (mg/plant)	Dry shoot weight (mg/plant)	Fresh root weight (mg/plant)	Dry root weight (mg/plant)
NAT001	3.33^{a}	7.45^{abc}	245.57^{ef}	23.15^{abcde}	42.61^{ab}	4.13^{ab}
NAT002	3.07^{a}	7.16^{bcd}	269.49^{bc}	25.14^{ab}	43.31^{ab}	4.04^{abc}
NAT004	3.00^{ab}	8.09^{a}	272.01^{bc}	24.01^{abcde}	45.47^{a}	3.67^{abcde}
NAT005	3.13^{a}	6.20^{e}	245.72^{ef}	21.31^{cdef}	41.20^{abc}	3.55^{abcdef}
NAT006	3.07^{a}	6.31^{e}	211.36^{h}	18.056^{f}	35.31^{ef}	2.99^{def}
NAT009	3.00^{ab}	7.33^{abc}	247.76^{e}	21.35^{cdef}	35.73^{def}	3.32^{cdef}
NAT012	2.60^{bc}	6.41^{de}	229.86^{g}	22.04^{bcde}	28.65^{gh}	2.916^{ef}
NAT015	2.93^{ab}	7.11^{bcd}	259.15^{d}	20.54^{ef}	43.98^{ab}	3.96^{abc}
KBT001	3.27^{a}	7.37^{abc}	258.25^{d}	25.97^{a}	39.88^{bcde}	4.26^{a}
KBT003	3.00^{ab}	7.95^{a}	270.27^{bc}	23.18^{abcde}	40.43^{bcd}	3.47^{bcdef}
KBT004	3.13^{a}	7.38^{abc}	273.27^{bc}	23.21^{abcde}	41.73^{ab}	3.76^{abcd}
KBT005	3.20^{a}	7.82^{ab}	269.02^{c}	25.32^{ab}	36.48^{cdef}	3.59^{abcdef}
KBT006	3.27^{a}	8.03^{a}	277.80^{a}	25.00^{abc}	36.38^{cdef}	3.28^{cdef}
KBT010	3.00^{ab}	7.77^{ab}	273.56^{ab}	24.44^{abcd}	39.55^{bcde}	3.56^{abcdef}
KBT011	3.20^{a}	8.08^{a}	259.63^{d}	23.30^{abcde}	33.29^{fg}	2.99^{def}
CONTROL	2.33^{c}	5.30^{f}	174.34^{i}	15.71^{g}	17.11^{i}	2.30^{g}
CD(0.05)	0.45	0.79	4.41	3.75	4.84	0.79
Treatment Vs control	S	S	S	S	S	S

Means followed by a common letter are not significantly different at 5% level of significance.

Table 9. Seed vigour index and germination per cent of chilli

Isolates	Germination (%) *	Seed vigour index
NAC001	78.75(8.93)	7000^{f}
NAC002	78.75(8.93)	5820^{g}
NAC003	73.99(8.66)	6720^{f}
NAC004	84.01(9.22)	5930^{g}
NAC005	73.99(8.66)	7335^{e}
NAC006	68.72(8.35)	7730^{d}
NAC007	84.01(9.22)	4500^{ij}
NAC008	89.06(9.49)	3900^{k}
NAC009	63.96(8.06)	4725^{hi}
NAC010	89.06(9.49)	8175^{c}
NAC011	84.01(9.22)	9180^{a}
NAC012	69.06(8.37)	7535de
TSAC001	73.99(8.66)	8745^{b}
TSAC002	68.72(8.35)	4970^{h}
CONTROL	53.76(7.4)	4375^{j}
CD(0.05)	NS	301

Means followed by a common letter are not significantly different at 5% level of significance

*Values in the parenthesis are square root transformed values.

Table. 10 Biometric observations of chilli seedling treated with the endophytes

Isolates	No. of leaves	shoot length (cm)	Fresh shoot weight (mg/plant)	Dry shoot weight (mg/plant)	Fresh root weight (mg/plant)	Dry root weight (mg/plant)
NAC001	4.20^{ef}	10.11^{ab}	579.44^{f}	52.15^{e}	123.88^{de}	11.15^{cd}
NAC002	4.67^{cd}	10.53^{a}	712.01^{bc}	64.08^{ab}	130.16^{cd}	11.71^{bc}
NAC003	4.00^{f}	8.94^{bcd}	449.39^{i}	40.44^{g}	89.53^{i}	7.70^{f}
NAC004	4.87^{abcd}	8.07^{de}	662.13^{e}	60.61^{bc}	114.01^{fg}	10.26^{cd}
NAC005	4.67^{cd}	10.11^{ab}	652.71^{e}	55.76^{d}	111.27^{gh}	10.80^{cd}
NAC006	5.20^{ab}	10.25^{ab}	650.69^{e}	60.59^{bc}	141.87^{b}	12.76^{b}
NAC007	4.80^{bcd}	10.08^{ab}	699.99^{cd}	61.76^{bc}	122.92^{e}	10.60^{cd}
NAC008	4.87^{abcd}	9.02^{bcd}	691.37^{d}	60.06^{c}	131.55^{c}	11.24^{bcd}
NAC009	3.47^{g}	8.26^{cde}	490.98^{h}	42.38^{g}	113.79^{fg}	8.70^{ef}
NAC010	5.07^{abc}	10.19^{ab}	737.39^{a}	66.56^{a}	173.76^{a}	14.61^{a}
NAC011	4.47^{de}	9.53^{abc}	559.08^{g}	47.98^{f}	93.50^{h}	8.68^{ef}
NAC012	4.20^{ef}	8.15^{de}	493.43^{h}	43.71^{g}	91.81^{i}	7.55^{f}
TSAC001	5.27^{a}	9.91^{ab}	718.14^{b}	61.96^{bc}	119.72^{ef}	10.31^{cd}
TSAC002	5.13^{ab}	9.31^{abcd}	698.33^{d}	62.63^{bc}	106.37^{h}	9.71^{de}
CONTROL	4.13^{ef}	6.95^{e}	309.76^{j}	27.87^{h}	52.71^{j}	4.52^{g}
CD (0.05)	0.41	1.32	13.04	3.56	6.99	1.54
Treatment Vs control	S	S	S	S	S	S

Means followed by a common letter are not significantly different at 5% level of significance.

Table 11. Seed vigour index and germination per cent of brinjal

Isotates	Germination (%) *	Seed vigor index
NAB001	78.74(8.93)[a]	4970[g]
NAB002	68.56(8.34)[abc]	4017[i]
NAB004	68.56(8.34)[abc]	5770[e]
NAB005	88.68(9.47)[a]	7730[a]
NAB006	73.82(8.65)[ab]	3917[i]
NAB007	48.42(7.03)[c]	4255[h]
TSAB001	88.01(9.47)[a]	6250[c]
TSAB002	84.01(9.22)[a]	6010[d]
TSAB003	84.01(9.22)[a]	5660[e]
TSAB004	68.56(8.34)[abc]	6550[b]
TSAB005	53.91(7.41)[bc]	3135[k]
TSAB006	84.01(9.22)[a]	5670[e]
TSAB007	78.92(8.94)[abc]	5430[f]
CONTROL	48.98(7.07)[c]	3605[j]
CD(0.05)	1.377	111.1

Means followed by a common letter are not significantly different at 5% level of significance.

*Values in the parenthesis are square root transformed values.

Table 12. Biometric observations of brinjal seedling treated with the endophytes

Isolates	No . of leaves	Shoot length (cm)	Fresh shoot weight (mg/plant)	Dry shoot weight (mg/plant)	Fresh root weight (mg/plant)	Dry root weight (mg/plant)
NAB001	4.33^{ab}	8.53^{g}	1105.79^{a}	99.52^{a}	187.17^{ab}	16.85^{ab}
NAB002	4.20^{abc}	9.17^{efg}	943.78b^{c}	84.94^{bc}	201.04^{a}	18.09^{a}
NAB004	4.40^{ab}	9.05^{fg}	922.01^{cd}	82.98^{cd}	201.70^{a}	18.15^{a}
NAB005	4.47^{a}	9.77^{abcde}	982.54^{b}	88.43^{b}	180.52^{bc}	16.25^{bc}
NAB006	3.73^{cd}	9.60^{bcdef}	922.62^{cd}	83.04^{cd}	106.78^{g}	9.61^{g}
NAB007	3.93^{bcd}	10.38^{a}	947.79^{bc}	85.30^{bc}	144.88^{de}	13.04^{de}
TSAB001	3.93^{bcd}	10.01^{abcd}	886.86^{de}	79.82^{de}	189.57^{ab}	17.06^{ab}
TSAB002	3.93^{bcd}	10.35^{a}	853.08^{ef}	76.78^{ef}	188.75^{ab}	16.98^{ab}
TSAB003	4.07^{abc}	10.26^{ab}	827.00^{f}	74.43^{f}	169.54^{c}	15.26^{c}
TSAB004	4.13^{abc}	9.41^{def}	896.31^{d}	80.67^{d}	147.38^{d}	13.26^{d}
TSAB005	4.07^{abc}	10.13^{abc}	817.91^{f}	73.61^{f}	190.70^{ab}	11.43^{f}
TSAB006	3.80^{cd}	9.46^{cdef}	955.66^{bc}	86.01^{bc}	128.69^{ef}	11.58^{ef}
TSAB007	3.53^{d}	9.40^{def}	640.84^{g}	57.68^{g}	112.99^{fg}	10.17^{fg}
CONTROL	3.53^{d}	9.52^{cdef}	607.46^{g}	54.67^{g}	82.22^{h}	7.40^{h}
CD(0.05)	0.50	0.68	42.90	3.86	16.56	1.47
Treatment Vs control	S	S	S	S	S	S

Means followed by a common letter are not significantly different at 5% level of significance.

Table .13 Compatibility of selected endophytes of tomato with *P. indica*

Isolates	Compatibility with *P.indica*	Zone of inhibition (cm)
NAT001	+	Nil
NAT002	-	1.32
NAT004	-	1.00
NAT005	-	1.21
NAT006	-	1.43
NAT009	-	1.31
NAT012	-	0.81
NAT015	-	0.90
KBT001	+	Nil
KBT003	-	1.11
KBT004	+	Nil
KBT005	-	1.32
KBT006	+	Nil
KBT010	-	1.21
KBT011	-	1.4

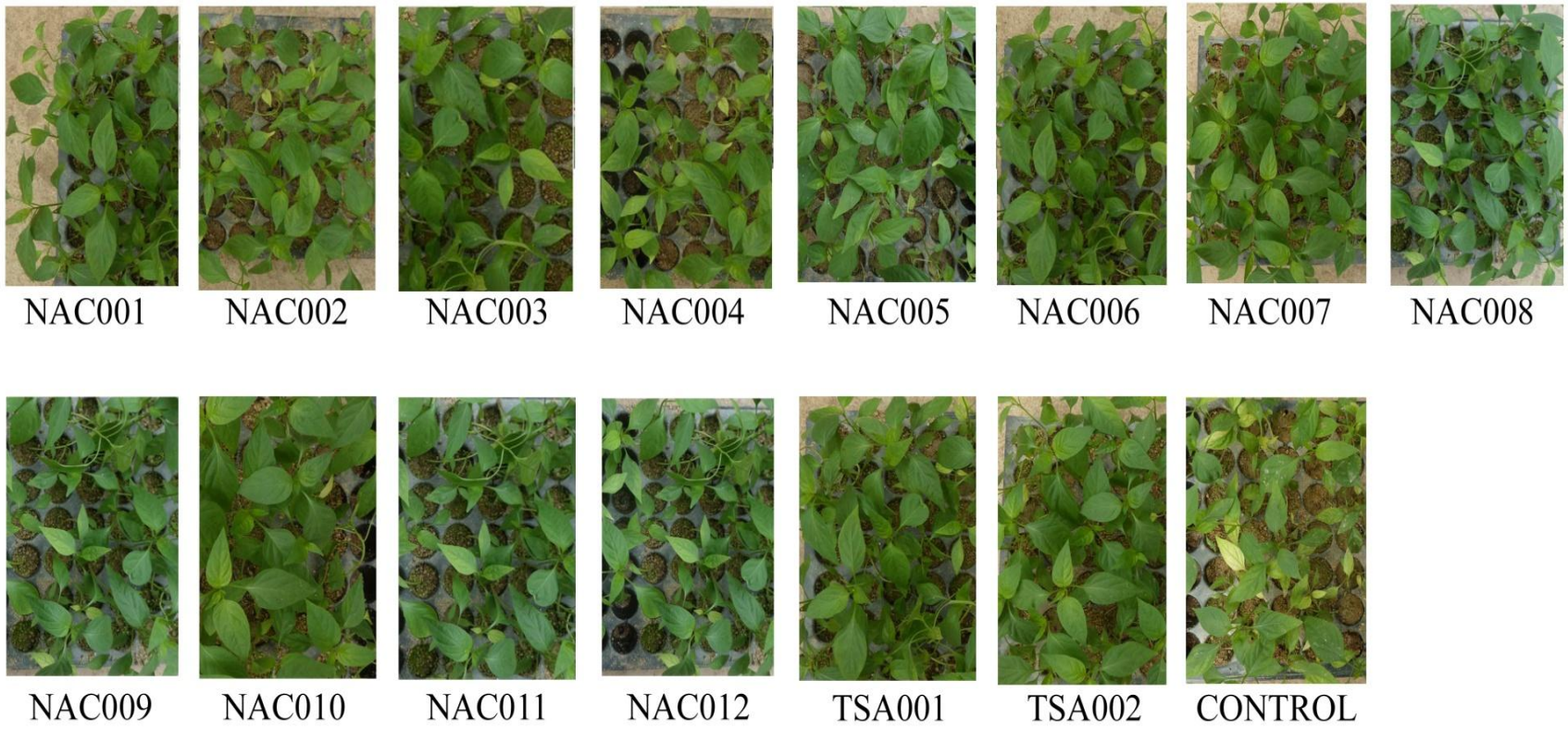
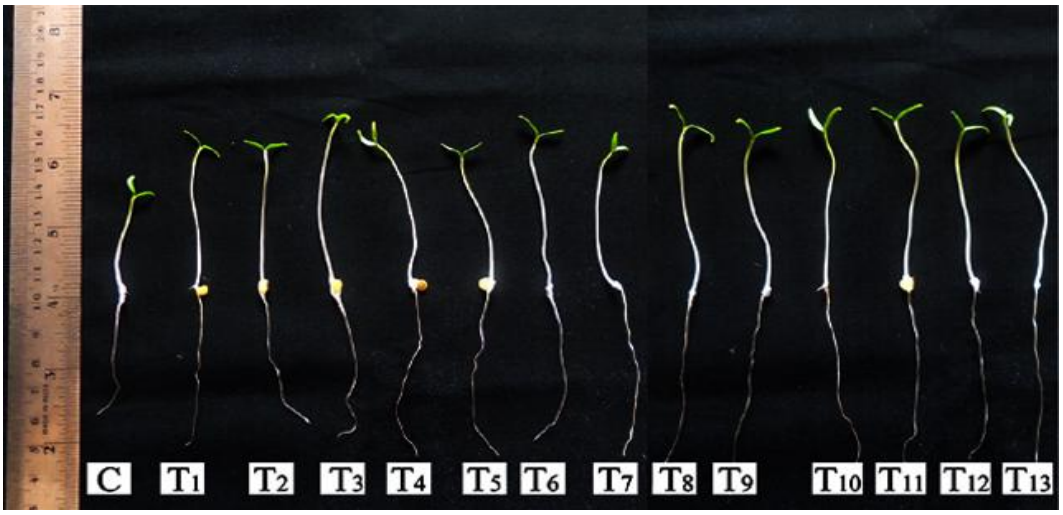
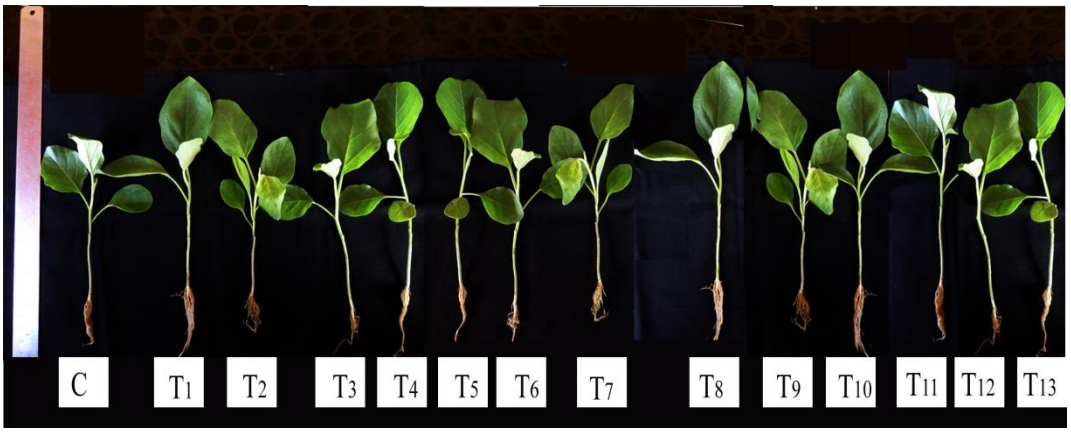


Plate 5. Preliminary screening of endophytes for growth promotion on chilli seedlings



Seed vigour index of brinjal seedlings treated with different isolates.



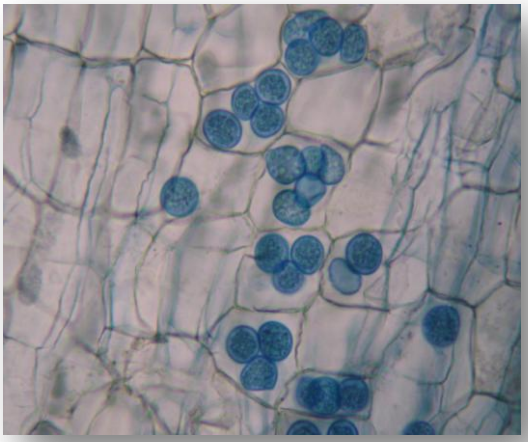
Preliminary screening of endophytes for growth promotion on brinjal seedlings

C- control, T_1 – NAB001 T_2- NAB002 T_3- NAB004 T_4- NAB005 T_5- NAB006 T_6- NAB007 T_7- TSAB001 T_8- TSAB002 T_9- TSAB003 T_{10}- TSAB004 T_{11}-TSAB005 T_{12}- TSAB006 T_{13}- TSAB007

Plate 6. Seed vigour index and preliminary screening of endophytes in brinjal seedlings.

Plate 7. Preliminary screening of endophytes for growth promotion on brinjal seedlings

Culture plate showing the growth of *P. indica* on PDA



Chlamydospore and mycelial growth in root tissues

Plate 8. Growth of *P. indica* on PDA, chlamydospore and mycelial growth in root tissues.

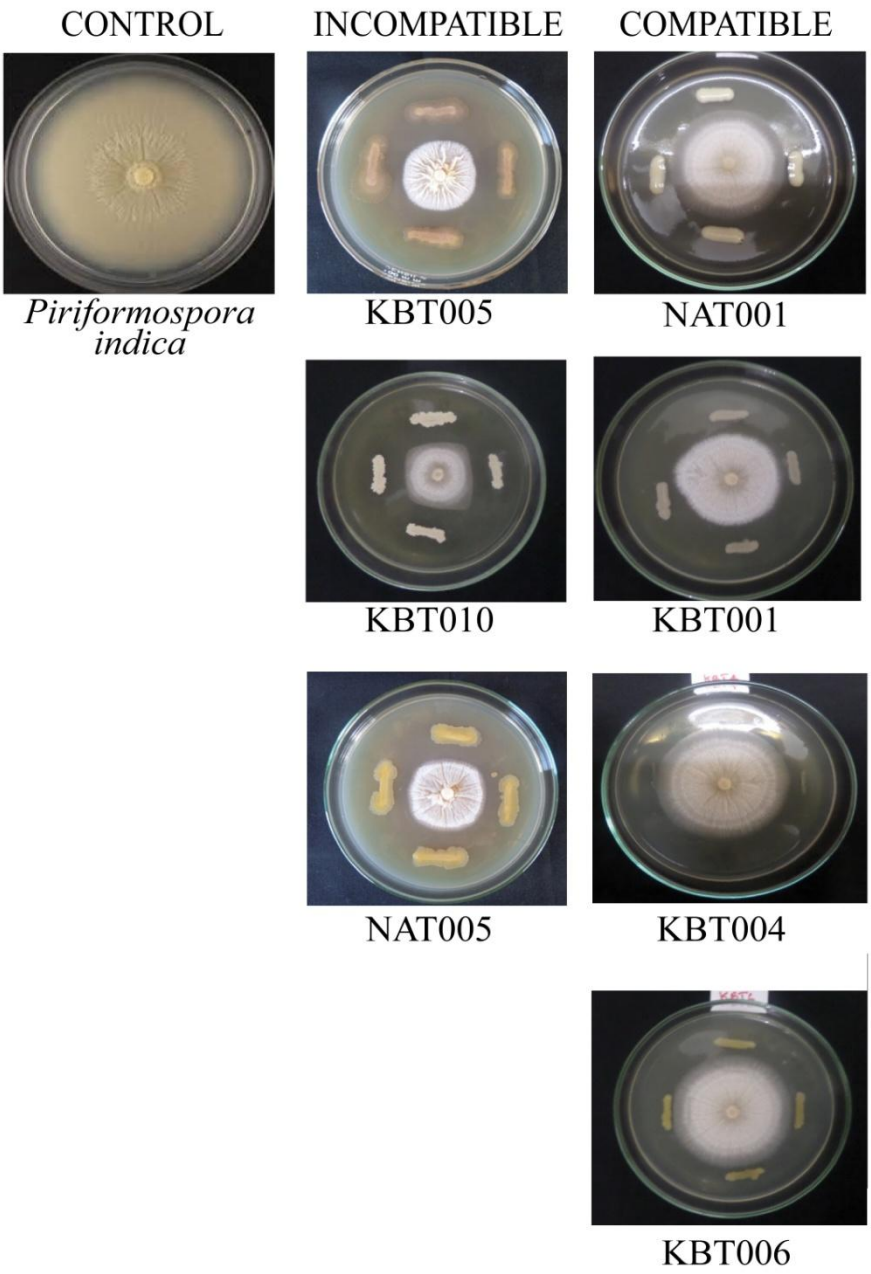


Plate 9. Compatibility of selected endophytes in tomato with *P. indica*

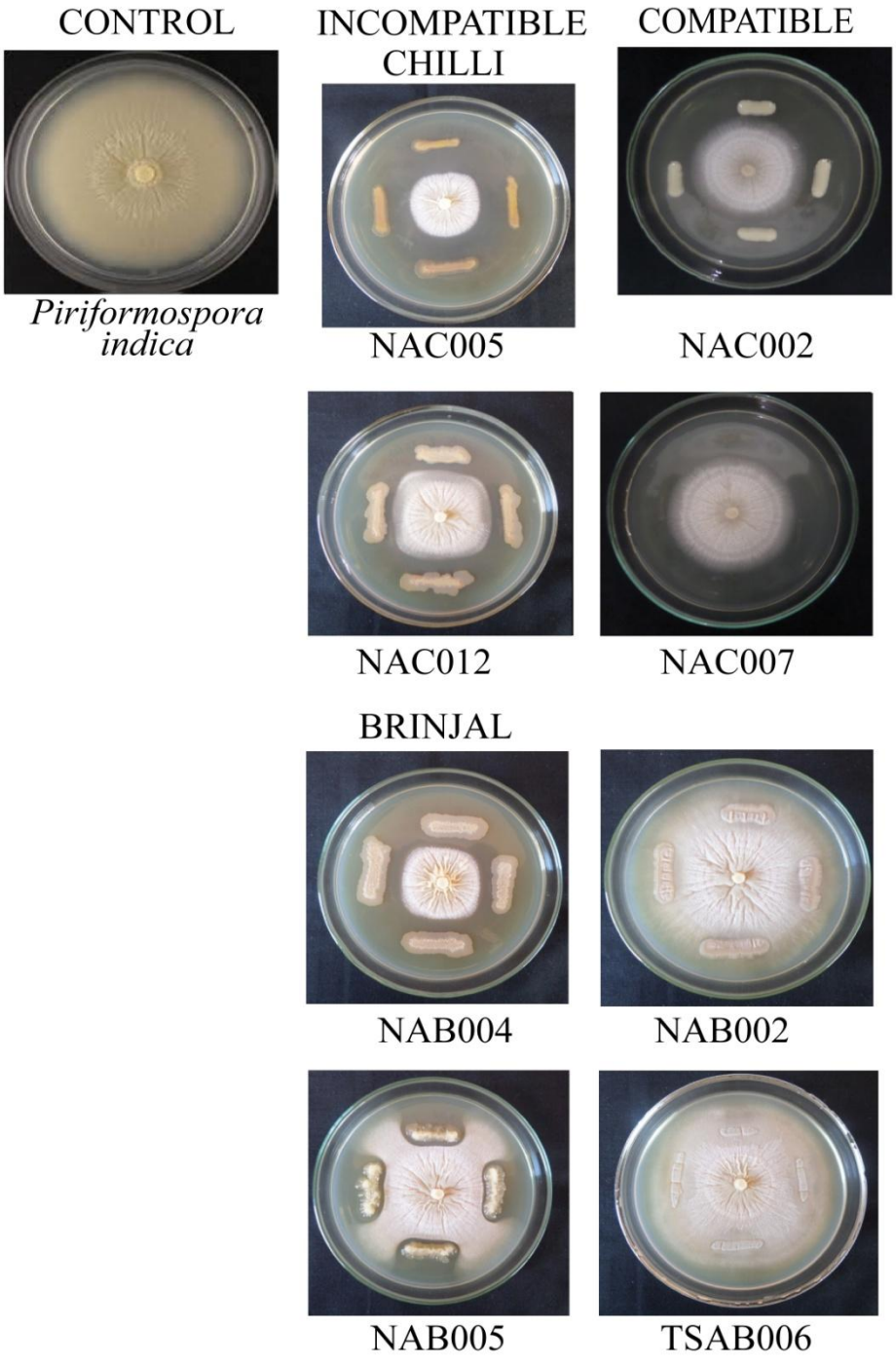


Plate 10. Compatibility of selected endophytes in chilli and brinjal with *P. indica*

4.3.4 Characterization of the selected endophytes

By the dual culture experiment, eight compatible endophytes with *P.indica* were selected for further studies and were characterized and identified (Table 16). The colony morphology and Gram's staining character of selected endophytes were shown in Plates 11 and 12.

4.3.5 Molecular Characterization

16S rRNA sequence of selected isolates obtained are presented in Table 17. The BLAST details of the most matching sequence are presented in Table 18.

Endophytes NAT001, KBT001, KBT004, KBT006, NAC002, NAC007, NAB002 and TSAB006 were identified as *Bacillus megaterium, Alcaligenes faecalis Streptomyces leeuwenhoekii, Bacillus pumilus, Bacillus megaterium, Bacillus licheniformis, Bacillus thuringiensis, Bacillus thuringiensis* respectively (Appendix III a – h).

4.4 PLANT GROWTH PROMOTION OF VEGETABLE TRANSPLANTS

4.4.1 Plant growth promotion in tomato by *P. indica* and selected endophytes

Efficacy of different isolates selected after preliminary screening on the growth promotion of the tomato seedlings were studied under portray conditions. Treatments had significant effect on the leaf number of tomato seedlings (Table 19). The treatment KBT006 (6.47) was found to be superior to all other individual and combined treatments.

Data given in Table 19 hinted that there was significant effect on the shoot length of tomato treated with the endophytes. The maximum mean height of 15.23 cm was observed by the treatment KBT001 + Pi .

Analysis of data implied that the treatments significantly influenced on the fresh shoot weight of tomato seedlings. The combination treatment KBT004 + Pi was found to be significantly superior with 1764.54 mg/plant over the other treatments.

Treatments had significant effect on the dry shoot weight of tomato seedlings. The maximum mean dry shoot weight of 134.86 mg/plant was observed by treatment NAT001.

Effect of endophytes on the mean fresh root weight of tomato seedlings are presented in Table 19 (Plate 13). There was significant difference among treatments over control with respect to the fresh root weight. The best treatment KBT004 + Pi found to have a mean fresh root weight of 332.88 mg/plant which was significantly superior.

Efficacy of endophytes on the dry root weight of the tomato seedlings are presented in the Table 19 (Plate 14). The combination treatment KBT004 + Pi (26.45 mg/plant) was found to be superior, which was on par with KBT004(24.47 mg/plant) and KBT006 + Pi(24.15 mg/plant).

4.4.1.1 Percentage root colonization by P. indica .in tomato

The percentage colonization by *P.indica* in tomato in the presence of different endophytes is presented in the Table 20 (Plate 17). There was no significant difference between the treatments. Highest value was observed in the combination treatmemt with NAT001 of 84.01per cent which was closely followed by KBT001 + Pi (83.09 %), KBT004 + Pi (83.01 %), KBT006 + Pi (81.08 %) and *P.indica* alone (81.81%).

4.4.2 Plant growth promotion in chilli by *P. indica* and selected endophytes

The data on the biometric observation of chilli plants treated with *P. indica*, endophytes and their combinations are given in Table 21 (Plate 15). The treatments had significant effect on the leaf number of chilli seedlings. Seed

treated with the endophytic isolate NAC002 (5.66) was found to be superior to all the other treatments in having the maximum number of leaves. All the other treatments including the combined application had less number of leaves (5.00) though it was higher than that of the control.

Analysis of the data hinted that there was significant effect on the shoot length of chilli treated with the endophytes. The maximum mean height of 11.93 cm was observed by the treatment NAC002 followed by NAC007 (11.20 cm). The least shoot length was observed with plant without any inoculation.

Significant effect was observed on the fresh shoot weight of chilli seedlings treated with the endophytes. The treatment NAC002 (855.20 mg/plant) was found to be significantly superior with all other individual and combined treatments.

The data presented in Table 21 revealed that the treatments had significant effect on the dry shoot weight. The maximum mean dry shoot weight of 87.97 mg/plant was observed by treatment NAC002 followed by NAC002 + Pi (66.19 mg/plant). Dry shoot weight was minimum for the untreated control (30.78 mg/plant).

Effect of endophytes on the mean fresh root weight of chilli seedlings was significantly different among treatments. The best treatment NAC007 found to have a mean fresh root weight of 90.82 mg/plant which was significantly superior to all other treatments.

The combination treatment NAC002 + Pi was found to be having maximum root dry weight (18.92 mg/plant). All the treatments had significantly higher values for root dry weight when compared to the control treatment (Plate16).

4.4.2.1 Percentage root colonization by P. indica .in chilli

Percentage colonization by *P.indica* in chilli was assessed and it was found that the highest percentage colonization was by *P.indica* alone (42.03%)

followed by the combination treatments with the endophyte NAC002 (40.99 %) and NAC007 (38.81%) (Table 22).

4.4.2 Plant growth promotion in brinjal by *P. indica* and selected endophytes

Efficacy of different isolates on the growth promotion of the brinjal seedlings are given in Table 23 (Plate 15 and Plate 16) . Treatments had no significant effect on the leaf number of brinjal seedlings. The treatment NAB002 (3.33) was found to have superior value and the least value was observed by control (2.80).

Analysis of the data on Table 23 hinted that there was significant effect on the shoot length of brinjal treated with the endophytes. The mean superior height of 6.95cm was observed by *P.indica*.

There was significant difference among the treatments with respect to the fresh shoot weight of brinjal seedlings treated with the endophytes (Table 23). The endophyte NAB002 alone (844.27 mg/plant) was found to be significantly superior.

Treatments had significant effect on the dry shoot weight of brinjal seedlings . The maximum mean dry shoot weight of 64.84 mg/plant was observed in treatment with TSAB006.

Effect of endophytes on the mean fresh root weight of brinjal seedlings are presented in Table 23. There was significant difference among treatments over control with respect to the fresh root weight. The best treatment NAB002 found to have a mean fresh root weight of 83.03 mg/plant which was significantly superior.

Efficacy of endophytes on the dry root weight were implied in the Table 23. The combination treatment NAB002 + Pi (7.72 mg/plant) was found to be superior which was on par with all the other treatments.

4.4.3.1 Percentage root colonization by P. indica .in brinjal

The percentage colonization of *P.indica* in brinjal with different endophytes is presented in the Table 24 (Plate 18) . There was no significant difference between the treatments, highest value was observed by the combination treatmemt with NAB002 of 44.83 per cent which was closely followed by *P.indica* alone (42.03%), TSAB006 + Pi (41.77%).

Table .14 Compatibility of selected endophytes of chilli with *P. indica*

Isolates	Compatibility with *P.indica*	Zone of inhibition (cm)
NAC001	-	1.3
NAC002	+	Nil
NAC003	-	1.2
NAC004	-	1
NAC005	-	1.2
NAC006	-	0.8
NAC007	+	Nil
NAC008	-	1
NAC009	-	1
NAC010	-	1.3
NAC011	-	0.9
NAC012	-	1.2
TSAC001	-	1
TSAC002	-	1.1

Table .15 Compatibility of selected endophytes of brinjal with *P. indica*

Isolates	Compatibility with *P. indica*	Zone of inhibition(cm)
NAB001	-	1.52
NAB002	+	Nil
NAB004	-	1.32
NAB005	-	1.21
NAB006	-	0.76
NAB007	-	1.00
TSAB001	-	1.11
TSAB002	-	1.38
TSAB003	-	1.42
TSAB004	-	1.23
TSAB005	-	1.01
TSAB006	+	Nil
TSAB007	-	0.82

Table 16. Morphological characteristics of selected endophytes

Isolates	Crop	Cell shape	Arrangements	Growth pattern in NA	Colony morphology	Colony color	Growth at 28 °C	Gram's staining
NAT001	Tomato	Rod	Single	Fast	Round	Off white	+	G+
KBT001	Tomato	Rod	Single	Fast	Round ,dry	White	+	G+
KBT004	Tomato	Rod	Single	Fast	Round	Yellow	+	G+
KBT006	Tomato	Rod	Single	Fast	Round	White	+	G+
NAC002	Chilli	Rod	Single	Fast	Round	White	+	G+
NAC007	Chilli	Rod	Single	Fast	Round	Orange	+	G+
NAB002	Brinjal	Rod	Paired	Fast	Round	Milky white	+	G+
TSAB006	Brinjal	Rod	Single	Fast	Round	White	+	G+

Table 17. 16S rRNA sequence of isolated endophytes obtained with universal primer

Isolates	SEQUENCE
NAT001	GCCCCTGAAGGCTCATGCTACGACTCACCCATCATCTGTCC CACCTTAGGCGGCTAGCTCCTTACGGTTACTCCACCGACTT CGGGTGTTACAAACTCTCGTGGTGTGACGGGCGGTGTGTAC AAGGCCCGGGAACGTATTCACCGCGGCATGCTGATCCGCG ATTACTAGCGATTCCAGCTTCATGTAGGCGAGTTGCAGCCT ACAATCCGAACTGAGAATGGTTTTATGGGATTGGCTTGACC TCGCGGTCTTGCAGCCCTTTGTACCATCCATTGTAGCACGT GTGTAGCCCAGGTCATAAGGGGCATGATGATTTGACGTCAT CCCCACCTTCCTCCGGTTTGTCACCGGCAGTCACCTTAGAG TGCCCAACTAAATGCTGGCAACTAAGATCAAGGGTTGCGCT CGTTGCGGGACTTAACCCAACATCTCACGACACGAGCTGAC GACAACCATGCACCACCTGTCACTCTGTCCCCCGAAGGGGA ACGCTCTATCTCTAGAGTTGTCAGAGGATGTCAAGACCTGG TAAGGTTCTTCGCGTTGCTTCGAATTAAACCACATGCTCCA CCGCTTGTGCGGGCCCCCGTCAATTCCTTTGAGTTTCAGTCT TGCGACCGTACTCCCCAGGCGGAGTGCTTAATGCGTTAGCT GCAGCACTAAAGGGCGGAAACCCTCTAACACTTAGCACTC ATCGTTTACGGCGTGTCTACCAGGGTATCTAATCCTGTTTG CTCCCCACGCTTTCGCGCCTCAGCGTCAGTTACAGACCAAA AAGCCGCCCTTCGCCACTGGTGTTCCTCCACATCTCTACGC ATTTCACCGCTACACGTGAATTCCGCTTTTCTCTTCTGCACT CAAGTTCCCCAGTTTCCAATGACCCCTCCACGGTTGGAGCC GTGGGCTTTCACATCAGACTTAAGAAAACCGCTGGCGCGCC GCTTTACGCCAATAATTCCGGATAACGCTTGCCAACTACGT ATTACCGCGGCTGCTGGCACGTATTAGCCGTGGCTTTCTGG GTAGGTACCGTCAGTAACAAGCAGTACCTCTTGTACTTGTT CCTCCCTTAACAACAGAGTTACGGACCGAAGCCTCATCACC TCACGCGGGGTTTCCGGTCGGAACTTTCGGTCAATTGCGGA GATCCCATCTTGCTAGCACTCCGGTGAGCAGGATTCTTGGA GCACCCGT
KBT001	GGGTGCACAAGATAGACTTCAGCGAGTCATGATCCCACGG TGGTGAGCGGTCTCATTGCGGTTGACCCCTACCTAGGCGCC GGCTGACTGGCGTGGGGTGGGGCTGCTAAAACAATTGTAC GGTCCCGCCACATTCTGGTTTGCGATTACAACAGCATTCCC ACTTGGACCACCTGCTTGCAAACTGCGAACGCGCACTAGTG AACGCGTTTCTCGAGATTGGATCCTGCTCCCGGCCTGACCA ACCTATGTTCCTGACAGATGGATGCCGTGTGAAGACCCACG CCATAAAGTCTCTTGACCAACCTATACTATAGGACTTGACG TCATCCCCACCTTCCTCCGGTTTGTCACCGGGCAGTCTCATT AGAGTGCTCATTGCGTAGGAACTAAAGACAAAGGCTGCGC TTGTTCGCGGTACTTAACCTAACATCTCACGACACGAGCTG ACGATAGCGATGCAAACCTGTGTTCCGGTTCGCTTGCCAAC

	ACAGAAAACTCTTTTCGGGTTTTGAGACATGTCTATGGGGA AGCAAGGTTTTTCCTCGCGTGCATCATAATTAATCCCACAT CATCCACCGCCTGGTGGGGGCCCCCCCATTTCTTTTGATTTT TTATCTTGCGACCGTACTCCCCCAGGAGGCAAAGTTCTTGT CGTTAGCTGCGCTACAAAGG GCCTAGCG
KBT004	CAGCACACTCTCGACTGCTGCTGCTCGGAGGCCTGCTTCGA CAGCTCCTCCACCAGGAAGTGGCCCCCGGGTGGTGGTGGCT CCTAATTGGACGTGACGCGCGGTGTGTACATACCCCGGGA ATGTATTCAGCGCAGCTGTGGATCTCTGCGAATACTAGCAA CTCTACACTTCATGGTCGAGAGTTGCAGACCCCAATCCAAA CGGAAACGGGCTTTTTGAGATTCGTCCAACCTCACGGAATC GCAGCTCTTTGTACCGGCAATTGTAGCACGTGTGCAGCCCC AAGACATAAGGGGGCATGATGACTTGACGTCGTCCCCACC TTCCTCCGAGTTGACCCCGGCGGTCTCCCGTGAGTCCCCAG CACCACAAGGGCCTGCTGGCAACACGGGACAAGGGTTGCG CTCGTTGCGGGACTTAACCCAACATCTCACGACACGAGCTG ACGACAGCCATGCACCACCTGTACACCGACCACAAGGGGG CGCCCATCTCTGGACGTTTCCGGTGTATGTCAAGCCTTGGT AAGGTTCTTCGCGTTGCGTCGAATTAAGCCACATGCTCCGC CGCTTGTGCGGGCCCCCGTCAATTCCTTTGAGTTTTAGCCTT GCGGCCGTACTCCCCAGGCGGGCACTTAATGCGTTAGCTGC GGCACGGACGACGTGGAATGTCGCCACACCTAGTGCCCAC CGTTTACGGCGTGACTACCAGGGTATCTAATCATGTCGCTC CCACGCTTTCGCTCTCAGCGTCAGTATCGGCCCAGAGATCG CTTCGCAACGTGTCTCTGATATCTGCGCATTCACGCTACAC AGGATTCGATCTCCCTTACGGACTCTAGCTGCCGGTATCGA ATGCAGATCAGGGTAGCCCCGGACTTACAATCCGACGTGA CAAGCGCTACAACTCTTACGCGATATCGACAGCTTGCCCTA GATAACGCGCTCTGCCGAGTTAGCGGCTTCTTCAGTACGTA CTTGGCTTTTCTGTGAGAGTTACCGAGGCGATCTCTCAGCA GTCAGTTCAGGTGCAAGTTGCAATCCATGTCCTGAGAGTGC CGT

KBT006	TGCAGTGGGCGCTGCTATAATGCAGTCGAGCGGACAGAAG GGAGCTTGCTCCCGGATGTTAGCGGCGGACGGGTGAGTAA CACGTGGGTAACCTGCCTGTAAGACTGGGATAACTCCGGG AAACCGGAGCTAATACCGGATAGTTCCTTGAACCGCATGGT TCAAGGATGAAAGACGGTTTCGGCTGTCACTTACAGATGG ACCCGCGGCGCATTAGCTAGTTGGTGAGGTAACGGCTCACC AAGGCGACGATGCGTAGCCGACCTGAGAGGGTGATCGGCC ACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGC AGCAGTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGG AGCAACGCCGCGTGAGTGATGAAGGTTTTCGGATCGTAAA GCTCTGTTGTTAGGGAAGAACAAGTGCAAGAGTAACTGCTT GCACCATGACGGTACCTAACCAGAAAGCCACGGCTAACTA CGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTG TCCGGAATTATTGGACGTAAAGGACTCGCAGGCGGTTTCTA AAGTCTGATAGAAAGCCCCCGCCTCAACCGGGGAGGGTCA TGGGAAACTGGGAAACTTGAGTGCAGAAGAGAGAGTGGAA TTCCACGTGTAGCGGTGAAATGCGTAGAGATGTGGAGGAA CACCAGTGGCGAAAGCGACTCTCTGGTCTGTAACTGACGCT GAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGATACCC TGGTAGTCCACGCCGTAAACGATGAGTGCTAAGTGTTAGG GGGTTTCCGCCCCTTATGCTGCAGCTAACGCATTAAACACT CCGCCTGGGGAGTACGGTCGCAGACTGAAACTCAAAGGAT TGACGGGGGCCGCAAAAACCGTGGAGCATGTGGTTTAATT CGAAGCAACGCGAAGAACCTTACCAGGTCTTGACATCCTCT GACAACCCTAGAGATAGGGCTTTCCCTTCGGGGACAGAGT GACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGAT GTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGATCTTAG TTGCCAGCATTCAGTTGGGCACTCTAAGGTGACTGCCGGTG ACAAACCGGAGGAAGGTGGGGATGACGTCAAATCATCATG CCCCTTATGACCTGGGCTACACACGTGCTACAATGGACAGA ACAAAGGGCTGCGAGACCGCAAGGTTTAGCCAATCCCACA AATCTGTTCTCAGTTCGGATCGCAGTCTGCAACTCGACTGC GTGAAGCTGGAATCGCTAGTAATCGCGGATCAGCATGCCG CGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCAC ACCACGAGAGTTTGCAACACCCGAAGTCGGTGAGGTAACC TTTATGGAGCCAGCCGCCGAAGGTGGGGCAGATGATTGGG GTGAAGTCGTATCATGAGGCATTGGG
NAC002	GGCATGCGGCTGCTATAATGCAGTCGAGCGAACTGATTAG AAGCTTGCTGATTGAAAGATGGTTTCGGCTATCACTTACAG ATGGGCCCGCGGTGCATTAGCTAGTTGGTGAGGTAACGGCT CACCAAGGCAACGATGCATAGCCGACCTGAGAGGGTGATC GGCCACACTGGAACTGAGAACCGCCAGATCCTACCGAAGC GCAGCAAGGAATCTCCGCATGGACGAAGTCGGACGGAGCA CGCCGCGTGAGTGATGAAGCTTTCGGTCGTAAAACTTGTTG TTAGGGAAGAACAAGTACGAGAGTACTGCTCGTACTTGAC GGTACCTAACCAGAAAGCCACGGCTAACTACGTGCCAGCA

	GCGCGGTAATACGTAGGTGGCAAGCGTATCCGGAATATGG CCGTAAAGCGCGCGCAGGCGGTTTCTTAAGTCTGATGTGAA AGCCCACGGCTCAACCGTGGAGGGTCATTGGAAACTGGGG AACTTGAGTGCAGAAGAGAAAAGCGGAATTCCACGTGTAG CGGTGAAATGCGTAGAGATGTGGAGGAACACCAGTGGCGA AGGCGGCTTTTTGGTCTGTAACTGACGCTGAGGCGCGAAAG CGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACG CCGTAAACGATGAGTGCTAAGTGTTAGAGGGTTTCCGCCCT TTAGTGCTGCAGCTAACGCATTAAGCACTCCGCCTGGGGAG TACGGTCGCAAGACTGAAACTCAAAGGAATTGACGGGGGC CCGCACAGCGGTGGAGCATGTGATTTAATTCGAAGCAACG CGAAGAACCTTACCAGGTCTTGACATCCTCTGACAACTCTA GAGATAGAGCGTTCCCCTTCGGGGACGAGTGACAGGTGTG CATGATGTCGTCAGCTCGTGTCTGAGATGGTGGGTTAGTCC CGCAACGAGCGCAACCTGGATCTAGTGCCAGCAATTAGCT GCACTCTAAGTGACTGCGTGACAACGAGAGTGGGATGACG TCATCTCATGCCCCTTATGACCTGGCTAAACACGTGCTACA TGGATGGTACAAAGGGCTGCAAGACCGCGAGGTCAAGCCA ATCCCATAAAACCATTCTCAGTTCGGATTGTAGGCTGCAAC TCGCCTACATGAAGCTGGAATCGCTAGTAATCGCGGATCAG CATGCCGCGGTGAATACGTTCCCGGGCCTTGTACACACCGC CCGTCACACCACGAGAGTTTGTAACACCCGAAGTCGGTGG AGTAACCGTAAGGAGCTAGCCGCCTAAGGTGGGACAGATG ATGGGGTGAAGTCGTAGATGAGCCTAGGGG
NAC007	GTGAGTGCGGCAGCTATAATGCAGTCGAGCGGATTTTGGG AAACCTAGTTTCCCCTTAATCAGCGGCGGACGGGTGAATAA CACGTGGGTAACCTGCCTGTAACACTGGGATAACTCCGGG AAACCGGGGCTAATACCGGATAACTCATTTCCTCGCATGAA GAAGTGTTGAAAGGTGGCTTTCCTCTACCACTTACAGATGG ACCCGCGGCGCATTATCTATTTGGTGAGGTAACGGCTCACC AGGGCAACGATGCATACCCGACCTGAGAGGGTGATCGGCC ACACTGGGACTGACAAACGGCCCACATCCTACGGGCAGCA ATAGGATCTTCCGCAATGACGAGTCTGACAGAACAACGCC GCGTGAGGGAAAAAGTTTTCGATCGTAAACTCTGTTGTTAG GGAAGAACAAGTGCCGTTCGAATAGGGCGGCACCTTGACG GTACCTACCAGAAAGCACGGCTAACTACGTGCCAACAGCC GCGGTAATACATAGGTGGCAAGCGTTGTCCGGATTTATTGC GCGTAAAGCGCGCGCAGGTGGTTTCTTAACTCTGATGTGAA AGCCCACGGCTCAACCGTGGAGGGTCATTGGAAACTGGAG AACTTGAGTGCAGAAGAGGAAAGTGGAATTCCAAGTGTAG CGGTGAAATGCATAGATATTTGGAGGAACACCAGTGGCGA ACGAGACTTTCTGGTCTGTAACTGACACTGACGCGCGAAAG CGTGGGGAGCAAACAAGATTAGATACCCTGATACTCCACG CCATAAACGATGAGTGCTAAGTGTTAGAGGGTTTCCGCCCT TTAGTGCTGCAGCTAACGCAATAAGCACTCCCCCTGGGGAG TACGGTCGCAAGACTGAAACTCAAAGGAATTGACGGGGGC

	CCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAACCAAC GCAAAAAACCTTACCAGGTCTTGACATCCTCAGACAACCCA AGAAATAGGGCTTCCCCCTTCGGGGGACAGAGTGACAGGG GGTGCATGGATGTCCTCAGCTCGTGTCGTGAAATGGTGGGT AAAGTCCCGCAACGAGCGCAACCCTTGATCTTAGTTGCCAG CATTCAGTTGGCACTCAAGATGACTGCCGGTACAAACCGG AGGAAAAGGGGGATGACCTCAACACCATGCCTTAAGACCT GGCTCACAAGGGCTACAATGGACGGTACAAAGGGCAGCGA GACCGCGAGGTTTAGCCAATCCCATAAAACCGTTCTCAGTT CGGATTGCAGGCTGCAACTCGCCTGCATGAAGCTGGAATC GCTAGTAATCGCGGATCAGCATGCCGCGGTGAATACGTTCC CGGGCCTTGTACACACCGCCCGTCACACCACGAGAGTTTGT AACACCCGAAGTCGGTGAGGTAACCTTTTGGAGCCAGCCG CCTAAGGTGGGACAGATGATGGGGTGAAGTCGTATCATGT GGCCTACGGG
NAB002	GCATACGGGTGCTATACATGCAGTCGAGCGATGGATTAAG AGCTTGCTCTTATGAAGTTAGCGGCGGACGGGTGAGTAAC ACGTGGGTAACCTGCCCATAAGACTGGGATAACTCCGGGA AACCGGGGCTAATACCGGATAACATTTTGAACCGCATGGTT CGAAATTGAAAGGCGGCTTCGGCTGTCACTTATGGATGGAC CCGCGTCGCATTAGCTAGTTGGTGAGGTAACGGCTCACCAA GGCAACGATGCGTAGCCGACCTGAGAGGGTGATCGGCCAC ACTGGGACTGAGACACGGCCCAGACGCCTACACGAGACAG AACCAGCAAACCACCAGCAACGGACAAAACCATGACGAGC AACGCCGCGTGAATGATGAGCTCGGTCGTAAACTCTGTTGT AGAGAACAGTGCTAGTGATAAGCTGCACTGACGGTACCTA ACAGAAAGCACGCTACTACGTGCAGCAGCGCGGTAATACG TAGTGGCAAGCGTATCGGATATGGGCGTAAAGCGCGCGCA GGTGGTTTCTTAGTCTGATGTGAAAGCCACGCTCACCGTGA GGGTCATTGGAAACTGGAGACTTGAGTGCAGAAGAGAAAG TGAATTCCATGTGTAGCGGTGAAATGCGTAGAGATATGGA GGAACACCAGTGGCGAAGGCGACTTTCTGGTCTGTAACTG ACACTGAGGCGCGAAAGCGTGGGAGCAAACAGGATTAGAT ACCCTGGTAGTCCACGCCGTAAACGATGAGTGCTAAGTGTT AGAGGGTTTCCGCCCTTTAGTGCTGAAGTTAACGCATTAAG CACTCCGCCTGGGGAGTACGGCCGCAAGGCTGAACTCAAA GGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGT TTAATTCGAAGCAACGCGAAGAACCTTACCAGTCTTGACAT CCTCTGACACCCTAGAGATAGGGCTTCTCCTTCGGAGCAGA GTGACAGGTGTGCATGATGTCGTCAGCTCGTGTCGTGAGAT GTTGGTAGTCCCGCAACGAGCGCAACCCTTGAATCTAGTT GCATCATTAGTTGGCAACTCTAGTGACTGCGTGAAAACCGA GAGGTGGATGACGTCAATCTCATGCTAGGACCTGCTAACAC GTGCTACAATGGACGGTACAAAGAGCTGCAAGACCGCGAG GTGGAGCTAATTTCATAAAACCGTTTTCAGTTCGGATTGTA

	GGCTGCAACTTGCCTACATGAAGCTGGAATCGCTAGTAATC GCGGACAGCATGCCGCGGTGAATACGTTCCCGGGCCTTGTA CACCCCGCCCGTCACACCCCGAGAGTTTGTAACACCCGAAG TCGGTGGGGTAACCTTTTTGGAGCCAGCCGCCTAAGGTGGA CAGATGATGGGTGAAGTCGAGCACTGGGCTTTGGG
TSAB006	TAGAACAACTATCTCCGGGCTGCAGTCGAGCTGAATGGATTAAG AGCTTGCTCTTATGAAGTTAGCGGCGGACGGGTGAGTAACACGT GGGTAACCTGCCCATAAGACTGGGATAACTCCGGGAAACCGGGG CTAATACCGGATAACATTTTGAACCGCATGGTTCGAAATTGAAA GGCGGCTTCGGCTGTCACTTATGGATGGACCCGCGTCGCATTAGC TAGTTGGTGAGGTAACGGCTCACCAAGGCAACGATGCGTAGCCG ACCTGAGAGGTGATCGCCAACTGGAACTGAACGGCCAGATCTAC CGGAAGCAGCAGTAGGAAATCTTCGCAATGAACGAAGTCGACGA AGCAACGCCGCGTGAGTGATGAGGCTTCGGGTCGTAAACTTGTT GTTAGGGAAGAACAAGTGCTAGTGAATAAGCTGCACCTTGACGG TACCTAACCAGAAAGCACGGCTAACTACGTGCCAGCAGCCGCGG TAATACGTAGGTGGCAAGCGTTATCCGGAATTATTGGGCGTAAA GCGCGCGCAGGTGGTTTCTTAAGTCTGATGTGAAAGCCCACGGC TCAACCGTGGAGGGTCATTGGAAACTGGGAGACTTGAGTGCAGA AGAGGAAAGTGGAATTCCATGTGTAGCGGTGAAATGCGTAGAGA TATGGAGGAACACCAGTGGCGAAGGCGACTTTCTGGTCTGTAAC TGACACTGAGGCGCGAAAGCGTGGGGAGCAAACAGGATTAGAT ACCCTGGTAGTCCACGCCGTAAACGATGAGTGCTAAGTGTTAGA GGGTTTCCGCCCTTTAGTGCTGAAGTTAACGCATTAAGCACTCCG CCTGGGGAGTACGGCCGCAAGGCTGAAACTCAAAGGAATTGACG GGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAA CGCGAAGAACCTTACCAGGTCTTGACATCCTCTGACACCCTAGA GATAGGGCTTCTCCTTCGGGAGCAGAGTGACAGGTGGTGCATGA TTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAGTCCCGCACGA GCGCACCCTTGATCTAGTGCCATCAATTAGTGGCACTCTAAGTGA CTGCCGTGACAACGAGAAGGTGGGATGACGTCAATCATCATGCC CTTAGACTGGCTACACTGCTACATGGACGGTACAAAGAGCCGC AACACCGCGAGGTGGAGCTAATCTCATAAAACCGTTCTCAGTTC GGATTGTAGGCTGCAACTCGCCTACATGAAGCTGGAATCGCTAG TAATCGCGGATCAGCATGCCGCGGTGAATACGTTCCCGGGCCTT GTACACACCGCCCGTCACACCACGAGAGTTTGTAACACCCGAAG TCGGTGGGGTAACCTTTTTGGAGCCAGCCGCCTAAGGTGGGACA GTATGATGGTGTAAGAACGAGAGACAGACCGGTTGCCAA

Table 18. BLAST search details of the sequences producing most significant alignment of the endophytes

Isolate	Description	Max score	Total score	Query cover (%)	E value	Identity (%)	Accession no.
NAT001	*Bacillus megaterium* Q3. Complete genome	1877	24294	94	0.0	96	NZ CP010586.1
KBT001	*Alcaligenes faecalis* ZD02. Complete genome	390	1172	80	3e-105	80	NZ CP013119.1
KBT004	*Streptomyces leeuwenhoekii* C34 Complete genome	1136	6804	77	0.0	91	NZ LN831790.1
KBT006	*Bacillus pumilus* NJ-V2. Complete genome	2571	15429	98	0.0	98	NZ CP012482.1
NAC002	*Bacillus megaterium* DSM319 Complete genome	2034	22980	98	0.0	95	NC 014103.1
NAC007	*Bacillus licheniformis* BL- 09 Complete genome	1897	13226	95	0.0	91	NZ CP010524.1
NAB002	*Bacillus thuringiensis* Al Hakam Complete genome	2058	28699	98	0.0	93	NC 008600.1
TSAB006	*Bacillus thuringiensis* HD 1011 Complete genome	2314	32241	96	0.0	96	NZ CP009335.1

Table. 19 Biometric observations of tomato seedling treated with the endophytes and *Piriformospora indica*

Treatments	Leaf no.	Shoot length (cm)	Fresh shoot weight (mg/plan)	Dry shoot weight (mg/plant)	Fresh root weight (mg/plant)	Dry root weight (mg/plant)
NAT001	6.00^{bc}	13.13^{bcd}	1444.11^{b}	134.86^{a}	261.77^{c}	22.88^{b}
KBT001	6.33^{ab}	14.21^{abc}	1332.25^{cd}	113.26^{c}	323.59^{b}	22.86^{b}
KBT004	6.20^{ab}	13.61^{bcd}	1383.25^{bc}	126.07^{b}	214.64^{e}	24.47^{ab}
KBT006	6.47^{a}	14.43^{ab}	1480.73^{b}	113.13^{c}	228.18^{d}	19.39^{cd}
NAT001+Pi	6.20^{ab}	12.83^{cd}	1129.82^{e}	125.54^{b}	213.95^{e}	16.27^{d}
KBT001 + Pi	6.27^{ab}	15.23^{a}	1274.99^{d}	123.20^{b}	257.56^{c}	21.91^{bc}
KBT004 + Pi	6.33^{ab}	14.45^{ab}	1764.54^{a}	114.05^{c}	332.88^{a}	26.45^{a}
KBT006 + Pi	6.40^{a}	12.60^{d}	980.89^{f}	98.04^{e}	231.93^{d}	24.15^{ab}
P.indica	6.20^{ab}	13.71^{bcd}	1128.13^{e}	105.29^{d}	145.42^{f}	17.64^{d}
Control	5.73^{c}	9.00^{e}	715.12^{g}	71.71^{f}	121.66^{g}	11.97^{e}
CD(0.05)	0.39	1.42	99.25	4.74	5.15	3.32
Treatment Vs control	S	S	S	S	S	S

Means followed by a common letter are not significantly different at 5% level of significance.

Table . 20 Percentage root colonization by *Piriformospora indica* in tomato

Treatments	colonization (%) *
Piriformospora indica alone	81.81(9.10)
NAT001+Pi	84.01(9.22)
KBT001+Pi	83.09(9.17)
KBT004+Pi	83.01(9.22)
KBT006+Pi	81.08(9.06)
CD(0.05)	NS

*Values in the parenthesis are square root transformed values.

Table 21. Biometric observations of chilli seedling treated with the endophytes and *Piriformospora indica*

Isolates	No . of leaves	Shoot length (cm)	Fresh shoot weight (mg/plant)	Dry shoot weight (mg/plant)	Fresh root weight (mg/plant)	Dry root weight (mg/plant)
NAC002	5.66[a]	11.93[a]	855.20[a]	87.97[a]	87.03[b]	15.49[b]
NAC007	5.00[b]	11.20[b]	685.92[d]	65.51[b]	90.82[a]	13.74[c]
NAC002+Pi	5.00[b]	10.00[c]	749.03[b]	66.19[b]	33.33[e]	18.92[a]
NAC007+Pi	5.00[b]	10.06[c]	651.60[e]	58.78[c]	45.98[d]	18.60[a]
P.indica	5.00[b]	9.76[c]	703.32[c]	61.98[bc]	59.17[c]	17.86[a]
Control	4.33[c]	7.60[d]	317.12[f]	30.78[d]	28.37[f]	8.16[d]
CD(0.05)	0.59	0.68	6.64	4.62	3.25	1.56
Treatment Vs control	S	S	S	S	S	S

Means followed by a common letter are not significantly different at 5% level of significance.

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Table 22. Percentage root colonization by *Piriformospora indica* in chilli

Treatments	Colonization (%) *
Piriformospora indica	42.03(6.56)
NAC002+Pi	40.99(6.48)
NAC007+Pi	38.81(6.31)
CD (0.05)	NS

*Values in the parenthesis are square root transformed values.

Table. 23 Biometric observations of brinjal seedling treated with the endophytes and *Piriformospora indica*

Treatments	Leaf no.	Shoot length (cm)	Fresh shoot weight (mg/plant)	Dry shoot weight (mg/plant)	Fresh root weight (mg/plant)	Dry root weight (mg/plant)
TSAB006	2.93	6.23^{ab}	741.29^{d}	64.84^{a}	69.90^{c}	7.61^{a}
NAB002	3.33	6.74^{a}	844.27^{a}	61.06^{b}	83.03^{a}	7.71^{a}
TSAB006+Pi	3.20	6.27^{ab}	701.28^{e}	61.58^{b}	62.24^{d}	6.79^{a}
NAB002+Pi	3.00	5.89^{b}	762.79^{c}	60.80^{b}	74.58^{b}	7.72^{a}
P.indica	3.27	6.95^{a}	828.01^{b}	61.98^{b}	62.96^{d}	6.67^{a}
Control	2.80	5.79^{b}	494.90^{f}	34.64^{c}	46.02^{e}	3.34^{b}
CD (0.05)	NS	0.733	13.465	2.116	3.458	1.404
Treatment Vs control	S	S	S	S	S	S

Means followed by a common letter are not significantly different at 5% level of significance.

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Table . 24 Percentage root colonization by *Piriformospora indica* in brinjal

Treatments	Colonization (%) *
Piriformospora indica alone	42.03(6.56)
NAB002+ Pi	44.83(6.77)
TSAB006+ Pi	41.77(6.54)
CD (0.05)	NS

*Values in the parenthesis are square root transformed values.

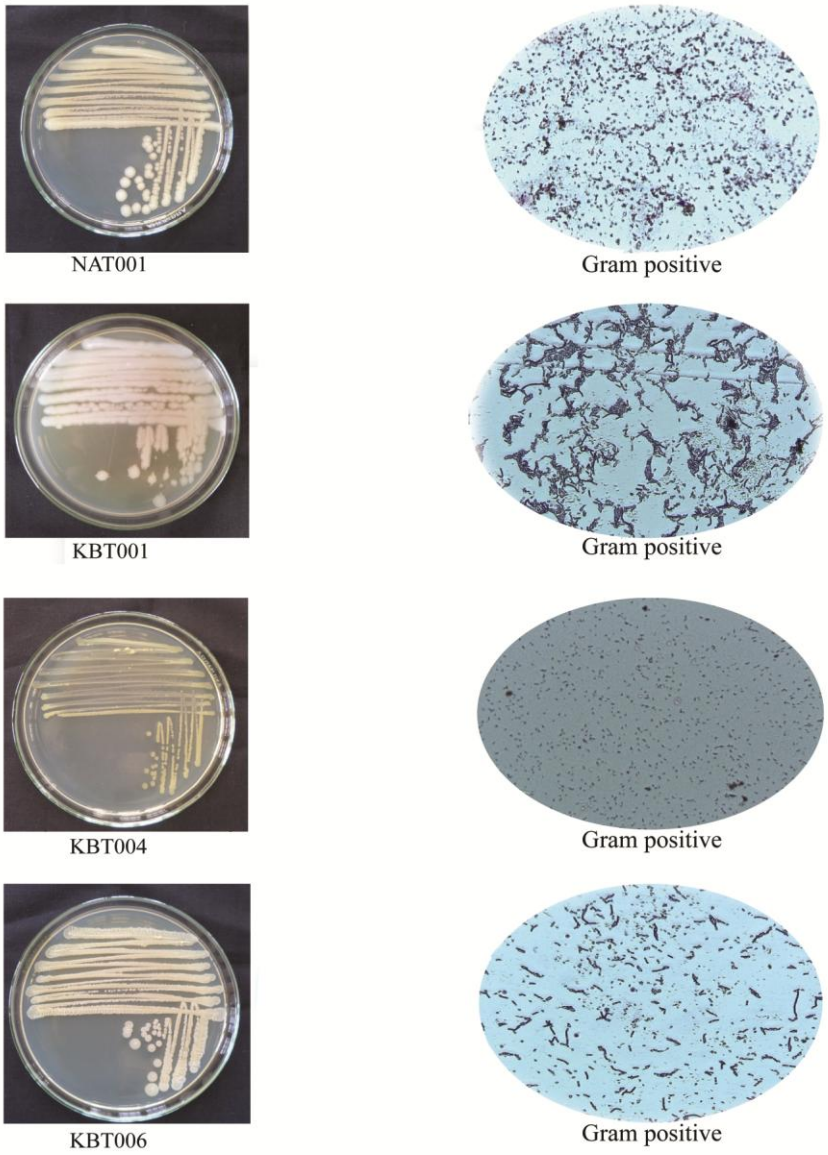


Plate 11. Colony morphology and Gram's reaction of endophytes from tomato.

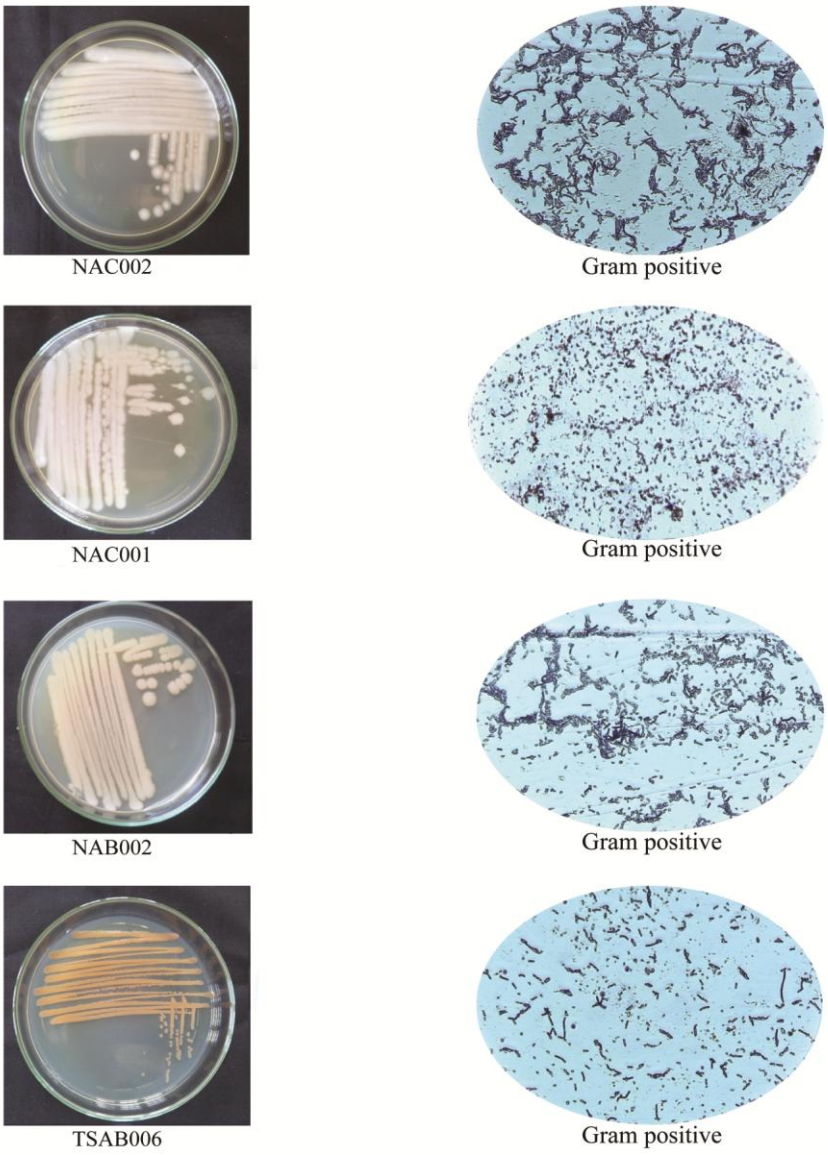


Plate 12. Colony morphology and Gram's reaction of endophytes from chilli and brinjal.

C-un inoculated control T_1-NAT001 T_2- KBT001 T_3- KBT004 T_4- KBT006 T_5- NAT001+Pi T_6- KBT001+ Pi T_7- KBT004 + Pi T_8-KBT006 + Pi T_9- *P. indica*

Plate 13. Endophytes are treated for growth promotion in tomato seedlings.

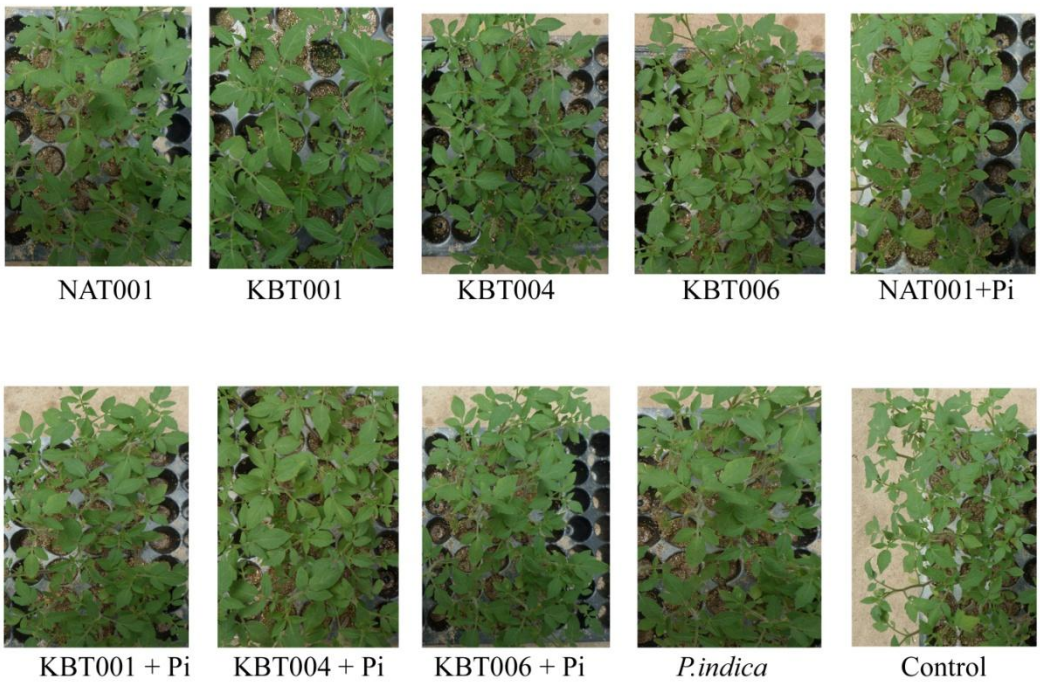
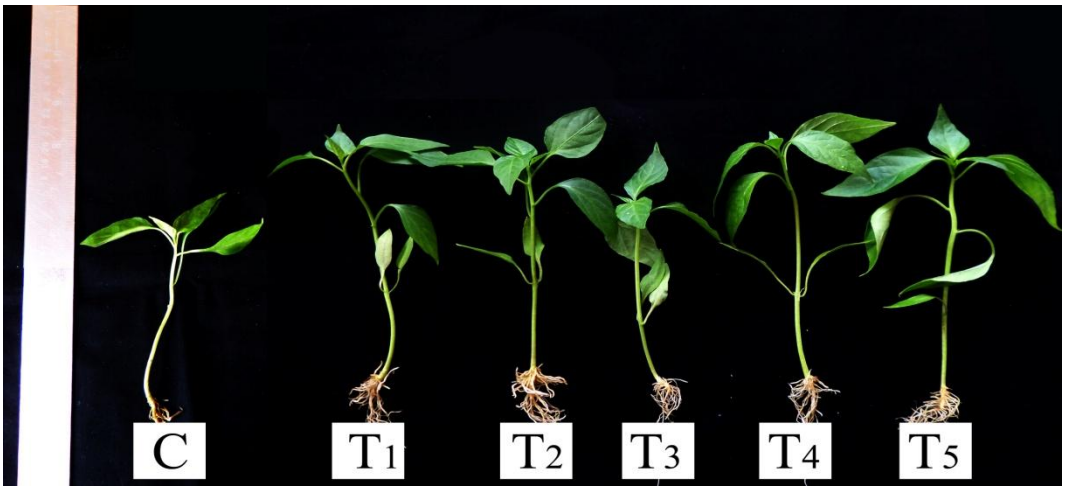
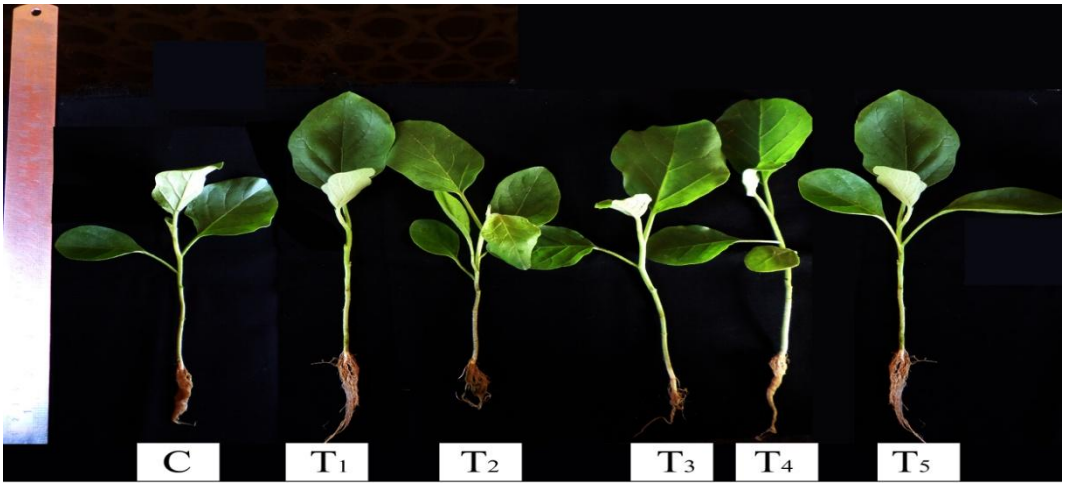


Plate 14. Endophytes are treated for growth promotion in tomato seedlings.



Endophytes are treated for growth promotion in chilli seedlings

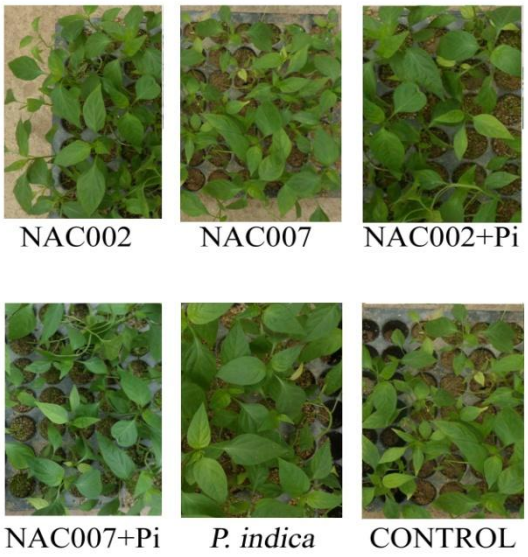
C- un inoculated control T_1- NAC002 T_2- NAC007 T_3- NAC002 + Pi T_4- NAC007 + Pi T_5- *P. indica*



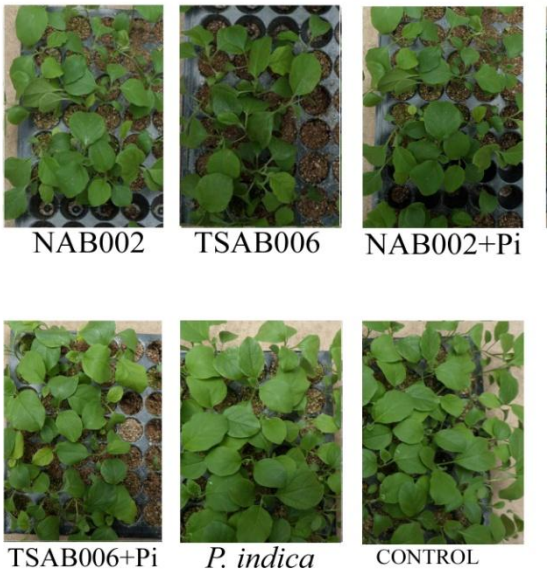
Endophytes are treated for growth promotion in brinjal seedlings

C- un inoculated control T_1- NAB002 T_2- TSAB006 T_3- NAB002 + Pi T_4- TSAB006 + Pi T_5 - *P. indica*

Plate 15. Endophytes are treated for growth promotion in chilli and brinjal seedlings.

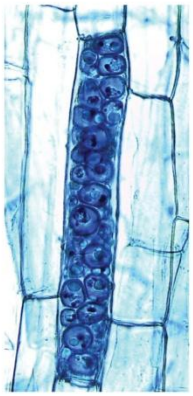


Endophytes are treated for growth promotion on chilli seedlings

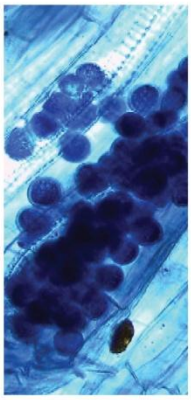


Endophytes are treated for growth promotion on brinjal seedlings

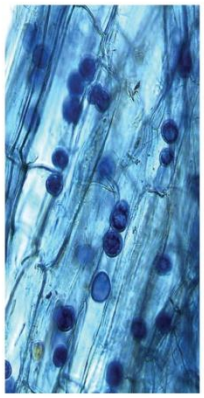
Plate 16. Endophytes are treated for growth promotion on chilli and brinjal seedlings.



Piriformospora indica

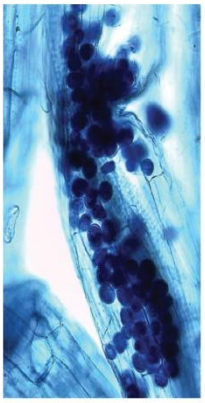


NAT001+Pi



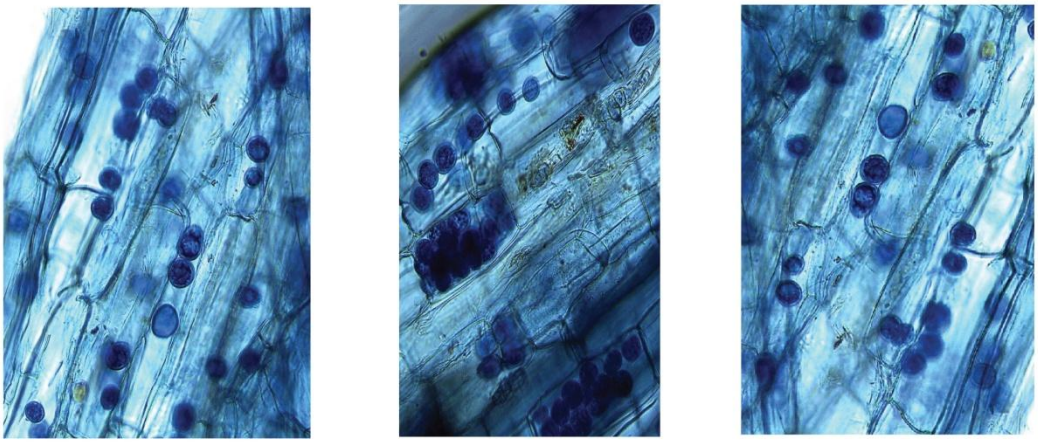
KBT001+Pi

KBT004+Pi

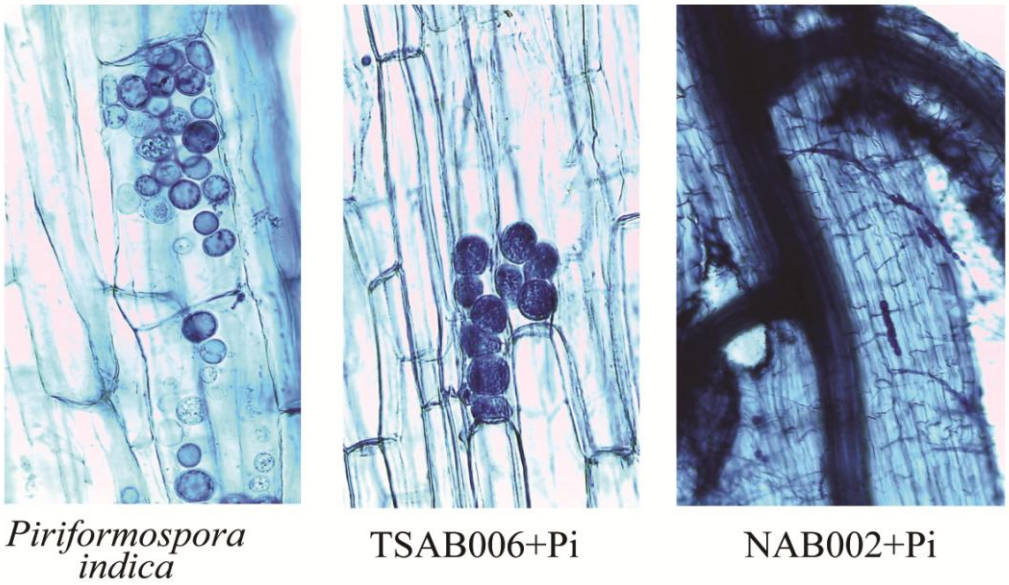


KBT006+Pi

Plate 17. Colonization by *P. indica* in tomato.



Colonization by *P. indica* in chilli.



Colonization by *P. indica* in brinjal.

Plate 18. Colonization by *P. indica* in chilli and brinjal.

Discussion

5 DISCUSSION

Use of transplants to establish vegetable crops in the field is an accepted practice all over the world. Researchers have focused on ways to produce transplants that meet mechanization requirements, survive field establishment, and contribute to plant health that could affect yield of plants (Damato and Trotta, 2000; de Grazia *et al*, 2002; Russo 2004). Transplants allow the grower to establish near perfect stands, optimal spacing and uniform physiological plant age during transplanting (Vavrina, 1998). Pro-tray seedlings enable less transplanting shock and quicker re-establishment. Russo (2005) developed a system in which chilli transplants were grown using conventional methods.

Plant growth promoting rhizobacteria are universal mutualists of higher plants, which enhance the adaptative potential of their hosts through a number of mechanisms such as the fixation of molecular nitrogen, the mobilization of recalcitrant soil nutrients and the synthesis of phytohormones, and control of phytopathogens (Van Peer and Schippers, 1989; Lugtenberg *et al*., 1991; Weller and Thomashow, 1994).

Induced systemic resistance (ISR) has been reported as one of the mechanisms by which PGPR reduce plant disease, through the manipulation of the host plant's physical and biochemical properties (Pieterse *et al*., 2002). PGPR elicited-ISR has been demonstrated in many plant species, including Arabidopsis sp., bean, carnation, cucumber, radish, tobacco, tomato etc. (Van Loon *et al*., 1998).

Plant growth-promoting rhizobacteria when grown in association with the host plants can stimulate the growth of the host (Kloepper and Schroth, 1981) Application for rhizobacteria in transplant production increases plant growth and reduce disease (Kloepper *et al*., 2004). Direct plant growth promotion by

microbes is based on improved nutrient acquisition and hormonal stimulation (Gabriele Berg, 2009). In organic system, addition of beneficial bacteria enhanced tomato yield and quality comparable with a conventional production system (Rippy *et al*., 2004)

Endophytes promote plant growth and yield, suppress pathogens, may helpto remove contaminants, solubilize phosphate, or contribute assimilable nitrogen to plants. (Rosenblueth and Martínez-Romero, 2006). Endophytic bacteria in a single plant host are not restricted to a single species but comprise several genera and species. A group of endophytes likely have established mutualistic relationship with the host by promoting plant growth and by inducing resistance against biotic and abiotic stressors (Podolich *et al*., 2007; Ryan *et al*., 2008; Yang *et al*., 2009).

Endophytic bacteria induce growth promotion and wilt disease suppression in oilseed rape and tomato (Nejad and Johnson, 2000). Plant growth promoting bacteria can interact synergistically with mycorrhizal fungi to increase root colonization by both nodulation of roots and amount of nutrients available to plants (Suresh and Bagyaraj, 2002). *Bacillus subtillis* when added to transplant mixes, seedling vigour of chilli, tomato and cucurbits were improved (Kokalis-Burelle *et al*., 1999).

Combined application of the biological product LS213 with *Bacillus*, *Pseudomonas* or Chryseobacterium for growth promotion and biological control of soil-borne diseases in chilli and tomato (Domenech *et al*., 2006).

In the present study endophytic bacteria from roots of vigorously growing seedlings of tomato, chilli and brinjal were isolated by trituration after surface sanitization. This result was in agreement with findings of Amaresan *et al*. (2012) that 87 endophytic bacteria were isolated from tomato and chilli plants. Similar result was reported by Achari and Ramesh (2014) that 167 isolates were obtained from the Xylem of Eggplant, chilli, and *S. torvum*.

15 bacterial isolates were obtained from tomato, out of which 11 were Gram positive. Similarly 14 and 13 isolates were obtained from chilli and brinjal respectively, out of which 9 and 11 were Gram positive. Bacterial isolates were subjected to a preliminary screening on their respective hosts for plant growth promotion.

Seedling vigour was assessed under green house condition in portrays using sterile planting medium. Six endophytes from tomato namely NAT001, NAT004, KBT001, KBT004, KBT006 and KBT011were selected based on the biometric observations like shoot length, fresh shoot weight, dry shoot weight, fresh root weight, dry root weight. The highest mean plant height was observed in plants treated with NAT004 followed by KBT003, KBT006 and KBT011 (Figure 1). The best treatment KBT006 found to have maximum fresh shoot weight and was on par with KBT010 (Figure 2). Plants treated with KBT001 yielded maximum dry shoot weight per plant followed by KBT005 (Figure 3). Plants treated with NAT004 yielded maximum fresh root weight followed by NAT001 (Figure 4). The maximum mean root dry weight was observed in KBT001 (Figure 5). Similar result was reported by Amaresan *et al.* (2012) in tomato resulted in greater enhancement of shoot growth, as compared with the root growth and dry biomass weight.

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Similarly four endophytes from chilli namely NAC002, NAC005, NAC007and NAC010 were selected on the growth parameters . This result was in agreement with findings of Amaresan *et al*. (2012) that 87 endophytic bacteria were isolated from tomato and chilli plants. Out of which isolates BETL13, BETL9, BECS1, BECL8 and BECS7 showed plant growth promotion in terms of an increase in root and shoot length and the number of secondary roots with respect to their host. The maximum shoot length was obtained in seedling that received the endophytic treatment with NAC002 (Figure 6). Maximum mean fresh shoot weight was observed with the treatment NAC010 followed by NAC002 (Figure 7). The seeds treated with the isolate NAC010 was notably

higher in the mean dry shoot weight which was on par with the treatment NAC002 followed by NAC007 (Figure 8). NAC010 was found to be statistically superior in fresh and dry root weight (Figure 9 and 10). Similar result was reported by Amaresan *et al.* (2012) in tomato significant increase in the root and shoot biomass was also observed in endophyte applied plants.

Five endophytes from brinjal were selected (NAB002, NAB005, NAB007, TSAB002 and TSAB006) based on the biometric observations . The highest mean plant height of was observed in NAB007 followed by TSAB002 (Figure 11). The best treatment NAB001 found to have a highest mean fresh shoot weight and was statistically superior over all other treatments followed by NAB002 and TSAB006 (Figure 12). Plants treated with NAB001 yielded maximum dry shoot weight per plant which was followed by NAB002 and TSAB006 (Figure 13). Plants treated with NAB004 yielded maximum fresh and dry root weight which was on par with NAB002 (Figure 14 and 15). *Pseudomonas* spp. and *Serratia* spp increased shoot dry weight of tomato and oilseed rape compared to control plants (Nejad and Johnson, 2000).

Endophytes with plant growth promoting ability selected through the preliminary screening were assessed under *in vitro* condition using dual culture plate assay for assessing the compatibility with *Piriformospora indica* (Pi).
Piriformospora indica, a member of the newly created order Sebacinales, is extremely versatile in its mycorrhizal associations and its ability to promote plant growth. Piriformospora indica was obtained from the rhizosphere soils of the woody shrubs *Prosopis juliflora* (Swartz) DC. and *Zizyphus nummularia* (Burm. fil.) Wt. & Arn. in the sandy desert soils of Rajasthan, India (Varma *et al.*,1999). *P. indica* is widely distributed as a symptomless root endophyte, and it colonizes members of bryophytes, pteridophytes, gymnosperms and angiosperms. Root colonization by *P. indica* results in an increase in plant growth, early flowering, higher seed yield, alteration in the secondary metabolites, and adaptation to

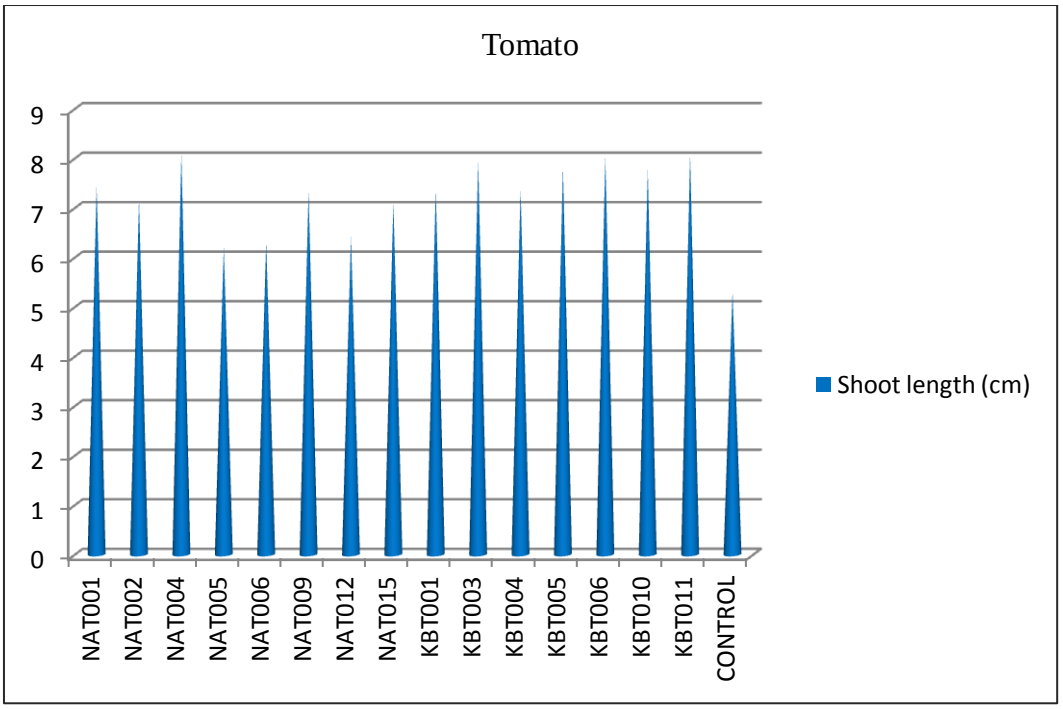


Figure 1. Effect of endophytes on shoot length of tomato seedlings.

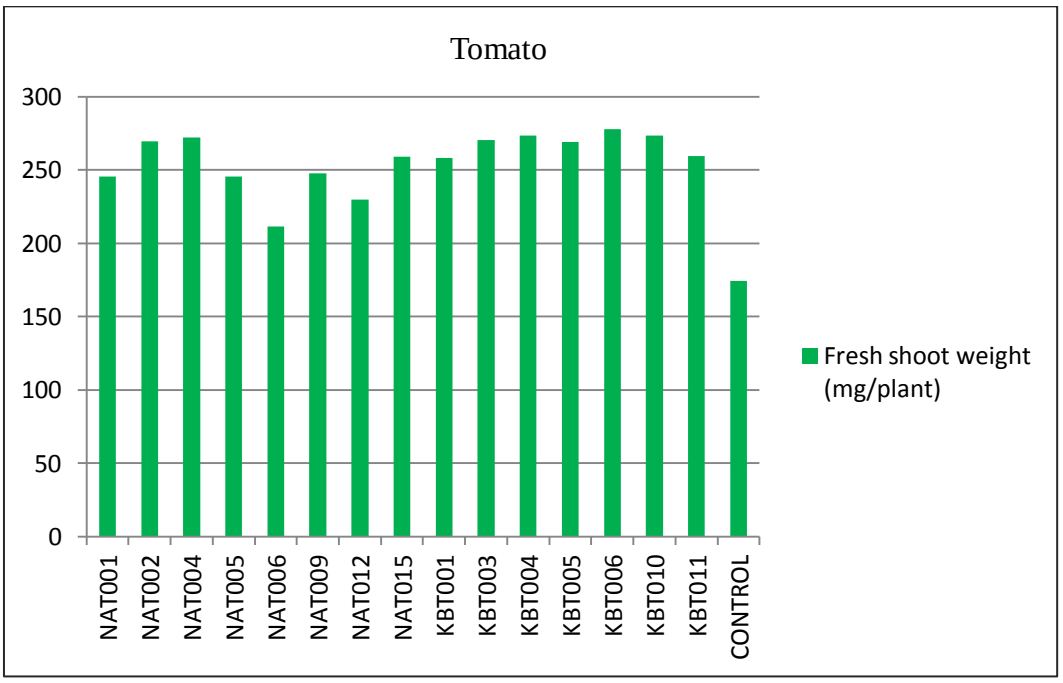


Figure 2. Effect of endophytes on fresh shoot weight of tomato seedlings.

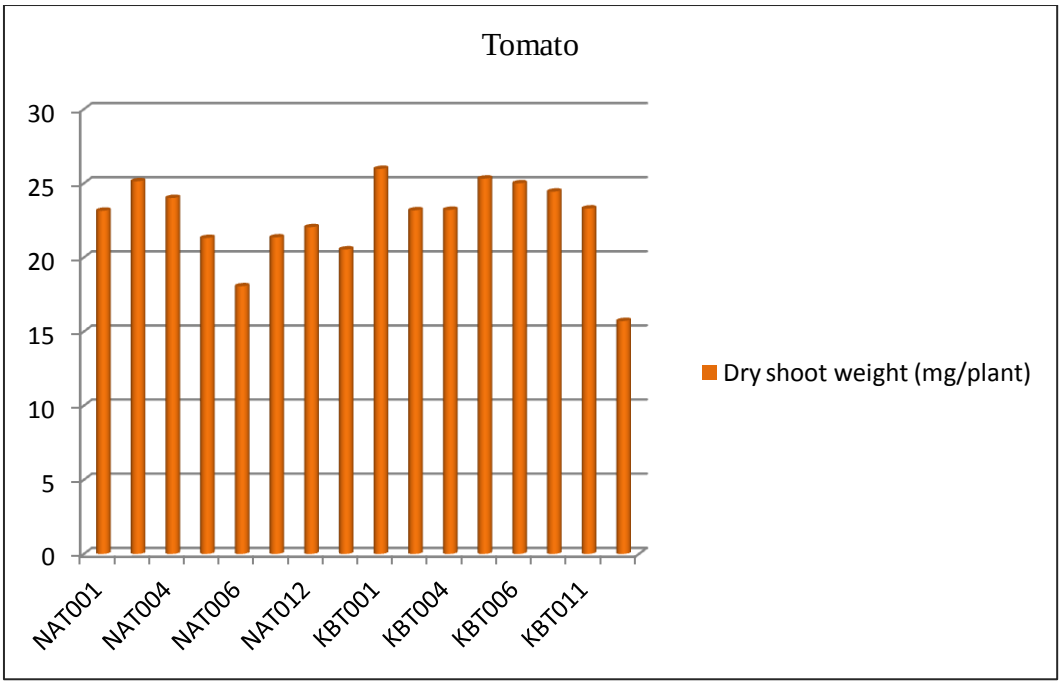


Figure 3. Effect of endophytes on dry shoot weight of tomato seedlings.

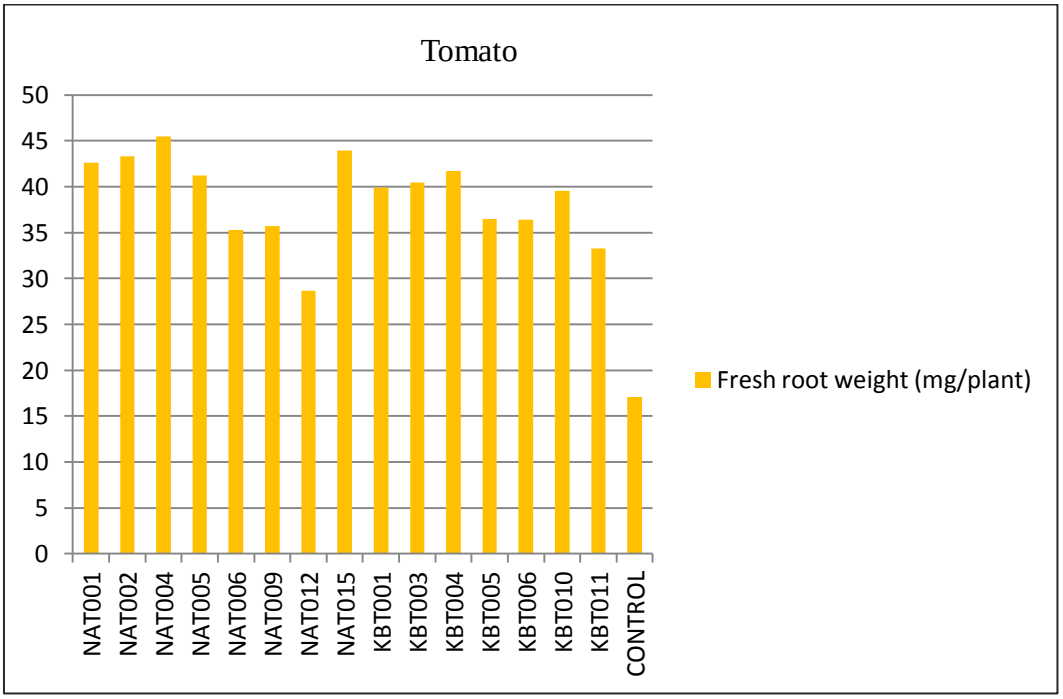


Figure 4. Effect of endophytes on fresh root weight of tomato seedlings.

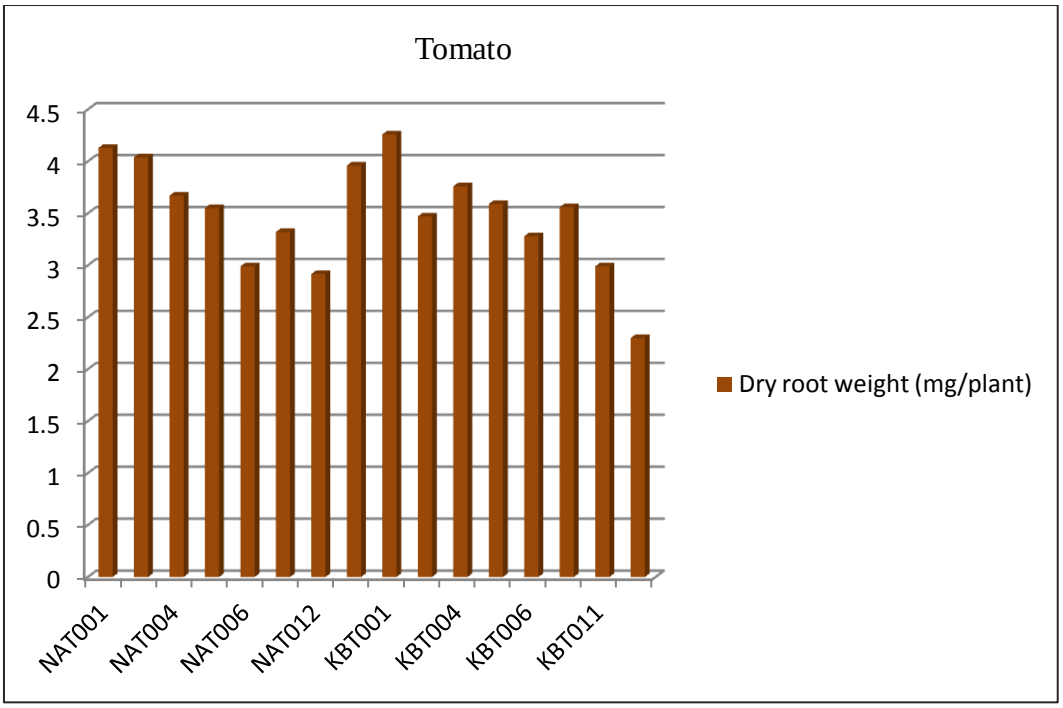


Figure 5. Effect of endophytes on dry root weight of tomato seedlings.

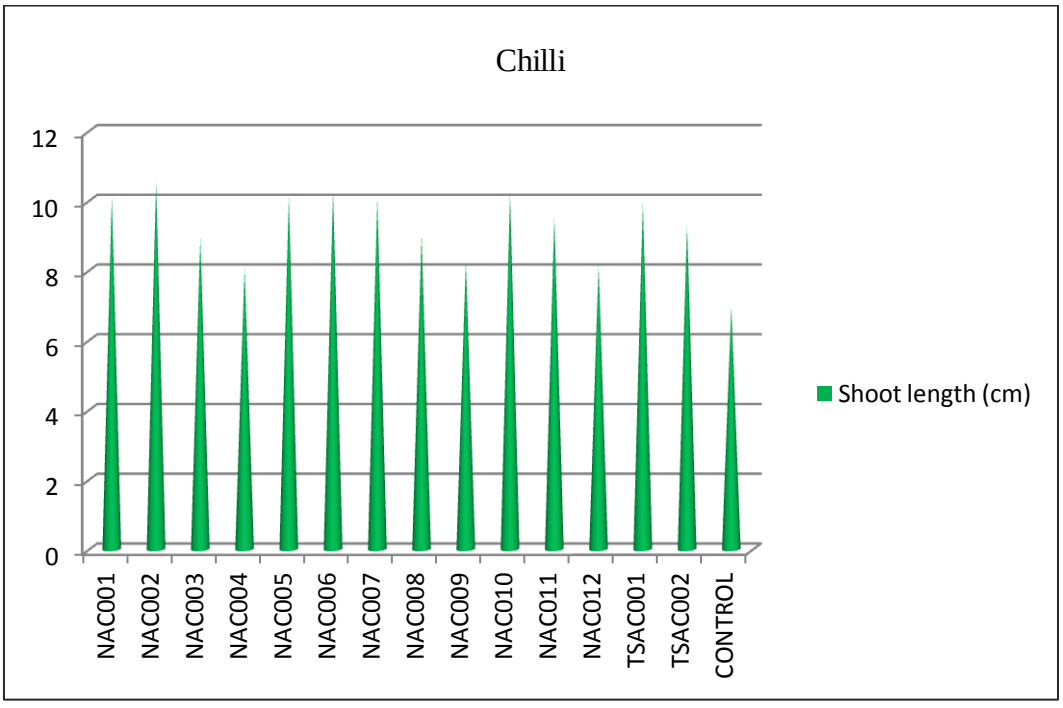


Figure 6. Effect of endophytes on shoot length of chilli seedlings

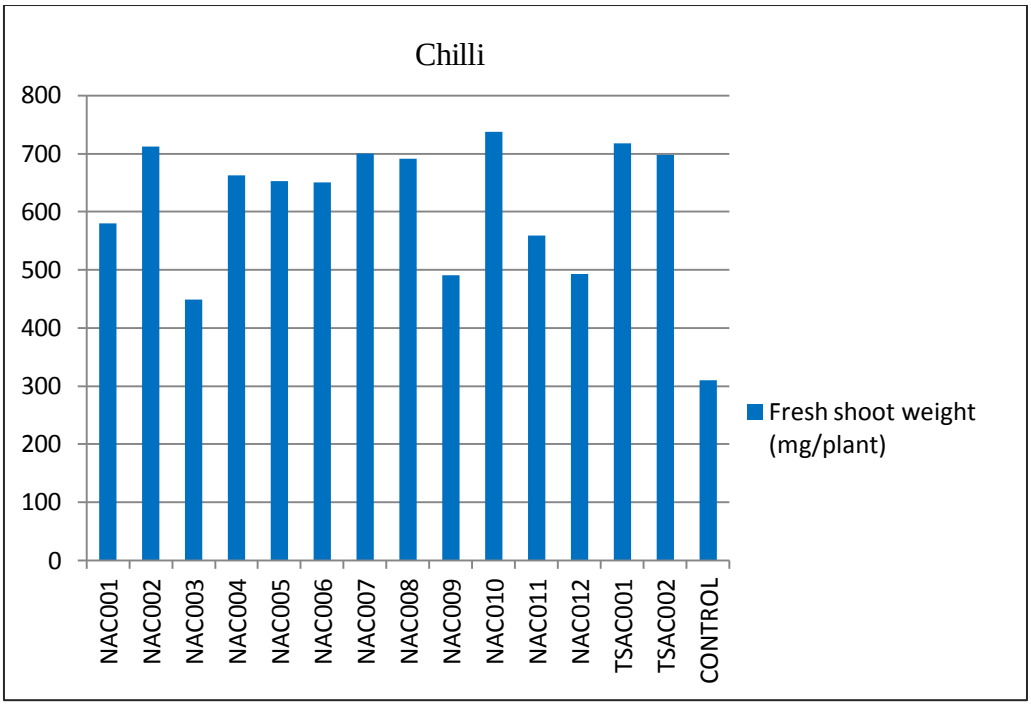


Figure 7. Effect of endophytes on fresh shoot weight of chilli seedlings.

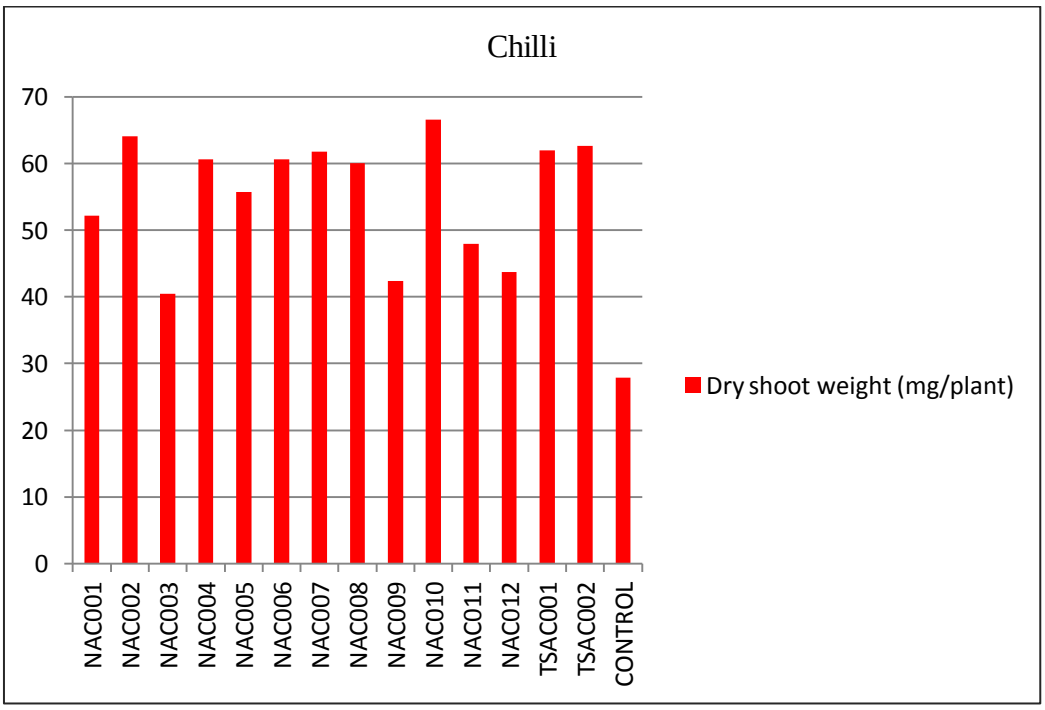


Figure 8. Effect of endophytes on dry shoot weight of chilli seedlings.

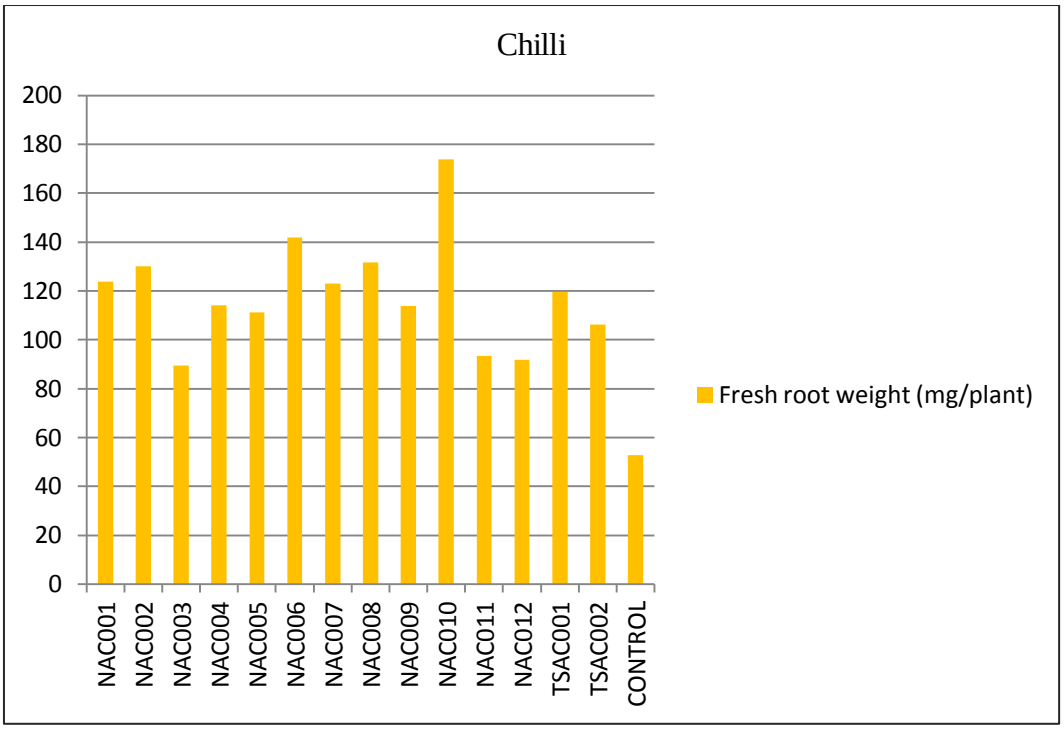


Figure 9. Effect of endophytes on freh root weight of chilli seedlings.

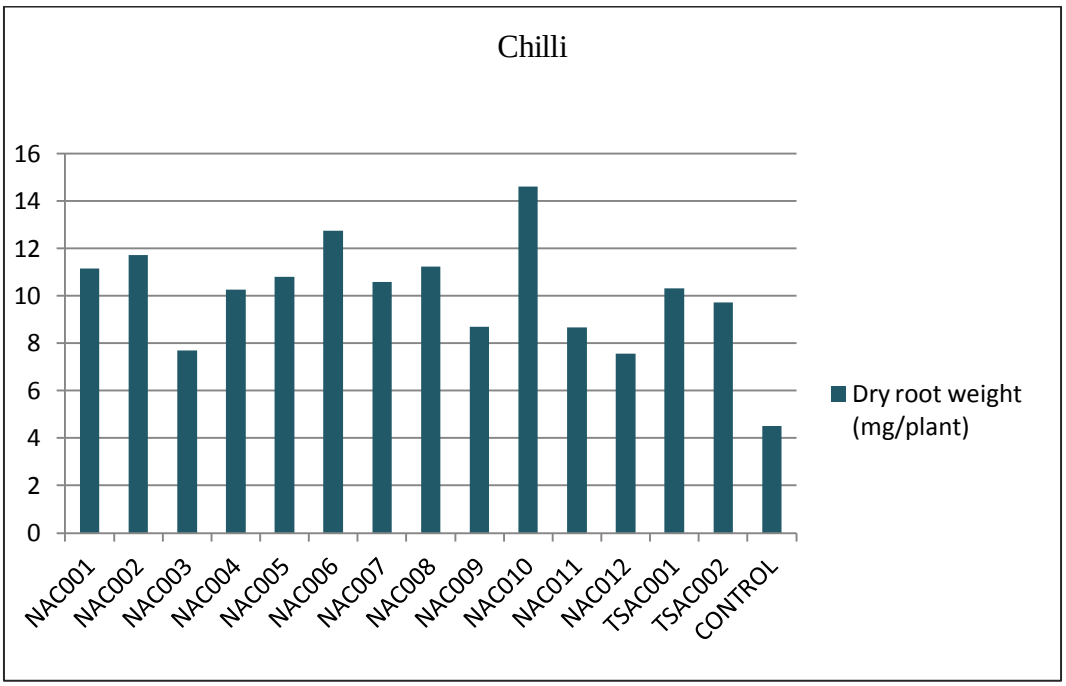


Figure 10. Effect of endophytes on dry root weight of chilli seedlings.

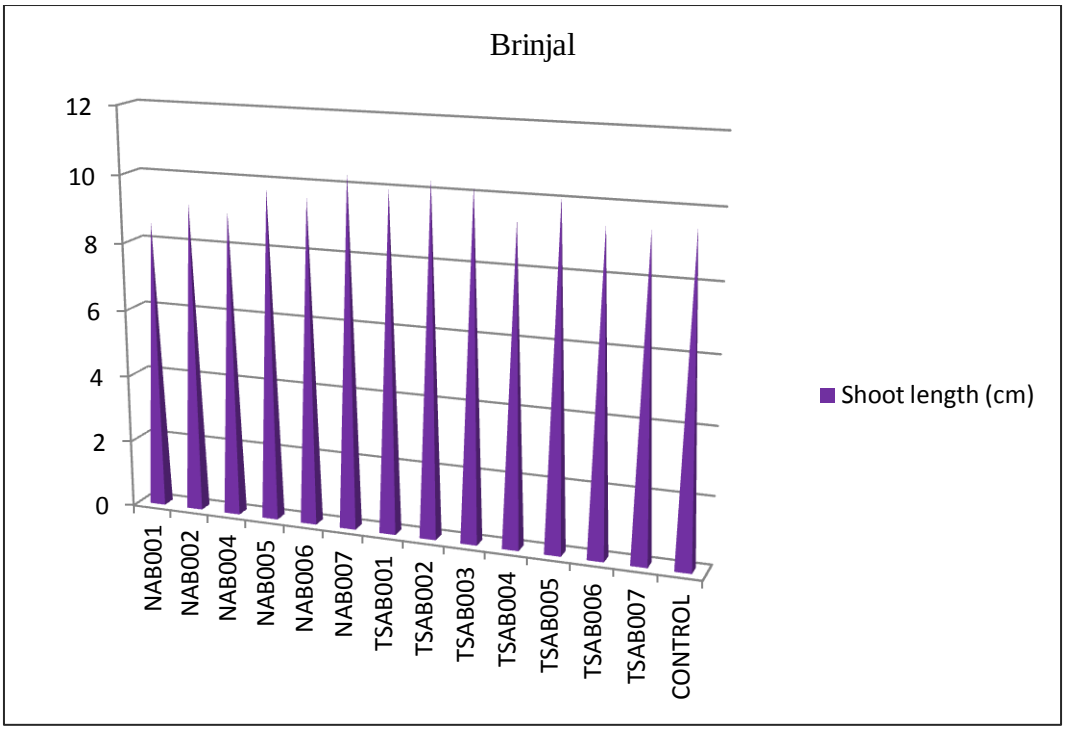


Figure 11. Effect of endophytes on shoot length of brinjal seedlings

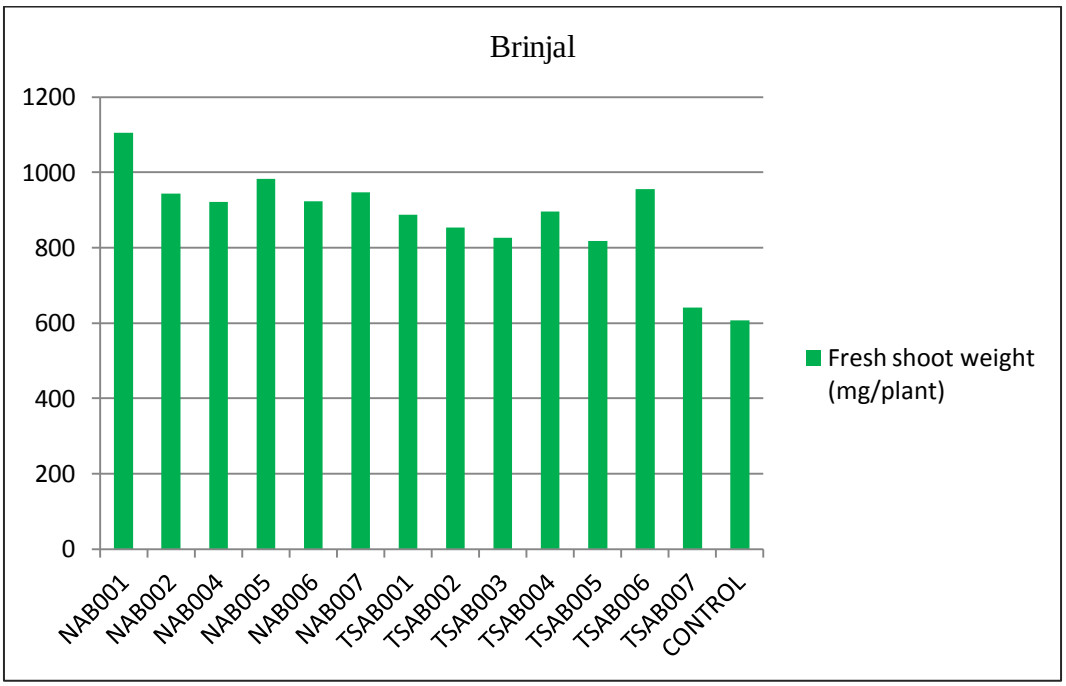


Figure 12. Effect of endophytes on fresh shoot weight of brinjal seedlings.

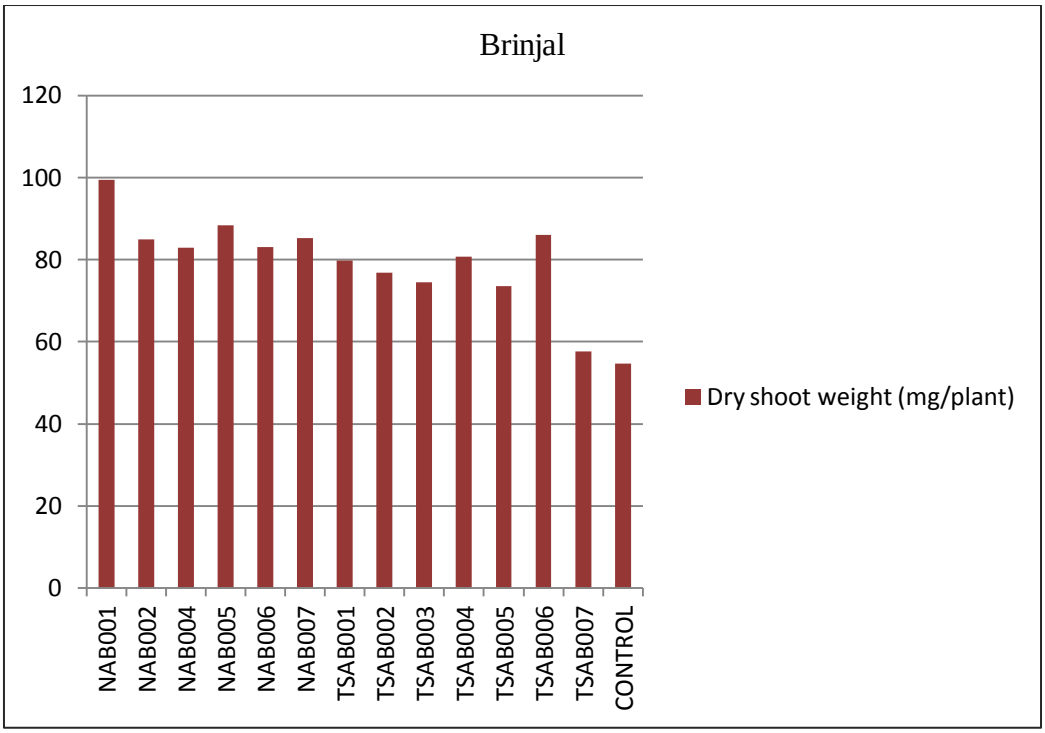


Figure 13. Effect of endophytes on dry shoot weight of brinjal seedlings.

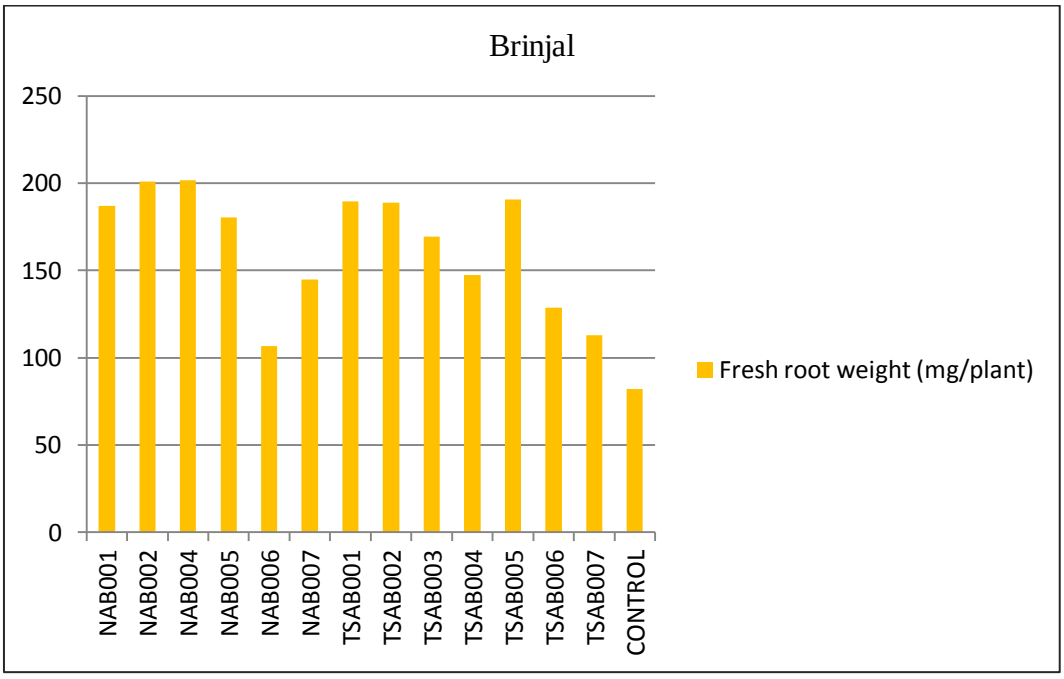


Figure 14. Effect of endophytes on fresh root weight of brinjal seedlings.

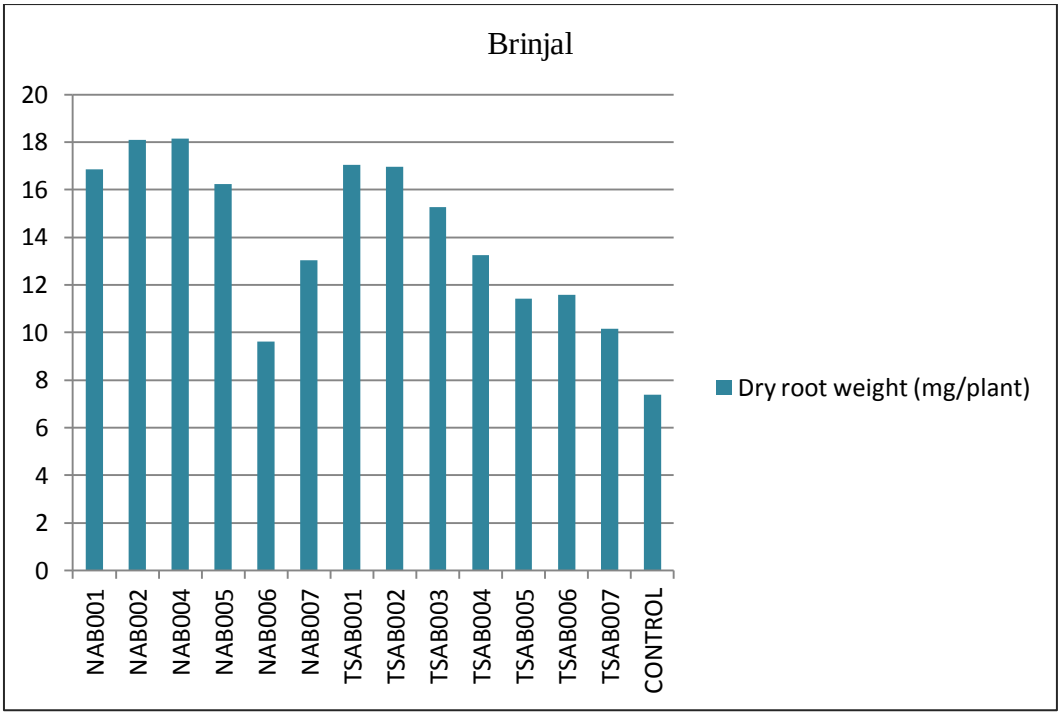


Figure 15. Effect of endophytes on dry root weight of brinjal seedlings.

abiotic and biotic stresses (Varma *et al.*,1999). *P. indica* promises to be an excellent candidate for biological hardening of micropropagated plantlets as the fungus rendered more than 90 per cent survival rate of the transferred plantlets of (*Nicotiana tabacum* L. and *Bacopa monniera* L. Wett). Studies on *P. indica* have shown fungal-mediated uptake of radiolabelled phosphorus from the medium and its translocation to the host in an energy-dependent process, evident by a sharp increase in its content in the shoot (Sahay and Varma., 1999) . The young mycelia of *P. indica* are white and almost hyaline, but inconspicuous zonations are observed in older cultures. The mycelia are mostly flat and submerged into the substratum . The hyphae are thin walled and of different diameters ranging from 0.7 to 3.5µm. The mycelia are often interwined and overlap each other. Chlamydospores are formed from thin-walled vesicles at the tips of the hyphae. The chlamydospores appear singly or in clusters and are distinctive because of their pear-shaped structure. The fungus associates with roots of various plant species, where it promotes plant growth. Hosts include the cereal crops rice, wheat, and barley as well as many Dicotyledoneae, including *Arabidopsis* (Varma *et al.*, 2012).

The plant growth-promoting activity of the endophytes were assessed based on the seedling vigour index (SVI) by the standard roll towel method. (Abdul-Baki and Anderson, 1973). Similar methodology was used by Sundaramoorty and Balabaskar (2012) during analyzing SVI by *Bacillus subtilis* in tomato. Endophytes with plant growth promoting ability selected through the preliminary screening were assessed under *in vitro* condition using dual culture plate assay for assessing the compatibility with the root endophytic fungus, *Piriformospora indica* (Pi). Eight compatible bacterial endophytes (four from tomato, two from chilli and two from brinjal) were further evaluated for their growth promoting ability individually and in combination with *P. indica*. Coinoculation with *P. indica* was found to stimulate endophytic colonization of *Pseudomonas striata* in both maize and mungbean (Singh *et al*., 2009).

The isolates were identified and of the eight isolates, six belong to the genus *Bacillus* and two were *Alcaligenes* and *Streptomyces*. This was in agreement with an earlier study of endophytes in tomato and chilli showing six isolates belongs to genus *Bacillus* and two are *Serratia*. (Amaresan *et al*., 2012).

Endophytes NAT001, KBT001, KBT004, KBT006, NAC002, NAC007, NAB002 and TSAB006 were identified as *Bacillus megaterium, Alcaligenes faecalis, Streptomyces leeuwenhoekii, Bacillus pumilus, Bacillus megaterium, Bacillus licheniformis, Bacillus thuringiensis, Bacillus thuringiensis* based on 16S rRNA sequence homology. *Bacillus cereus, Bacillus pumilus* and *Bacillus* spp. were reported in tomato and *Bacillus megaterium* was reported in chilli earlier (Amaresan *et al*., 2012). *Bacillus* sp., *Bacillus thuringiensis* and *Streptomyces* sp. were reported in chilli, brinjal and *Solanum torvum* respectively (Achari and Ramesh 2014). *Bacillus megaterium* was reported in maize, citrus plants and carrot (McInroy and Kloepper, 1995., Araujo *et al*., 2001; Surette *et al*., 2003) and *Streptomyces* spp. was reported in wheat (Coombs and Franco, 2003). *Pseudomonas* spp. and *Serratia* spp. were reported in oilseed rape and tomato (Nejad and Johnson, 2000).

Bacillus subtilis and *Bacillus sp.* were found to be compatible with *P. indica* (Varma *et al*., 2012). *Bacillus pumilus* was reported to be compatible with *P. indica* (Anith *et al.,* 2015).

The use of a combination of biocontrol agents intends to achieve better results based on the fact that each biocontrol agent may use a different mechanism to fight the pathogen, thus better results would be achieved than with a single one (de Boer *et al*., 1999).

There was no significant difference observed in the germination per cent of tomato on treating with different endophytes. The treatment KBT003 was found to be significantly superior over the rest of the treatments . Similar result

was obtained in consortial effect of endophytic and plant growth promoting rhizobacteria *P. fluorescens* and *B. subtilis* for the management of early blight of tomato incited by *Alternaria solani* (Sundaramoorthy and Balabaskar, 2012).

The maximum mean height of was observed by the combination treatment of *Alcaligenes faecalis* KBT001 with *P.indica for 28 days* (Figure 16). Similar result was reported by Amaresan *et al.* (2012) in tomato plants inoculated with tomato isolates *Bacillus pumilus* and *Bacillus sp.* The combination treatmet *Streptomyces leeuwenhoekii* KBT004 + Pi was found to be significantly superior over the other treatments in fresh shoot weight (Figure 17). Similar result was reported by Amaresan *et al.* (2012) in tomato significant increase in the root and shoot biomass was also observed in endophyte applied plants.

The maximum mean dry shoot weight was observed by treatment *Bacillus megaterium* NAT001 (Figure 18). Similar result was reported by Amaresan *et al.* (2012) in tomato resulted in greater enhancement of shoot growth, as compared with the root growth and dry biomass weight. *Pseudomonas* spp. and *Serratia* spp increased shoot dry weight of tomato and oilseed rape compared to control plants (Nejad and Johnson, 2000).

The best treatment *Streptomyces leeuwenhoekii* KBT004 + Pi found to have a mean fresh root weight which was significantly superior (Figure 19). The fresh and dry weight of shoots and roots of *Adhatoda vasica* plants inoculated with *P.indica* was higher than that of the controls (Rai and Varma, 2005).

The combination treatment *Streptomyces leeuwenhoekii* KBT004 + Pi was found to be superior which was on par with *Streptomyces leeuwenhoekii* KBT004 alone and *Bacullus pumilus* KBT006 + Pi in dry root weight (Figure 20). Anith *et al*., (2015) reported the enhanced growth of tomato seedlings inoculated with co-cultivated *P. indica* and *B. pumilus.*

The treatment *Bacillus megaterium* NAC002 was found to have maximum mean height, fresh and dry shoot weight followed by the combination

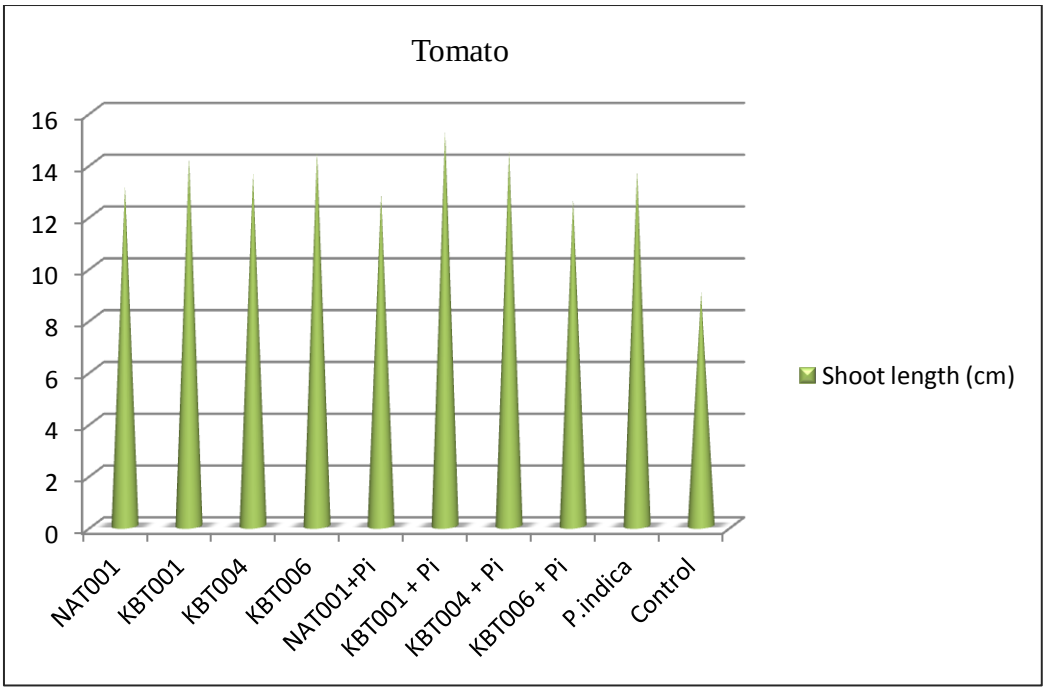


Figure. 16 Effect of endophytes on shoot length of tomato plants

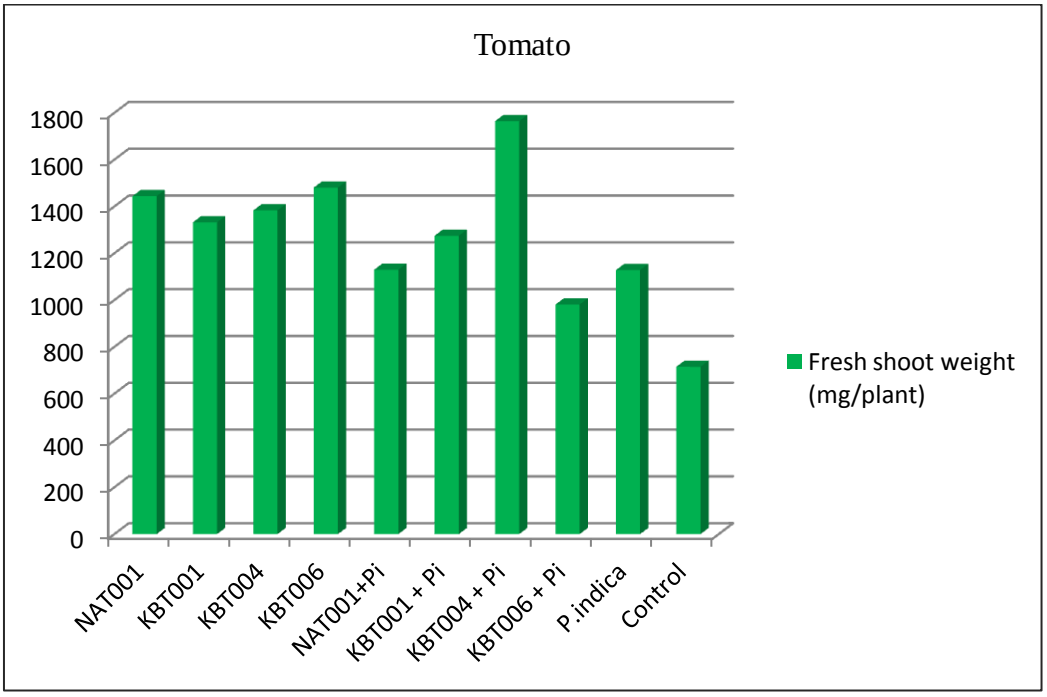


Figure 17. Effect of endophytes on fresh shoot weight of tomato plants

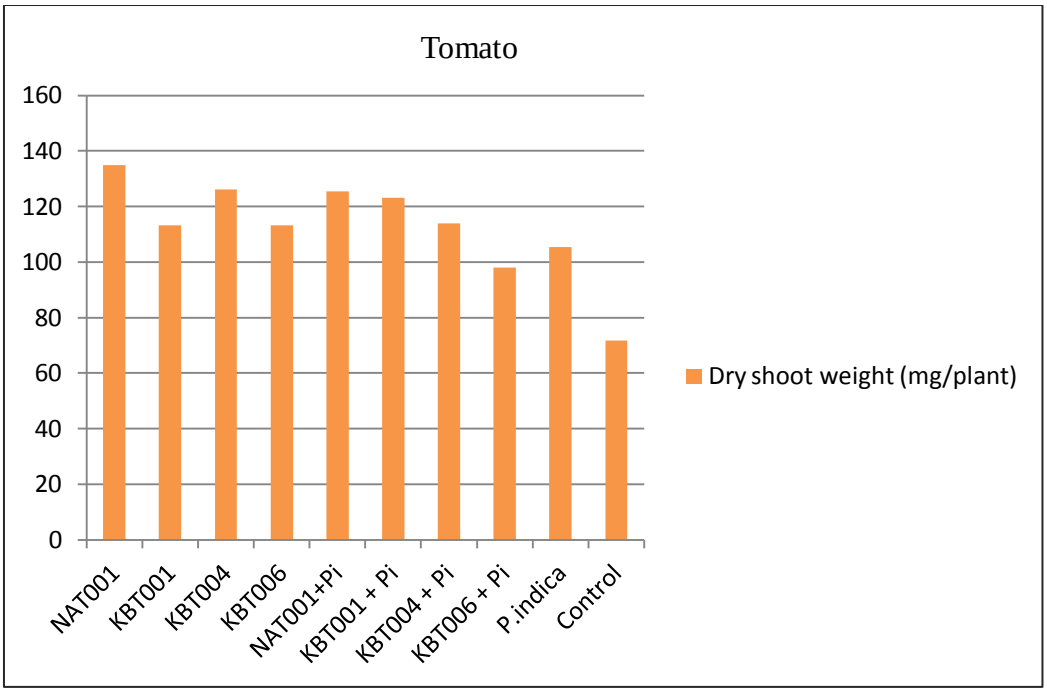


Figure 18. Effect of endophytes on dry shoot weight of tomato plants

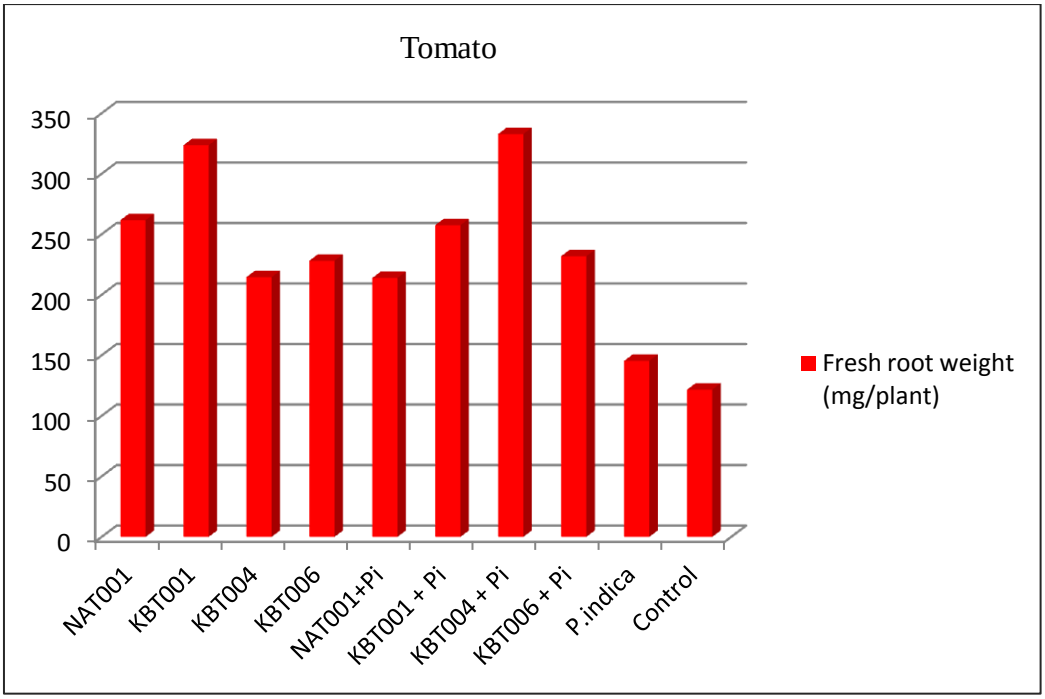


Figure 19. Effect of endophytes on fresh root weight of tomato plants

treatment of *Bacillus megaterium* NAC002 and *P. indica* (Figure 21, 22 and 23). The best treatment *Bacillus licheniformis* NAC007 found to have a mean fresh root weight of was significantly superior to all other treatments (Figure 24). The combination treatment *Bacillus megaterium* NAC002 + Pi was found to be having maximum root dry weight (Figure 25).
The mean superior height of was observed by *P.indica.* The endophyte *Bacillus thuringiensis* NAB002 alone was found to be significantly superior (Figure 26).

The endophyte NAB002 alone was found to be significantly superior in the fresh shoot weight of brinjal seedlings (Figure 27). The maximum mean dry shoot weight was observed by treatment *Bacillus thuringiensis* TSAB006 (Figure 28). The best treatment *Bacillus thuringiensis* NAB002 found to have a highest mean fresh root weight which was significantly superior (Figure 29). The combination treatment *Bacillus thuringiensis* NAB002 + Pi was found to be superior which was on par with all the other treatments in dry root weight (Figure 30). Nejad and Johnson, (2000) found that *Pseudomonas* sp. and *Serratia* sp. significantly improved seed germination, seedling length, and plant growth of oilseed rape and tomato. Rai *et al.* (2001) reported growth increase in *Withania somnifera* and *Spilanthes calva* by *P.indica. P. striata* and *P. indica* dual inoculation was reported to be an attractive and efficient biological system to augment micro/macro nutrient and water availability to the plants (Singh *et al.*, 2009). Endophytic isolates of *Pseudomonas* sp. and *Methylobacterium* sp. promoted growth of potato shoots (Pavlo *et al.*, 2011).

The percentage colonization of *P.indica* in tomato highest value was observed by the combination treatmemt with *Bacillus megatherium* NAT001 of 84.01per cent. Highest value in chilli was observed by *P.indica* alone (42.03%) and brinjal was observed by the combination treatmemt with *Bacillus thuringiensis* NAB002 of 44.83per cent. Similar result was reported endophytic colonization of the added *P. striata* following co-inoculation with *P. indica* was noticed in maize cultivars Him 129 and Mahikanchan and significant promotion

of endophytic counts of *P. striata* was recorded in the roots of P-Vishal and PS-16 following co-inoculation with *P. indica*. (Singh *et al.*, 2009).

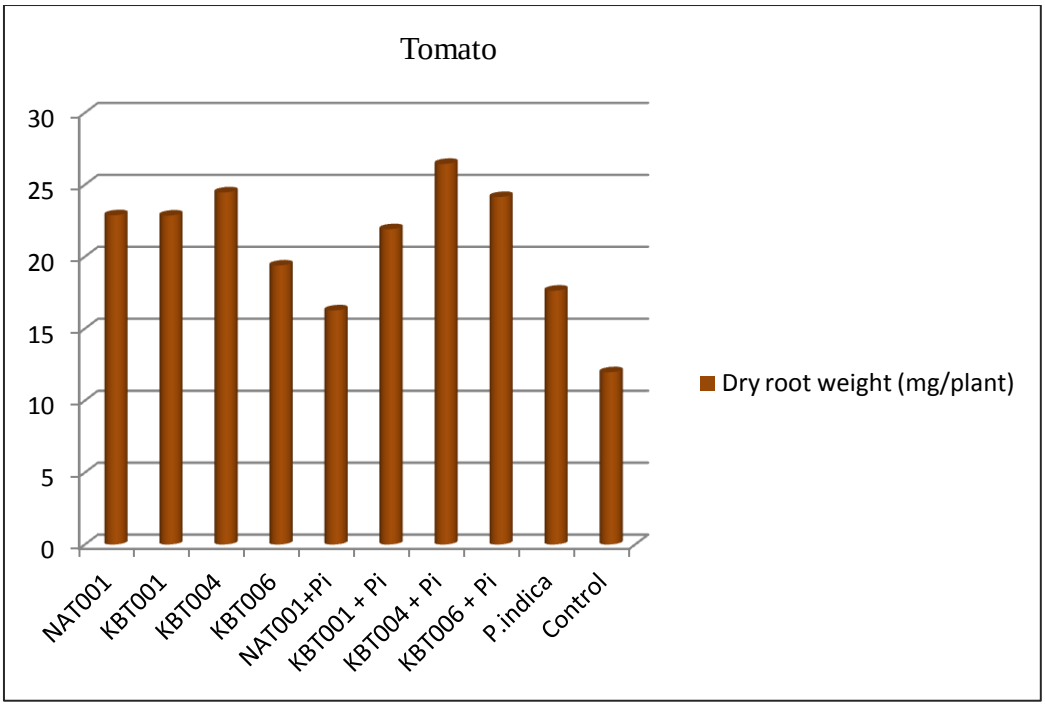


Figure 20. Effect of endophytes on dry root weight of tomato plants

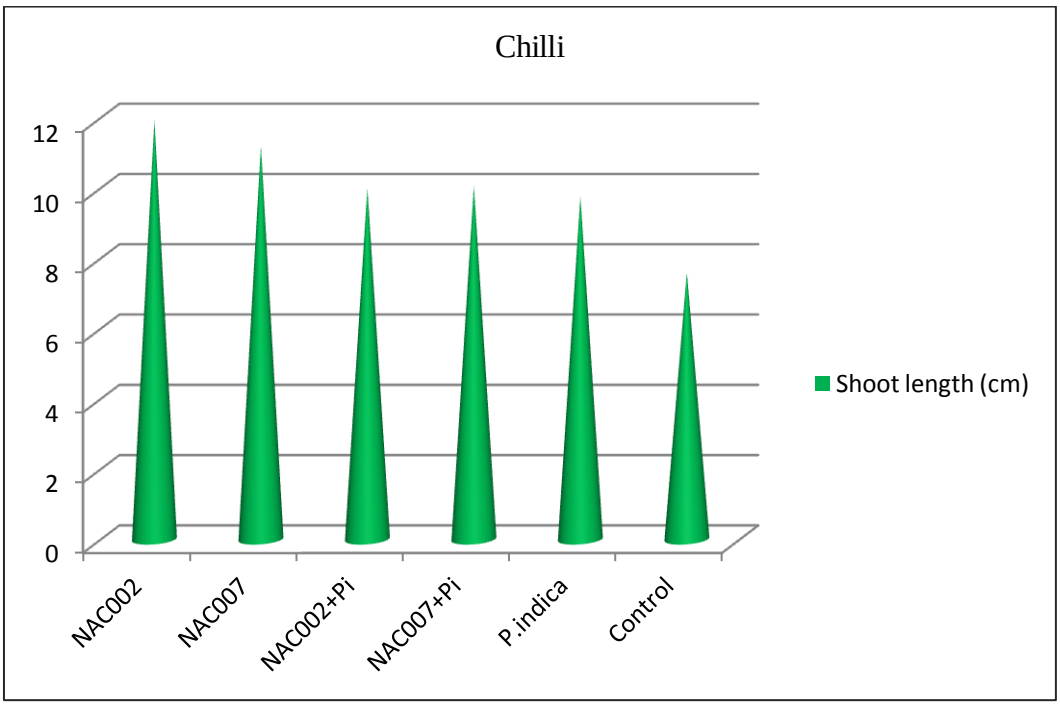


Figure. 21 Effect of endophytes on shoot length of chilli plants

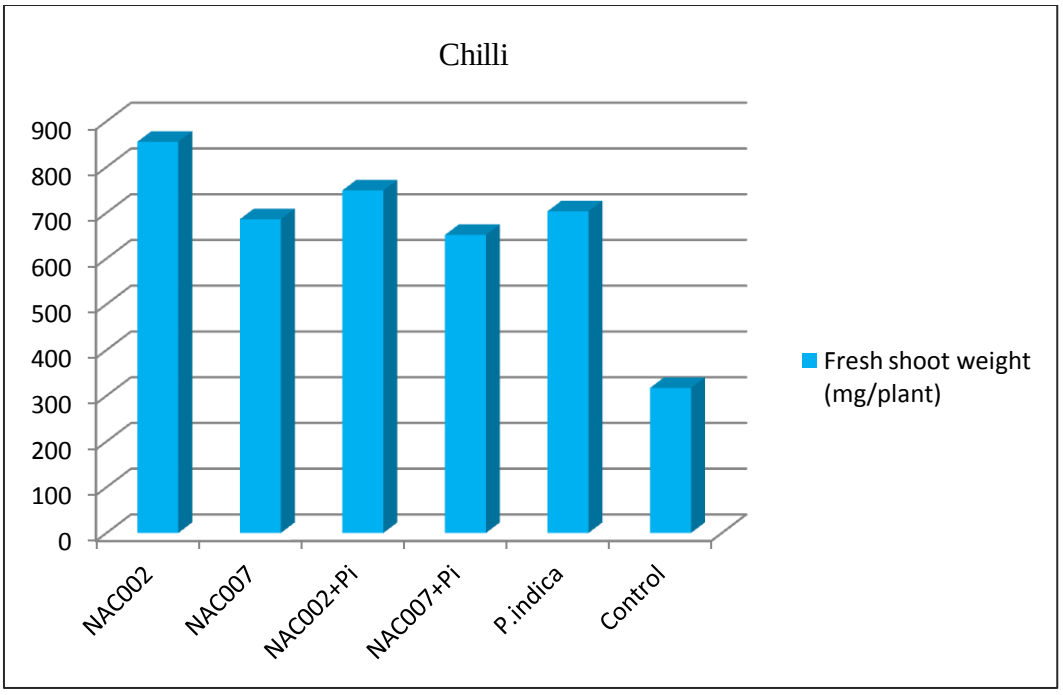


Figure 22. Effect of endophytes on fresh shoot weight of chilli plants

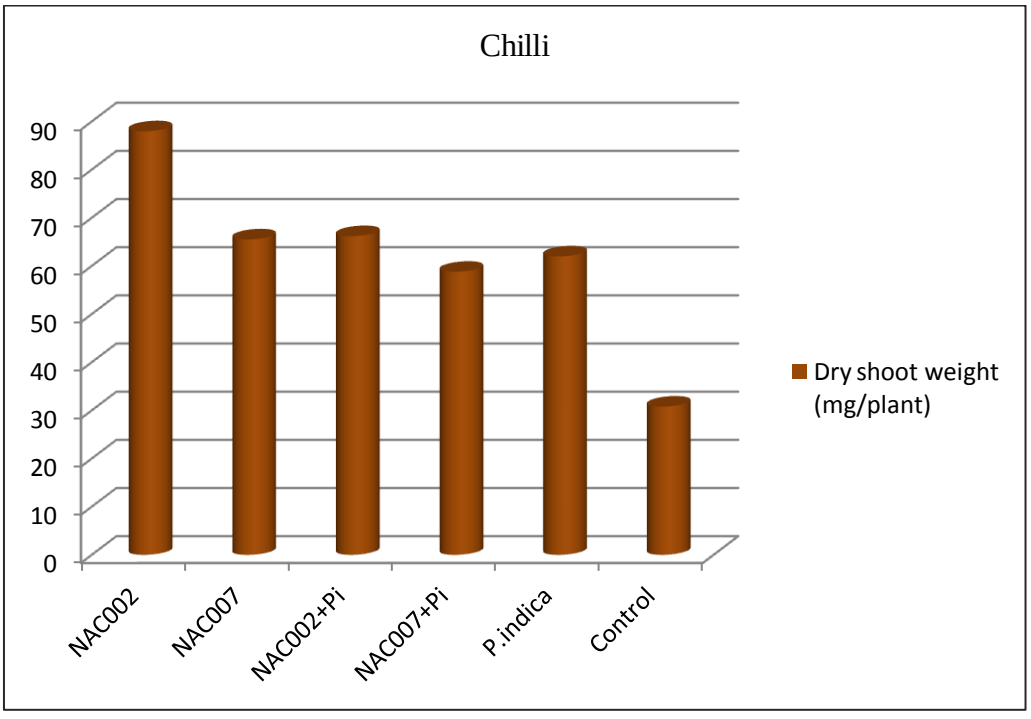


Figure 23. Effect of endophytes on dry shoot weight of chilli plants

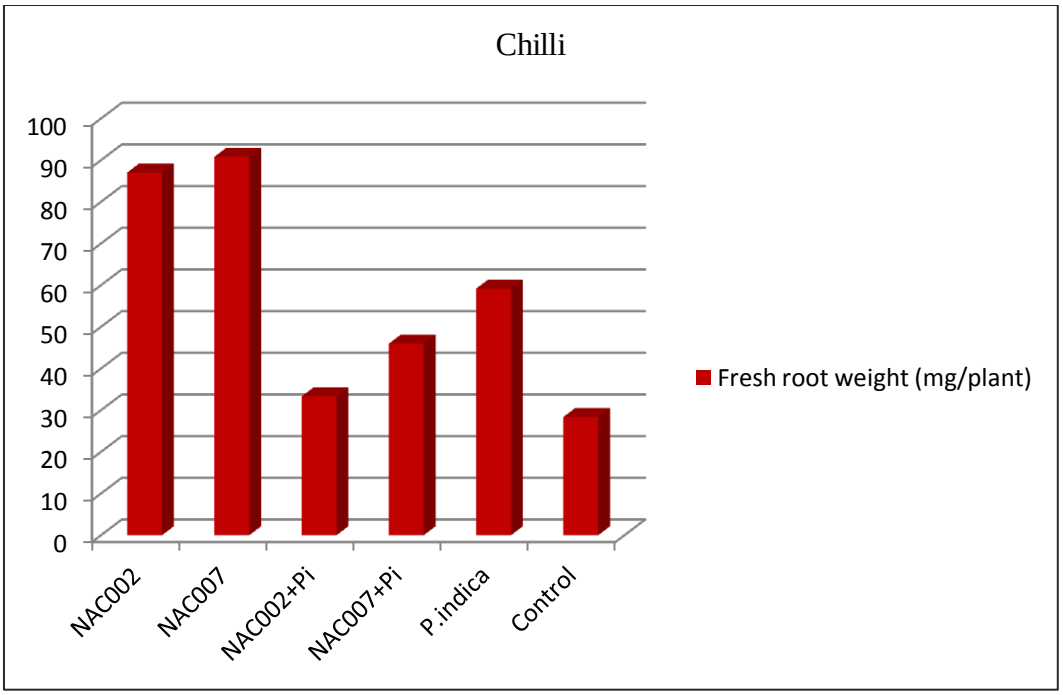


Figure 24. Effect of endophytes on fresh root weight of chilli plants

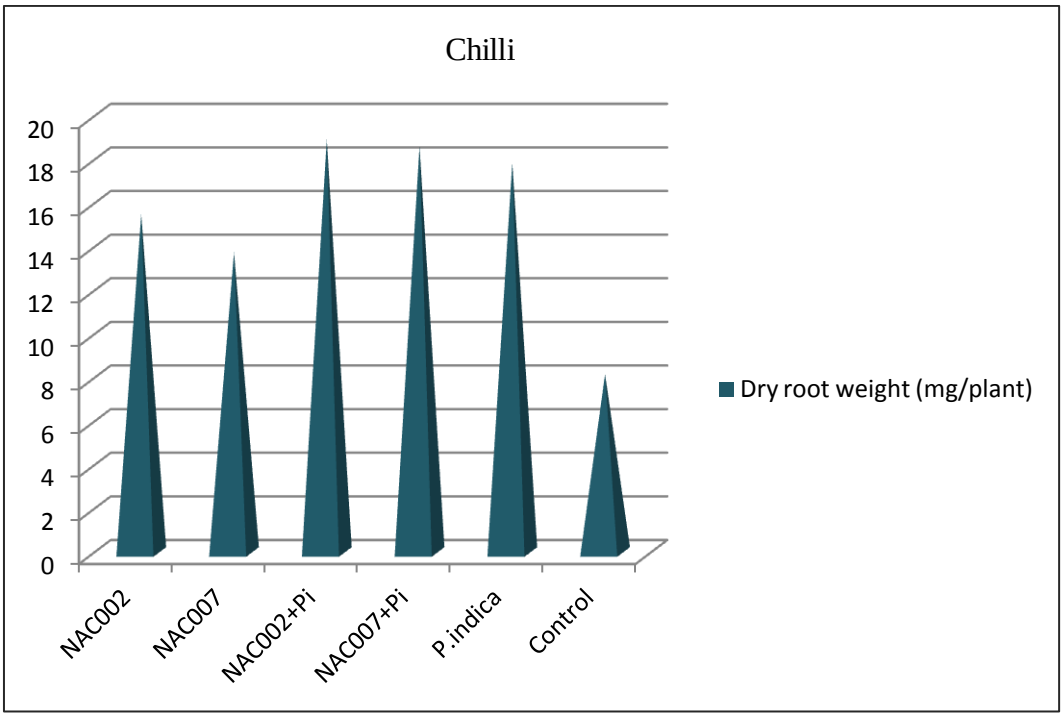


Figure 25. Effect of endophytes on dry root weight of chilli plants

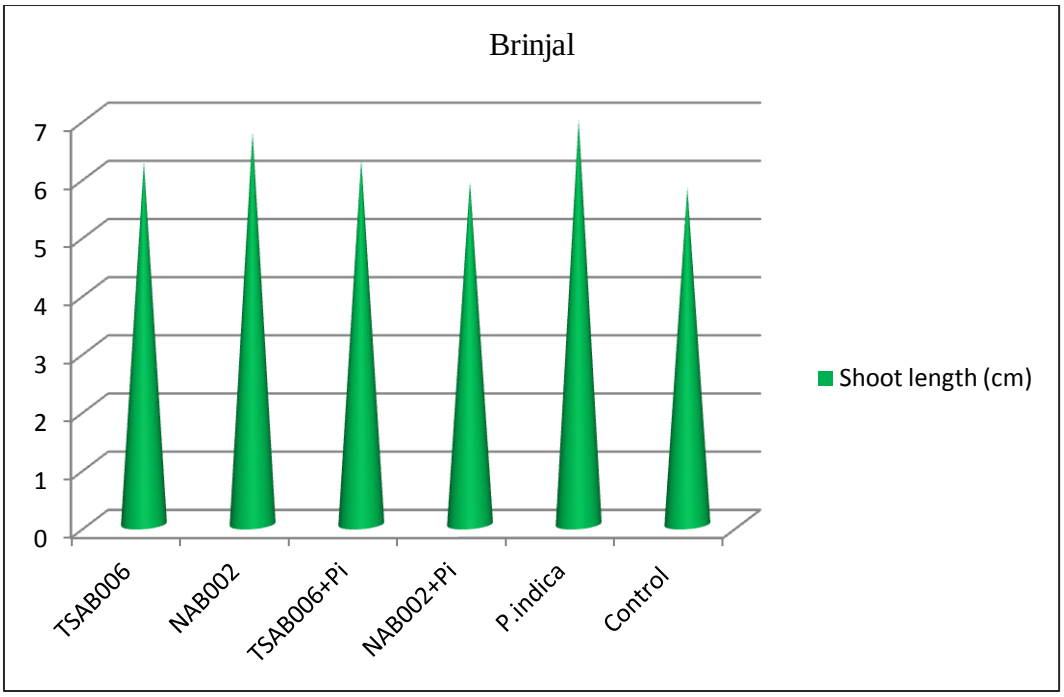


Figure. 26 Effect of endophytes on shoot length of brinjal plants

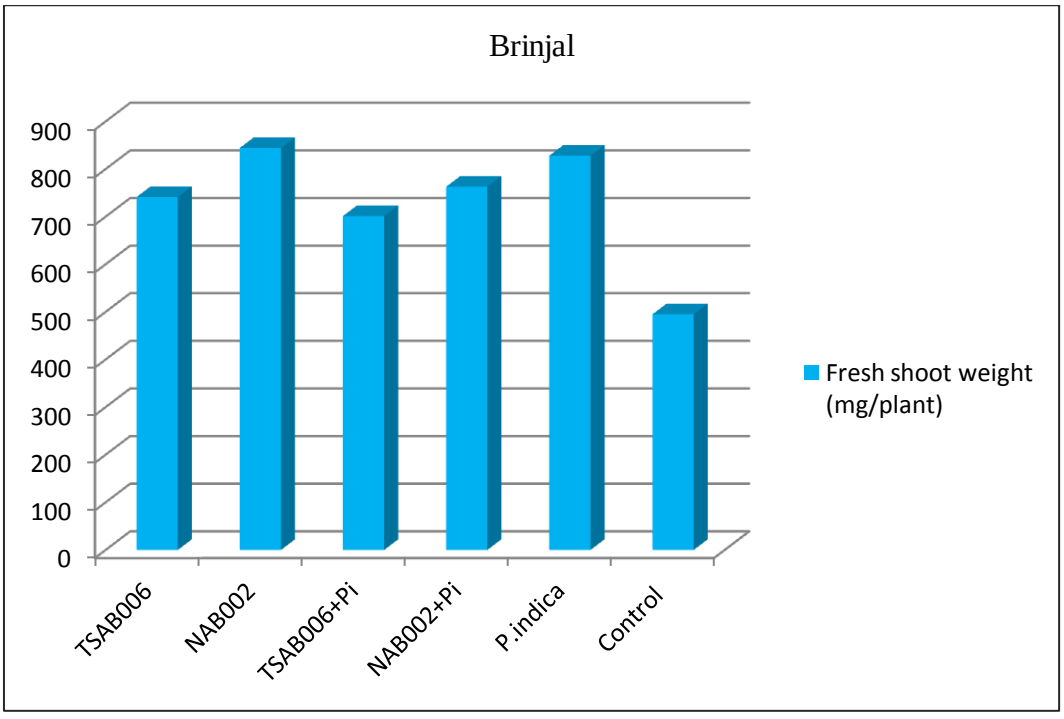


Figure 27. Effect of endophytes on fresh shoot weight of brinjal plants

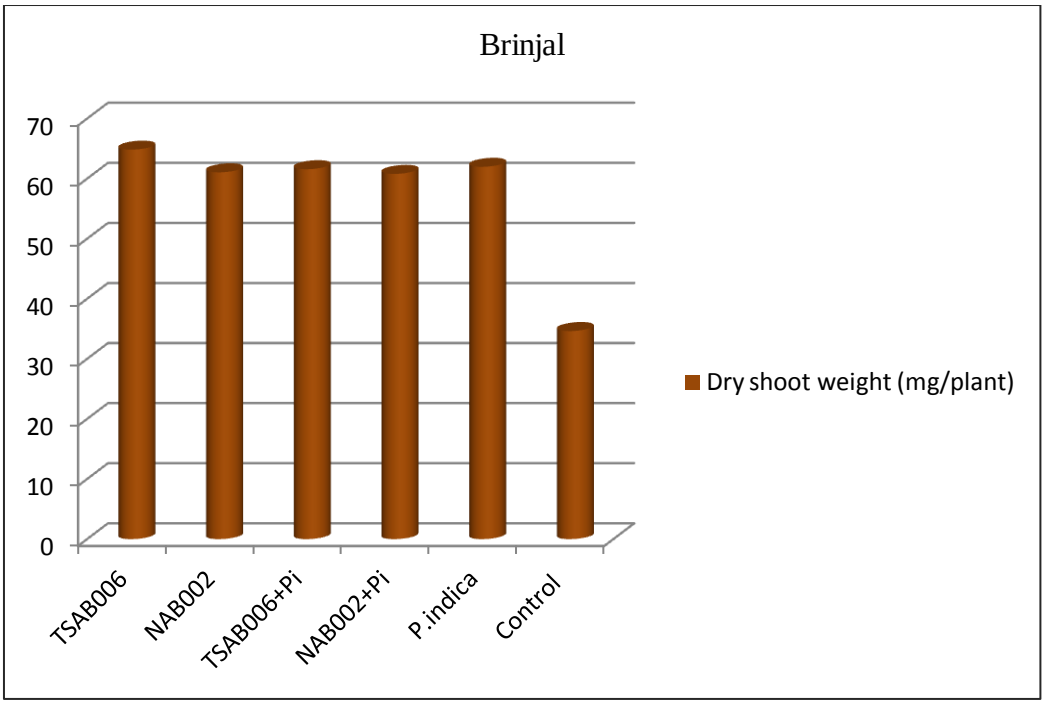


Figure 28. Effect of endophytes on dry shoot weight of brinjal plants

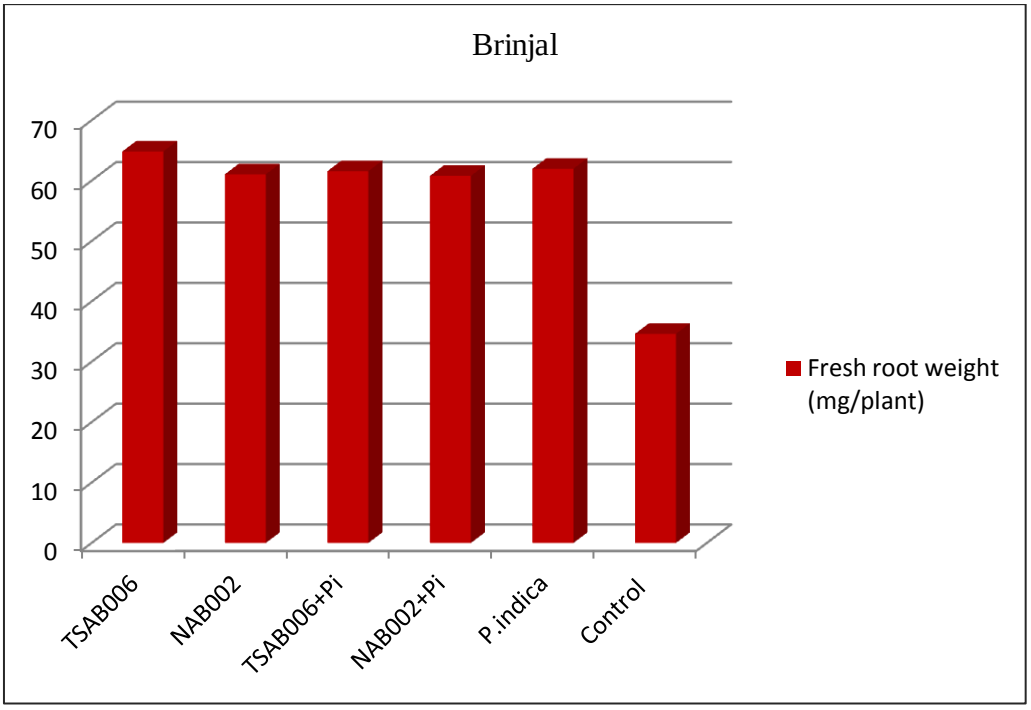


Figure 29. Effect of endophytes on fresh root weight of brinjal plants

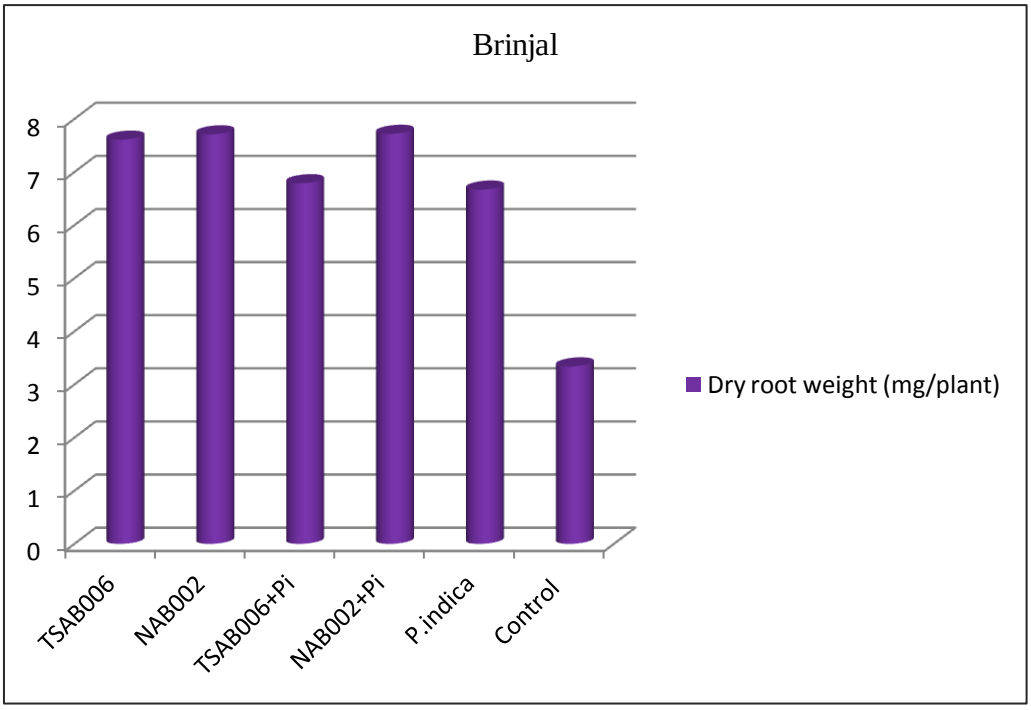


Figure 30. Effect of endophytes on dry root weight of brinjal plants

Summary

6. SUMMARY

A balanced diet should contain adequate energy source, nutrients and vitamins, mineras, carbohydrates, fats, protein etc. Vegetable are the reliable source for many dietary factors. As vegetable contain many of the dietary factors like vitamins, minerals and amino acids they are considered as protective supplementary food. Solanaceous vegetables plays a key role in this aspect.They contributes to the major share of vegetable production. So there is a need for the better establishment solanaceous vegetables a part from the conventional methods. In this ground the work entitled "Development of root endophytic plant growth-promoters as bio-inoculants for pro-tray seedlings" was undertaken at Department of Agriculltural Microbiology, College of Agriculture, Vellayani during 2013 – 2015.

The main objective of the present study was to develop microbial root endophytic plant growth-promoters as bio-inoculants in pro-tray seedling production of major solanaceous vegetable crops like chilli, tomato and brinjal.

The salient findings of the present study are as follows

Microorganisms were isolated by triturating the roots of vigorously growing seedlings of tomato, brinjal and chilli after surface sanitization. Bacterial isolates were subjected to a preliminary screening on their respective hosts for plant growth promotion. Seedling vigour was assessed under green house condition in portrays using sterile planting medium.

In this new study, we planned to combine one strain of bacterial root endophytes and fungal endophytes which induced plant growth promotion . These PGPR mixtures would then by added to the planting material . Development of such an integrated biological preparation could enhance growth and health of vegetable transplants. We chose to incorporate the components of the biological system into the soil-less media used to grow transplants, rather than to use the traditional approach of seed treatment for applying PGPR.

Improving the consistency of beneficial effects is a goal for PGPR research and development. Most approaches for biocontrol of plant diseases and plant growthpromotion have used applications of single PGPR strains. Because one strain is not likely to be active in all soil environments or against all pathogens that attack the host plant, the use of a single strain may partially account for the reported inconsistent performance by PGPR. Mixtures of PGPR provided greater activity against a broader range of plant pathogens than did single strains.

Endophytes with plant growth promoting ability selected through the preliminary screening were assessed under *in vitro* condition using dual culture plate assay for assessing the compatibility with *Piriformospora indica* (Pi). Eight compatible bacterial endophytes (four from tomato, two from chilli and two from brinjal) were further evaluated for their growth promoting ability individually and in combination with *P. indica*. Bacterial inoculants were provided as seed treatment and the fungal inoculant as additive in the transplant medium.

The plant growth promoting experiments in tomato indicated that the combination treatment of bacterial isolate KBT004 with Pi was found to be statistically superior in shoot fresh weight, shoot dry weight root fresh weight and root dry weight (1764.536 mg, 134.05mg, 332.881 mg and 26.452 mg). Treatment KBT001 + Pi was found to be statistically superior in shoot length (15.233 cm) followed by the treatment KBT004 + Pi (14.447 cm). All the treatment were found to be superior over control. Root colonization by *P. indica* was not found to be influenced by the combined application with endophytic bacteria.

By assessing the plant growth promotion in brinjal, significantly higher values with respect to shoot length, shoot fresh weight, and root fresh weight (6.947 cm, 828.013 mg, 61.979 mg) were observed with the plant treated with endophytic bacterial isolate NAB002. However the combination treatment of endophytic isolates with *P. indica* showed superior values compared to control.

Analising the efficacy of the endophytic isolates in chilli for plant growth promotion indicated that treatment with the endophytic isolate NAC002 was found to be have significantly superior values in leaf number, shoot length, shoot fresh weight, and shoot dry weight (5.67, 11.93 cm, 855.203 mg and 87.97 mg). All the treatments including the combinations were found to be superior to control.

The specific goal of this project was to determine if an integrated biological preparation could enhance growth promotion protect vegetable transplants against diseases for several weeks after being transplanted into the field. The broader purpose was to accelerate development of vegetable transplant plugs and to increase plant health.

P. indica has capability to induce resistance against biotic and abiotic stress, including drought, salinity resistance and bacterial, fungal and virus infection in plants. The current experiment suggest that native root endophytic bacteria can be used in combination with *P. indica* as far as plant growth is concerned. Further studies are required to assess the potential of such combinations in combating plant diseases and helping the plant overcome drought, salinity etc.

Reference

7. REFERENCE

Abdul-Baki, A. A. and Anderson, J. D. 1973. Vigor determination in soybean seed by multiple criteria. *Crop Sci.* 13: 630-633.

Achari, G. A. and Ramesh, R. 2014. Diversity, biocontrol, and plant growth promoting abilities of xylem residing bacteria from solanaceous crops. *Int. J. Microbiol*. [e- journal]. Available: http://www.hindawi.com/ Volume 2014, Article ID 296521, 14 pages [5 March 2015].

Amaresan, N., Jayakumar, V., Kumar, K., and Thajuddin, N. 2012. Isolation and characterization of plant growth promoting endophytic bacteria and their effect on tomato (*Lycopersicon esculentum*) and chilli (*Capsicum annuum*) seedling growth. *Ann. Microbiol*. 62:805–810.

Anith, K. N. 2009. Mature coconut as a bio-fermentor for multiplication of plant growth promoting rhizobacteria. *Curr. Sci.*, 97: 1647-1653.

Anith, K. N. and Manomohandas, T. P. 2001. Combined application of *Trichoderama* and *Alcaligenes* sp. strain AMB 8 for controlling nursery rot (*Phytophthora capsici*) of black pepper (*Piper nigrum*). *Indian Phytopathol*., 54: 335-339.

Anith, K. N., Faseela, K.M., Archana, P. A. and Prathapan, K. D. 2011.

Compatibility of *Piriformospora indica* and *Trichoderma harzianum* as dual inoculants in black pepper (*Piper nigrum* L.). *Symbiosis*, 55: 11-17

Anith, K. N., Momol, M. T., Kloepper, J. W., Marois, J. J., Olson, S. M. and

Jones, J. B. 2004. Efficacy of plant growth-promoting rhizobacteria, Acibenzolar-*S*-methyl and soil ammendment for integrated management of bacterial wilt on tomato. *Plant Dis.*, 88: 669-673.

Anith, K. N., Anjana, S., Sreekumar, J. 2015. The growth of tomato seedlings inoculated with co-cultivated *Piriformospora indica* and *Bacillus pumilus*. *Symbiosis* 65: 9-16.

Araujo, W. L., Maccheroni, W., Jr., Aguilar-Vildoso, C. I., Barroso, P. A.

V., Saridakis, H. O., and Azevedo, J. L. 2001. Variability and interactions between endophytic bacteria and fungi isolated from leaf tissues of citrus rootstocks. *Can. J. Microbiol*. 47:229-236.

Beecher, G.R. 1998. Nutrient content of tomatoes and tomato products, Nutrient content of tomatoes. *Proc. Soc. Exp. Biol. Med*. 218: 98–100.

Bonfante, P. and Anca, I-A.. 2009. Plants, Mycorrhizal fungi and bacteria: A net work of interactions. *Annu. Rev. Microbiol.* 63: 363- 383.

Bu¨tehorn, B., and Franken, P. 1998. Piriformospora indica, a new root colonizing fungus. *Mycologia.* 90:896–903

Cao, G., Sofic, E., and Prior, R.L. 1996. Antioxidant capacity of tea and common vegetables. *J. Agric. Food Chem*. 44: 3426–3431.

Coombs, J. T., and Franco, C. M. M. 2003. Visualization of an endophytic *Streptomyces* species in wheat seed. *Appl. Environ. Microbiol*. 69:4260-4262.

Damato, G. and Trotta, L. 2000. Cell shape, transplant age, cultivars and yield in broccoli. *Acta Hort.* 533:153–160.

Davies, J.N. and Hobson, G.E. 1981. The constituents of tomato fruit–The influence of environment, nutrition, and genotype. *CRC Crit. Rev. Food Sci. Nutr.*15: 205–280.

Deshmukh, S. and Kogel K-H. 2007. *Piriformospora indica* protects barley from root rot caused by *Fusarium graminearum*. *Plant Dis. Prot*., 114: 263-268.

De Boer, M., der Sluis, V.I., Loon Van, L.C., and Bakker, P.A.H.M .1999. Combining *Pseudomonas fluorescent* spp. strains to enhance suppression of Fusarium wilt of radish. *Eur. J. Plant Pathol*. 105: 201–210.

De Grazia, J., Tittonell, P. and Chiesa. A. 2002. Pepper (*Capsicum annuum* L.) transplant growth as affected by growing medium compression and cell size. *Agron*. 22:503–509.

Domenech, J. Reddy,M. S., Kloepper, J.W.,2, b. Ramos, B and Gutierrez-man˜

ero, J. 2006. Combined application of the biological product LS213 with Bacillus, Pseudomonas or Chryseobacterium for growth promotion and biological control of soil-borne diseases in pepper and tomato. *Bio. Control.* 51:245–258

Druedge, U., Baltruschat, H. and Franken, P. 2007. *Piriformospora indica*

promotes adventitious root formation in cuttings. *Sci Hortic.*, 112: 422-426.

Duponnois, R., Garbaye, J., Bouchard, D. and Churin, J. L. 1993. The fungus

specificity of mycorrhization helper bacteria (MHBs) used as an alternative to soil fumigation for ectomycorrhizal inoculation of bare-root Douglas-fir planting stocks with *Laccaria laccata*. *Plant Soil*, 157**:** 257–262.

Fakhro, A., Linares, D. R. A., Bargen, S. V., Bandet, M., Buttener, C., Grosch, R.,

Schwarzz, D. and Franken, P. 2009. Impact of *Piriformospora indica* on tomato growth and interaction with fungal and viral pathogen. *Mycorrhiza*, 20**:** 191-200.

Franceschi, S., Bidoli, E., La Vecchia, C., Talamini, R., D'Avanzo, B., and Negri, E. 1994. Tomatoes and risk of digestive tract cancers. *Int.J. Cancer* 59, 181–184.

Gabriele, B. 2009. Plant–microbe interactions promoting plant growth and health: perspectives for controlled use of microorganisms in agriculture. *Appl Microbiol Biotechnol.* 84:11–18.

Gerster, H. 1997. The potential role of lycopene in human health. *J. Am.Coll. Nutr*.16: 109–126.

Gutierrez-Manero, F.J., Ramos-Solano, B., Probanza. A., Mehouachi, J., Tadeo, F.R., and Talon, M. 2001. The plant-growth promoting rhizobacteria *Bacillus pumilus* and *Bacillus licheniformis* produce high amounts of physiologically active gibberellins. Physiol Plantarum 111:206–211.

Hallmann, J., Quadt-Hallmann, A., Mahaffee, W.F. and Kloepper, J.W. 1997.

Bacterial endophytes in agricultural crops. *Can. J. Microbiol*., 43: 895–914.

Huang, H. Y., Chang, C. K., Tso, T. K., Huang, J. J., Chang, W. W., and Tsai, W. C. 2004. Antioxidant activities of various fruits and vegetables produced in Taiwan. *Int. J.Food Sci.Nutri*. 55: 423–429

Ibiza, V.P., J. Blanca, J., Ca˜nizares, J., and Nuez, F. 2012. Taxonomy and genetic diversity of domesticated Capsicum species in the Andean region. *Genet. Resour. Crop. Evol.* 59: 1077–1088.

Johri, B. N. 2006. Endophytes to the rescue of plants. *Curr. Sci.,* 90: 1315-1316.

Johri, B. N. 2006. Endophytes to the rescue of plants. *Curr. Sci.,* 90: 1315-1316.

Kaur, C. and Kapoor, H. C. 2001. Antioxidants in fruits and vegetables – the Millennium's health. *Int.J. Food Sci. Technol*. 36: 703–725.

Kim, K.S., Koo, M.S., Jeon, J.W., Park, H.S., and Seung, I.S. 2002. Capsicum plaster at the Korean hand acupuncture point reduces postopera-tive nausea and vomiting after abdominal hysterectomy. *Anesth. Analg*. 95: 1103–1107.

Kloepper, J. W., Leang, J., Tuntze, M. and Schroth, M. N. 1980. Enhanced plant-growth by siderophores produced by plant growth promoting rhizobacteria. *Nature*, 286: 885-886.

Kloepper, J. W., Reddy, M. S., Rodríguez-Kabana, R., Kenney, D. S., Kokalis-Burelle, N., Martinez-Ochoa, N., Vavrina, C. S. 2004. Application for Rhizobacteria in Transplant Production and Yield Enhancement. *Acta Hort.*, 631: 217-229.

Kloepper, J.W. and Schroth, M.N. 1981. Relationship of in vitro antibiosis of plant growth promoting rhizobacteria to plant growth and the displacement of root microflora. *Phytopathol*. 71:1020–1024.

Kokalis-Burelle, N., Klopper, J.W., Rodriguez-Ka´bana, R., Vavrina, C.S. Rosskopf, E.N and Kenney, D.S. 1999. Evaluation of amended transplant

mixes for fruit and vegetable production. [e- journal] Available : http : // www . epa.gov/ozone/strathome.html. [3 May. 2015].

Lavelli, V., Peri, C., and Rizzolo, A. 2000. Antioxidant activity of tomato products as studied by model reactions using xanthine oxidase, myeloperoxidase, and copper-induced lipid peroxidation. *J. Agric. Food Chem.* 48: 1442–1448.

Leonardi, C., Ambrosino, P., Esposito, F., and Fogliano, V. 2000. Antioxidant activity and carotenoid and tomatine contents in different typologies of fresh consumption tomatoes. *J. Agric. Food Chem*. 48 :4723–4727.

Lugtenberg, B.J.J.,. De Weger, L.A and Benett, J.W. 1991. Microbial stimulation of plant growth and protection from disease. *Curr. Opinion Microbiol*. 2: 457- 464.

Madhavi, D.L., Salunkhe, D.K. 1998. Tomato. Handbook of vegetable science and technology. In: Salunkhe, D.K., Kadam, S.S. (Eds.), Production, Composition, Storage, and Processing. Marcel Dekker, New York, pp. 171–201.

McInroy, J. A., and Kloepper, J. W. 1995. Survey of indigenous bacterial endophytes from cotton and sweet corn. *Plant Soil* 173:337-342.

Nejad, P. and Johnson, P.A. 2000. Endophytic bacteria induce growth promotion and wilt disease suppression in oilseed rape and tomato. *Biol. Control* 18**:** 208–215.

Oelmuller, R., Sherameti, I., Tripathi, S. and Varma, A. 2009. *Piriformospora indica*, a cultivable root endophyte with multiple biotechnological applications. *Symbiosis*, 49: 1-17.

Pandey, D.K., Shekelle, R., Selwyn, B.J., Tangney, C., and Stamler, J. 1995. Dietary vitamin C and beta carotene and risk of death in middle-aged men. *Am. J. Epidemiol*. 142: 1269–1278.

Panse, V.G. and Sukhatme, P.V. 1967. *Statistical Method for Agricultural Workers.* Second edition. Indian Council of Agricultural Research. New Delhi, 381 p.

Park, H.S., Kim, K.S., Min, H.K., and Kim, D.W. 2004. Prevention of postop-

erative sore throat using capsicum plaster applied at the Korean hand acupuncture point. *Anaesth.* 59: 647–651.

Pavlo, A., Leonid, O., Iryna, Z., Natalia, K. and Maria, P. A. 2011. Endophytic bacteria enhancing growth and disease resistance of potato (Solanum tuberosum L.). *Biol. Control* 56: 43–49.

Peer Van, R. and Schippers, B.1989. Plant growth responses to bacterization and rhizosphere microbial development in hydroponic cultures. *Can J. Microbiol*. 35: 456–463.

Pieterse, C.M.J., Wees Van, S.C.M., Ton, J., J.A. Pelt Van, J.A., and Loon Van, L.C. 2002. Signalling in rhizobacteria-induced systemic resistance in *Arabidopsis thaliana. Plant Biol*. 4: 535–544.

Podolich, O.V., Ardanov, P.E., Voznyuk, T.M., Kovalchuk, M.V., Danylchenko, O.V.,Laschevskyi, V.V., Lyaschenko, S.A., and Kozyrovska, N.O. 2007. Endophytic bacteria from potato in vitro activated by exogenic non-pathogenic bacteria. *Biopolim. Kletka*. 23: 21–27.

Rai, M and Varma, A. 2005. Arbuscular mycorrhiza-like biotechnological potential of *Piriformospora indica*, which promotes the growth of *Adhatoda vasica* Nees. Electronic Journal of Biotechnology [e- journal] 8(1)Available:http://www.ejbiotechnology.info/content/vol8/issue1/full/5/index.html. ISSN: 0717-3458 [8 May 2015].

Rai, M., Acharya, D., Singh, A. and Varma, A. 2001. Positive growth responses of the medicinal plants *Spilanthes calva* and *Withania somnifera* to inoculation by *Piriformospora indica* in a field trial. *Mycorrhiza*. 11:123-128.

Rippy, J.F.M., Peet, M.M., Louws, F.J., Nelson, P.V., Orr, D.B., and. Sorensen. K.A. 2004. Plant development and harvest yield of greenhouse tomatoes in six organic growing systems. *Hortic.Sci*. 39:223–229.

Rosenblueth, M., Martínez-Romero, E., 2006. Bacterial endophytes and their interactions with the hosts. *Mol. Plant–Microbe Interactions*. 19: 827–837.

Russo, V. M. 2006. Biological amendment, fertilizer rate and irrigation frequency for organic bell pepper production. *HortScience*, 41: 1402-1407.

Russo, V.M. 2004. Greenhouse-grown transplants as an alternative to bare-root transplants for onion. *Hortic. Sci*. 39:1267–1271.

Russo, V.M. 2005. Organic vegetable transplant production. *Hortic.Sci*. 40:623–628.

Ryan, R.P., Germaine, K., Franks, A., Ryan, D.J., and Dowling, D.N., 2008. Bacterial endophytes: recent developments and applications. *FEMS Microbiol. Lett.* 278, 1–9.

Sahay, N. S. and Varma, A. 1999. *Piriformospora indica*: a new biological hardening tool for micropropagated plants. *FEMS Microbiol. Lett.*, 181: 297-302.

Satheesan, J., Anith, K. N., Sakunthala, M. 2012. Induction of root colonization by *Piriformospora indica* leads to enhanced asiaticoside production in *Centella asiatica*. *Mycorrhiza.* 22: 195-202.

Sanchez, M. C., Valencia, C., Ciruelos, A., Latorre, A., and Gallegos, C. 2003. Rheological properties of tomato paste: Influence of the addition of tomato slurry. *J. Food Sci*. 68: 551–554.

Singh, G., N. Singh, N., and Marwahl, T.S. 2009. Crop genotype and a novel symbiotic fungus influences the root endophytic colonization potential of plant growth promoting rhizobacteria. *Physiol. Mol. Biol*. 15:1-4.

Spiller, F., Alves, M. K., Vieira, S. M., Carvalho, T.A., Leite, C.E., Lunardelli, A., Poloni, J.A., Cunha, F.Q., and de Oliveira, J.R. 2008. Anti-inflammatory effects of red pepper (*Capsicum baccatum*) on carrageenan and antigen-induced inflammation. *J. Pharm.Pharmacol*. 60 : 473–478.

Stommel, J.R., and Whitaker, B.D. 2003. Phenolic acid content and composition of eggplant fruit in a germplasm core subset. *J. Am. Soc. Hortic. Sci*. 128 : 704–710.

Sundaramoorthy , S and Balabaskar, P. 2012. Consortial effect of endophytic and plant growth promoting rhizobacteria for the management of early blight of tomato incited by *Alternaria solani*. *J.Plant Pathol. Microbiol.* [e-

journal] 3 (7) Available : http://www.dx.doi. org/10.4172/ vol3/ issue7/ full/4/index.html. ISSN:2157-7471 JPPM 1000145 [5 March 2015].

Suresh, C.K. and Bagyaraj. D.J. 2002. Mycorrhiza– microbe interactions: Effect on rhizosphere, p. 7–28. In: Sharma, A.K. and Johri, B.N. (eds.). Arbuscular mycorrhizae. Interactions in plants, rhizosphere and soils. Science Publishers, Enfield, NH.

Surette, M. A., Sturz, A. V., Lada, R. R., and Nowak, J. 2003. Bacterial endophytes in processing carrots (*Daucus carota* L. var. sativus): Their localization, population density, biodiversity and their effects on plant growth. *Plant Soil* 253:381-390.

Tolan, I., Ragoobirsingh, D., and Morrison, E.Y. 2004. Isolation and purifi-cation of the hypoglycaemic principle present in *Capsicum frutescens*. *Phytother. Res.* 18 : 95–96.

Van Loon, L.C., Bakker, P.A.H.M and. Pieterse, C.M.J. 1998. Systemic resistance induced by rhizosphere bacteria. *Annu. Rev. Phytopathol*. 36: 453–483.

Varma, A., Bakshi, M., Lou, B., Hartmann, A. and Oelmueller, R. 2012.

Piriformospora indica : A Novel Plant Growth-Promoting Mycorrhizal Fungus. *Agric. Res.*, 1: 117-131.

Varma, A., Verma, S., Sudha., Sahay, N., Butehorn, B. and Franken, P.1999.

Piriformospora indica, a cultivable plant growth promoting root endophyte. *Appl. Environ. Microbiol.*, 65: 2741-2744.

Vavrina, C. S. 1998. Transplant age in vegetable crops. *HortTechnology*, 8: 550-555.

Verma, S., Varma, A., Rexer, K., Hassel, A., Kost, G., Sarbhoy, A., Bisen,

P.,Yan, Z., Reddy, M. S., Kloepper, J. W., 2003. Survival and colonization of rhizobacteria in a tomato transplant system. *Can. J. Microbiol.* 49: 383-389.

Verma, S., Varma, A., Rexer, K., Hassel, A., Kost, G., Sarbhoy, A., Bisen, P., Bütehorn, B., and Franken, P. 1998. *Piriformospora indica*, a new root-colonizing fungus. *Mycologia*. 90:896–903.

Yang, J., Kloepper, J.W., and Ryu, C.M. 2009. Rhizosphere bacteria help plants tolerate abiotic stress. *Trends in Plant Sci*. 14: 1–4.

Weller, D.M. and Thomashow, L.S. 1994. Current challenges in introducing beneficial microorganisms into the rhizosphere microorganisms. In: F. O'Gara (eds), et al. Molecular Ecology of Rhizosphere Microorganisms. VCH, New York. pp. 1–18

Weisburger, J.H. 1998. Evaluation of the evidence on the role of tomato products in disease prevention. *Proc. Soc.Exp. Biol. Med*. 218: 140–143.

Appendix

APPENDIX - I

COMPOSITION OF MEDIA USED

1. Nutrient Agar

Peptone	- 5g
NaCl	- 5g
Beef extract	- 3g
Agar	- 20g
Distilled water	- 1000 ml

Peptone, NaCl and beef extract were dissolved in 500 ml distilled water and volume made up to 1000 ml. 20 g agar-agar was added into this mixture and autoclaved at 15 lsb pressure and 121 ºC for 15 min.

2. King's Medium B

Peptone	- 20g
K_2HPO_4	- 1.5g
$MgSO_4$	- 1.5g
Glycerol	- 10 ml
Agar	- 20g
Distilled water	- 1000 ml

Peptone, K_2HPO_4 and $MgSO_4$ were dissolved in distil water containing glycerol. Agar-agar was added into this mixture and autoclaved at 15 lbs pressure and 121 ºC for 15 min.

3. Potato Dextrose Agar

Peeled and sliced potatoes	- 200g
Dextrose ($C_6H_{12}O_6$)	- 20g
Agar-agar	- 20g

Distilled water - 1000 ml

Potatoes were boiled in 500 ml of distilled water and the extract was collected by filtering through a muslin cloth. Agar-agar was dissolved separately in 500 ml of distilled water. The potato extract was mixed in the molten agar and 20 g of dextrose was dissolved in to the mixture. The volume was made up to 1000 ml with distilled water and medium was sterilized at 15 lsb pressure and 121 ^{o}C for 15 min.

APPENDIX - II

COMPOSITION OF STAIN USED

1. Crystal violet

One volume saturated alcohol solution of crystal violet in four volumes of one per cent aqueous ammonium oxalate.

2. Gram's iodine

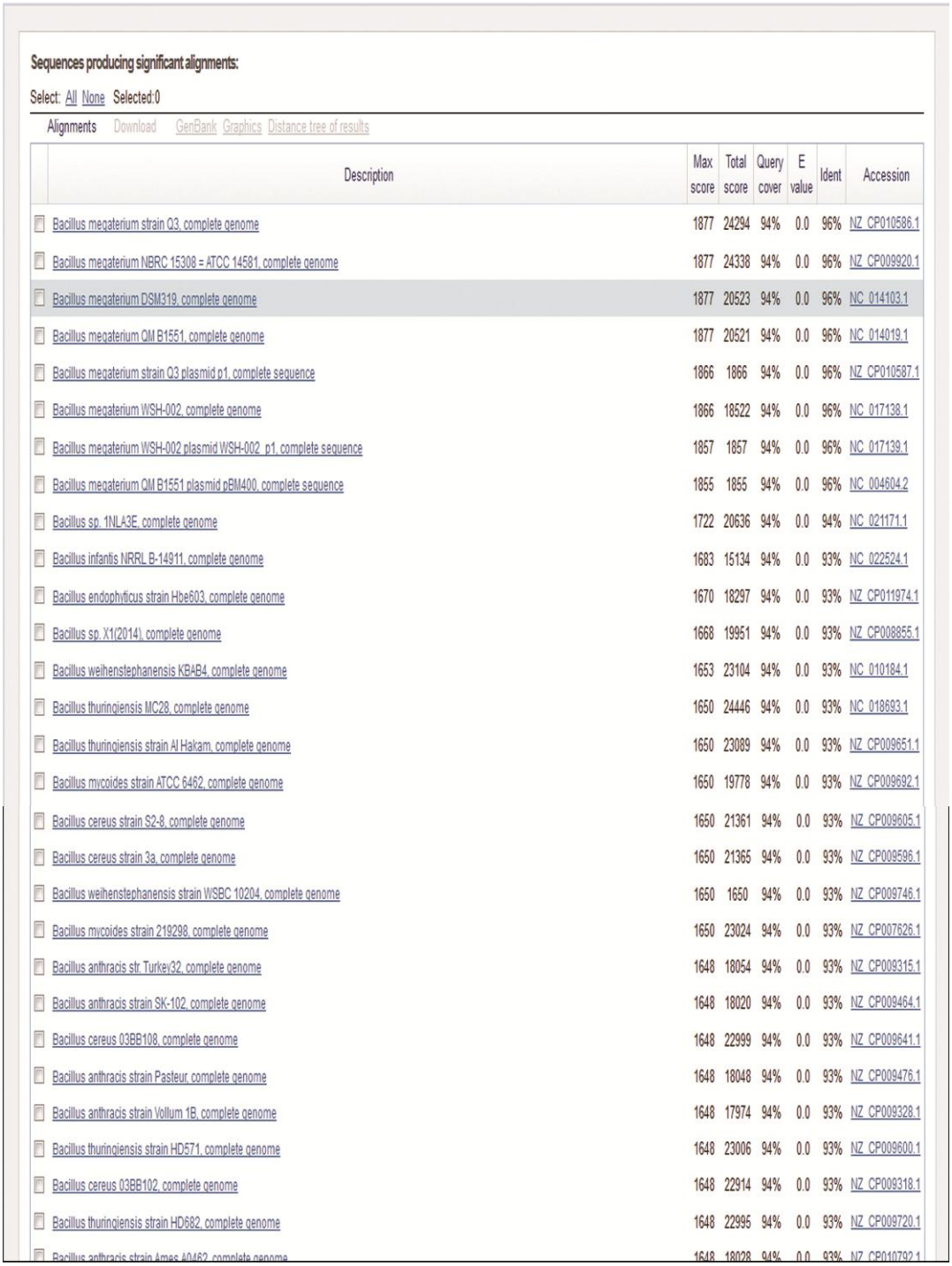
Iodine crystals	- 1.0g
Potassium iodide	- 2.0g
Distilled water	- 300ml

3. Safranin

Ten ml saturated solution of safranin in 100 ml distilled water.

APPENDIX – III

a. NAT001



Sequences producing significant alignments:

Select: All None Selected:0

Alignments Download GenBank Graphics Distance tree of results

Description	Max score	Total score	Query cover	E value	Ident	Accession
Bacillus megaterium strain Q3, complete genome	1877	24294	94%	0.0	96%	NZ_CP010586.1
Bacillus megaterium NBRC 15308 = ATCC 14581, complete genome	1877	24338	94%	0.0	96%	NZ_CP009920.1
Bacillus megaterium DSM319, complete genome	1877	20523	94%	0.0	96%	NC_014103.1
Bacillus megaterium QM B1551, complete genome	1877	20521	94%	0.0	96%	NC_014019.1
Bacillus megaterium strain Q3 plasmid p1, complete sequence	1866	1866	94%	0.0	96%	NZ_CP010587.1
Bacillus megaterium WSH-002, complete genome	1866	18522	94%	0.0	96%	NC_017138.1
Bacillus megaterium WSH-002 plasmid WSH-002_p1, complete sequence	1857	1857	94%	0.0	96%	NC_017139.1
Bacillus megaterium QM B1551 plasmid pBM400, complete sequence	1855	1855	94%	0.0	96%	NC_004604.2
Bacillus sp. 1NLA3E, complete genome	1722	20636	94%	0.0	94%	NC_021171.1
Bacillus infantis NRRL B-14911, complete genome	1683	15134	94%	0.0	93%	NC_022524.1
Bacillus endophyticus strain Hbe603, complete genome	1670	18297	94%	0.0	93%	NZ_CP011974.1
Bacillus sp. X1(2014), complete genome	1668	19951	94%	0.0	93%	NZ_CP008855.1
Bacillus weihenstephanensis KBAB4, complete genome	1653	23104	94%	0.0	93%	NC_010184.1
Bacillus thuringiensis MC28, complete genome	1650	24446	94%	0.0	93%	NC_018693.1
Bacillus thuringiensis strain Al Hakam, complete genome	1650	23089	94%	0.0	93%	NZ_CP009651.1
Bacillus mycoides strain ATCC 6462, complete genome	1650	19778	94%	0.0	93%	NZ_CP009692.1
Bacillus cereus strain S2-8, complete genome	1650	21361	94%	0.0	93%	NZ_CP009605.1
Bacillus cereus strain 3a, complete genome	1650	21365	94%	0.0	93%	NZ_CP009596.1
Bacillus weihenstephanensis strain WSBC 10204, complete genome	1650	1650	94%	0.0	93%	NZ_CP009746.1
Bacillus mycoides strain 219298, complete genome	1650	23024	94%	0.0	93%	NZ_CP007626.1
Bacillus anthracis str. Turkey32, complete genome	1648	18054	94%	0.0	93%	NZ_CP009315.1
Bacillus anthracis strain SK-102, complete genome	1648	18020	94%	0.0	93%	NZ_CP009464.1
Bacillus cereus 03BB108, complete genome	1648	22999	94%	0.0	93%	NZ_CP009641.1
Bacillus anthracis strain Pasteur, complete genome	1648	18048	94%	0.0	93%	NZ_CP009476.1
Bacillus anthracis strain Vollum 1B, complete genome	1648	17974	94%	0.0	93%	NZ_CP009328.1
Bacillus thuringiensis strain HD571, complete genome	1648	23006	94%	0.0	93%	NZ_CP009600.1
Bacillus cereus 03BB102, complete genome	1648	22914	94%	0.0	93%	NZ_CP009318.1
Bacillus thuringiensis strain HD682, complete genome	1648	22995	94%	0.0	93%	NZ_CP009720.1
Bacillus anthracis strain Ames A0462, complete genome	1648	18028	94%	0.0	93%	NZ_CP010792.1

c. KBT004

Sequences producing significant alignments:

Select: All None Selected:0

Alignments Download GenBank Graphics Distance tree of results

Description	Max score	Total score	Query cover	E value	Ident	Accession
Streptomyces leeuwenhoekii genome assembly sleC34, chromosome : chromosome	1136	6804	77%	0.0	91%	NZ_LN831790.1
Streptomyces cyaneogriseus subsp. noncyanogenus strain NMWT 1, complete genome	1136	6800	82%	0.0	91%	NZ_CP010849.1
Streptomyces glaucescens strain GLA.O, complete genome	1125	6754	77%	0.0	91%	NZ_CP009438.1
Streptomyces albus J1074, complete genome	1114	7791	77%	0.0	91%	NC_020990.1
Streptomyces cattleya DSM 46488, complete genome	1083	6405	77%	0.0	90%	NC_017586.1
Streptomyces bingchenggensis BCW-1, complete genome	1083	6499	77%	0.0	90%	NC_016582.1
Streptomyces lividans TK24, complete genome	1083	6499	77%	0.0	90%	NZ_CP009124.1
Streptomyces cattleya NRRL 8057 = DSM 46488, complete genome	1083	6344	77%	0.0	90%	NC_016111.1
Streptomyces albus strain DSM 41398, complete genome	1081	6488	77%	0.0	90%	NZ_CP010519.1
Streptomyces ambofaciens ATCC 23877, complete genome	1077	6460	77%	0.0	90%	NZ_CP012382.1
Streptomyces violaceusniger Tu 4113, complete genome	1075	6364	77%	0.0	90%	NC_015957.1
Streptomyces sp. CNQ-509, complete genome	1074	5283	77%	0.0	90%	NZ_CP011492.1
Streptomyces xiamenensis strain 318, complete genome	1062	5314	77%	0.0	90%	NZ_CP009922.2
Streptomyces collinus Tu 365, complete genome	1055	6333	77%	0.0	90%	NC_021985.1
Streptomyces lydicus A02, complete genome	1050	5352	77%	0.0	89%	NZ_CP007699.1
Streptomyces hygroscopicus subsp. limoneus strain KCTC 1717 chromosome I	1050	6272	77%	0.0	89%	NZ_CP013219.1
Streptomyces albulus strain NK660, complete genome	1050	7309	77%	0.0	89%	NZ_CP007574.1

b. KBT001

Sequences producing significant alignments:

Select: All None Selected:0

Alignments Download GenBank Graphics Distance tree of results

Description	Max score	Total score	Query cover	E value	Ident	Accession
Alcaligenes faecalis strain ZD02, complete genome	390	1172	80%	3e-105	80%	NZ_CP013119.1
Bordetella pertussis 18323 complete genome	363	1089	72%	6e-97	81%	NC_018518.1
Bordetella hinzii strain H568, complete genome	363	1089	72%	6e-97	81%	NZ_CP012077.1
Bordetella hinzii strain F582, complete genome	363	1089	72%	6e-97	81%	NZ_CP012076.1
Bordetella bronchiseptica DNA, complete genome, strain: S798	363	1089	72%	6e-97	81%	NZ_AP014582.1
Bordetella pertussis B1917, complete genome	363	1089	72%	6e-97	81%	NZ_CP009751.1
Bordetella holmesii ATCC 51541, complete genome	363	1089	72%	6e-97	81%	NZ_CP007494.1
Bordetella pertussis CS, complete genome	363	1089	72%	6e-97	81%	NC_017223.1
Achromobacter xylosoxidans genome assembly NCTC10807, chromosome : 1	363	1089	72%	6e-97	81%	NZ_LN831029.1
Bordetella bronchiseptica 253, complete genome	363	1089	72%	6e-97	81%	NC_019382.1
Bordetella avium 197N complete genome	363	1089	72%	6e-97	81%	NC_010645.1
Bordetella bronchiseptica MO149 complete genome	363	1089	72%	6e-97	81%	NC_018929.1
Bordetella pertussis strain B3621	363	1089	72%	6e-97	81%	NZ_CP011401.1
Bordetella pertussis strain B3629	363	1089	72%	6e-97	81%	NZ_CP011400.1
Bordetella pertussis strain B3921, complete genome	363	1089	72%	6e-97	81%	NZ_CP011448.1
Bordetella pertussis strain B3913, complete genome	363	1089	72%	6e-97	81%	NZ_CP011447.1

d. KBT006

Sequences producing significant alignments:

Select: All None Selected:0

Alignments Download GenBank Graphics Distance tree of results

Description	Max score	Total score	Query cover	E value	Ident	Accession
Bacillus pumilus strain NJ-V2, complete genome	2571	15429	98%	0.0	98%	NZ_CP012482.1
Bacillus pumilus strain NJ-M2, complete genome	2571	17994	98%	0.0	98%	NZ_CP012329.1
Bacillus pumilus strain GR-8, complete genome	2571	20202	98%	0.0	98%	NZ_CP009108.1
Bacillus pumilus strain MTCC B6033, complete genome	2571	20495	98%	0.0	98%	NZ_CP007436.1
Bacillus pumilus strain W3, complete genome	2571	17658	98%	0.0	98%	NZ_CP011150.1
Bacillus sp. WP8, complete genome	2532	20263	98%	0.0	98%	NZ_CP010075.1
Bacillus pumilus SAFR-032, complete genome	2521	17647	98%	0.0	98%	NC_009848.1
Bacillus atrophaeus 1942, complete genome	2374	16601	98%	0.0	96%	NC_014639.1
Bacillus atrophaeus strain NRS 1221A, complete genome	2374	18970	98%	0.0	96%	NZ_CP010778.1
Bacillus amyloliquefaciens subsp. plantarum UCMB5033, complete genome	2362	23563	98%	0.0	96%	NC_022075.1
Bacillus methylotrophicus strain YJ11-1-4, complete genome	2362	21161	98%	0.0	96%	NZ_CP011347.1
Bacillus amyloliquefaciens SQR9, complete genome	2362	16513	98%	0.0	96%	NZ_CP006890.1
Bacillus subtilis strain Bs-916, complete genome	2362	21228	98%	0.0	96%	NZ_CP009611.1
Bacillus amyloliquefaciens subsp. plantarum TrigoCor1448, complete genome	2362	18767	98%	0.0	96%	NZ_CP007244.1
Bacillus subtilis strain ATCC 19217, complete genome	2362	16502	98%	0.0	96%	NZ_CP009749.1
Bacillus sp. Pc3, complete genome	2362	21244	98%	0.0	96%	NZ_CP010406.1

e. NAC002

Sequences producing significant alignments:

Select: All None Selected:0

Alignments Download GenBank Graphics Distance tree of results

Description	Max score	Total score	Query cover	E value	Ident	Accession
Bacillus megaterium DSM319, complete genome	2034	22980	98%	0.0	95%	NC_014103.1
Bacillus megaterium QM B1551, complete genome	2034	22859	98%	0.0	95%	NC_014019.1
Bacillus megaterium strain Q3, complete genome	2034	27079	98%	0.0	95%	NZ_CP010586.1
Bacillus megaterium NBRC 15308 = ATCC 14581, complete genome	2034	27054	98%	0.0	95%	NZ_CP009920.1
Bacillus megaterium WSH-002, complete genome	2034	20797	98%	0.0	95%	NC_017138.1
Bacillus megaterium strain Q3 plasmid p1, complete sequence	2034	2092	98%	0.0	95%	NZ_CP010587.1
Bacillus megaterium WSH-002 plasmid WSH-002_p1, complete sequence	2019	2077	98%	0.0	95%	NC_017139.1
Bacillus megaterium QM B1551 plasmid pBM400, complete sequence	2017	2076	98%	0.0	95%	NC_004604.2
Bacillus sp. 1NLA3E, complete genome	1838	21971	95%	0.0	93%	NC_021171.1
Bacillus infantis NRRL B-14911, complete genome	1821	16391	95%	0.0	92%	NC_022524.1
Bacillus endophyticus strain Hbe603, complete genome	1755	19271	94%	0.0	92%	NZ_CP011974.1
Bacillus cereus AH820, complete genome	1751	20884	95%	0.0	92%	NC_011773.1
Bacillus anthracis strain Ames_BA1004, complete genome	1751	19075	95%	0.0	91%	NZ_CP009981.1
Bacillus cereus strain 3a, complete genome	1748	22637	95%	0.0	91%	NZ_CP009596.1
Bacillus cereus biovar anthracis str. CI, complete genome	1748	19151	95%	0.0	91%	NC_014335.1
Bacillus anthracis str. Turkey32, complete genome	1748	19132	95%	0.0	91%	NZ_CP009315.1
Bacillus sp. X1(2014), complete genome	1748	20913	95%	0.0	91%	NZ_CP008855.1
Bacillus cereus strain S2-8, complete genome	1748	22634	95%	0.0	91%	NZ_CP009605.1

f. NAC007

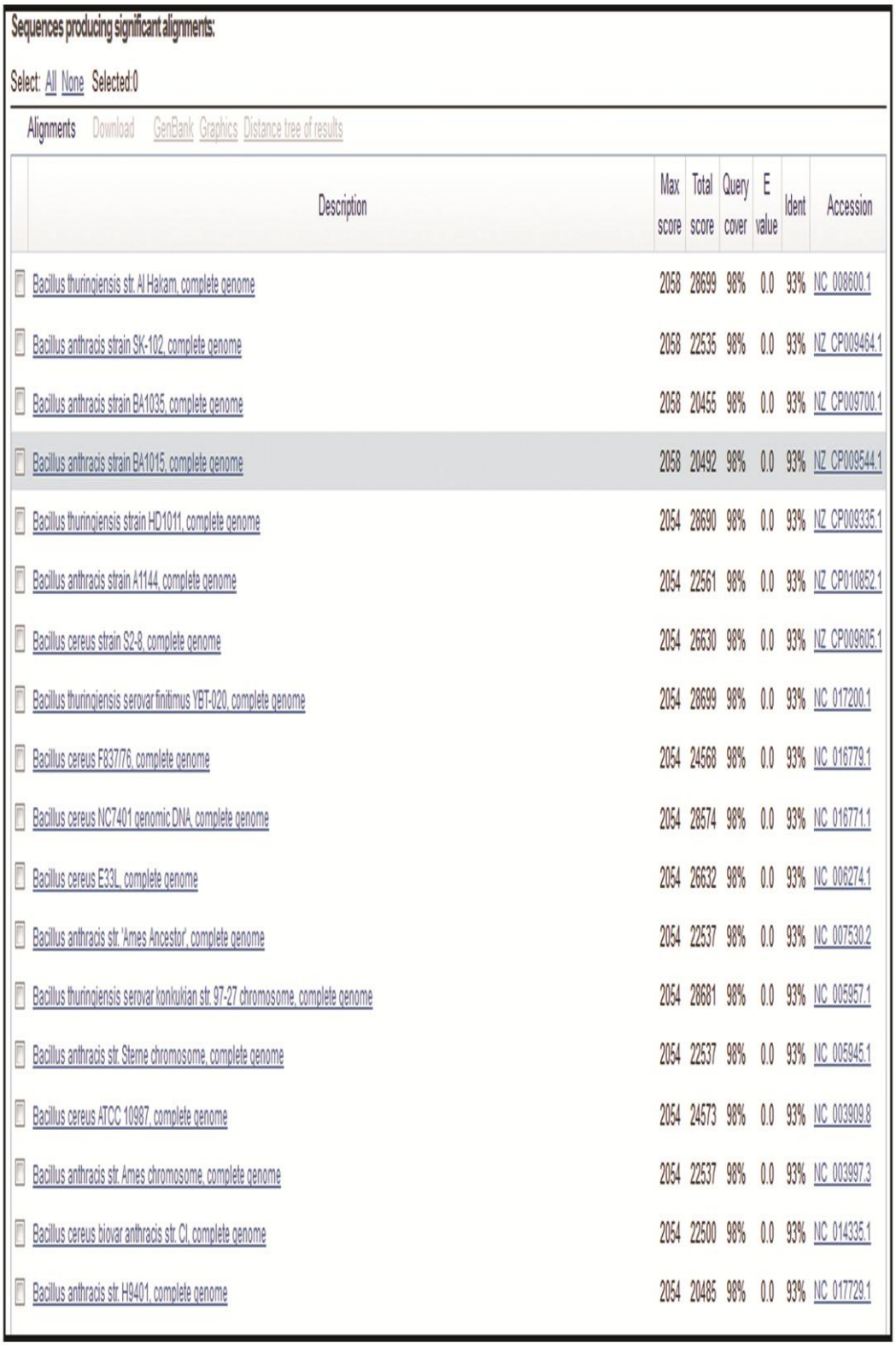
Sequences producing significant alignments:

Select: All None Selected:0

Alignments Download GenBank Graphics Distance tree of results

Description	Max score	Total score	Query cover	E value	Ident	Accession
Bacillus licheniformis strain BL-09, complete genome	1897	13226	95%	0.0	91%	NZ_CP010524.1
Bacillus licheniformis ATCC 14580, complete genome	1895	13216	95%	0.0	91%	NC_006270.3
Bacillus licheniformis DSM 13 = ATCC 14580, complete genome	1895	13216	95%	0.0	91%	NC_006322.1
Bacillus licheniformis 9945A, complete genome	1893	13183	95%	0.0	91%	NC_021362.1
Bacillus infantis NRRL B-14911, complete genome	1877	16868	95%	0.0	91%	NC_022524.1
Bacillus atrophaeus 1942, complete genome	1877	13119	94%	0.0	91%	NC_014639.1
Bacillus atrophaeus strain NRS 1221A, complete genome	1877	14990	94%	0.0	91%	NZ_CP010778.1
Bacillus amyloliquefaciens subsp. plantarum YAU B9601-Y2 complete genome	1866	18594	94%	0.0	91%	NC_017061.1
Bacillus amyloliquefaciens Y2, complete genome	1866	17537	94%	0.0	91%	NC_017912.1
Bacillus sp. BH072, complete genome	1866	16725	94%	0.0	91%	NZ_CP009938.1
Bacillus subtilis strain UD1022, complete genome	1860	18472	94%	0.0	91%	NZ_CP011534.1
Bacillus amyloliquefaciens subsp. plantarum str. FZB42, complete genome	1860	17404	94%	0.0	91%	NC_009725.1
Bacillus amyloliquefaciens subsp. plantarum UCMB5113, complete genome	1860	17428	94%	0.0	91%	NC_022081.1
Bacillus amyloliquefaciens subsp. plantarum UCMB5033, complete genome	1860	18522	94%	0.0	91%	NC_022075.1
Bacillus amyloliquefaciens subsp. plantarum NAU-B3, complete genome	1860	18509	94%	0.0	91%	NC_022530.1
Bacillus amyloliquefaciens strain L-H15, complete genome	1860	15560	94%	0.0	91%	NZ_CP010556.1
Bacillus amyloliquefaciens LL3, complete genome	1860	12980	94%	0.0	91%	NC_017190.1
Bacillus methylotrophicus strain YJ11-1-4, complete genome	1860	16690	94%	0.0	91%	NZ_CP011347.1
Bacillus amyloliquefaciens CC178, complete genome	1860	16679	94%	0.0	91%	NC_022653.1
Bacillus amyloliquefaciens SQR9, complete genome	1860	12991	94%	0.0	91%	NZ_CP006890.1
Bacillus subtilis strain ATCC 13952, complete genome	1860	12958	94%	0.0	91%	NZ_CP009748.1
Bacillus amyloliquefaciens strain L-S60, complete genome	1860	15566	94%	0.0	91%	NZ_CP011278.1
Bacillus subtilis HJ5, complete genome	1860	12936	94%	0.0	91%	NZ_CP007173.1
Bacillus subtilis XF-1, complete genome	1860	16581	94%	0.0	91%	NC_020244.1
Bacillus amyloliquefaciens subsp. plantarum AS43.3, complete genome	1855	17199	94%	0.0	91%	NC_019842.1
Bacillus amyloliquefaciens XH7, complete genome	1855	13945	94%	0.0	91%	NC_017191.1
Bacillus amyloliquefaciens TA208, complete genome	1855	12114	94%	0.0	91%	NC_017188.1
Bacillus methylotrophicus strain JJ-D34, complete genome	1855	16646	94%	0.0	91%	NZ_CP011346.1
Bacillus amyloliquefaciens DSM7 complete genome	1855	18507	94%	0.0	91%	NC_014551.1
Bacillus methylotrophicus strain JS25R, complete genome	1855	12952	94%	0.0	91%	NZ_CP009679.1
Bacillus subtilis strain Bs-916, complete genome	1855	16657	94%	0.0	91%	NZ_CP009611.1
Bacillus amyloliquefaciens subsp. plantarum TrigoCor1448, complete genome	1855	14793	94%	0.0	91%	NZ_CP007244.1

g. NAB002



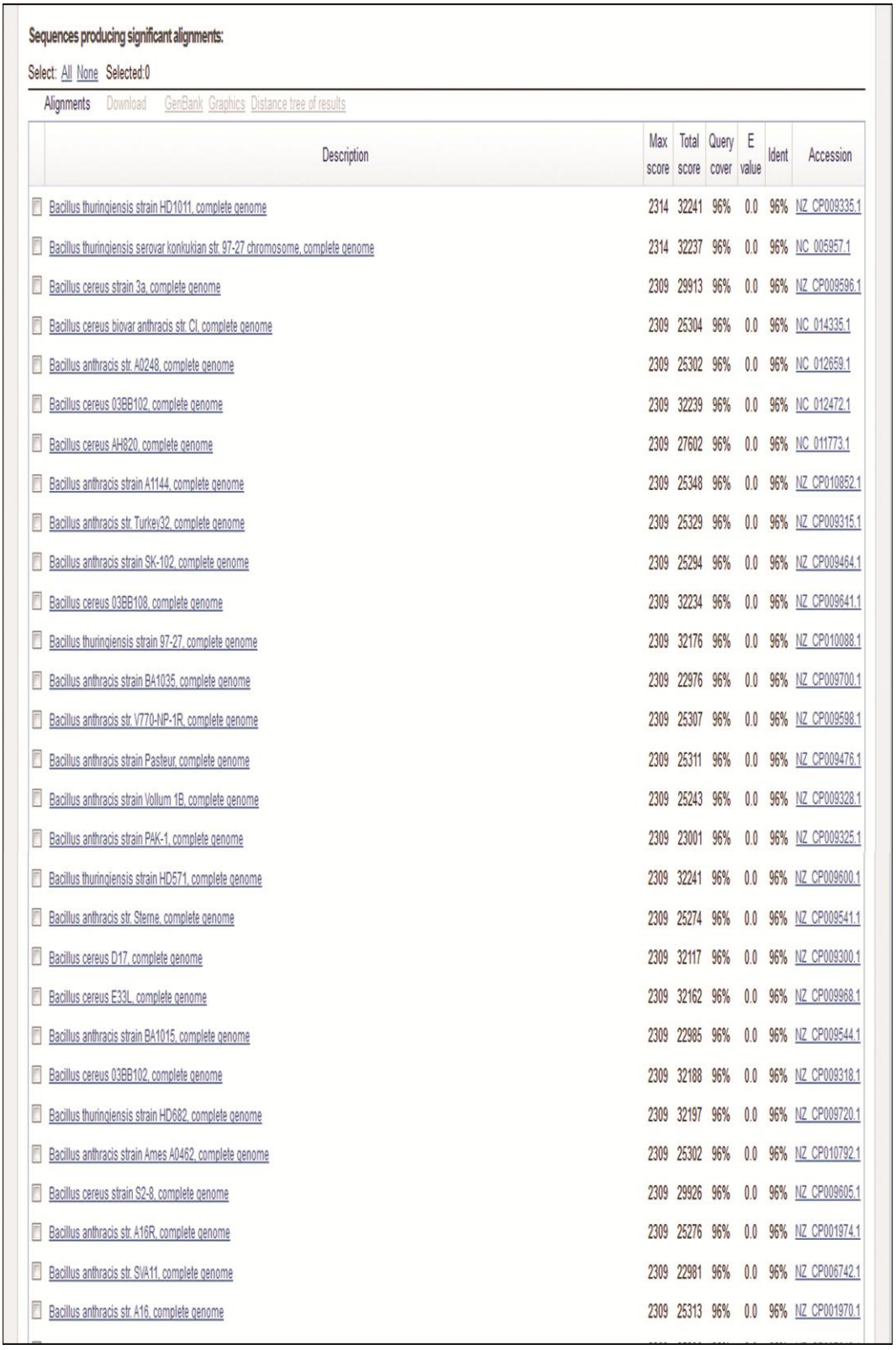
Sequences producing significant alignments:

Select: All None Selected:0

Alignments Download GenBank Graphics Distance tree of results

Description	Max score	Total score	Query cover	E value	Ident	Accession
Bacillus thuringiensis str. Al Hakam, complete genome	2058	28699	98%	0.0	93%	NC_008600.1
Bacillus anthracis strain SK-102, complete genome	2058	22535	98%	0.0	93%	NZ_CP009464.1
Bacillus anthracis strain BA1035, complete genome	2058	20455	98%	0.0	93%	NZ_CP009700.1
Bacillus anthracis strain BA1015, complete genome	2058	20492	98%	0.0	93%	NZ_CP009544.1
Bacillus thuringiensis strain HD1011, complete genome	2054	28690	98%	0.0	93%	NZ_CP009335.1
Bacillus anthracis strain A1144, complete genome	2054	22561	98%	0.0	93%	NZ_CP010852.1
Bacillus cereus strain S2-8, complete genome	2054	26630	98%	0.0	93%	NZ_CP009605.1
Bacillus thuringiensis serovar finitimus YBT-020, complete genome	2054	28699	98%	0.0	93%	NC_017200.1
Bacillus cereus F837/76, complete genome	2054	24568	98%	0.0	93%	NC_016779.1
Bacillus cereus NC7401 genomic DNA, complete genome	2054	28574	98%	0.0	93%	NC_016771.1
Bacillus cereus E33L, complete genome	2054	26632	98%	0.0	93%	NC_006274.1
Bacillus anthracis str. 'Ames Ancestor', complete genome	2054	22537	98%	0.0	93%	NC_007530.2
Bacillus thuringiensis serovar konkukian str. 97-27 chromosome, complete genome	2054	28681	98%	0.0	93%	NC_005957.1
Bacillus anthracis str. Sterne chromosome, complete genome	2054	22537	98%	0.0	93%	NC_005945.1
Bacillus cereus ATCC 10987, complete genome	2054	24573	98%	0.0	93%	NC_003909.8
Bacillus anthracis str. Ames chromosome, complete genome	2054	22537	98%	0.0	93%	NC_003997.3
Bacillus cereus biovar anthracis str. CI, complete genome	2054	22500	98%	0.0	93%	NC_014335.1
Bacillus anthracis str. H9401, complete genome	2054	20485	98%	0.0	93%	NC_017729.1

h. TSAB006



Sequences producing significant alignments:

Select: All None Selected:0

Alignments Download GenBank Graphics Distance tree of results

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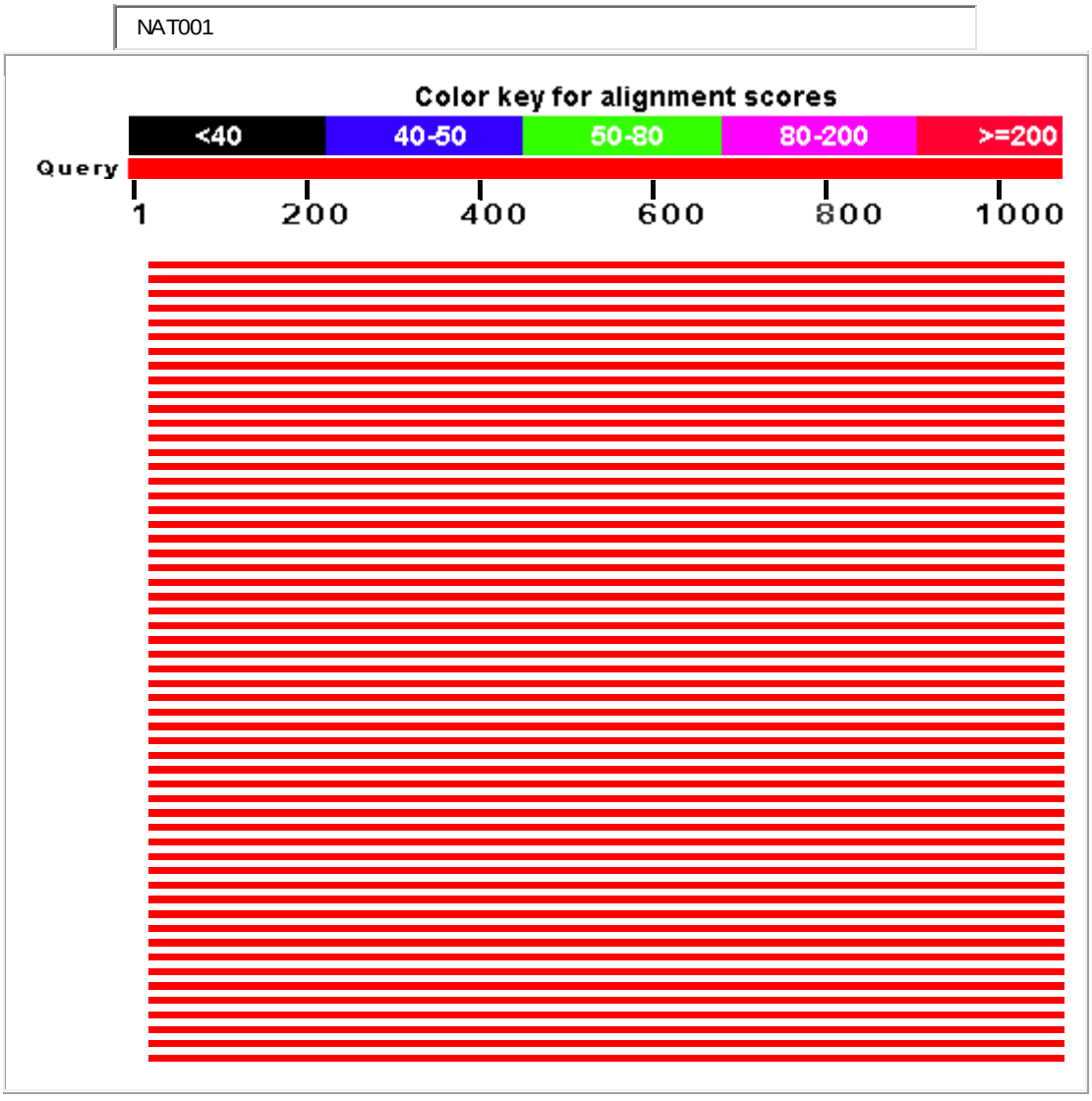
Bacillus thuringiensis strain HD1011, complete genome	2314	32241	96%	0.0	96%	NZ_CP009335.1
Bacillus thuringiensis serovar konkukian str. 97-27 chromosome, complete genome	2314	32237	96%	0.0	96%	NC_005957.1
Bacillus cereus strain 3a, complete genome	2309	29913	96%	0.0	96%	NZ_CP009596.1
Bacillus cereus biovar anthracis str. CI, complete genome	2309	25304	96%	0.0	96%	NC_014335.1
Bacillus anthracis str. A0248, complete genome	2309	25302	96%	0.0	96%	NC_012659.1
Bacillus cereus 03BB102, complete genome	2309	32239	96%	0.0	96%	NC_012472.1
Bacillus cereus AH820, complete genome	2309	27602	96%	0.0	96%	NC_011773.1
Bacillus anthracis strain A1144, complete genome	2309	25348	96%	0.0	96%	NZ_CP010852.1
Bacillus anthracis str. Turkey32, complete genome	2309	25329	96%	0.0	96%	NZ_CP009315.1
Bacillus anthracis strain SK-102, complete genome	2309	25294	96%	0.0	96%	NZ_CP009464.1
Bacillus cereus 03BB108, complete genome	2309	32234	96%	0.0	96%	NZ_CP009641.1
Bacillus thuringiensis strain 97-27, complete genome	2309	32176	96%	0.0	96%	NZ_CP010088.1
Bacillus anthracis strain BA1035, complete genome	2309	22976	96%	0.0	96%	NZ_CP009700.1
Bacillus anthracis str. V770-NP-1R, complete genome	2309	25307	96%	0.0	96%	NZ_CP009598.1
Bacillus anthracis strain Pasteur, complete genome	2309	25311	96%	0.0	96%	NZ_CP009476.1
Bacillus anthracis strain Vollum 1B, complete genome	2309	25243	96%	0.0	96%	NZ_CP009328.1
Bacillus anthracis strain PAK-1, complete genome	2309	23001	96%	0.0	96%	NZ_CP009325.1
Bacillus thuringiensis strain HD571, complete genome	2309	32241	96%	0.0	96%	NZ_CP009600.1
Bacillus anthracis str. Sterne, complete genome	2309	25274	96%	0.0	96%	NZ_CP009541.1
Bacillus cereus D17, complete genome	2309	32117	96%	0.0	96%	NZ_CP009300.1
Bacillus cereus E33L, complete genome	2309	32162	96%	0.0	96%	NZ_CP009968.1
Bacillus anthracis strain BA1015, complete genome	2309	22985	96%	0.0	96%	NZ_CP009544.1
Bacillus cereus 03BB102, complete genome	2309	32188	96%	0.0	96%	NZ_CP009318.1
Bacillus thuringiensis strain HD682, complete genome	2309	32197	96%	0.0	96%	NZ_CP009720.1
Bacillus anthracis strain Ames A0462, complete genome	2309	25302	96%	0.0	96%	NZ_CP010792.1
Bacillus cereus strain S2-8, complete genome	2309	29926	96%	0.0	96%	NZ_CP009605.1
Bacillus anthracis str. A16R, complete genome	2309	25276	96%	0.0	96%	NZ_CP001974.1
Bacillus anthracis str. SVA11, complete genome	2309	22981	96%	0.0	96%	NZ_CP006742.1
Bacillus anthracis str. A16, complete genome	2309	25313	96%	0.0	96%	NZ_CP001970.1

APPENDIX - IV

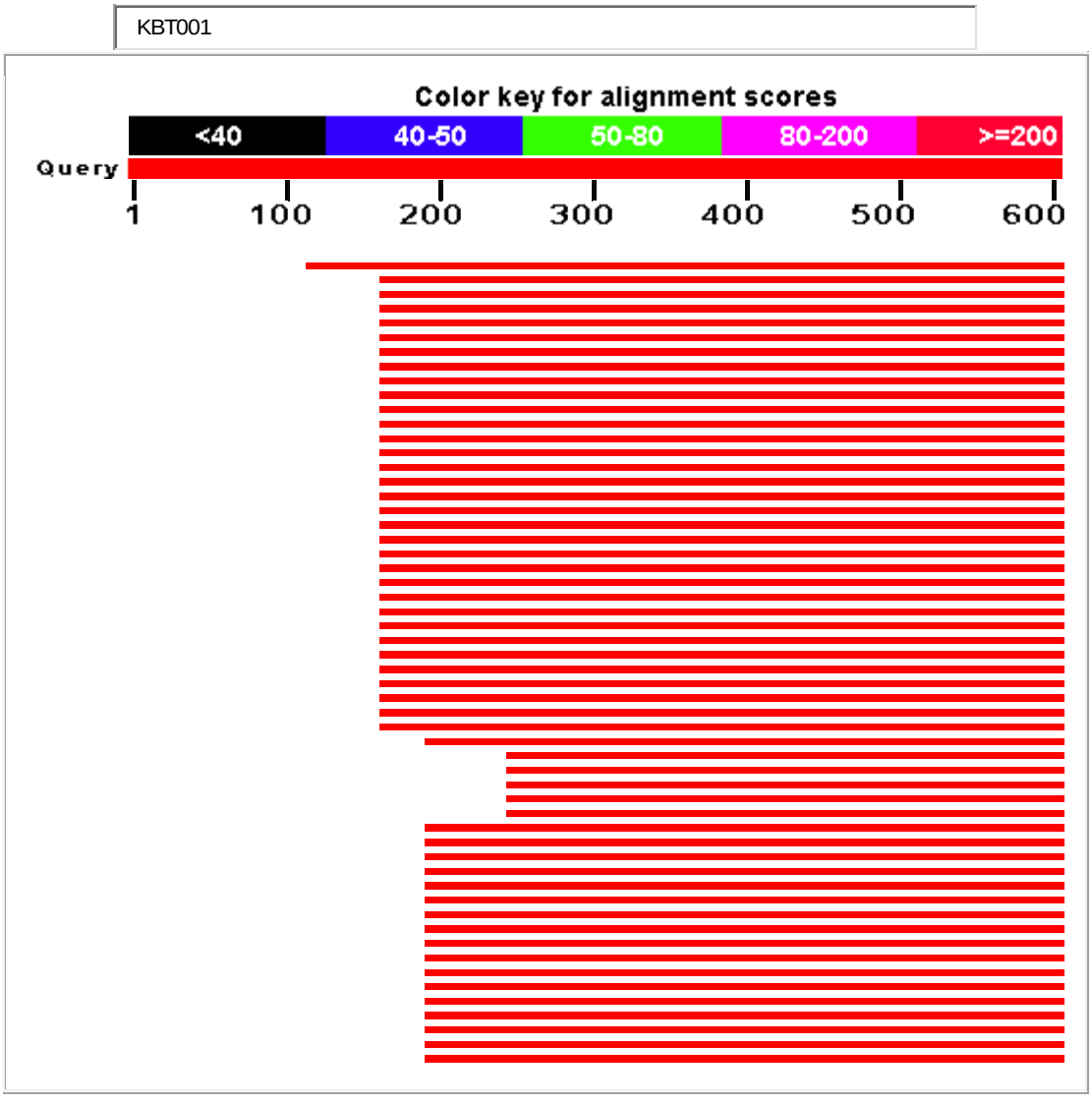
a. NAT001

Distribution of 200 Blast Hits on the Query Sequence



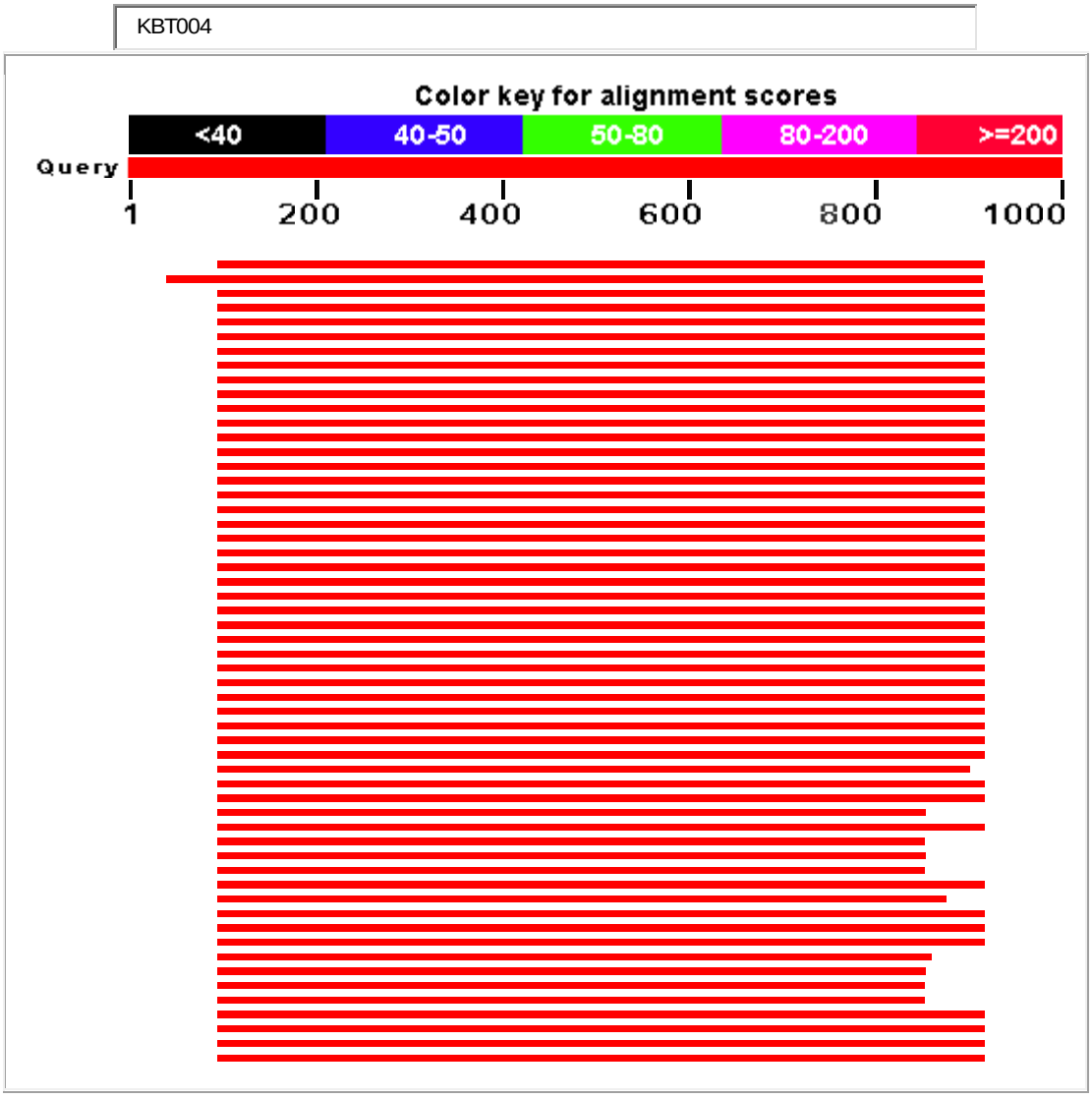
b. KBT001

Distribution of 235 Blast Hits on the Query Sequence



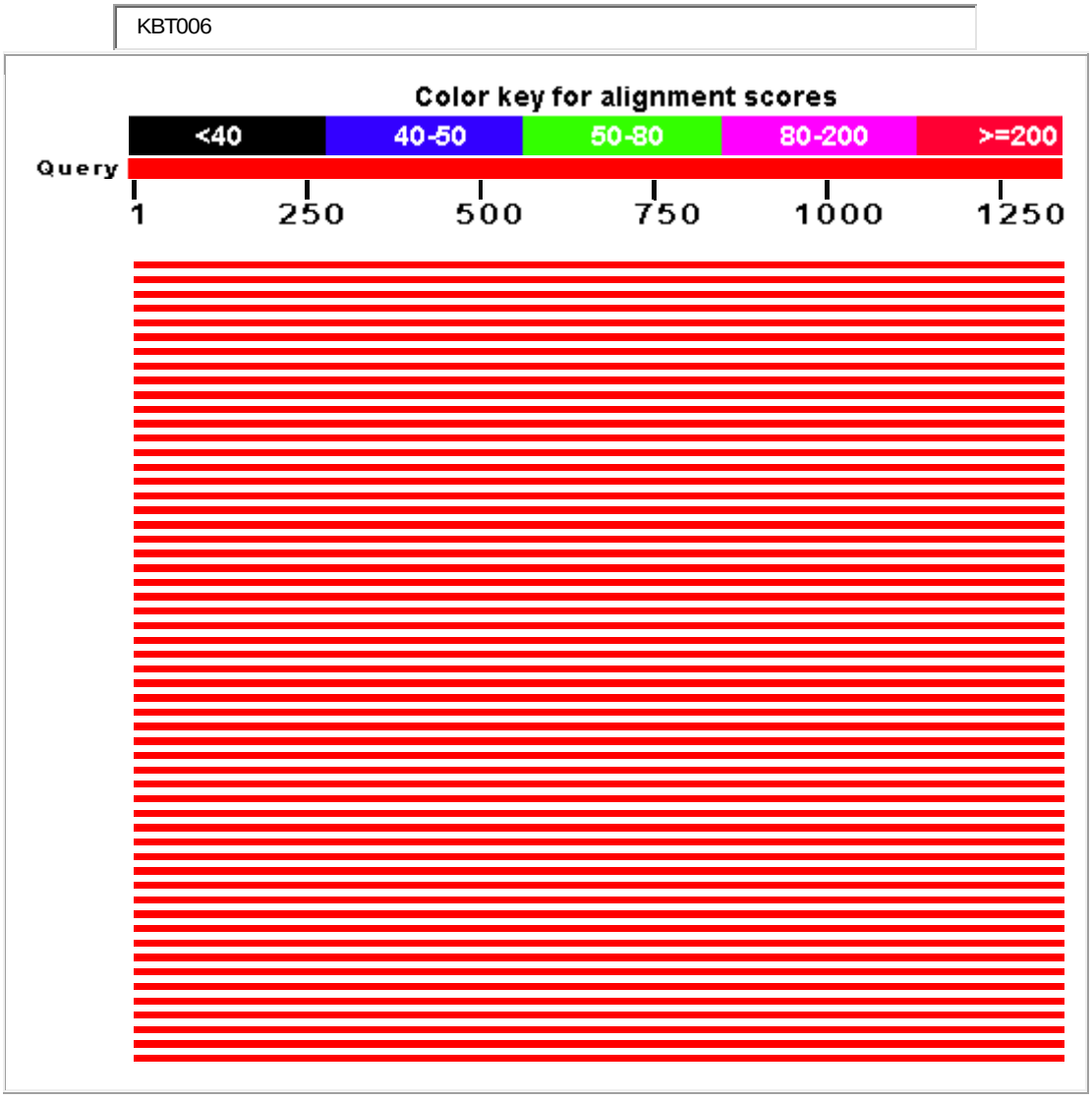
c. KBT004

Distribution of 200 Blast Hits on the Query Sequence



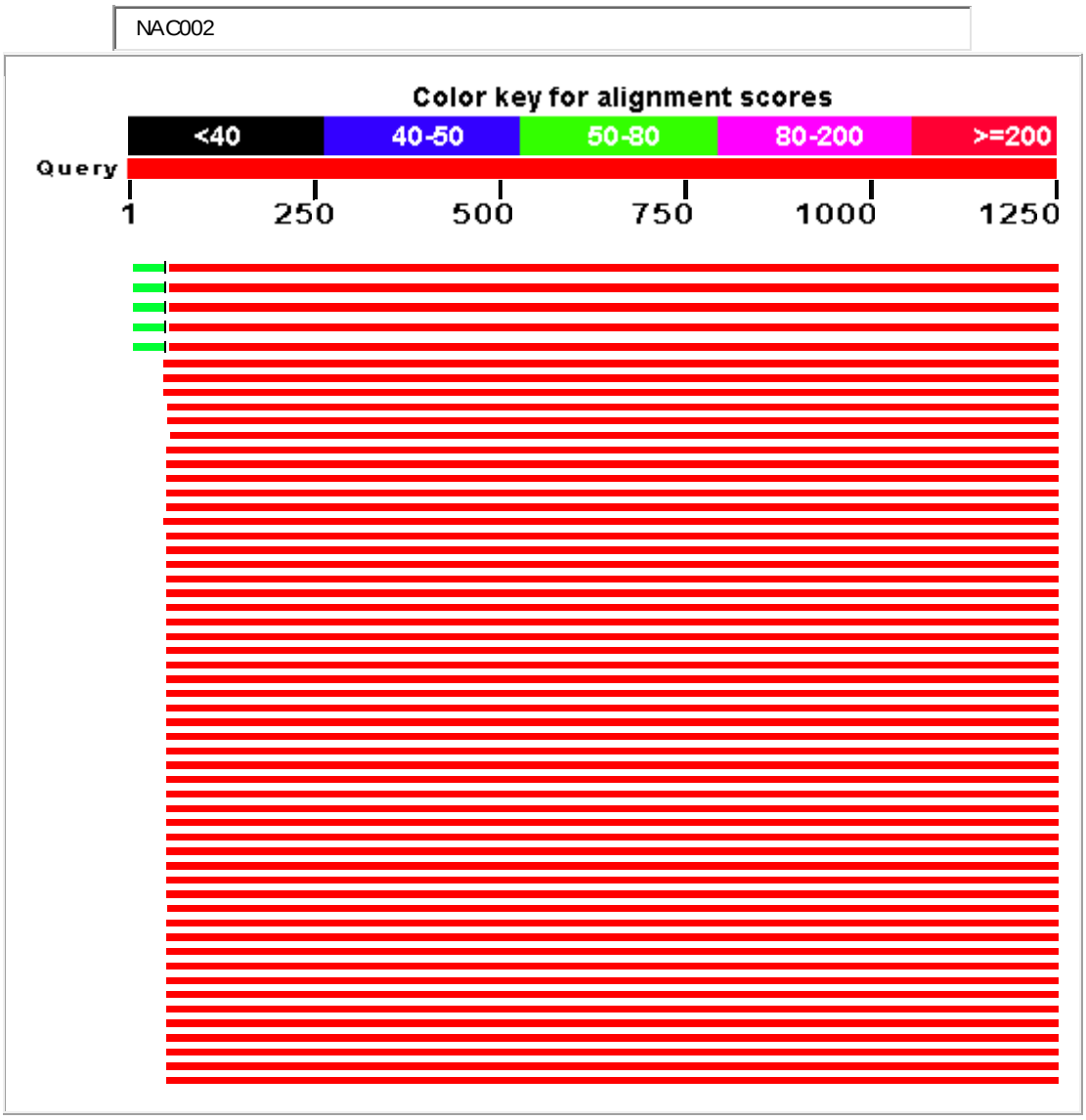
d. KBT006

Distribution of 200 Blast Hits on the Query Sequence



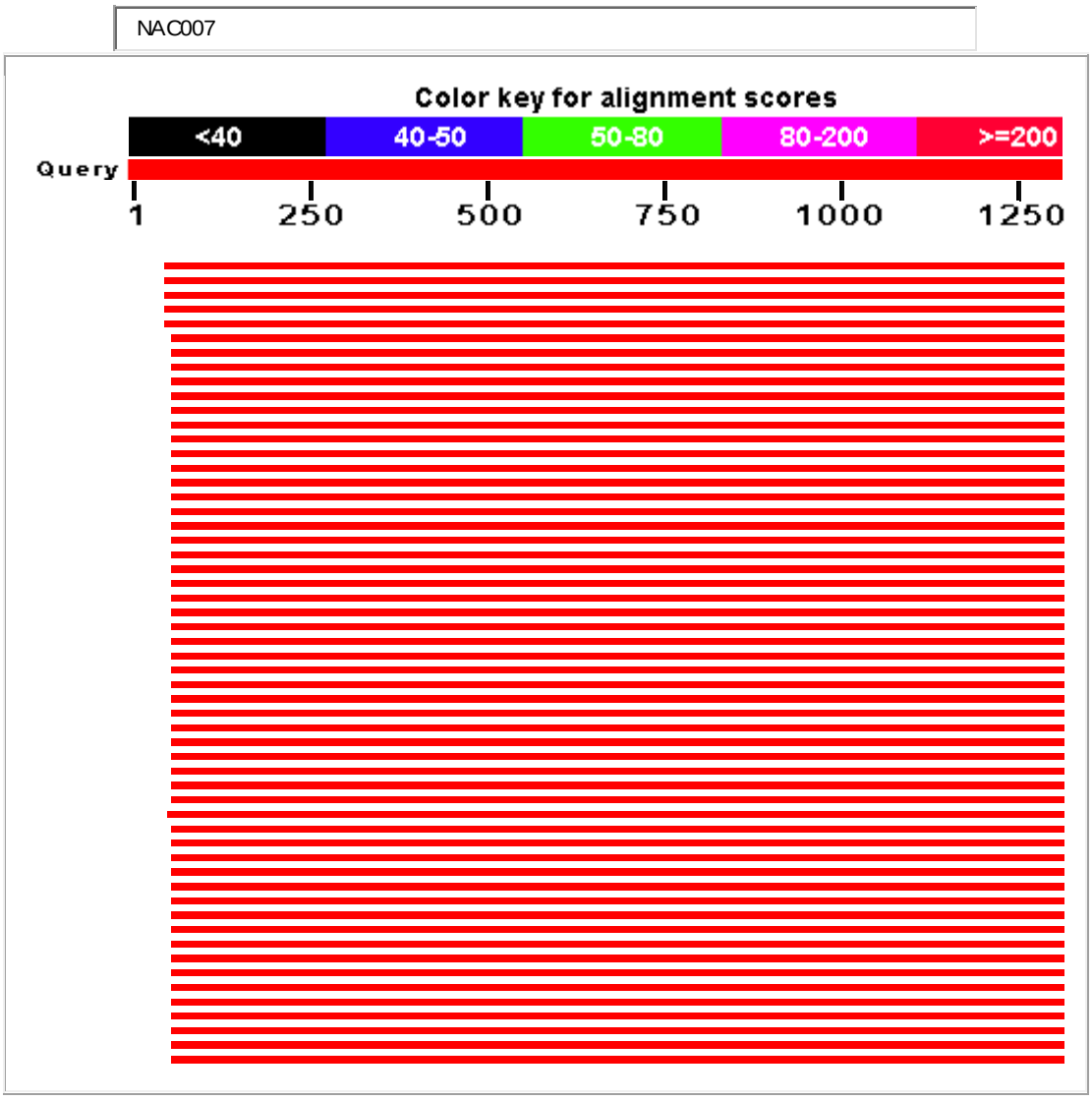
e. NAC002

Distribution of 200 Blast Hits on the Query Sequence



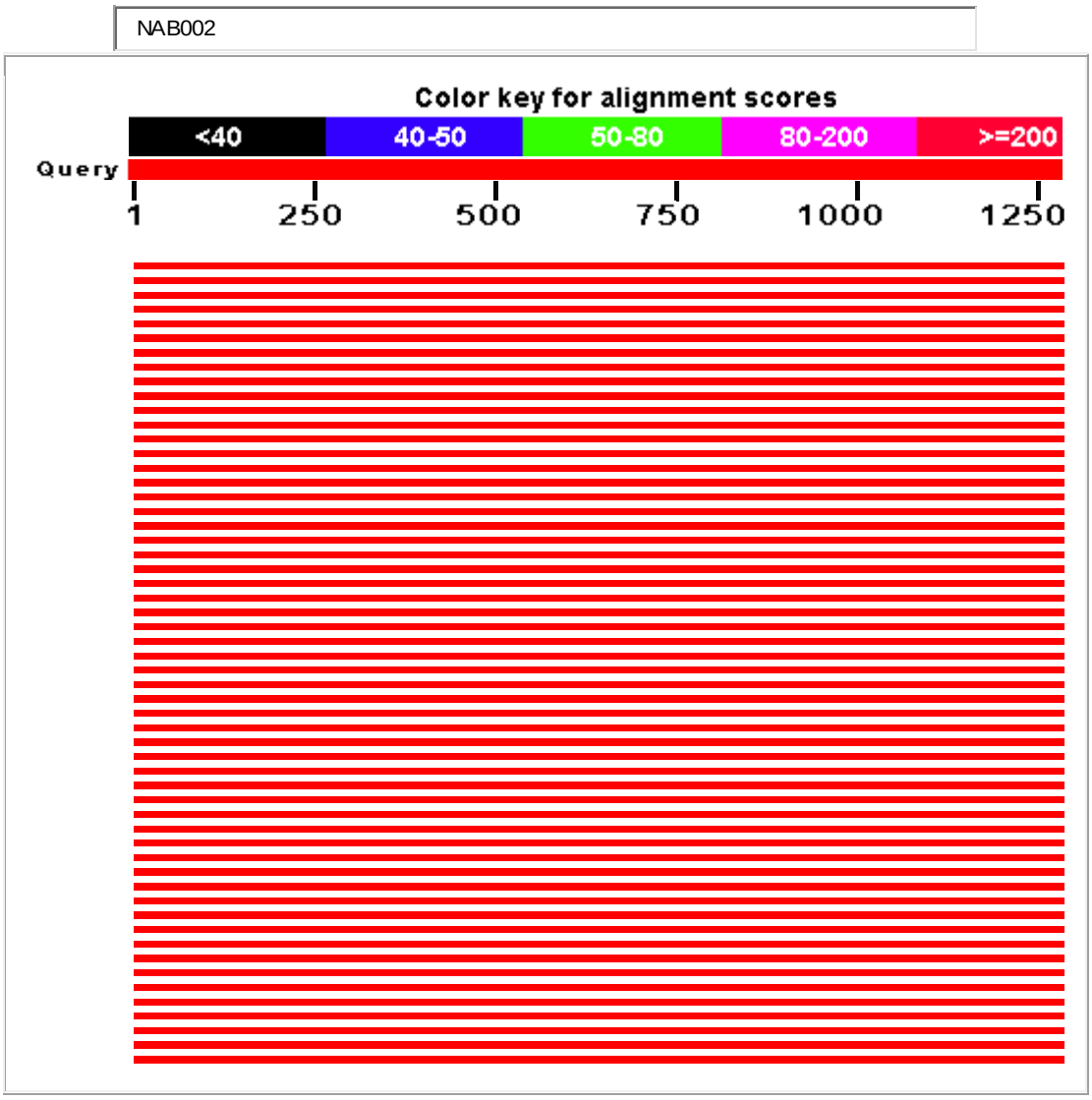
f. NAC007

Distribution of 200 Blast Hits on the Query Sequence



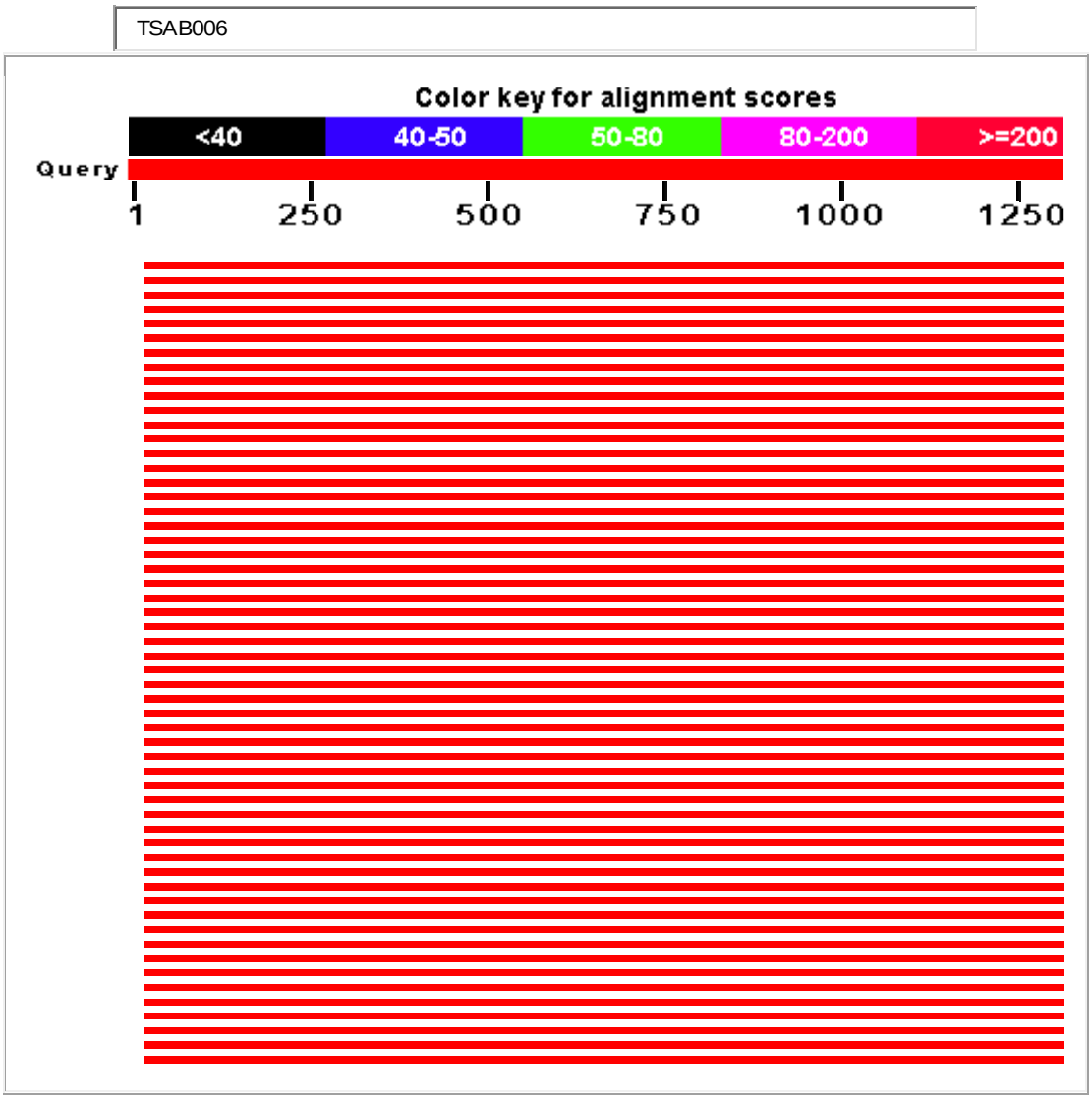
g. NAB002

Distribution of 200 Blast Hits on the Query Sequence



h. TSAB006

Distribution of 200 Blast Hits on the Query Sequence



Abstract

Development of root endophytic plant growth-promoters as bio-inoculants for pro-tray seedlings

by

VYSHAKHI. A. S.

(2013-11-159)

ABSTRACT

Submitted in partial fulfillment of the requirement for the degree of

MASTER OF SCIENCE IN AGRICULTURE

Faculty of Agriculture

Kerala Agricultural University

Department of Agricultural Microbiology

COLLEGE OF AGRICULTURE

VELLAYANI, THIRUVANANTHAPURAM- 695 522

KERALA, INDIA

2016

ABSTRACT

The study entitled "Development of root endophytic plant growth promoters as bio-inoculants for pro-tray seedlings" was conducted at College of Agriculture, Vellayani during the period 2013-15 with the objective to develop microbial root endophytic plant growth-promoters as bio-inoculants in pro-tray seedling production of major solanaceous vegetable crops chilli, tomato and brinjal.

Microorganisms were isolated by triturating the roots of vigorously growing seedlings of tomato, brinjal and chilli after surface sanitization. Bacterial isolates were subjected to a preliminary screening on their respective hosts for plant growth promotion. Seedling vigour was assessed under green house condition in portrays using sterile planting medium. Endophytes with plant growth promoting ability selected through the preliminary screening were assessed under *in vitro* condition using dual culture plate assay for assessing the compatibility with *Piriformospora indica* (Pi). *Piriformospora indica* is a wide host root colonizing endophytic fungus which allows the plants to grow under extreme physical and nutrient stress condition. It belongs to the Sebacinales in Basidiomycota. Eight compatible bacterial endophytes (four from tomato, two from chilli and two from brinjal) were further evaluated for their growth promoting ability individually and in combination with *P. indica*. Bacterial inoculants were provided as seed treatment and the fungal inoculant as additive in the transplant medium. The bacteria were identified as *Bacillus megaterium, Alcaligenes faecalis, Streptomyces leeuwenhoekii, Bacillus pumilus, Bacillus megaterium, Bacillus licheniformis, Bacillus thuringiensis* and *Bacillus thuringiensis* based on 16s rRNA sequence homology.

The plant growth promoting experiments in tomato indicated that the combination treatment of bacterial strain *Streptomyces leeuwenhoekii* with Pi was found to be statistically superior in shoot fresh weight, root fresh weight and root dry weight (1764.54 mg, 332.88 mg/plant and 26.45 mg/plant). Treatment *Alcaligenes faecalis* + Pi was found to be statistically superior in shoot length (15.23 cm) followed by the treatment *Streptomyces leeuwenhoekii* + Pi (14.45 cm). All the treatment were found to be superior over control. Root colonization by *P. indica* was not found to be influenced by the combined application with endophytic bacteria.

By assessing the plant growth promotion in brinjal, significantly higher values with respect to shoot fresh weight, and root fresh weight (844.27 mg/plant, 83.03 mg/plant) were observed with the plant treated with endophytic bacterial isolate. *Bacillus thuringiensis* + *P. indica* showed superior mean height of 6.95 cm which was on par with *Bacillus thuringiensis* (6.74 cm). However the combination treatment of endophytic isolates with *P. indica* showed superior values compared to control.

Analising the efficacy of the endophytic isolates in chilli for plant growth promotion indicated that treatment with the endophytic isolate *Bacillus megaterium* was found to be have significantly superior values in leaf number, shoot length, shoot fresh weight, and shoot dry weight (5.66, 11.93 cm, 855.20 mg/plant and 87.97 mg/plant). All the treatments including the combinations were found to be superior to control.

P. indica has capability to induce resistance against biotic and abiotic stress, including drought, salinity resistance and bacterial, fungal and virus infection in plants. The current experiment suggest that native root endophytic bacteria can be used in combination with *P. indica* as far as plant growth is concerned. Further studies are required to assess the potential of such combinations in combating plant diseases and helping the plant overcome drought, salinity etc.